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Cystic fibrosis newborn screening programs: implications of the *CFTR* variant spectrum in nonwhite patients

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Purpose: Cystic fibrosis newborn screening (CFNBS) has been offered across the United States since 2010. However, as compared with white patients with CF, *CFTR* variant identification in nonwhite populations remains inequitable. Utilizing the recent characterization of the nonwhite CF variant spectrum, we examined the effectiveness of current CFNBS molecular panels in identifying affected nonwhite newborns.

Methods: Based on a cross-sectional evaluation of genotyping data from the CF Foundation Patient Registry that compared 3,496 nonwhite with 22,206 white CF patients, the current CFNBS algorithms used in the 50 states and the District of Columbia were analyzed. We assessed the percentage of CF patients of Hispanic, African, Asian, and Native American heritage who would not be identified by the molecular panels most commonly used.

Results: Compared with whites, variant detection was significantly lower in Hispanic, black, and Asian newborns ($P \leq 0.0001$ each), as well as in Native American newborns (P values ranged from 0.001 to 0.0003), for the most common CFNBS panels.

Conclusion: This study provides a perspective on the applicability of current panels to a diverse population and enables CFNBS programs to consider more inclusive test approaches to facilitate diagnosis, timely clinical intervention, and enhanced prognosis for CF patients of nonwhite and mixed ethnicities.

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Key Words: CFTR; cystic fibrosis; mutation detection; newborn screening; nonwhite

INTRODUCTION

Cystic fibrosis (CF; OMIM 219700) is an autosomal recessive condition caused by mutations in the *CFTR* gene (OMIM *602421) that disrupt the production of the cystic fibrosis transmembrane conductance regulator (CFTR), a protein critical for chloride ion channel formation in the exocrine epithelial cells of multiple tissues and organs. A hallmark of classic CF, and the major contributor to morbidity and mortality, is pulmonary disease, which manifests as chronic respiratory inflammation and infection.[1] Additional clinical features include failure to thrive, pancreatic insufficiency, intestinal obstruction, and infertility. The birth prevalence of CF in the United States approximates 1:3,500 overall, but Ashkenazi Jews and non-Hispanic whites are most frequently affected, with respective prevalences of ~1:2,270 and up to 1:2,500 individuals.[2–5] CF is reported in ~1:15,000 blacks, ~1:35,000 Asians, and ~1:10,900 Native Americans.[5,6]

Clinically, CF can be established by the presence of at least one distinctive phenotypic feature as well as laboratory confirmation of functional CFTR abnormality, primarily by verification of an elevated sweat chloride (Cl^-) concentration and/or the identification of a pathogenic sequence change in each of the two copies of the *CFTR* gene.[7] Early recognition and diagnosis can be challenging, however, because presenting symptoms are not specific to CF.[8] Consequently, a CF diagnosis predicated on the manifestation of clinical symptoms (excluding newborns diagnosed with meconium ileus or with a positive family history) is delayed, on average, until well into the second year of life, which commonly results in compromised clinical status as compared with early diagnosis and intervention.[9–11] In an effort to minimize the potential for diagnostic delay, CF newborn screening (NBS) programs were implemented across the United States, in all 50 states and the District of Columbia, by 2010.[12] The median age of CF diagnosis has since been advanced to ~2–4 weeks of life.[13]

Although CF newborn screening (CFNBS) methodologies vary between programs, the first step always involves the measurement of immunoreactive trypsinogen (IRT) in dried blood spots. When an elevated IRT level is detected, secondary testing consisting of a repeat IRT, DNA testing (most often comprising a mutation panel), or a combination of the two is performed. Newborns with a second occurrence of elevated IRT and/or a single identified *CFTR* variant are usually considered screening test–positive and may receive more extensive molecular analysis or are referred for clinical evaluation and sweat chloride measurement. The *CFTR* variants chosen for the initial DNA test affect the clinical sensitivity of NBS algorithms. Because the distribution and frequency of *CFTR* sequence changes vary with respect to specific ethnic groups and geographic locations,[5,14,15] panel composition is an important consideration for

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NBS programs, particularly in the genetically diverse and ethnically admixed population of the United States.

We recently published a cross-sectional analysis comparing 3,496 nonwhite patients to 22,206 white CF patients using the genotyping data of patients from all ethnicities in the CF Foundation Patient Registry.[16] In that study, the spectrum and allele frequencies of *CFTR* sequence variants in nonwhite CF patients were categorized by ancestry and the most frequent sequence changes were tabulated.[16] The objective of the present study, in contrast, was twofold: first, to determine which algorithms and molecular analyses are currently used for CFNBS in the United States and, second, to utilize our characterization of the nonwhite CF variant spectrum to analyze the impact of different panels on the detection of *CFTR* variants in nonwhite CF patients as compared with whites.

