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## Diagnosis of Cystic Fibrosis in the Kindred of an Infant With *CFTR*-Related Metabolic Syndrome: Importance of Follow-Up that Includes Monitoring Sweat Chloride Concentrations Over Time

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**Summary.** Newly implemented newborn screening (NBS) programs in California have resulted in a large subset of patients in whom at least two cystic fibrosis transmembrane conductance regulator (*CFTR*) mutations are identified, but subsequent sweat chloride analysis reveals normal or indeterminate values. These patients are diagnosed with *CFTR*-Related Metabolic Syndrome (CRMS). However, the natural progression and management of these patients are not clearly understood and frequently after the age of 1-year these patients are lost to follow-up with Cystic Fibrosis (CF) Centers. We present the first case of an infant who was referred to Miller Children's Hospital for a NBS positive for CF and subsequent discovery of identical mutations in six of his seven older brothers. Several siblings had positive sweat chloride results on repeat testing after the age of 3 years. We suggest the need for continued follow-up of CRMS in a CF center with diagnostic evaluation including repeat sweat chloride testing, beyond the currently recommended period. *Pediatr Pulmonol.* 2014; 49:E103–E108. © 2013 Wiley Periodicals, Inc.

**Key words:** cystic fibrosis; *CFTR* Mutations; sweat chloride testing.

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### INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene that cause chronic sinopulmonary infections and inflammation. Diagnosis is confirmed by clinical manifestations and sweat chloride (SC) analysis.<sup>1</sup> Newborn screening (NBS) utilizing immunoreactive trypsinogen (IRT) has allowed for earlier detection of infants with potential CF. Since 2007, infants with elevated IRT in the state of California, undergo *CFTR* mutation analysis by 40-mutation panel with reflex to sequencing (if only one mutation is detected) and SC testing

to confirm the diagnosis.<sup>2</sup> Those with two *CFTR* mutations *in trans* and positive SC are expected to have CF. Those with intermediate SC and one mutation or normal SC and two mutations *in trans* are considered *CFTR*-related metabolic syndrome (CRMS).<sup>3</sup> To monitor for development of CF, SC testing is repeated at two and 6 months of age.<sup>3</sup> However, the natural history of these infants and the progression of *CFTR* dysfunction (as reflected by SC), beyond age 6 months remains ambiguous.<sup>4</sup> We present a family where evaluation of the youngest in a sibship of eight led to the discovery of identical *CFTR* genotype in seven, three with positive SCs upon repeat testing.

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**TABLE 1A—Description of the *CFTR* Genotype, Age, and Results of Sweat Chloride Testing in the Index Case (Patient A) and his Seven Siblings (Patients B through H)**

	<i>CFTR</i> mutations	IRT results (ng/ml)	Age (months)	Sweat chloride* (mMol/L)	Sweat volume (μl)	Date performed
Patient A	I507del/(TG)12-5T	96.9	2	24	75	11/2009
DOB: 9/2009			4	20	30	01/2010
			12	30	60	09/2010
Patient B	I507del/(TG)12-5T	N/A	40	None induced	0	07/2010
DOB: 3/2007			<b>42</b>	<b>71</b>	25	09/2010
Patient C	I507del/(TG)12-5T	N/A	60	45	25	07/2010
DOB: 7/2005			<b>63</b>	<b>76</b>	15	10/2010
Patient D	I507del/(TG)12-5T	N/A	75	45	30	07/2010
DOB: 4/2004			78	26	30	10/2010
			89	42	30	09/2011
Patient E	I507del/(TG)12-5T	N/A	93	50	15	02/2011
DOB: 5/2003			<b>98</b>	<b>64</b>	60	07/2011
Patient F	I507del/(TG)12-5T	N/A	125	32	15	06/2011
DOB: 1/2001			140	52	15	09/2012
Patient G	I507del/(TG)12-5T	N/A	165	45	60	06/2011
DOB: 9/1997						
Patient H	Wild type/(TG)12-5T	N/A	Not performed	Not performed	N/A	N/A
DOB: 3/1995						

Positive sweat chloride results are bolded. \*All sweat testing was performed via quantitative pilocarpine iontophoresis. DOB, date of birth; IRT, immunoreactive trypsinogen.

