Role of *CFTR* mutation analysis in the diagnostic algorithm for cystic fibrosis

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Background: **The cystic fibrosis transmembrane conductance regulator (*CFTR*) gene mutation identification is being used with increased frequency to aid in the diagnosis of cystic fibrosis (CF) in those suspected with CF. Aim of this study was to identify diagnostic outcomes when *CFTR* mutational analysis was used in CF diagnosis. *CFTR* mutational analysis results were also compared with sweat chloride results.**

Methods: **This study was done on all patients at our institution who had *CFTR* mutation analysis over a seven-year period since August 2006.**

Results: **A total of 315 patients underwent *CFTR* mutational analysis. Fifty-one (16.2%) patients had two mutations identified. Among them 32 had positive sweat chloride levels (≥60 mmol/L), while seven had borderline sweat chloride levels (40-59 mmol/L). An additional 70 patients (22.3%) had only one mutation identified. Among them eight had positive sweat chloride levels, and 17 had borderline sweat chloride levels. Fifty-five patients (17.5%) without *CFTR* mutations had either borderline (*n*=45) or positive (*n*=10) sweat chloride results. Three patients with a CF phenotype had negative *CFTR* analysis but elevated sweat chloride levels. In eighty-three patients (26.4%) *CFTR* mutational analysis was done without corresponding sweat chloride testing.**

Conclusions: **Although *CFTR* mutation analysis has improved the diagnostic capability for CF, its use either as the first step or the only test to diagnose *CFTR* dysfunction should be discouraged and CF diagnostic guidelines need to be followed.**

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Original article

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease in the Caucasian population but can occur in other ethnicities.[1] Mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene result in defective chloride transport across epithelial cells in multiple organ systems.[2] Pulmonary manifestations include acute and chronic infections and chronic inflammation leading to progressive lung damage.[1] Since the discovery of the *CFTR* gene in 1989, more than 2000 mutations have been identified.[3,4] The F508del mutation is the most commonly identified mutation in the United States occurring in 90% of patients with CF.[2]

CFTR mutation identification is being utilized with increasing frequency to aid in the diagnosis of CF either in infants with positive CF newborn screen results (usually prior to onset of clinical disease) or in older patients with clinical suspicion for CF. Genetic testing is also used to confirm mutations in those with classic CF disease, as new mutation-specific therapies become available. The large number of identified mutations has made interpretation of *CFTR* test results increasingly challenging. While about 270 of the most common mutations have been well-characterized,[5] the clinical implication of the majority of the remaining ones is not well-described.[4,6]

Diagnosis of CF is straight-forward in patients who present with typical disease manifestations, elevated sweat chloride level and the presence of two disease-causing mutations in *CFTR*.[7] However, some children and adults present with a non-classic clinical picture, borderline sweat chloride results and/or the absence of two known

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disease-causing *CFTR* mutations. It is recommended that in suspected patients, the first test needs to be a sweat chloride test in an accredited CF center; only in cases with an intermediate sweat test, a *CFTR* mutation analysis may be helpful.[7] The aim of this study was to understand the use of *CFTR* mutational analysis for CF diagnosis at our institution with the goal to make sure CF diagnostic guidelines by the Cystic Fibrosis Foundation are followed throughout our institution. The goal was also to understand how the use of *CFTR* mutation analysis can be helpful in the diagnostic evaluation of CF.

Methods

This study was approved by local Institutional Review Board at our Children's Hospital (IRB13-00556). Data from the medical records of all patients who had *CFTR* mutation testing between August 2006 and June 2013 was collected. Entry into the study was based on having a mutation analysis completed. Newborn screening for CF in the state of Ohio was implemented in August 2006. Infants with positive newborn screening for CF are included in this study. While the present study coincides with the implementation of newborn screening for CF in Ohio, the patients in this study include all patients with *CFTR* mutation analysis, regardless of clinical indication. At our institution, *CFTR* diagnostic testing can be ordered by any clinician, including pulmonary (both CF specialists and non-CF providers), gastroenterology, ear, nose and throat, and primary care. *CFTR* gene sequence analysis using Sanger sequencing, deletion/duplication identification and poly T variant status (CF amplified) was sent to an outside laboratory specializing in genetic testing (Ambry Genetics, Aliso Viejo, California). Results were interpreted at Ambry laboratory and confirmed by physicians and genetic counselors at our institution in accordance with the *CFTR*1 (http://www.genet.sickkids.on.ca/CFTR/Home.html) and *CFTR*2 (http://CFTR2.org/) mutation prediction databases.[4] Sweat chloride tests were measured at Nationwide Children's Hospital laboratory, which is accredited by the Cystic Fibrosis Foundation, using the same collecting, processing and analysis techniques approved by the Cystic Fibrosis Foundation. Sweat chloride results that are ≥60 mmol/L are considered positive. Sweat chloride results that are 40-59 mmol/L (or 30-59 mmol/L in infants less than six months of age) are considered borderline. Sweat chloride results that are <40 mmol/L (or <30 mmol in infants less than six months of age) are considered normal.

