Newborn Screening for Cystic Fibrosis in California

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abstract

OBJECTIVES: This article describes the methods used and the program performance results for the first 5 years of newborn screening for cystic fibrosis (CF) in California.

METHODS: From July 16, 2007, to June 30, 2012, a total of 2,573,293 newborns were screened for CF by using a 3-step model: (1) measuring immunoreactive trypsinogen in all dried blood spot specimens; (2) testing 28 to 40 selected cystic fibrosis transmembrane conductance regulator (CFTR) mutations in specimens with immunoreactive trypsinogen values ≥62 ng/mL (top 1.6%); and (3) performing DNA sequencing on specimens found to have only 1 mutation in step 2. Infants with ≥2 mutations/variants were referred to CF care centers for diagnostic evaluation and follow-up. Infants with 1 mutation were considered carriers and their parents offered telephone genetic counseling.

RESULTS: Overall, 345 CF cases, 533 CFTR-related metabolic syndrome cases, and 1,617 carriers were detected; 28 cases of CF were missed. Of the 345 CF cases, 20 (5.8%) infants were initially assessed as having CFTR-related metabolic syndrome, and their CF diagnosis occurred after age 6 months (median follow-up: 4.5 years). Program sensitivity was 92%, and the positive predictive value was 34%. CF prevalence was 1 in 6,899 births. A total of 303 CFTR mutations were identified, including 78 novel variants. The median age at referral to a CF care center was 34 days (18 and 37 days for step 2 and 3 screening test-positive infants, respectively).

CONCLUSIONS: The 3-step model had high detection and low false-positive levels in this diverse population.

WHAT'S KNOWN ON THIS SUBJECT: Several newborn screening models for cystic fibrosis (CF) exist, including DNA-based models that use mutation panels. There is limited experience with models (such as used in California) that include comprehensive DNA sequence testing methods as part of newborn screening.

WHAT THIS STUDY ADDS: California's 3-step newborn screening model for CF showed high efficiency, sensitivity, and positive predictive value. More than 300 mutations were found, reflecting the state's diverse population. Some CF transmembrane conductance regulator–related metabolic syndrome cases converted to CF over time.
Cystic fibrosis (CF) is the most common life-limiting autosomal recessively inherited disease in white populations. It is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene that encodes the CFTR protein, which participates in fluid homeostasis across mucosal surfaces. In 2004, the Centers for Disease Control and Prevention concluded that newborn screening (NBS) for CF was justified to minimize the impact of nutritional deficiencies and poor growth (and possibly lung disease) caused by CF through early detection and proper care. By 2010, NBS for CF had been instituted throughout the United States.

During development of NBS for CF in California (2000–2005), the California Department of Public Health Genetic Disease Screening Program (GDSP) established 6 requirements and goals (Fig 1). One challenge that California faced was a poor understanding of common CFTR mutations within its large and heterogeneous population. After establishing a CF registry and researching the CFTR mutations and immunoreactive trypsinogen (IRT) levels in CF case subjects and control subjects from 3 main race/ethnic groups in California, GDSP developed a 3-step model (IRT–mutation panel–DNA sequencing) for CF NBS.

The goal of this article is to present the methods used and program performance results for the first 5 years of routine CF NBS in California after 2 to 7 years (average: 4.5 years) of follow-up. We discuss how well the California model met its goals and the implications of the findings on our current understanding of CF and cystic fibrosis transmembrane conductance regulator–related metabolic syndrome (CRMS) and on other commonly used CF NBS models.