## CASE PRESENTATION

Patient A was born at term and immediately developed pneumothorax, requiring a 5-day neonatal intensive care unit hospitalization. He was referred to our CF center at 7 weeks due to a positive NBS. IRT was 96.9 ng/ml, sequencing/rearrangement testing revealed I507del and the intron 8 variant (TG) 12-5T, which has been reported as a CF-causing mutation<sup>5</sup>; initial SC was normal (24 mMol/L) (Table 1A). Sweating was induced via pilocarpine iontophoresis and the Macroduct coil collection method was used. In accordance with the CF Foundation guidelines for infants up to and including 6 months of age, SC results of 0–29 mMol/L was considered normal, 30–59 mMol/L intermediate and 60 mMol/L or greater positive or indicative of CF. For individuals older than 6 months of age, SC results of 0–39 mMol/L was considered normal, 40–59 mMol/L intermediate and 60 mMol/L or greater positive or indicative of CF.<sup>6</sup>

### ABBREVIATION:

BAL	bronchoalveolar lavage
CF	cystic fibrosis
<i>CFTR</i>	cystic fibrosis transmembrane conductance regulator
CRMS	cystic fibrosis transmembrane conductance regulator metabolic syndrome
IRT	immunoreactive trypsinogen
NBS	newborn screen
NPD	nasal potential difference
SC	sweat chloride
PS	pancreatic sufficiency

Parental testing revealed that mom carried I507del, and identified paternal homozygosity for (TG)12-5T. The father is clearly fertile and has never had any symptoms of CF; he has never had a sweat test.

By age three, patient A was hospitalized four times for respiratory symptoms. Table 1B describes his sputum culture, bronchoalveolar lavage (BAL) samples and chest roentgenogram. Bronchoscopy at age 17 months revealed mucus plugs and airway inflammation. Since his last hospitalization he remains well with minimal respiratory issues. He remains pancreatic sufficient (PS) (fecal elastase > 500 μg/g) and has had no other organ system involvement. He has had two additional normal SC analyses (20 and 30 mMol/L).

The other brothers were born prior to the implementation of NBS for CF in California in 2007. The three youngest (patients B, C, and D) had histories of recurrent respiratory symptoms but were never referred for evaluation of CF. After evaluating patient A, we assessed patients B, C, and D with *CFTR* sequencing/rearrangement and SC testing. All three had genotypes identical to patient A and either positive (≥60 mMol/L) or intermediate (40–59 mMol/L) SC results (Table 1A).

Patient B has been hospitalized three times for abdominal pain. GI work-up revealed only GERD. He remains PS (fecal elastase > 500 μg/g). Patient C has been hospitalized once for abdominal pain and sinusitis. Patient D had a clinical course similar to patient B, which included hospitalizations for abdominal pain and GI work-up notable for GERD. The results of sputum culture

TABLE 1B—Clinical Characteristics for Patients A—H

	Sputum cultures	Bronchoscopy findings	BAL cultures	Chest X-ray findings	CT chest findings	Hospitalizations	Pulmonary function test
Patient A	<i>S. aureus</i> <i>H. influenzae</i> <i>Enterobacter</i>	Thick mucus plugs bilaterally. Diffuse airway inflammation	Light growth normal upper respiratory tract flora	2010—basilar pneumonia 2010—minimal changes of cystic fibrosis 2011—new infiltrate in right middle lobe	None	4 hospitalizations for pulmonary exacerbations	
Patient B	<i>S. aureus</i> <i>H. influenzae</i>	Moderate airway inflammation bilaterally. Diffuse mucus impaction	2010—moderate growth moraxella catarrhalis 2011—penicillium species	Perihilar peribronchial thickening	Hyperexpansion, mild diffuse peribronchial thickening, scattered atelectasis	4 hospitalizations for abdominal pain	
Patient C	<i>S. aureus</i> <i>H. influenzae</i>	2010—Moderate mucus plugs bilaterally 2012—Minimal airway inflammation, no mucus plugs	2010—moderate growth normal upper tract flora, rare growth aspergillus niger 2012—rare growth normal upper respiratory tract flora	Normal chest	None	1 hospitalization for abdominal pain and sinusitis	Age: 6 years Ht: 111 cm, Wt: 22 kg FVC (% predicted) 136 FEV <sub>1</sub> (% predicted) 134 FEF <sub>25-75</sub> (% predicted) 129
Patient D	<i>S. aureus</i> <i>Candida albicans</i>	2010—Erythema of the airways, minimal mucus hypertrophy. 2011—Adenoidal hypertrophy. Minimal mucus plugs	2010—no growth 2011—s. aureus, h. influenzae	2010—hyperexpansion 2011 (AXR)—retained stool	None	3 hospitalizations for abdominal pain and constipation	Age: 7 years Ht: 119 cm, Wt: 21.8 kg FVC (% predicted) 118 FEV <sub>1</sub> (% predicted) 104 FEF <sub>25-75</sub> (% predicted) 78
Patient E	<i>S. aureus</i> <i>Klebsiella</i> <i>H. influenzae</i>	Mild airway inflammation, no mucoid impaction	2011—no growth	Mild hyperexpansion	None	None	Age: 8 years  Ht: 124.5 cm, Wt: 27.4 kg FVC (% predicted) 118 FEV <sub>1</sub> (% predicted) 108 FEF <sub>25-75</sub> (% predicted) 79
Patient F	<i>S. aureus</i>	None	None	Increased peribronchial markings	Nonspecific soft tissue structure w/n anterior mediastinum, bronchi thickening	None	Age: 11 years

