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OPTIMAL DNA TIER FOR THE IRT/DNA ALGORITHM DETERMINED BY CFTR MUTATION RESULTS OVER 14 YEARS OF NEWBORN SCREENING

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Abstract

Background—There has been great variation and uncertainty about how many and what *CFTR* mutations to include in cystic fibrosis (CF) newborn screening algorithms, and very little research on this topic using large populations of newborns.

Methods—We reviewed Wisconsin screening results for 1994–2008 to identify an ideal panel.

Results—Upon analyzing approximately 1 million screening results, we found it optimal to use a 23 *CFTR* mutation panel as a second tier when an immunoreactive trypsinogen (IRT)/DNA algorithm was applied for CF screening. This panel in association with a 96th percentile IRT cutoff gave a sensitivity of 97.3%, but restricting the DNA tier to F508del was associated with 90% (P<. 0001).

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Conclusions—Although *CFTR* panel selection has been challenging, our data show that a 23 mutation method optimizes sensitivity and is practically advantageous. The IRT cutoff value, however, is actually more critical than DNA in determining CF newborn screening sensitivity.

Keywords

Cystic Fibrosis Transmembrane Conductance Regulator; immunoreactive trypsinogen; sensitivity

Newborn screening for cystic fibrosis (CF) became feasible in 1979 after Crossley et al [1] demonstrated the elevation of immunoreactive trypsinogen (IRT) in the blood of infants eventually diagnosed with CF. This led to regional programmes using IRT/IRT screening [2, 3]. However, due to concerns regarding suboptimal screening sensitivity of the initial high IRT cutoff and observations that the second specimens of some CF patients showed precipitous decreases in IRT [4], the IRT/DNA-F508del method [5–7] was developed to incorporate a lower IRT cutoff and a strategy applying the discovery of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene [8]. While many studies have shown that CF NBS is beneficial [9], only limited work has been done to evaluate the long term effectiveness of the screening tests. Yet during 2010, many newborns in Europe and most babies in North America and Australasia are being screened with an IRT/DNA algorithm that now often employs *CFTR* multi-mutation analyses [10, 11]. However, the number of mutations in the screening panel and the methods used for mutation detection vary greatly with some regions still testing only for F508del [11] and others for as many as 400 mutations [12] or performing expanded genetic analysis by gene scanning and selective sequencing.

Although there has been a trend in recent years to add more *CFTR* mutations for screening [13], published guidance is currently available from only two sources: 1) the revised carrier screening recommendations of American College of Medical Genetics (ACMG) to use 23 alleles [14]; and 2) the following suggestion in the “European best practice guidelines for cystic fibrosis neonatal screening” published from a consensus conference [15] with qualifications: “A second tier NBS panel should be designed in order to include well known CF-causing mutations represented in the local CF population, including those alleles frequently occurring in ethnic minorities.” The important issue of how many CFTR mutations in IRT/DNA screening remains unresolved due in part to a lack of published data. Thus, we evaluated DNA results from screening almost 1 million newborns during 1994 to 2008 in Wisconsin.

METHODS

Wisconsin has used an IRT/DNA screening algorithm in a CF routine NBS program since July 1994 when our randomized controlled trial was completed [16]. IRT was measured throughout the 14.5 years of this study with DELFIA kits manufactured by PerkinElmer (Wallac Oy, Finland). CFTR mutations were identified as F508del alone in screening period 1 (July, 1994 – February, 2002) or by CFTR multiple mutation analysis in period 2 (March, 2002 – December, 2008) using the Roche Diagnostic Corp (Indianapolis, IN) linear array kit, or Hologic, Inc. (Madison, WI) Invader chemistry. Both assays detect the 23 mutations currently recommended by ACMG after revision of their original 25 mutation panel recommendation [14].

Infants with positive screening (i.e., IRT ≥ 96th percentile and 1 or 2 CFTR mutations detected) are referred for a sweat test in a CF center. When a child with CF is diagnosed based on signs and symptoms after a negative IRT/DNA screen, the CF center reports the case via our ongoing surveillance programme [16,17], and the case is recorded as a

screening false negative. This study population consists of the CF screening positive cases with a confirmed CF diagnosis and screening false negatives born between July, 1994 and December, 2008. This study was approved by the University of Wisconsin-Madison Institutional Review Board.

