ORIGINAL ARTICLE

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

Chee Y Ooi,1,2,3 Annie Dupuis,4,5 Lynda Ellis,2 Keith Jarvi,6 Sheelagh Martin,2 Peter N Ray,7,8,9 Leslie Steele,8,9 Paul Kortan,10 Tanja Gonska,1,2 Ruslan Dorfman,7 Melinda Solomon,1,11 Julian Zielenksi,7 Mary Corey,4,5 Elizabeth Tullis,10,12 Peter Durie1,2

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 diagnostically 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 single-organ manifestations such as sino-pulmonary diseases, pancreatitis or obstructive azoosperma.2–5

The majority of these individuals are pancreatic insufficient (PI) CF patients.10 11 As the reference range for sweat chloride was originally defined by evaluating children presenting with classic multi-organ symptoms,12–13 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
limited by the fact that only 23 mutations were designated as CF causing.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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.\textsuperscript{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). The major- ity 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).

**Cystic fibrosis**

**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 major- ity 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,\textsuperscript{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 PAN C, one of 68 (1.5%) in RESP, and nine of 92 (9.8%) among AZOOSP patients. In short, genotyp- ing 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 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

---

**Cystic fibrosis**
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
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. Among undiagnosed symptomatic patients, fewer patients had borderline NPD results, 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 (10.4%) of 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 63%, 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 1  Subject characteristics and outcomes of sweat chloride and NPD testing

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>Age, mean±SD</th>
<th>Gender: male, n (%)</th>
<th>FEV1, % predicted, mean±SD*</th>
<th>Sweat chloride (mmol/L)</th>
<th>NPD: Cl-free+Iso (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Normal</td>
<td>Borderline</td>
</tr>
<tr>
<td>RESP</td>
<td>68</td>
<td>38.9±15.1</td>
<td>19 (27.9%)</td>
<td>80±21</td>
<td>46 (67.6%)</td>
<td>8 (11.8%)</td>
</tr>
<tr>
<td>PANC</td>
<td>42</td>
<td>24.3±12.8</td>
<td>16 (38.1%)</td>
<td>93±16</td>
<td>36 (85.7%)</td>
<td>4 (9.5%)</td>
</tr>
<tr>
<td>AZOOSP</td>
<td>92</td>
<td>34.8±5.3</td>
<td>92 (100%)</td>
<td>94±14</td>
<td>39 (42.4%)</td>
<td>34 (36.9%)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>104</td>
<td>31.7±8.2</td>
<td>51 (49.0%)</td>
<td>91±10</td>
<td>100 (96.2%)</td>
<td>4 (3.8%)</td>
</tr>
<tr>
<td>HETERO</td>
<td>52</td>
<td>38.9±8.6</td>
<td>21 (40.4%)</td>
<td>92±13</td>
<td>41 (78.8%)</td>
<td>11 (21.2%)</td>
</tr>
<tr>
<td>CFPS</td>
<td>64</td>
<td>32.3±12.5</td>
<td>33 (51.6%)</td>
<td>72±25</td>
<td>12 (18.8%)</td>
<td>11 (17.2%)</td>
</tr>
<tr>
<td>CFPI</td>
<td>43</td>
<td>22.5±10.8</td>
<td>31 (72.1%)</td>
<td>64±20</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*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 Gorska et al for additional clinical details.
‡Among the 12/64 (18.8%) CFPS subjects with normal sweat test, all except one had normal NPD: five had previous abnormal sweat test (four with normal 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.

**Table 2 Breakdown of CFTR mutations in all subjects**

<table>
<thead>
<tr>
<th>Groups</th>
<th>N</th>
<th>No of CFTR mutations*</th>
<th>Two CF-causing mutations†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Consensus</td>
<td>CFTR2‡</td>
</tr>
<tr>
<td>RESP</td>
<td>68</td>
<td>37 (54.4%)</td>
<td>15 (22.1%)</td>
</tr>
<tr>
<td>PANC</td>
<td>42</td>
<td>21 (50%)</td>
<td>9 (21.4%)</td>
</tr>
<tr>
<td>AZOOSP</td>
<td>92</td>
<td>12 (13%)</td>
<td>17 (18.5%)</td>
</tr>
<tr>
<td>All undiagnosed symptomatic subjects</td>
<td>202</td>
<td>70 (34.7%)</td>
<td>41 (20.3%)</td>
</tr>
<tr>
<td>CONTROL</td>
<td>104</td>
<td>84 (80.8%)</td>
<td>18 (17.3%)</td>
</tr>
<tr>
<td>HETERO</td>
<td>52</td>
<td>0</td>
<td>47 (90.4%)</td>
</tr>
<tr>
<td>CFPS</td>
<td>64</td>
<td>1 (1.5%)</td>
<td>9 (14.1%)</td>
</tr>
<tr>
<td>CFPI</td>
<td>43</td>
<td>1 (2.3%)</td>
<td>1 (2.3%)</td>
</tr>
</tbody>
</table>

*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.
Cystic fibrosis

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

<table>
<thead>
<tr>
<th>Group</th>
<th>All subjects</th>
<th>Subjects with borderline sweat test and/or NPD results excluded</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Observed agreement</td>
</tr>
<tr>
<td>RESP</td>
<td>68</td>
<td>0.65</td>
</tr>
<tr>
<td>PANC</td>
<td>42</td>
<td>0.55</td>
</tr>
<tr>
<td>AZOOSP</td>
<td>92</td>
<td>0.44</td>
</tr>
</tbody>
</table>

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 exclude 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.