**METHODS**

The study population included infants who underwent CF NBS in California from July 16, 2007, to June 30, 2012. The CF algorithm (Fig 2) uses the base program’s 1-time collection on filter paper of blood spots through a heel stick at ≥12 hours of age (median: 30 hours). The filter paper card is transported to 1 of 7 laboratories for analysis of serum IRT by using the AutoDELFI Neonatal IRT Kit (PerkinElmer, Waltham, MA). Quality control samples are incorporated into each assay batch on every analytical instrument used in CF screening. Daily quality control results are charted and examined for short- and long-term drift. Specimens with an IRT level ≥62 ng/mL (top 1.6%) are sent for analysis at the Stanford Molecular Pathology Laboratory (Stanford University, Palo Alto, CA) using a 28- to 40-CFTR mutation panel (Table 1). The IRT cutoff was determined by maximizing the sum of sensitivity and specificity in a California study of archived blood spots from 715 prescreening CF case subjects and 5026 control subjects. Mutations on the California panel were selected accordingly: (1) highest allelic frequencies from a second study of 1648 comprehensively genotyped, prescreening, California CF cases to achieve a race/ethnicity-specific rate ≥95% of CF case detection through mutation panel testing in Hispanic, non-Hispanic white, and African-American subjects; (2) include 1 prevalent gross deletion not detectable by using DNA sequence testing, CFTRdel2;3(21kb); and (3) include only mutations with clear CF-causing potential. For example, the common yet variable R117H mutation was not included on the panel.

After testing, IRT levels and mutation panel results (when IRT is positive) are reported at the same time as analyte results for other screened disorders. Newborns with 2 mutations identified after panel testing are referred to 1 of 17 California- and Cystic Fibrosis Foundation (CFF)-approved pediatric cystic fibrosis specialty care center (CFCs) for diagnostic evaluation and follow-up.

Specimens found to have only 1 mutation after panel testing are sent for either CFTR gene scanning and sequence analysis using the Ambry Test: CF (Ambry Genetics, Aliso Viejo, CA) from July 16, 2007, to June 30, 2010 or direct CFTR DNA Sanger sequencing at Stanford Molecular Pathology Laboratory from July 1, 2010, to June 30, 2012. These sequencing methods are highly comprehensive and capable of finding novel mutations. Sequencing results are reported via a supplemental report to hospitals and primary care providers. If only 1 mutation is identified, the newborn is considered a screening test–negative carrier, and parents and the primary care provider are sent letters describing the infant’s carrier status. Parents of carriers are offered free telephone genetic counseling in Spanish or English. If ≥2 mutations (as defined in Fig 3) are identified after sequencing, primary care providers are contacted by telephone to arrange referral to a CFC for genetic counseling of parents, and sweat chloride tests (SCTs) and other diagnostic tests of the child. Parents are sent a letter and pamphlet with information about the positive screening results and the need for confirmatory SCTs.

Pediatric CFCs in California routinely report all subjects diagnosed with CF with negative CF NBS test results to GDSP as part of their quality assurance procedures. The reasons why CF was missed are thoroughly investigated, and they may include...
performing multiplex ligation-dependent probe amplification\(^{13}\) (MRC-Holland, Amsterdam, The Netherlands) to determine gross deletions or duplications when 1 or no mutation was detected. These aforementioned methods meet or exceed implementation, design, and reporting guidelines for CF NBS programs.\(^{14}\)

SCT results and diagnostic and clinical follow-up data are collected from CFCs via GDSP’s secure, online screening information system. SCTs are performed by CFF-accredited laboratories according to current standards\(^{15}\) and guidelines.\(^{16}\) Parents are encouraged to provide salt supplementation and hydration to the infant before testing. Follow-up is conducted with the use of guidelines developed by CFCs and GDSP.\(^{17}\)

Diagnostic services and medical care for uninsured families are covered by California Children’s Services.