RESULTS

During the 14.5 years, a total of 986,997 infants were screened. In this population, 234 screening positives were confirmed with CF including 127 in period 1, and 107 in period 2. In 187 CF confirmed cases with two identified CFTR mutations (79.9%), 91 cases had F508del/F508del (38.9%), 87 cases had F508del and another mutant allele (37.1%), and 9 cases had other/other alleles (3.8%). In 46 CF confirmed cases with one identified CFTR mutation, 42 patients had F508del, and 4 cases had a different CFTR mutation. One CF confirmed patient is receiving care in a different state, and CFTR mutation information is not available. In 6 non-Caucasian cases (three African American, two Native American and one Hispanic), five had at least one F508del allele, and one was compound heterozygote of G542X/1812-1 G>A. There were 8 screening false negative cases, including 5 in period 1, and 3 in period 2. The CFTR mutation distributions and causes of false negatives, which are mainly due to low IRT levels, are listed in Table 1. Note that most of the *CFTR* mutations are associated with pancreatic insufficiency and that 6 of the 8 false negatives occurred after 2 months of age.

The frequencies of *CFTR* alleles included in ACMG 23 mutation panel in 107 CF cases identified in period 2 are listed in Table 2, which are similar to the predicted values from a previous study involving 360 CF patients receiving care in Wisconsin [6]. Note that 12 of the 23 mutations in the revised ACMG list were detected but that some (e.g., 3849+10KbC>T) did not add to the cumulative detection rate. The significant association of IRT elevations and CF risk in newborns with one CFTR mutant allele detected using ACMG 23 mutation panel are demonstrated in Table 3, which extends previous observations [6, 18]. These data provide an indication of the value of the 96th percentile IRT cutoff value; in fact, 13 of 45 (29%) patients diagnosed with a single mutant allele had IRT levels below the 99th percentile, which is commonly used in Europe [10].

DISCUSSION

The demographics of Wisconsin for live births are as follows: 74.4% white, 10.0% black, 9.8% Hispanic/Latino, 4.1% Asian American, and 1.6% American Indian (http://dhs.wisconsin.gov/births/pdf/08births.pdf), which resembles the U.S. demographics as a whole (quickfacts.census.gov/qfd/states/00000.html). After screening ~1 million newborns in Wisconsin over 14 years, we calculated a CF prevalence in Wisconsin of 1 in 4079 or 1 in 3112 Caucasian births, a rate similar to the entire U.S. [19] Based on 234 screening positives with confirmed CF and 8 actual screening false negatives, the calculated overall sensitivity of the screening program is 96.7% (96.2% in period 1, and 97.3% in period 2). Notably, during period 1, when F508del was the only CFTR mutation used in the second tier screening, 3 of 5 false negative cases had IRT level above the cutoff including two cases with at least one mutation listed in 23 CFTR mutation panel. Furthermore, if F508del alone also been used as the second tier test in period 2, we could have missed additional 8 cases, resulting in 90.0% screening sensitivity rather than the observed 97.3% (P<.0001 by McNemar's test). This demonstrates that the 23 CFTR mutation panel significantly improves CF screening sensitivity. In addition, identifying two mutant alleles by 1 week of age in ~80% of patients eventually diagnosed with CF provides several advantages such as expediting and facilitating follow-up and interpretation of intermediate sweat chloride test results [20]. On the other hand, increasing the number of mutations from F508del only to the

23 allele panel led to an average of 67 more sweat tests per year /year vs 213/year, which at ~1/week more is a readily manageable increase).

It is also obvious from the data presented in Table 1 that the IRT cutoff value plays is most significant in determining CF screening sensitivity because 5 out of 8 false negative cases, 2 from period 1 and 3 from period 2, had IRT levels below the DNA reflex cutoff. Interestingly, all but one had IRT values close to the cutoff. Thus, it remains a challenge to set a proper IRT cutoff to maximize CF screening sensitivity without generating more screening false positives.

Our DNA data demonstrate that it is satisfactory to use a 23 *CFTR* mutation panel as the second tier when an IRT/DNA algorithm is applied to the Wisconsin population for CF newborn screening. With this panel, only two patients had alleles that were not detected, less than 1% of the diagnosed cases in this study. Interestingly, the frequency of alleles closely aligns with our predictions [6] in 1997 when we began to evaluate transforming our DNA tier from F508del only to a *CFTR* multi-mutation analysis. As we reported originally [6] with fewer observations, the IRT levels provide valuable information to assess CF risk infants with one CFTR mutation detected. The relative risk of having CF increases significantly when IRT level is greater than 100 ng/mL and approaches 50% with IRT≥ 150 ng/mL (>99.8th percentile). This relationship can also help CF centres plan follow-up evaluations and management.