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. 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.

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.

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? 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 fulfilled the criteria for a ‘CFTR-related disorder’.

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

<table>
<thead>
<tr>
<th>NPD</th>
<th>Normal (n=97)</th>
<th>Borderline (n=38)</th>
<th>Abnormal (n=67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sweat chloride</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal (n=121)</td>
<td>73 (60%)</td>
<td>24 (20%)</td>
<td>24 (20%)</td>
</tr>
<tr>
<td>Borderline (n=46)</td>
<td>17 (37%)</td>
<td>10 (22%)</td>
<td>19 (41%)</td>
</tr>
<tr>
<td>Abnormal (n=35)</td>
<td>7 (20%)*</td>
<td>4 (11%)</td>
<td>24 (69%)†</td>
</tr>
</tbody>
</table>

*The seven subjects include one RESP, one PANC and five AZOOSP. t114/24 (58%) were not identified with two CF-causing mutations: 12 carried one CF-causing mutation (DF508/−(x3); DF508S T (x3); DF508B/D1152H; R75X/V562A; 1717IG>AQ1291H; 1717IG>AQ1291H; 1717IG>AQ1291H; 1717IG>AQ1291H; 1717IG>AQ1291H; 1717IG>AQ1291H) 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.
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. 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.

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. Respiratory disease at/after CF diagnosis has also been reported in patients with pancreatitis. In addition, there may be a future role for CFTR-assist therapies in patients with pancreatitis. The female preponderance among RESP and PANC subjects is consistent with previous reports.

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.

Author affiliations
1 Physiology and Experimental Medicine, Research Institute, The Hospital for Sick Children, Toronto, Canada
2 Department of Paediatrics, Division of Gastroenterology, Hepatology and Nutrition, The Hospital for Sick Children, Toronto, Canada
3 Discipline of Paediatrics, School of Women’s and Children’s Health, Faculty of Medicine, University of New South Wales and Department of Gastroenterology, Sydney Children’s Hospital Randwick, Sydney, Australia
4 Child Health Evaluative Sciences, The Hospital for Sick Children, Toronto, Canada
5 Dalla Lana School of Public Health, University of Toronto, Toronto, Canada

Figure 2 Sweat chloride measurements plotted against NPD (ΔCl-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: normal 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.
Division of Urology, Mount Sinai Hospital, Toronto, Canada

7 Departments of Genetics and Genome Biology, Research Institute, The Hospital for Sick Children, Toronto, Canada

8 Department of Paediatric Laboratory Medicine, The Hospital for Sick Children, Toronto, Canada

9 Department of Medical Genetics and Microbiology, University of Toronto, Toronto, Canada

10 Department of Medicine, University of Toronto, Toronto, Canada

11 Department of Paediatrics, Division of Respiratory, Hospital for Sick Children, Toronto, Canada

12 Division of Respiratory, Keenan Research Centre of Li Ka Shing Knowledge Institute, St. Michael’s Hospital, Toronto, Canada

Acknowledgements

The authors would like to thank the many research subjects who gave up time to participate in the project and acknowledge the support and assistance of Louise Taylor, Susan Carpenter, Thora St Cyr, Rachel Paul, Debbie Ryan, Lenny Chong, Lea Spencer, Xiao-Wei Yuan, Qiiju Huang, Satti Beharry and Wan Ip.

Contributors

CYO and PD contributed to the study design, data acquisition, statistical analysis and interpretation and drafting of the manuscript. LE, SM, TG, KJ, CYO and PD contributed to the study design, data acquisition, Lenny Chong, Leia Spencer, Xiao-Wei Yuan, Qiuju Huang, Satti Beharry and Wan Ip.

Funding

PD and ET were supported by research grants from Cystic Fibrosis Canada (formerly Canadian Cystic Fibrosis Foundation) and Genome Canada through the Ontario Genomics Institute as per research agreement 2004-OGI-3-05, the Ontario Research Foundation and from the Lloyd Carr-Harris Foundation. CYO and TG were funded by the Cystic Fibrosis Canada fellowship awards and CYO received a Canadian Child Health Clinician Scientist Program career enhancement award. PD was funded by a Canadian Institutes for Health Research–Ontario Women’s Health Council joint fellowship.

Competing interests

None.

Patient consent

Obtained.

Ethics approval

The research ethics board of The Hospital for Sick Children, St Michael’s Hospital and Mount Sinai Hospital gave authorisation for the study.

Provenance and peer review

Not commissioned; externally peer reviewed.

REFERENCES


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

Chee Y Ooi, Annie Dupuis, Lynda Ellis, Keith Jarvi, Sheelagh Martin, Peter N Ray, Leslie Steele, Paul Kortan, Tanja Gonska, Ruslan Dorfman, Melinda Solomon, Julian Zielenski, Mary Corey, Elizabeth Tullis and Peter Durie


Updated information and services can be found at: http://thorax.bmj.com/content/69/3/254

These include:

Supplementary Material
Supplementary material can be found at:
http://thorax.bmj.com/content/suppl/2013/10/18/thoraxjnl-2013-203832.DC1
http://thorax.bmj.com/content/suppl/2013/10/22/thoraxjnl-2013-203832.DC2

References
This article cites 32 articles, 5 of which you can access for free at:
http://thorax.bmj.com/content/69/3/254#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
Cystic fibrosis (525)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/