Based on the results of *CFTR* mutation analysis, patients were classified according to number of mutations and sweat chloride results. For patients with two mutations and those with one mutation and either a borderline or a positive sweat chloride result, additional demographic data such as age and gender were collected. Definition of borderline or positive sweat chloride results were according to the Cystic Fibrosis Foundation Consensus Report Guidelines for Diagnosis of CF.[7] All gene variants regardless of known clinical significance were included. Poly T variants were only included if associated with another mutation or an abnormal sweat chloride result. Mutations were referenced using the *CFTR*1 and *CFTR*2 databases, and those mutations that had been classified in the databases were described accordingly.[4]

A clinical diagnosis of CF is established by the presence of at least one characteristic phenotypic feature of CF and evidence of *CFTR* dysfunction. *CFTR* dysfunction is established by at least one of the following: two disease-causing *CFTR* mutations, two abnormal (>60 mEq/L) quantitative pilocarpine iontophoresis sweat chloride values or abnormal nasal potential difference measurements consistent with CF.[4] The diagnosis of non-classic CF is utilized for individuals who have clinical symptoms of CF in at least one organ system but normal or borderline sweat chloride values.[8]

Results

A total of 315 samples for *CFTR* mutational analysis were sent between August 2006 and June 2013. The median age was 6.1 years (range one month to 65 years) with a male:female ratio of 145:165. Fig. shows the details of mutation analysis results and associated sweat chloride findings based on the number of mutations identified. Fifty (16%) had two mutations and among them five had normal sweat chloride levels (Table 1), 32 had positive sweat chloride levels and additional eight had borderline sweat chloride levels (Table 2). Seventy-two (23%) had one mutation identified. Among them ten had positive sweat chloride levels (Table 3), and an additional 17 had borderline sweat chloride levels. One hundred and ninety-two (61.14%) had no mutations identified, of which ten had positive sweat chloride levels (Table 4), 45 had borderline sweat chloride levels, and 68 did not have sweat chloride tests performed. Results were unavailable in one patient (Fig.). In total, 77 patients (24.5%) had either two mutations identified (n=50) or had one mutation with either a borderline (n=17) or a positive (n=10) sweat chloride level. Thirty-two (42%) of those 77 patients carried a diagnosis of classic CF, and an additional 12 (15.7%) were followed for non-classic CF.

There were 119 (37.8%) patients with either borderline (n=70) or positive (n=52) sweat chloride

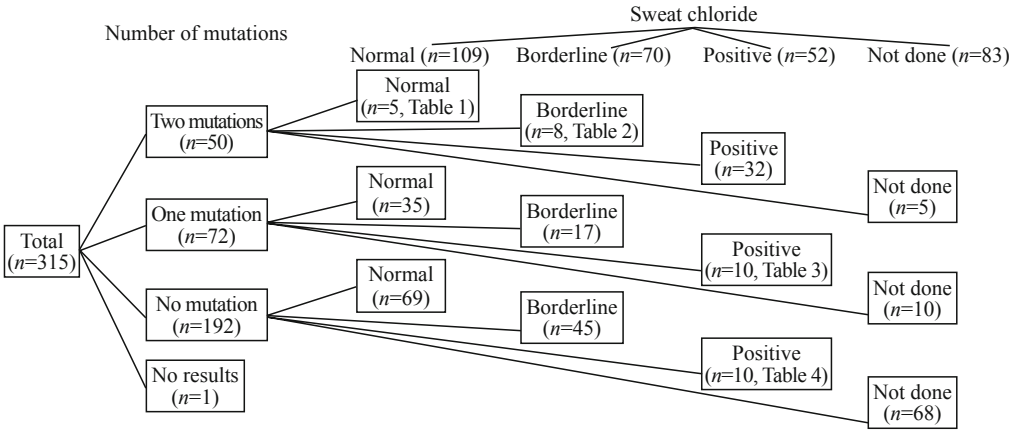

Fig. *CFTR* mutational analysis and sweat chloride test results. *CFTR:* cystic fibrosis transmembrane conductance regulator gene.