MATERIALS AND METHODS

CFTR genotyping and CF Foundation Patient Registry data analysis

The spectrum of genotypes in non-Hispanic whites and Ashkenazi Jews is well characterized because these populations are the most commonly affected with CF.[3–5] To more completely elucidate the spectrum and frequencies of *CFTR* variants in affected US minorities, and to facilitate early identification in these populations, we combined our genotyping results from nonwhite patients with genotype data from the CF Foundation Patient Registry to perform a cross-sectional analysis.[16] Nonwhite CF patients with incomplete *CFTR* genotypes (0 or 1 previously identified *CFTR* variants) as well as those who had no prior genotype testing who were enrolled in the CF Foundation Patient Registry through 161 US CF centers were eligible for genotyping in our study. Nonwhite patients, for the purposes of this study, were individuals who self-identified as black, Asian, Native American, East Indian, or Middle Eastern. White and Hispanic CF patients were excluded from the genotyping. CF patients of more than one race/ethnicity were included for genotyping unless they reported solely white and Hispanic ancestry. *CFTR* variants established to be benign were excluded. This assessment was performed by the following combined approaches: (i) reported variant allele frequency (>1%); (ii) information in public databases, such as dbSNP (http://www.ncbi.nlm.nih.gov/projects/SNP/); (iii) frequency of recurrence observed in our internal clinical diagnostic database; and (iv) reports in the literature regarding functional studies or segregation with disease. Sequence changes of uncertain clinical significance were included but not further evaluated to assess the degree of pathogenicity.

Genotyping was performed by the clinical diagnostic Stanford Molecular Pathology laboratory as previously described.[16] Leftover DNA samples with only one or no variant(s) found by sequencing were de-identified and assayed separately for *CFTR* copy-number variants by multiplex ligation-dependent probe amplification on a research basis. Breakpoints of identified rearrangements were also characterized. A total of 140 CF patients were enrolled and genotyped over the course of the study period, and their clinical diagnostic test results were appended to their CF Foundation Patient Registry entries by CF center staff. Thus, entries in the CF Foundation Patient Registry were enriched by the study. Using the genotyping data of patients from all ethnicities from the CF Foundation Patient Registry, a cross-sectional analysis comparing 3,496 nonwhite patients with 22,206 white CF patients was performed, and the spectrum of *CFTR* sequence variants and their allelic frequencies in nonwhite CF patients were characterized.[16]

Survey of state NBS programs and DNA testing comparisons

For the four molecular test approaches most widely implemented by CFNBS programs in the United States (**Table 1**), we assessed the number of current CF patients across ethnic ancestry groups in the 2013 CF Foundation Patient Registry who would not be identified by the given testing algorithm. For this study, we defined "current CF patients" as those who were alive and seen at a CF center within the past year of the data set. Patients with self-declared Hispanic-only ancestry were assigned to the "Hispanic Only" category. Nonwhite patients with any African, Asian, or Native American ancestry were categorized in the "Black–Any," "Asian–Any," and "Native–Any" groups, respectively. The CF Foundation Patient Registry database was analyzed using SAS statistical software (version 12.1; SAS Institute, Cary, NC), and, per ethnic group, the frequencies were determined for the number of sequence variants identified by each DNA testing method. Chi-squared statistics were performed and *P* values were calculated using "White Only" as the reference group to establish whether observed distribution differences between ethnic groups in the number of *CFTR* variants identified by each molecular test were statistically significant. Additional chi-squared analyses were completed to determine the statistical significance of observed differences between testing platforms in the percentages of each ethnic group for whom zero or two mutations would be detected. State CFNBS programs were contacted via telephone and e-mail regarding their CF screening methodologies. Each state's 2013 birth data[17] and details of their newborn CF screening, particularly in regard to DNA testing, were compiled in a list of DNA testing methods/panels (**Table 2**).