(Continued)

TABLE 1B. (Continued)

	Sputum cultures	Bronchoscopy findings	BAL cultures	Chest X-ray findings	CT chest findings	Hospitalizations	Pulmonary function test
Patient G	<i>S. aureus</i> <i>Candida albicans</i>	Minimal airway inflammation, no mucus plugs	no growth	None	None	None	Ht: 143.5 cm, Wt: 51.5 kg FVC (% predicted) 111 FEV <sub>1</sub> (% predicted) 104 FEF <sub>25-75</sub> (% predicted) 101 Age: 15 years
Patient H	No growth	None	None	None	None	None	Ht: 172 cm, Wt: 61.8 kg FVC (% predicted) 142 FEV <sub>1</sub> (% predicted) 128 FEF <sub>25-75</sub> (% predicted) 126

FVC, forced vital capacity; FEV<sub>1</sub>, forced expiratory volume in 1 sec; FEF<sub>25-75</sub>, forced expiratory flow 25-75%; TLC, total lung capacity; RV, residual volume.

and BAL samples, as well as imaging and bronchoscopy findings, for the three brothers are described in Table 1B. Further evaluation with SC and *CFTR* mutation panel was performed for the remaining brothers. Three (patients E, F, and G) were found to have an identical genotype to patient A; patient H does not carry I507del. Patients E, F, and G have never been hospitalized but have had intermittent respiratory symptoms previously managed as acute asthma exacerbations. Baseline spirometry demonstrated stable lung function and their sputum cultures, imaging, and bronchoscopy findings are also described in Table 1B. Patient H has never been hospitalized; his medical history is negative for pulmonary, GI or other CF-related symptoms, as would be expected by his genotype.

## DISCUSSION

Since the discovery of *CFTR* over 1,900 mutations have been reported,<sup>7</sup> but limited phenotypic information is available on most mutations. The incorporation of *CFTR* testing into NBS has resulted in a population of patients with a genetic diagnosis of disease without supporting clinical evidence. It remains unknown whether those with CRMS will subsequently develop CF, and what may be the associated predisposing factors. It is likely that both environmental influences and as-yet unidentified genetic modifiers play a role in the progression. This uncertainty has led to exploration of other modalities (lung clearance index, nasal potential difference (NPD), etc.) for earlier detection of subtle changes in *CFTR* dysfunction.<sup>8,9</sup> However, given limited genotype-phenotype correlations and the variability of alternate modalities, a CF diagnosis remains based on widely accepted clinical diagnostic criteria that includes SC testing, the gold standard for diagnosis.<sup>3,6,8,10-12</sup>

In this sibship of eight, seven have identical genotypes. This seemingly unlikely statistical occurrence is partially explained by the father's genotype, such that each conception had a 50% chance rather than the 25% chance typically seen in autosomal recessive inheritance. We are unable to explain why seven out of eight inherited I507del. There is no family history to suggest any chromosomal rearrangements that may favor inheritance of the I507del allele.

We believe that patients A-G have CF. Patients B, C, E have positive SC results, recurrent GI, and respiratory illness poorly controlled on prior non-CF directed therapy in addition to two *CFTR* mutations. Patients D, F, G have intermediate SC results in addition to two *CFTR* mutations, recurrent symptoms, and family history in regards to their siblings' CF diagnoses.