Table 1. Patients with two mutations and normal sweat chloride results

Case	Age	Gender	Race	*CFTR* analysis	Sweat chloride (mmol/L)	CF diagnosis	Additional information
1	2 mon	M	B	P718R*, 5'UTR-755C>T†	<10	No	Positive newborn screening, prematurity with bronchopulmonary dysplasia, PS, BMI 59%, colonization: none
2	7 y	M	W	I807M‡, 406-6T>C†	17	No	FEV1=102%, asthma, CXR: normal; no sinusitis, colonization: MSSA; PS, BMI 84%
3	1 mon	F	W	R117H†(7T/9T), L997F‡	13, 30	CRMS	Positive newborn screening, CXR: normal; colonization: MRSA; PS, BMI 52%
4	2 mon	F	W	F508del§, (TG)10-9T/(TG)12-5T†	31, 25	CRMS	Positive newborn screening, CXR: normal; colonization: MSSA; PS, BMI 54%
5	4 mon	F	Bi-racial	164+28A>G†, (TG)12-5T†, 4243-5C>T* (maternal)/R74W†, D1270N† (presumed paternal)	11, 31	Non-classic	Positive newborn screening, CXR: peribronchial thickening; chest CT: normal; colonization: MRSA, PA; PS, BMI 62%

*: not present in *CFTR*1/*CFTR*2 databases; †: mutation of varying consequences (per *CFTR*1/*CFTR*2 databases); ‡: not a disease-causing mutation; §: disease-causing mutation. CF: cystic fibrosis; *CFTR:* cystic fibrosis transmembrane conductance regulator gene; FEV1: forced expiratory volume in 1 second; PS: pancreatic sufficient; BMI: body mass index; PA: *Pseudomonas aeruginosa*; MSSA: methicillin sensitive *Staphylococcus aureus*; MRSA: methicillin resistant *Staphylococcus aureus*; CRMS: *CFTR*-related metabolic syndrome; CT: computed tomography; M: male; F: female; B: black; W: white; CXR: chest X-ray.

Table 2. Patients with two mutations and a borderline sweat chloride

Case	Age	Gender	Race	*CFTR* analysis	Sweat chloride (mmol/L)	CF diagnosis	Additional information
1	2 y	M	W	G576A, R668C (most often found in *cis*)	41, 11	No	FEV1=109%, asthma, PS, BMI 100%, colonization: none
2	3 y	F	W	F508del*, R117H†(7T/9T)	47/51, 41/47	Yes	FEV1=110%, bronchiectasis, sinusitis, colonization: PA, MSSA; PS, BMI 53%
3	5 y	F	W	F508del*, R117H†(7T/9T)	33, 40/45	Non-classic	FEV1=110%, CXR: normal; colonization: MSSA; PS, BMI 25%
4	3 mon	F	W	G551D*, P574H*	50/48, 41/45	Non-classic	Positive new born screening, CXR: normal; colonization: none; PS, BMI 87%
5	1 mon	F	W	F575Y‡, F508del*	37/34, 42/39	CRMS	Positive new born screening, FEV0.5=128%, CXR: normal; colonization: MSSA; PS, BMI 75%
6	44 y	F	W	I507del*, Q1291H§	42/44	Non-classic	FEV1=78%, bronchiectasis, sinusitis, colonization: PA, MSSA; PS, BMI 26%
7	11 y	F	W	R117H†, (TG)12-5T†, (TG)12-7T	58/48, 19/19, 13/14	No	FEV1=109%, CXR: normal; colonization: none; PS, BMI 35%
8	2 mon	F	W	F508del*, R117C*	34, 39	CRMS	FEV0.5=125%, CXR: normal; colonization: PA, *Klebsiella*; PS, BMI 78%

*: disease-causing mutation; †: mutation of varying consequences (per *CFTR*1/*CFTR*2 databases); ‡: not present in *CFTR*1/*CFTR*2 databases; §: not a disease-causing mutation. CF: cystic fibrosis; *CFTR:* cystic fibrosis transmembrane conductance regulator gene; FEV1: forced expiratory volume in 1 second; PS: pancreatic sufficient; BMI: bone mineral index; PA: *Pseudomonas aeruginosa*; MSSA: methicillin sensitive *Staphylococcus aureus*; M: male; F: female; W: white; CRMS: *CFTR*-related metabolic syndrome; CXR: chest X-ray.