CFCs make a determination for a final diagnosis of CF or CRMS by using published guidelines.\(^{5,18}\) DNA testing on biological parents of screening test-positive infants with nonelevated SCT values was recommended and then offered by GDSP starting July 1, 2010, to determine the \textit{cis/trans} mutation phase (ie, on same/different chromosomes, respectively). In 2014, all CF screening test-positive and false-negative CF cases were reviewed for accuracy and consistency to confirm the diagnoses of CF, CRMS, and CF carrier. Newborns with positive CF screening test results were considered to have CRMS unless there was evidence of \(\geq 1\) of the following CF diagnostic criteria: 2 identified CF-causing mutations (per the \textit{Clinical and Functional Translation of CFTR Project [CFTR2])}\(^{19}\), a SCT value \(\geq 60\) mmol/L, last fecal elastase value \(\leq 200\) µg/g, neonatal meconium ileus, a sibling with the same genotype and positive SCT results diagnosed with CF, or physician’s discretion. A carrier diagnosis was given when known mutations/variants were documented to be in the \textit{cis} phase according to parent studies or the literature.

The CF prevalence, detection rate, and positive predictive value were estimated overall and according to 5 race/ethnicity categories. Age at blood collection, IRT result, panel mutation result, sequencing result, referral, first evaluation, first SCT, diagnosis, and treatment initiation were reported overall and according to panel- and sequence-positive groups by using the 25th, 50th, and 75th percentiles. The study protocol

\begin{figure}
\centering
\includegraphics[width=\textwidth]{flowchart.png}
\caption{The California CF NBS program flowchart: July 16, 2007, to June 30, 2012. \textsuperscript{a}Missed CF cases. \textsuperscript{b}As determined by DNA sequencing laboratory. \textsuperscript{c}Began parent testing as part of the screening program to determine phase in July 2010. \textsuperscript{d}Stopped referring 1 panel mutation in combination with only a (TG)\textsubscript{11}–\textsubscript{5T} variant starting June 2011.}
\end{figure}
was approved by the California Health and Human Services Agency Committee for the Protection of Human Subjects (project no. 12-0600354).

RESULTS

Figure 2 presents the number of infants in each step of the program. During the first 5 years, 2,573,293 newborns had an IRT test completed, representing 98.8% of births. Of these, 40,646 (1.6%) had an IRT value ≥ 62 ng/mL and were tested for 28 to 40 CFTR mutations. The allelic frequency of these mutations is found in Supplemental Table 8. No panel mutations were identified in 38,149 (93.9%) hypertrypsinogenemic newborns. Two panel mutations were identified in 194 (0.5%), and these newborns were referred to a CFC for SCT and follow-up. Of the 174 (89.7%) infants with 2 panel mutations who underwent SCT, 162 (93.1%) had initial valid positive test results (≥ 60 mmol/L).

Of the hypertrypsinogenemic newborns, 2303 (5.7%) had 1 panel mutation, and their blood spots subsequently underwent DNA sequencing. After sequencing, 1485 (64.5%) newborns still had only 1 mutation identified, and telephone genetic counseling was offered to these parents. One or more parents of 180 (12.1%) infants received such counseling.

Two or more mutations were identified in 818 (35.5%) newborns (64 had ≥ 3 mutations) after sequencing and were referred to a CFC for SCT and follow-up. Of these, 77 (9.4%) had an initial valid positive SCT result, and 76 were diagnosed with CF at < 6 months of age (median age: 51 days; 25th–75th percentile: 42–64 days). Of the 818 newborns, 741 (90.6%) had a nonpositive initial valid SCT result (< 60 mmol/L) and were followed up by a CFC. To date, 132 (17.8%) of these newborns were determined to be CF carriers (Fig 2). Of the 741 newborns, 74 (10.0%) have been diagnosed with CF (median age: 129.5 days; 25th–75th percentile: 61–272 days) with the remaining noncarriers given an initial CRMS diagnosis. Twenty initial CRMS case subjects had their diagnosis changed to CF at > 6 months of age (Table 2). CRMS remained the diagnosis for 533 (71.9%) children; 47 (8.8%) of these children had a maximum SCT value in the intermediate range (40–59 mmol/L). A typical child with CRMS had a high IRT value, 1 panel mutation, and ≥ 1 mutation/variant from DNA sequencing in the trans phase and a maximum SCT result < 60 mmol/L.