Patient A is an example of how the reliance on SC analysis allows for a subset of infants to potentially be dismissed from further evaluation and treatment of CF

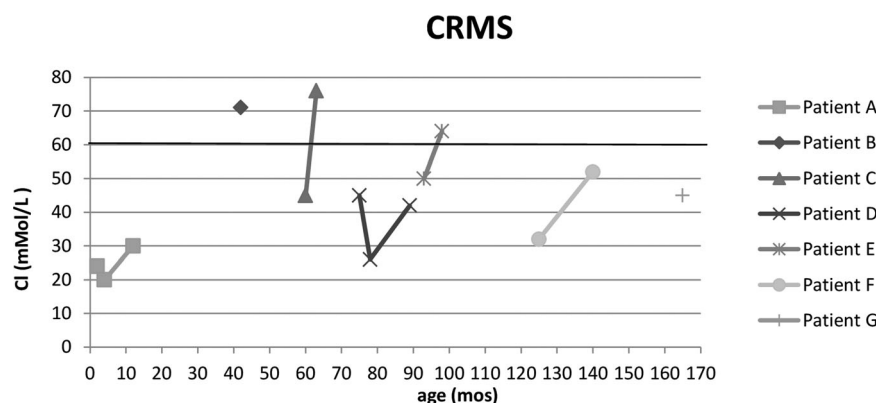


Fig. 1. Sweat chloride level and ages for patients A–H.

due to an initial diagnosis of CRMS. He benefited from the rare identification of siblings with identical genotypes who subsequently showed positive SC results on repeat testing performed after 40 months. He will continue to be followed at our CF Center and will have annual SC testing with the expectation that values will rise with time and concomitant changes to *CFTR* dysfunction, as was demonstrated by his brothers (Fig. 1).

Patient A received salt supplementation during his SC testing. We believe that salt intake at the time of testing allowed for optimal collection of SC results. CF patients are at higher risk for dehydration due to the loss of salt in their sweat and since dehydration is a known cause of falsely elevated SC levels, continuing salt supplementation would allow for a lower incidence of false positive results for patient A.

Patients A–E are followed in our CF center 4×/year with cultures at each visit. They continue on twice daily airway clearance regimen with albuterol, hypertonic saline, VEST, and pulmozyme daily. Annual labs are obtained inclusive of vitamin and IgE levels. Repeat pancreatic elastase levels are also continually monitored, particularly if they present with any new or changing GI symptoms. Patients F and G are also now seen in our clinic 4×/year, since their initial evaluations. They continue on twice daily airway clearance with albuterol, hypertonic saline with acapella and pulmozyme daily in addition to treatment for sinus disease which has been their major complaint.

To date the sibship remains PS and remains negative for pseudomonas. We suspect that the age of the patients during their times of evaluation mainly accounts for their lack of pseudomonal acquisition and more notable presence of *Staphylococcus aureus*. This is not surprising, given the higher prevalence for *S. aureus* during early childhood. We also attribute their current absence of pseudomonas, to their continued focus on airway clearance and adherence to bronchodilator and mucolytics as part of their daily care.

Adolescent boys with clinical and NPD findings consistent with CF, despite normal SC values during infancy, have been documented.<sup>13</sup> However, this is the first report of a patient followed from infancy and monitored for progression towards a CF phenotype with respective evaluation of *CFTR* dysfunction, as reflected by clinical status and progressive changes in SC. Had his brothers not subsequently tested positive for the same mutations he would not likely have had the same level of close monitoring for progression to CF, as this is not the norm for CRMS patients. We believe that this interesting family provides an excellent opportunity to point out the need for extended follow-up of SC analyses. We believe that infants with CRMS identified by NBS should be closely followed in a CF Center with re-testing of SC beyond the currently recommended ages; continued examination may be necessary in order to confirm a CF diagnosis. Definitive diagnosis will lead to better understanding of disease progression and will impact therapeutic decisions.<sup>14</sup> This case also illustrates the dilemma CF clinicians will face with the wide-spread use of genotyping. Patients may have two mutations of *CFTR* in *trans* location but a negative SC. Re-testing of SC may be needed to support the clinical diagnosis of CF.

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