RESULTS

All 50 states and the District of Columbia were surveyed regarding their CFNBS testing algorithms and, when applicable, the details of their molecular test methods (**Table 2** and **Figure 1**). Every program begins by measuring newborn IRT levels. An elevated IRT reading is followed by a repeat IRT measurement and/or DNA testing. Eight programs (8/51 = 15.7%) do not currently include DNA testing as part of their CFNBS; in these circumstances, two elevated IRT levels comprise a positive CFNBS result. The remaining programs (43/51 = 84.3%), however, incorporate either one or two tiers of DNA testing into their algorithms. Three commercially available panels represent the most commonly implemented approaches: the InPlex CF

Table 1 Variants included in the molecular testing panels most frequently used by cystic fibrosis newborn screening programs in the United States

Variants[a]	p.Phe508del (*N* = 1 variant)	Hologic InPlex CF molecular test[b] (*N* = 23 variants)	Luminex xTAG CF39 kit v2 (*N* = 39 variants)	Hologic CF InPlex card (*N* = 41 variants)
p.F508del	•	•	•	•
p.A455E		•	•	•
p.G85E		•	•	•
p.G542X		•	•	•
p.G551D		•	•	•
p.I507del		•	•	•
p.N1303K		•	•	•
p.R117H		•	•	•
p.R334W		•	•	•
p.R347P		•	•	•
p.R553X		•	•	•
p.R560T		•	•	•
p.R1162X		•	•	•
p.W1282X		•	•	•
621+1G>T		•	•	•
711+1G>T		•	•	•
1717-1G>A		•	•	•
1898+1G>A		•	•	•
2184delA		•	•	•
2789+5G>A		•	•	•
3120+1G>A		•	•	•
3659delC		•	•	•
3849+10kbC>T		•	•	•
p.A559T			•	
p.D1152H				•
p.D1270N				•
p.E60X				•
p.F508C		*	•	•
p.I148T				•
p.I506V			•	
p.I507V			•	
p.M1101K			•	
p.Q493X				•
p.R347H			•	•
p.S549N			•	•
p.S549R			•	
p.S549R (A>C)				•
p.S549R (T>G)				•
p.S1255X			•	
p.V520F			•	•
p.Y122X			•	•
p.Y1092X			•	
p.Y1092X (C>A)				•
p.Y1092X (C>G)				•
394delTT			•	•
1078delT			•	•
1898+5G>T			•	
2183AA>G		*	•	•
2307insA			•	
3849+4G>A				•
3876delA			•	•
3905insT			•	•
5/7/9T[c]		•	•	•

[a]Variants are listed according to legacy nomenclature. ACMG 23 Carrier Screening Panel variants are shown in italics. The p.F508C, p.I507V, and p.I506V benign variants and the p.I148T polymorphism are shown in bold. [b]Variants used for analytical interpretation only are indicated by an asterisk. [c]Polypyrimidine tract variants in intron 8 (IVS8; intron 9, IVS9 in sequential nomenclature).

Table 2 Cystic fibrosis newborn screening programs in the United States (as of 25 August 2015)