Table 3 displays the median age at blood collection, IRT test results, panel mutation test results, and sequencing results for all CF NBS screening test-positive children; the data are stratified according to screening step. Typically, newborns had an IRT result by 5 days of age, and if the IRT level was ≥ 62 ng/mL, the mutation panel was completed by...
A mutation is defined as any of the following:
1. Variant listed in the CF Mutation Database® or published in the literature.
2. IVS Poly SYT-1® of any TG tract length
3. Novel variant
(except:
Variants documented to be benign polymorphisms from the literature, the Exome Aggregation Consortium, the NCB; Short Genetic Variations database, and other databases)

**FIGURE 3**
Definition of a CFTR mutation from DNA sequence testing used by the California NBS program: July 16, 2007, to June 30, 2012. NCBI, National Center for Biotechnology Information.

16 days. For those with only 1 panel mutation, sequencing results were evaluated at a CFC when the child was ≤30 days old, with a median age at diagnosis and treatment initiation of 25 and 24 days, respectively. Seventy-five percent of infants identified according to sequencing results were evaluated at a CFC by 60 days of age, with a median age at diagnosis of 148 days.

Thirteen deaths occurred among the 1012 children who were CF screening test-positive. Five died (of prematurity and/or malformations) before follow-up could be completed, and 8 died after referral. Four of these deaths were likely CF related (ages 1, 8, 12, and 36 months) (Supplemental Table 9).

In 5 years, GDSP has thus far identified 1617 CF carriers (1485 screening test-negative and 132 screening test-positive children); 533 CRMs cases; and 373 CF cases (194 (~39 per year) with 2 mutations from the panel in step 2, 151 (~30 per year) infants by sequencing in step 3 (allelic mutation frequency in Supplemental Table 10), and 28 (~6 per year) screening test-negative).

**TABLE 2**
Genotype of 20 Children According to Age Diagnosis Was Changed From CRMS to CF in the California NBS Program: July 16, 2007, to June 30, 2012

<table>
<thead>
<tr>
<th>Genotypea cDNA Name</th>
<th>No. of Patients</th>
<th>Age in Years at Diagnostic Changeb</th>
</tr>
</thead>
<tbody>
<tr>
<td>c.1521_1523delCTT (F508del) / c.[1210-12(5)][1210-34TG(13)] (IVS8 (TG)13-St)c</td>
<td>5</td>
<td>1 (n = 2)</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.3454G&gt;C (D1152H)d</td>
<td>2</td>
<td>0.5 (n = 1)</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.350G&gt;A (R117H)e</td>
<td>2</td>
<td>1 (n = 1)</td>
</tr>
<tr>
<td>c.223C&gt;T (R75X) / c.[1210-12(5)][1210-34TG(13)] (IVS8 (TG)13-St)f</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>c.2988+1G&gt;A (3120+1G&gt;A) / c.164+28A&gt;G (296+28A&gt;G)g</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.226C&gt;A (L32M)h</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.3475T&gt;C (S1159P)i</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c.933_935delCTT (delF311)e / c.[1210-12(5)][1210-34TG(11)] (IVS8 (TG)11-St)j</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>c.531delT (IS5del)/ c.314T&gt;A (I105N)k</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.1841A&gt;G (D614G)l</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>c.1521_1523delCTT (F508del) / c.298T&gt;C (V97A)m</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>c.1519_212delATC (IS07del) / c.[1210-12(5)][1210-34TG(12)] (IVS8 (TG)12-St)n</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>c.1624C&gt;A (G542X) / c.3454G&gt;C (D1152H)o</td>
<td>1</td>
<td>5</td>
</tr>
<tr>
<td>c.2988+1G&gt;A (3120+1G&gt;A) / c.[1210-12(5)][1210-34TG(12)] (IVS8 (TG)12-St)</td>
<td>1</td>
<td>5</td>
</tr>
</tbody>
</table>

a) CF-carrying mutation according to CFTR2® unless noted.
b) Diagnosis change from CRMS to CF due to positive SCT results (>60 mmol/L), unless noted.
c) Mutation of varying clinical consequence according to CFTR2®.
d) Diagnosis change due to abnormal fecal elastase results (<200 µg/g).
e) Mutation not evaluated by CFTR2®.
f) Mutation is non-CF-causing according to CFTR2®.