levels. Most of these patients (*n*=82) had sweat chloride testing repeated on at least one other occasion to confirm these levels. Among patients who had *CFTR* mutation analysis done (*n*=315), 52 (16%) had positive sweat chloride levels, of which 32 had two mutations, 10 had one mutation (Table 3) and remaining 10 had no mutations identified (Table 4). An additional 70 (22%) had borderline sweat chloride levels, of which eight had two mutations (Table 2), 17 had one mutation and remaining 45 had no mutation identified (Fig.). One hundred and nine (34.7%) patients had normal sweat chloride levels, of which five had two mutations (Table 1), 35 had one mutation and remaining 69 had no mutation identified. The remaining 83 (26.4%) patients had *CFTR* mutation analysis done without a corresponding sweat chloride test.

Fifteen infants with positive newborn screening had *CFTR* mutation analysis done (Table 5), of which four were diagnosed with classic CF, one with non-classical CF and five with *CFTR*-related metabolic syndrome (CRMS). In three patients, CF was ruled out and the remaining one patient relocated and was lost to follow up.

Table 3. Patients with 1 mutation and a positive sweat chloride

Case	Age (y)	Gender	Race	*CFTR* analysis	Sweat chloride (mmol/L)	CF diagnosis	Additional information
1	23	F	W	F508del*	85	Unknown	Lost to follow-up
2	15	M	W	R668C† (TG)11-5T‡/(TG)10-7T	30, 68	No	Anoxic brain injury, tracheostomy, pneumonia on CXR, no sinus disease, colonization: PA, *Klebsiella*; PS, BMI 97%
3	6	M	W	R170H‡	70, 45	No	CXR: pneumonia; colonization: none; PS, BMI 31%, renal transplant
4	42	F	W	F508del*	67, 53, 41	No	CXR: normal; sinusitis+, colonization: none; PS, BMI 20%
5	7	M	W	621+1G>T*	70, 62	Non-classic	FEV1=111%, chronic pancreatitis, CXR: normal; no sinusitis, colonization: MRSA; PS, BMI 39%
6	14	F	B	5'UTR-755C>T§	71, 69	Non-classic	FEV1=126%, CXR: normal; no sinusitis, colonization: MSSA; PS, BMI 96%
7	14	M	W	S549R(T>G)*	57, 64	Non-classic	FEV1=89%, asthma, CXR: normal; no sinusitis, colonization: MSSA; PS, BMI 52%
8	2	M	W	F508del*	72, 46	Non-classic	FEV1=103%, asthma, CXR: normal; no sinusitis, colonization: MSSA; PS, BMI 19%
9	12	F	B	(TG)11-5T‡/(TG)10-7T	87	No	FEV1=85%, CXR: normal; no sinusitis, colonization: none; PS, BMI 96%
10	1 mon	F	W	F508del*	99	Classic CF	Meconium ileus, mild bronchiectasis, no sinusitis, colonization: PA, MSSA; PI, BMI 19%

*: disease-causing mutation; †: not a disease-causing mutation; ‡: mutation of varying consequences (per *CFTR*1/*CFTR*2 databases); §: not present in *CFTR*1/*CFTR*2 databases. CF: cystic fibrosis; *CFTR*: cystic fibrosis transmembrane conductance regulator gene; FEV1: forced expiratory volume in 1 second; PS: pancreatic sufficient; PI: pancreatic insufficient; BMI: body mass index; PA: *Pseudomonas aeruginosa*; MSSA: methicillin sensitive *Staphylococcus aureus*; M: male; F: female; B: black; W: white; CXR: chest X-ray.