Program	No. of births, 2013 (ref. 21)	Percentage of total US births, 2013 (ref. 21)	Testing algorithm	DNA testing method	No. of variants tested[a]	5/7/9T testing[b]
Alabama	58,167	1.5%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Alaska	11,446	0.3%	IRT/IRT	N/A	N/A	N/A
Arizona	85,600	2.2%	IRT/DNA	Hologic CF InPlex card	41	Always reported
Arkansas	37,832	1.0%	IRT/DNA	Hologic CF InPlex card	41	Reflex test, reported with p.R117H
California	494,705	12.6%	IRT/DNA/ DNA	Modified Luminex/full *CFTR* sequencing	40/full sequencing	N/A
Colorado	65,007	1.7%	IRT/IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Connecticut	36,085	0.9%	IRT/DNA	Hologic CF InPlex card	40	Reported to CF center only
Delaware	10,831	0.3%	IRT/IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
District of Columbia	9,288	0.2%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Florida	215,407	5.5%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Georgia	128,748	3.3%	IRT/DNA	Hologic InPlex CF molecular test	23	Reflex test, reported with p.R117H
Hawaii	18,987	0.5%	IRT/IRT/DNA/ DNA	Integrated Genetics Cfplus/full *CFTR* sequencing	97/full sequencing	N/A
Idaho	22,383	0.6%	IRT/IRT	N/A	N/A	N/A
Illinois	156,931	4.0%	IRT/DNA	Hologic CF InPlex card	41	Reflex test, reported with p.R117H
Indiana	83,102	2.1%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Iowa	39,094	1.0%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H[c]
Kansas	38,839	1.0%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Kentucky	55,686	1.4%	IRT/DNA	Hologic InPlex CF molecular test	23	Reflex test, reported with p.R117H
Louisiana	63,201	1.6%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Always reported
Maine	12,776	0.3%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Maryland	71,953	1.8%	IRT/IRT	N/A	N/A	N/A
Massachusetts	71,788	1.8%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Michigan	113,489	2.9%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Minnesota	69,159	1.8%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Mississippi	38,634	1.0%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Missouri	75,296	1.9%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Montana	12,377	0.3%	IRT/IRT/ DNA[d]	Hologic InPlex CF molecular test	23	Not reported
Nebraska	26,095	0.7%	IRT/IRT/DNA/ DNA	p.Phe508del/ Luminex xTAG CF39 kit v2	1/39	Reflex test, reported with p.R117H
Nevada	35,030	0.9%	IRT/IRT	N/A	N/A	N/A
New Hampshire	12,396	0.3%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
New Jersey	102,575	2.6%	IRT/IRT/DNA	p.Phe508del	1	N/A
New Mexico	26,354	0.7%	IRT/IRT	N/A	N/A	N/A
New York	236,980	6.0%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
North Carolina	119,002	3.0%	IRT/DNA	Hologic CF InPlex card	41	Reflex test, reported with p.R117H
North Dakota	10,599	0.3%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H[c]
Ohio	138,936	3.5%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Oklahoma	53,369	1.4%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Oregon	45,155	1.1%	IRT/IRT	N/A	N/A	N/A
Pennsylvania	140,921	3.6%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Rhode Island	10,809	0.3%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
South Carolina	56,795	1.4%	IRT/IRT	N/A	N/A	N/A
South Dakota	12,248	0.3%	IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H[c]
Tennessee	79,992	2.0%	IRT/IRT	N/A	N/A	N/A
Texas	387,340	9.9%	IRT/IRT/DNA	Hologic CF InPlex card	40	Reflex test, reported with p.R117H
Utah	50,957	1.3%	IRT/IRT/DNA	Ambry Genetics	32	N/A
Vermont	5,975	0.2%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H
Virginia	102,147	2.6%	IRT/DNA	Luminex xTAG CF39 kit v2	39	Always reported
Washington	86,577	2.2%	IRT/IRT/ DNA[d]	p.Phe508del	1	N/A
West Virginia	20,825	0.5%	IRT/DNA	Hologic CF InPlex card	41	Always reported
Wisconsin	66,649	1.7%	IRT/DNA	Hologic InPlex CF molecular test	23	Not reported
Wyoming	7,644	0.2%	IRT/IRT/DNA	Luminex xTAG CF39 kit v2	39	Reflex test, reported with p.R117H

IRT, immunoreactive trypsinogen; N/A, not applicable.

[a]The number of variants tested does not include the p.I148T polymorphism or variants that are typically reflex-tested, such as p.F508C, p.I507V, p.I506V, and 3199del6. Some states have opted to drop the p.D1270N variant from the Hologic CF InPlex Card; as a result, they are testing for only 40 of 41 variants. [b]Polypyrimidine tract variants in intron 8 (IVS8; intron 9, IVS9 in sequential nomenclature). [c]Iowa, North Dakota, and South Dakota report the p.R117H variant only if 5T is also present. [d]Montana and Washington perform limited DNA testing of low-birth-weight/premature infants.

Molecular Test and the CF InPlex Card (Hologic, Bedford, MA) and the xTAG Cystic Fibrosis 39 Kit v2 (Luminex, Austin, TX). Of the 43 programs performing DNA testing, 19 (44.2%; 19/51 = 37.3% overall) use the Hologic CF InPlex Card, 14 (32.6%; 14/51 = 27.5% overall) offer the Luminex xTAG CF39 Kit v2, and four (9.3%; 4/51 = 7.8% overall) apply the Hologic InPlex CF Molecular Test. In addition, two programs (4.7%; 2/51 = 3.9% overall) test only the most common *CFTR* mutation, p.Phe508del (delF508 by legacy nomenclature), which accounts for ~66% of identified mutant alleles worldwide.[14] The remaining four programs (9.3%; 4/51 = 7.8% overall), grouped together in the "Other" category, use two-tier DNA testing that combines two separate DNA testing methods (California, Hawaii, and Nebraska) and/or a less common testing panel (California, Hawaii, and Utah). Regardless of the laboratory testing algorithm ultimately selected, CFNBS programs refer their screening-positive newborns to CF centers for sweat chloride testing, further clinical evaluation, and additional molecular testing if clinically indicated.