Of these missed cases, 14 (50%) had an IRT value below the cutoff, 9 (32.1%) had no panel mutations identified, and 5 (17.9%) did not have a second mutation detected after sequencing (Table 4).

Table 5 illustrates the 373 CF cases distributed according to the 6 diagnostic review criteria used. A total of 70.8% of the CF cases met ≥2 criteria. Small percentages had at least 1 SCT result ≥60 mmol/L (11.5%) or 2 CF-causing mutations (8.9%) as the sole criterion. The remainder of the CF cases (8.8%) were diagnosed based solely on other clinical evidence or physician’s discretion. Overall, 53 (14.2%) of CF case subjects had meconium ileus (43 detected and 10 missed by the screening program).

Table 6 provides selected population and screening statistics. CF birth prevalence was 1 in 6899 overall, 1 in 4162 in non-Hispanic white subjects, 1 in 9259 in Hispanic subjects, and 1 in 9071 in African-American subjects. The overall case detection rate and positive predictive value for the program were 92% and 34%, respectively. Detection rates were 91% to 100% for California’s 3 main race/ethnicity groups.

Over the study period, 303 different CFTR mutations were identified, including 78 novel variants. Of 85 children carrying a novel variant, 21 (24.7%) have been diagnosed with CF to date.

**DISCUSSION**
This 5-year analysis from the California CF program has many strengths. The findings were derived from a diverse and large number of screened newborns (~2.5 million), and genotyping as part of screening was comprehensive in terms of mutations and Intron 8 Poly (T) Tract (IVS8) status before referral to CFCs. This screening provided timely, high-quality genotype information to CFC staff so that important decisions could be made regarding clinical...
care, testing, and treatment, especially now that mutation-specific therapy technology is advancing. The clinical diagnosis of CF was reviewed and verified by using a set of standard criteria. The length of the follow-up period for screening test-positive individuals extended into early childhood, with CF diagnosed in children as old as 7 years. GDSP made repeated efforts to ensure thorough reporting of all CF case reports. Despite these efforts, follow-up of CRMS cases was less complete than for CF cases after 1 year of age. Because children who become symptomatic presumably return to a CFC, thereby triggering reporting to

**TABLE 3** Time From Birth to Critical Screening Steps and Critical Follow-up Steps According to CF Screening Test–Positive Step in the California NBS Program: July 16, 2007, to June 30, 2012

<table>
<thead>
<tr>
<th>Screening and Follow-up Step</th>
<th>Total (N = 1012)</th>
<th>Panel Positive (n = 194)</th>
<th>Sequencing Positive (n = 818)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median 25th–75th%</td>
<td>Median 25th–75th%</td>
<td>Median 25th–75th%</td>
</tr>
<tr>
<td>Time from birth to critical screening steps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at blood spot collection, h</td>
<td>26 23–35</td>
<td>29 24–41</td>
<td>26 23–34</td>
</tr>
<tr>
<td>Age at IRT test result (step 1)</td>
<td>5 4–8</td>
<td>5 4–7</td>
<td>5 4–8</td>
</tr>
<tr>
<td>Age at panel result (step 2)</td>
<td>16 14–18</td>
<td>16 14–19</td>
<td>16 14–19</td>
</tr>
<tr>
<td>Age at sequencing result (step 3)</td>
<td>— —</td>
<td>— —</td>
<td>36 29–43</td>
</tr>
<tr>
<td>Time from birth to critical follow-up steps</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age at referral</td>
<td>34 26–43</td>
<td>18 16–20</td>
<td>37 31–45</td>
</tr>
<tr>
<td>Age at first evaluation</td>
<td>47 35–61</td>
<td>24 18–52</td>
<td>51 41–64</td>
</tr>
<tr>
<td>Age at first SCT</td>
<td>56 42–78</td>
<td>46.5 28–85</td>
<td>57 44–77</td>
</tr>
<tr>
<td>Age at diagnosis</td>
<td>88 46–248</td>
<td>25 18–41</td>
<td>148 60–296</td>
</tr>
<tr>
<td>Age at treatment initiation</td>
<td>53 34–94</td>
<td>24 19–35</td>
<td>67 48–152</td>
</tr>
</tbody>
</table>

All values are in days unless otherwise noted. —, not applicable.