Table 4. Patients with negative genetic screening but positive sweat chloride

Case	Age (y)	Gender	Race	*CFTR* analysis	Sweat chloride (mmol/L)	CF diagnosis	Additional information
1	12	M	W	None	71, 53	No	Failure to thrive, FEV1=101%, CXR: normal; no sinus disease, colonization: none; PS, BMI 1%
2	8	F	W	461A>G*	68	No	Failure to thrive, FEV1=127%, CXR: normal; no sinus disease, colonization: none; PS, BMI 15%
3	1	M	W	None	66, 45	No	Failure to thrive, CXR: normal; colonization: none; PS, BMI 15%
4	10	F	Hispanic	None	65, 51	No	Asthma, CXR: normal; colonization: MSSA; PS, BMI 10%
5	8	F	W	(TG)11-5T†	64	No	FEV1=104%, CXR: normal; colonization: none; PS, BMI 50%
6	6	F	W	None	66, 45	No	FEV1=82%, CXR: normal; no sinusitis, colonization: none; PS, BMI 97%
7	6	M	W	None	65, 69	CF	Chronic cough, FEV1=104%, asthma, CXR: normal; chronic sinusitis+, colonization: MSSA; PI, BMI 50%
8	8	M	W	None	58, 85	CF	Recurrent pneumonia, FEV1=111%, CXR: normal; chronic sinusitis, colonization: MSSA; PS, BMI 50%
9	7	F	W	None	62, 60	Non-classical	Chronic cough, FEV1=98%, CXR: normal; chronic sinusitis, colonization: none; PS, BMI 50%
10	39	M	W	None	106, 55	CF	Chronic cough, FEV1=68%, CXR: bronchiectasis; chronic sinusitis, colonization: MSSA; PI, BMI 75%, NPD abnormal

*: not a disease-causing mutation; †: mutation of varying consequences. CF: cystic fibrosis; *CFTR*: cystic fibrosis transmembrane conductance regulator gene; FEV1: forced expiratory volume in 1 second; PS: pancreatic sufficient; PI: pancreatic insufficient; BMI: body mass index; MSSA: methicillin sensitive *Staphylococcus aureus*; NPD: nasal potential difference; M: male; F: female; W: white; CXR: chest X-ray.

Table 5. Patients with abnormal newborn screening who underwent *CFTR* mutational analysis

Case	Age (mon)	Gender	Race	*CFTR* analysis	Sweat chloride (mmol/L)	CF diagnosis	Additional information
1	3	M	W	R1438Q*, (TG)12-5T†/(TG)10-7T	None	Unknown	Lost to follow-up, CXR: normal; PS
2	1	F	W	F508del‡, F575Y*	37/34, 42/39	CRMS	CXR: PBT; colonization: MSSA; BMI 75%, PS
3	2	F	Biracial	F508del‡, Y913X‡	97/100	Yes	CXR: PBT; colonization: PA, MSSA; PI, BMI 66%, FEV0.5=101%,
4	2	F	W	I507del‡, 2622+1G>A‡	98/95	Yes	CXR: PBT; colonization: PA, MSSA; FEV0.5=108, PI, BMI 27%
5	2	M	Biracial	R764X‡, F508del‡	74, 65	Yes	CXR: normal; FEV0.5=92%, colonization: PA, MSSA; PI, BMI 36%
6	2	F	W	F508del‡, R117C‡	34/37, 39/39	CRMS	FEV0.5=125%, CXR: normal; colonization: PA; PS, BMI 78%
7	2	F	W	F508del‡, G542X‡	109/114	Yes	CXR: PBT; colonization: PA, MSSA; PI, BMI 59%
8	2	F	W	G542X‡	39/44, 28/27	No	CXR: normal; colonization: none; PS, BMI 74%
9	2	F	W	F508del‡, (TG)10-9T/(TG)12- 5T†	31/30, 25/18	CRMS	CXR: normal; colonization: MSSA; PS, BMI 54%
10	1	F	W	R117H†(7T/9T), L997F§	13/14, 30/30	CRMS	CXR: normal; colonization: MRSA; PS, BMI 52%
11	2	M	B	5'UTR-755C>T*, P718R*	<10/11	No	CXR: PBT; colonization: none; PS, BMI 59%
12	3	F	W	G551D‡, P574H‡	50/48, 41/45	CRMS	CXR: none; colonization: none; PS, BMI 87%
13	4	F	Biracial	164+28A>G†, (TG)12-5T†, 4243-5C>T* (maternal), R74W†, D1270N† (presumed paternal)	11/10, 31/32	Non-classic CF	CXR: PBT; chest CT: normal; colonization: PA, MRSA; PS, BMI 62%
14	8	M	W	F508del‡	<10/<10	No	CXR: PBT; colonization: acinebacter; PS, BMI 61%
15	9	F	B	E193X‡, N1303K‡	96/95. 85/92	Yes	CXR: none; colonization: MRSA; PI, BMI 93%