The frequency of CF-causing alleles reported herein (Table 2) is similar to U.S. data available from the CF Foundation (www.cff.org/UploadedFiles/research; http://www.cftr2.org/index1.php). This suggests that our conclusions are generally applicable to the nation as a whole, although at least one state (California) with a large proportion of Hispanic-Latino births might need other alleles in the *CFTR* panel. In addition, because the frequency of the major mutations in Canada is similar [21], we believe that the ACMG-23 panel would be applicable to almost all of North America. Moreover, the methods and much of the data reported herein may be applicable to Western European countries. Certainly, all CF newborn screening programmes worldwide need to decide on the optimal number of *CFTR* mutations, and the quantitative distribution of the major alleles is similar in typical European populations and their derivatives such as Euro-Americans, as described previously [21]. Nevertheless, an important issue in decisions regarding *CFTR* mutation panels relates to the clinical consequences of mutant alleles, as has been pointed out for R117H [22]. Although our data are insufficient to reach a conclusion about this international policy issue, it should be emphasized that missed cases often have pancreatic insufficiency, as we found, and that even children with pancreatic sufficiency develop CF lung disease and are susceptible to salt depletion [23]. Consequently, this challenging topic must be addressed with more long term clinical data and a formal policy development process.

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Table 1

CF Screening False Negatives and the Cause of Delayed Diagnosis after IRT/DNA Analyses

Year	Mutation 1	Mutation 2	IRT[a]	Age at Diagnosis	Sweat Test Results (mmol/L)	Cause of False Negative
1995	p. R553X	Unknown	53 (56)	4 months old	94	IRT below the cut-off
1996	p. R553X	p. R1161X	64 (56)	6 weeks old	109	F508del alone as the 2nd tier
1997	p. R347P	Unknown	82 (56)	7 weeks old	108	F508del alone as the 2nd tier
2000	3007delG	Unknown	99 (64)	9 months old	110	Rare mutations
2001	Unknown	Unknown	44 (66)	7 years old	59	IRT below the cut-off
2002	p. G551D	p. Q1291H[c]	53 (64)	4 years old	77	IRT below the cut-off
2003	F508del	F508del	45 (51)	1 year old	121	IRT below the cut-off
2004	p. R170H[c]	Unknown	13 (62)	3 years old	66	IRT below the cut-off

[a] IRT was reported as ng/mL. The value reported in the parenthesis was the IRT cutoff value for that testing date. In 1999, Wisconsin changed from a fixed cutoff value for IRT to a floating cutoff calculated on a daily basis as described elsewhere [17].

[b] Note that all sweat chloride values were diagnostic of CF [20] and that 6 of the 8 patients were identified after 2 months of age.

[c] CFTR mutation associated with pancreatic sufficiency, while all other alleles list are associated with pancreatic insufficiency

Table 2

CFTR Mutations from ACMG 23 Mutation Panel in 107 CF Patients Identified via Newborn Screening Programme

CFTR Mutation[a]	Proportion of Allele	Frequency of Allele (%)	Cumulative Detection (%)[b]
F508del	137/214	64.02	92.52
3849+10KbC>T	6/214	2.80	92.52[c]
G542X	5/214	2.34	94.39
N1303K	4/214	1.87	98.13
R117H	4/214	1.87	99.07
R553X	3/214	1.40	99.07
1717-1G>A	2/214	0.93	99.07
G551D	1/214	0.47	100
R347P	1/214	0.47	100
A455E	1/214	0.47	100
W1282X	1/214	0.47	100
621+1 G>T	1/214	0.47	100

[a]The other 11 mutations in ACMG 23 mutation panel are G85E, 711+1 G>T, R334W, I507del, R560T, 1898+1 G>A, 2184delA, 2789+5 G>A, 3120+1 G>A, R1162X and 3659delC.

[b]The identification of either one or two mutant alleles prompts further confirmatory evaluations, so some mutant alleles do not contribute to the detection rate when they are in compound heterozygote.

[c]Note that 3849+10KbC>T did not contribute to the cumulative detection rate because all 6 of the patients with this allele also had a F508del mutation.

Table 3

Correlation of IRT Level and CF Risk in Newborns with One Mutant Allele Detected Using the ACMG *CFTR* 23 Mutation Panel

IRT level (ng/mL)	IRT percentile	No. CF/No. infants	CF risk, % (binomial exact 95% CI)	Relative risk, (95% CI) compare with IRT<99	P-value compare with IRT<99
<99	96.0–98.9th	13/1029	1.3 (0.7, 2.2)		
100–124	99.0–99.3th	9/147	6.1 (2.8, 11.3)	4.8 (2.1, 11.1)	<0.0001
125–149	99.4–99.7th	8/48	16.7 (7.5, 30.2)	13.2 (5.7, 30.3)	<0.0001
≥150	99.8–100th	15/31	48.4 (30.2, 66.9)	38.3 (20.0, 73.4)	<0.0001