**TABLE 4** Numbers and Selected Characteristics of 28 False-Negative CF Cases According to Screening Step in the California NBS Program: July 16, 2007, to June 30, 2012

<table>
<thead>
<tr>
<th>Screening Step</th>
<th>N</th>
<th>IRT Level (ng/mL)</th>
<th>Genotype, cDNA Name (Legacy Name)</th>
<th>Race/Ethnicity</th>
<th>Reason for CF Diagnosis</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. IRT below cutoff</td>
<td>14</td>
<td>9 / (mutations not identified)</td>
<td>White (n = 5)</td>
<td>Meconium ileus (n = 2)</td>
<td></td>
</tr>
<tr>
<td>2. No mutations on panel</td>
<td>9</td>
<td>2822delT/ (n = 3)</td>
<td>Hispanic (n = 5)</td>
<td>Family history (n = 2)</td>
<td></td>
</tr>
<tr>
<td>3. Second mutation not detected by using DNA sequencing</td>
<td>5</td>
<td>1521_1523delCTT (F508del)/ (Ex6b_10dup)</td>
<td>Other/multiple (n = 6)</td>
<td>Symptoms (n = 12)</td>
<td></td>
</tr>
</tbody>
</table>

**cDNA, complementary DNA.**

| a | IRT level listed only when below the cutoff value (62 ng/mL). |
| b | Case missed before mutation was added to the California mutation panel. |
| c | Case missed initially by the DNA sequencing because testing was incomplete for Intron 12 (legacy Intron 11) but subsequently found on retesting of these blood spots. |
| d | Case missed by the DNA sequencing test for unknown reasons. |
GDSP, underreporting of CF was probably small.

Unlike traditional 2-step IRT-DNA programs, which consider any hypertrypsinogenemic newborn with \( \geq 1 \) CFTR mutation as screening test–positive, the California program required \( \geq 2 \) mutations to be considered positive. CFTR sequencing, as a third step conducted in <1 in 1000 infants screened, reduced the number of CF carriers referred for SCT by two-thirds (1485 fewer newborns) compared with the 2-step model.

Using a broad definition for a second CFTR mutation, 35% of hypertrypsinogenemic infants with 1 mutation from the California panel had a second mutation/variant (or more) identified from sequencing. The findings of 303 different CFTR mutations, including 78 novel variants, indicate great population heterogeneity and novelty. Previously, an in-depth analysis of a 3-year subset of the current data showed that a significant portion (10 of 55 [18%]) of the novel mutations were likely CF-causing, a finding that is consistent with this 5-year study.

The presence of \( \geq 2 \) mutations in all screening test–positive infants by definition prompted CFCs to implement a diagnostic follow-up period of at least 1 year in asymptomatic infants with initial SCT values <60 mmol/L. This method resulted in detection of 74 (27.3%) more CF cases, the diagnosis in 20 occurring after 6 months of age; it also revealed that in a portion of screening test–positive infants, SCT and/or fecal elastase values changed into the CF diagnostic range as the child aged. Many of the mutations associated with this change were of varying clinical consequence (Table 2). A recent study of newborns with an initial CRMS diagnosis followed up for 3 years by 8 CFCs outside California found that 2 (3%) of 75 obtained a subsequent diagnosis of CF due to elevated SCT values.
To clarify the value of identifying infants with CRMS as well as continue to adjust the referral algorithm by removing benign variants, it is essential to conduct further follow-up studies (probably into adulthood) to better understand the factors that contribute to the evolution of the CF phenotype.