Positive sweat chloride: ≥60 mmol/L; borderline sweat chloride: 40-59 mmol/L (or 30-59 mmol/L in infants less than six months of age); normal sweat chloride: <40 mmol/L (or <30 mmol in infants less than six months of age). In Europe cut off for normal sweat chloride test is 30 for all and sweat chloride between 31-59 mmol/L are considered borderline. *: not present in *CFTR*1/*CFTR*2 databases; †: mutation of varying consequences (per *CFTR*1/*CFTR*2 databases); ‡: disease-causing mutation; §: not a disease-causing mutation. CF: cystic fibrosis; *CFTR*: cystic fibrosis transmembrane conductance regulator gene; FEV1: forced expiratory volume in 1 second; PS: pancreatic sufficient; PI: pancreatic insufficient; BMI: body mass index; PBT: peribronchial thickening; PA: *pseudomonas aeruginosa*; MSSA: methicillin sensitive staph aureus; CRMS: *CFTR*-related metabolic syndrome; MRSA: methicillin resistant *Staphylococcus aureus*; CT: computed tomography; M: male; F: female; B: black; W: white; CXR: chest X-ray.

Discussion

Diagnosing CF in certain clinical scenarios can be relatively straightforward in the typical clinical setting and evidence of *CFTR* dysfunction, either by elevated sweat chloride (≥60 mmol/L) and/or two *CFTR* mutations or abnormal nasal potential difference.[7] However, there are some patients who do not fit the standard criteria but have some evidence of either CF disease or *CFTR* dysfunction.[5,6,8] For diagnostic purposes, it is recommended that sweat chloride should be the first test for *CFTR* dysfunction in suspected patients and *CFTR* mutation analysis should be done if sweat chloride testing is inconclusive. Our group with one identified mutation and a positive sweat chloride levels (Table 3) serves as an example of patients who would have been missed if *CFTR* mutation analysis is used in place of sweat chloride testing. This also illustrates that despite the extensive number of mutations in *CFTR* that have been identified, there are still abnormalities that can lead to classic CF manifestations that have not yet been identified, which highlights the potential limitation of *CFTR* mutation analysis in making a diagnosis of CF. Some of the cases also illustrate the questionable use of *CFTR* mutation analysis as the patient lacked any obvious clinical symptoms of CF.

Our group with no mutations and a borderline or positive sweat chloride is an example of patients who would have been missed if *CFTR* mutation analysis alone had been relied upon to confirm a diagnosis of CF (Table 4). This group of patients is a primary reason why genetic screening should be reserved for the patients with clinical suspicion of CF and inconclusive sweat chloride testing and should not be used either as an initial or as the only diagnostic testing for *CFTR* dysfunction. In our review of 315 patients, 83 (26%) had *CFTR* mutation analysis done without a sweat chloride test, and in this group CF was excluded based only on results of *CFTR* mutation analysis. This is not consistent with diagnostic guidelines as some patients with CF can be missed if *CFTR* mutation analysis is used as the only diagnostic test for *CFTR* dysfunction. This suggests that many healthcare providers are not aware of the CF diagnostic guidelines and are relying on *CFTR* mutation analysis either as the first or the only diagnostic test for CF, which is not consistent with the CF diagnostic guidelines and such practice should be discouraged. Our basic assumption of this study was to identify these deviations from the guidelines. This emphasizes area for potential improvement, stressing the need for clinical evaluation at a CF center in children

in whom a diagnosis of CF is being considered prior to ordering genetic testing and/or sweat chloride testing.

The implementation of newborn screening allows for early detection of affected individuals. However, a small percentage of affected individuals, especially those with a pancreatic-sufficient, non-classical presentation, will not be detected by newborn screening. Because of this, a negative newborn screening should not exclude CF from consideration as a diagnosis in the appropriate clinical setting. Kirk[9] looked at sweat chloride results in a population of infants identified by newborn screen in Scotland and identified newborns with two mutations and normal sweat chloride levels, while other infants had no identified *CFTR* mutations but had CF confirmed by abnormal sweat chloride levels, affirming the continuing role of sweat chloride testing in diagnosis of CF.