In an attempt to focus screening on severe CF cases, we split mutation testing into 2 steps (panel and sequencing) and restricted panel mutations to those that we found to be clearly CF-causing in the California population. Theoretically, this approach has maximized severe CF case identification in the panel step, compared with most other programs. According to CFTR2, 37 panel mutations are CF-causing, and 3 have not yet been evaluated; this outcome highlights the importance at a regional level of including mutations found in confirmed clinical cases in the screened population and not solely basing the choice of mutations on those evaluated by CFTR2. In the sequencing step, a broad definition of mutation was used (Fig 3); although this definition enhanced sensitivity, its use led to a diagnosis of CRMS 1.5 times more common than CF in our screening test–positive population (533 vs 345, respectively). With traditional IRT-DNA algorithms, these same CRMS cases and others are considered screening test–positive and are referred for SCT; however, after receiving SCT results <30 mmol/L, most are misdiagnosed as carriers because of a lack of comprehensive genotyping. It is potentially very stressful for parents to learn that their child is screening test–positive and then be told by the CFC that their child does not currently exhibit signs and symptoms of CF but may do so in the future. California uses follow-up guidelines that encourage CFC visits quarterly for at least 1 year for children with CRMS; thus, SCTs can be repeated, symptoms evaluated, and other testing and monitoring performed. The short- and long-term psychosocial effects on parents and costs to families of caring for a child with CRMS must be evaluated, along with efforts to reduce psychosocial distress. By continuing to limit the definition of screening test–positive results to only those genotypes that cause CF using knowledge gained over time from the screening program, CFTR2, and elsewhere, the California algorithm will be able to reduce the cost of identifying infants with CRMS as well as continue to adjust the referral algorithm by removing benign variants.

No CF NBS algorithm will detect all cases of CF. The detection rate of CF in California (total 345 [69 per year]) was 92%, exceeding program expectations of 90%. This rate includes infants with meconium ileus. Because cases of meconium ileus are identified in the absence of screening, removing these infants from the calculation produces a detection rate of 95%. Reports from other programs typically range from 92% to 98% (Table 7); however, these rates are likely underestimated due to shorter follow-up periods and/or less rigorous identification of missed cases than the present study. One-half of the missed cases (14 of 28) had an IRT value below the cutoff of 62 ng/mL, corresponding to the top 1.6th percentile. Lowering the cutoff to 49.4 ng/mL, which corresponds to the top fourth percentile used by many states, would have resulted in 4 fewer cases being missed by the IRT step (or <1 per year). The costs

<table>
<thead>
<tr>
<th>Location</th>
<th>Algorithm</th>
<th>CF Case Detection Rate, %</th>
<th>Positive Predictive Value, %</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>New South Wales, Australia</td>
<td>IRT-IRT</td>
<td>92</td>
<td>5</td>
<td>V. Wiley, PhD, personal communication, 2015; B. Wilcken, MD, personal communication, 2015</td>
</tr>
<tr>
<td>New South Wales, Australia</td>
<td>IRT-DNA</td>
<td>94</td>
<td>28</td>
<td>25</td>
</tr>
<tr>
<td>Massachusetts</td>
<td>IRT-DNA</td>
<td>98</td>
<td>9</td>
<td>26</td>
</tr>
<tr>
<td>Wisconsin</td>
<td>IRT-DNA</td>
<td>95</td>
<td>9</td>
<td>27</td>
</tr>
<tr>
<td>Colorado</td>
<td>IRT-IRT</td>
<td>93</td>
<td>5</td>
<td>28</td>
</tr>
<tr>
<td>New York</td>
<td>IRT-DNA</td>
<td>98</td>
<td>3</td>
<td>29</td>
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* Based on a c.1521_1523delCTT (F508del) only mutation panel.
* Figures exclude meconium ileus cases.
and inefficiencies to the program that would come with lowering the IRT cutoff value to the top fourth percentile (estimated by GDSP to be approximately twice the costs for mutation testing and genetic counseling for carriers and likely higher costs for additional referrals) are considered excessive compared with the small gain in sensitivity (1%–2%); they would also conflict with the program’s goal of equally maximizing both sensitivity and specificity. In the first 5 years, there was 1 mutation missed by the panel that appeared in >1 unrelated subjects (c.2822delT/c.2822delT found in 3 Hispanic subjects from 2 unrelated families) (Table 4), suggesting that the program could improve sensitivity ~1% by adding this mutation to California’s 40-mutation panel. The DNA sequencing step missed only a small number of subjects with unique gross insertions or deletions as the second mutation.