In our cohort, the group with two mutations and a borderline sweat chloride (Table 2) illustrates the potential limitations of sweat chloride testing and shows that genetic testing can help confirm a diagnosis of CF in a patient with clinical findings consistent with CF but non-diagnostic sweat chloride testing. Similarly, the group with two mutations and normal sweat chloride levels (Table 1) also highlights the benefits of *CFTR* mutation analysis. Identification of two mutations in patients with a clinical history suspected of CF but normal sweat chloride levels allowed for a diagnosis of non-classic CF. On the other hand, identification of two mutations in asymptomatic patients with positive newborn screening but normal sweat chloride levels allowed for a diagnosis of CRMS. The initiation of preventive therapies, close monitoring at a CF center and follow-up over time may benefit these patients and prevent complications.

The use of *CFTR* mutation analysis without proper interpretation also has its pitfalls. In some cases *CFTR* mutation analysis is ordered by health care providers for non-specific pulmonary symptoms such as chronic cough or uncontrolled asthma, before a sweat chloride test is even performed (#2, Table 1). This is further illustrated by mutations like G576A and R668C, which do not meet diagnostic criteria for CF as these mutations are typically located on the same chromosome and therefore act as a single mutation (Table 2, patient 1 - parents were not available to confirm *cis* configuration). However, if interpretation of these results were left to someone without access to CF-specific genetic counseling, this might lead to a false positive diagnosis or at least significant patient and parental anxiety until the patient was evaluated at a CF center. In these cases, a negative sweat chloride level is very reassuring.

At our institution, *CFTR* mutation analysis can be ordered by any physician with the majority of the tests being ordered by pulmonary, gastroenterology, otolaryngology or primary care services primarily for suspected sinu-pulmonary and gastrointestinal symptoms of disease. With other services ordering the genetic testing, some patients are not evaluated by the pulmonary service, which may in part be responsible for unnecessary genetic testing without following diagnostic guidelines. In this cohort CF was excluded in many (n=83, 26.4%) based only on results of *CFTR* mutation analysis without even a sweat test. Of note, many of these patients did not have an evaluation or counseling by a CF specialist neither pre-testing nor post-testing. It is possible that the diagnosis of CF could have been missed in some of these patients. On the other hand, clinicians should also keep in mind that cost of a sweat chloride test is approximately $200-$250 at most CF centers whereas *CFTR* sequence analysis and deletion/duplication studies can cost $3000 or more. Many health care insurance companies may not fully reimburse, potentially adding significant cost to the families. Specifically in the 83 patients without sweat chloride testing, the cost of *CFTR* mutation analysis as a whole was ~$250 000. Comparatively, the cost of sweat chloride for this cohort would have been ~$17 000 with many (if not nearly all) likely having normal testing, making *CFTR* mutation analysis not indicated. Further, within this cohort, 111 patients with a negative sweat chloride test had *CFTR* mutation analysis, costing an additional ~$330 000, many of whom did not meet clinical diagnostic criteria. Stricter utilization of genetic testing could have saved >$500 000 in healthcare costs at our institution over this time period.

The findings of this study further illustrate that the first diagnostic test should continue to be a sweat chloride test and not *CFTR* mutation analysis. If sweat chloride testing is non-diagnostic (either borderline or normal) and clinical disease is suggestive of CF, then *CFTR* mutation analysis can be more useful in diagnosis. In these cases we also recommend that if *CFTR* mutation analysis is indicated, these patients would also benefit from a referral to a CF center prior to proceeding. In this case, the appropriate need for such testing can be determined and, if needed, genetic counseling would be available to help families understand the results of testing and its impact on their families. *CFTR* mutation analysis as an initial diagnostic test can lead to many diagnostic dilemmas thus complicating the diagnosis and causing more anxiety for many families, and such counseling may not be feasible in a primary care setting due to the comfort level of providers. Specifically at our institution, since this testing can be ordered by any clinician, we have recommended further development of institution-level checkpoints in the test ordering process to vet the appropriateness of *CFTR* mutation analysis in individual cases, including requirement of a baseline sweat chloride

test and possibly a clinical evaluation by the CF center.

This study highlights the complexity of the diagnosis of CF and the importance of knowing the limitations of the diagnostic studies testing commonly used. *CFTR* mutation analysis should not replace sweat chloride testing or the importance of a careful correlation of clinical symptoms when making the diagnosis of CF. A sweat chloride test should be part of the evaluation for any child in whom a diagnosis of CF is being considered prior to *CFTR* mutation analysis.

In conclusion, although *CFTR* mutation analysis has improved the diagnostic evaluation of patients suspected to have CF, sweat chloride testing continues to have its primary role. Sweat chloride testing is less complex and less expensive to perform than genetic testing, affirming it as the better initial test.

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