The positive predictive value of California’s 3-step program is 34% (31% excluding infants with meconium ileus). Reports from other programs typically range from 5% to 9% (Table 7). Reducing false-positive findings produces large savings in program, medical, family, and societal costs, despite costs of US $500 to $1000 incurred by the program per DNA sequencing test. The California findings are consistent with those modeled in a cost-effectiveness study of 4 different CF NBS algorithms. However, 2 recent studies suggest that IRT/pancreatitis-associated protein algorithms may be even more cost-effective than California’s 3-step algorithm.

Age at reporting CF NBS results and at making a CF diagnosis are 2 challenging areas for the California program. CFF guidelines instruct that SCT should be performed by 2 to 4 weeks of age and diagnosis should occur by 1 to 2 months of age. The literature suggests that subjects with CF diagnosed within 2 months of life are most likely to benefit from early interventions. In California, 74.5% of CF NBS-positive newborns were seen by CFCs before age 2 months; this time frame was largely influenced by the 2 to 3 weeks needed to complete DNA sequencing. Improvements have been made to reduce this time by conducting both mutation panel and DNA sequence testing in the same physical location and in reducing assay testing time. New technologies, such as next-generation sequencing, may be valuable in further shortening this time.

The uptake of genetic counseling by parents of nearly 1500 nonreferred CF carriers identified by the program was 12.1%. It is unknown why so many parents are not using the telephone genetic counseling service, designed according to California’s NBS follow-up of hemoglobinopathy traits. Given the large scale of prenatal CF carrier testing being conducted, many parents may have already received CF genetic counseling. The program has not evaluated the effectiveness of the telephone genetic counseling program, although such a review is being considered.

Given that an additional 6.2% of CF cases identified to date were diagnosed after 6 months of age, CF NBS programs that do not conduct comprehensive genotyping (and which rely mainly on elevated SCT values and symptoms at initial follow-up to make a diagnosis of CF) may be mislabeling some infants who actually have CF as CF carriers. It is important that the genetic counseling that follows diagnostic testing emphasize that CF is still possible in these infants and that the appearance of any suggestive CF signs and symptoms as the child ages should result in prompt referral to a CFC for evaluation and comprehensive genotyping.

CONCLUSIONS

After 5 years, the 3-step (IRT–40-mutation panel–DNA sequencing) CF NBS model used in a racially and ethnically diverse California population is meeting its goals of high detection and low false-positive results. The follow-up of newborns with ≥2 mutations showed that CF cases are not always apparent in the first few months of life. Reliance on an initial SCT result ≥60 mmol/L (or even ≥30 mmol/L) to distinguish true CF cases from carriers in the absence of comprehensive genotyping is likely to miss a small portion of CF cases.

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REFERENCES


ABBREVIATIONS

CF: cystic fibrosis
CFC: cystic fibrosis specialty care center
CFF: Cystic Fibrosis Foundation
CFTR: cystic fibrosis transmembrane conductance regulator
CFTR2: The Clinical and Functional Translation of CFTR Project
CRMS: cystic fibrosis transmembrane conductance regulator–related metabolic syndrome
GDSP: California Department of Public Health Genetic Disease Screening Program
IRT: immunoreactive trypsinogen
NBS: newborn screening
SCT: sweat chloride test

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