In 2001, the American College of Medical Genetics (ACMG, now the American College of Medical Genetics and Genomics) recommended a panel of 25 *CFTR* variants designed specifically for CF carrier screening in the general population; the panel was revised to 23 variants in 2004.[4,18] Contrary to its original intended purpose, use of this panel has been extended to CF diagnostic testing and CFNBS. Not surprisingly, the 23 ACMG-recommended variants have also been incorporated into the three most common commercial panels used for CFNBS (**Table 1**). The composition of the Hologic InPlex CF Molecular Test, a US Food and Drug Administration–cleared genotyping test used in the CFNBS programs of four states, corresponds exactly to the ACMG panel. Expanded panels ranging from 32 to 97 variants, including the Hologic CF InPlex Card and the Luminex xTAG CF39 Kit v2, are used in 37 of 43 (86.0%) CFNBS programs with a molecular tier (37/51 = 72.5% overall) (**Table 2**). These panels all contain the ACMG-recommended variants with one exception: the CFNBS program in California includes only 15 of the 23 ACMG variants in favor of a modified panel that better reflects the *CFTR* alleles most frequently identified in CF patients in that state.[19]

Each of the four most common molecular CFNBS components (p.Phe508del testing, the InPlex CF Molecular Test, the CF InPlex Card, and the xTAG CF39 Kit v2) was evaluated to assess the percentages of individual ethnic ancestry groups in the "current" 2013 CF population who would not be identified (**Table 3**, shaded rows). As expected, of the four methodologies, testing solely for p.Phe508del results in the highest percentage of CF patients with no detected *CFTR* variants, ranging from 10% of whites to 40% of Asians. The InPlex CF Molecular Test (comprising the 23 ACMG-recommended variants for carrier screening) reduced the number of CF individuals with two unidentified variants across all groups; however, individuals of Hispanic (16%), black (18%), Asian (28%), and Native American (8%) backgrounds were more likely to carry no ACMG variants when compared with whites (3%) ($P \leq 0.0001$ for Hispanic, black, and Asian patients and $P = 0.0003$ for Native Americans). Use of the 41-variant CF InPlex Card did not significantly lower the percentage of individuals with zero detected variants when compared with the 23-variant InPlex CF Molecular Test. Implementation of the 39-variant xTAG Cystic Fibrosis panel, however, resulted in modest, yet statistically significant ($P \leq 0.001$), decreases by 3% in Hispanics and blacks each, for whom no variants are detected, as compared with the InPlex CF Molecular Test. The xTAG CF39 panel is also predicted to identify a two-allele genotype in a higher percentage of Hispanic and black individuals with CF (52 and 46%, respectively) versus the InPlex CF Molecular Test (48 and 37%, respectively; $P \leq 0.001$). With all panels, Native American individuals were closest in percentage to whites.

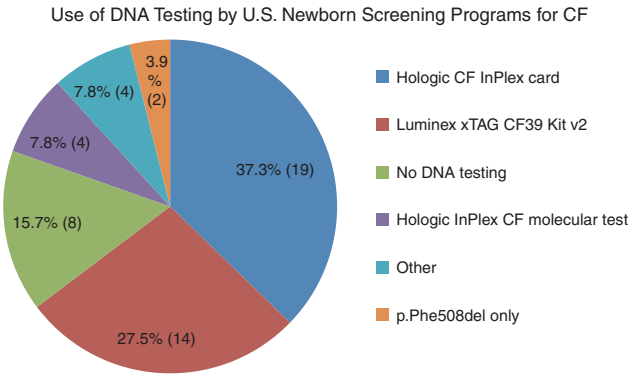

Figure 1 Use of a DNA testing component in cystic fibrosis newborn screening (CFNBS) testing algorithms is summarized for all 50 states and the District of Columbia in the United States (*N* = 51 programs). In the eight states (15.7%) that do not currently include DNA testing as part of their CFNBS, two elevated IRT level results are used to consider a newborn screen-positive. The remaining states (84.3%) incorporate DNA testing into their algorithms. In the context of CFNBS, three commercially available panels are frequently used. The "Other" category represents states that utilize two-tier DNA testing, which combines two separate DNA testing methods, and/or use a less common molecular panel.

DISCUSSION

Delays in making a clinical and molecular diagnosis of CF are likely to negatively impact the health of an affected child and the well-being of his or her family.[10,11,20,21] With a median diagnostic age of 2 to 4 weeks, CFNBS programs have markedly reduced the overall time to diagnosis;[13] however, to make that benefit inclusive of all participating newborns, programs must carefully consider the composition of their testing algorithms. In the United States, the selection of CFNBS algorithms is made by individual states and the District of Columbia based on prioritization decisions and allotment of resources. Every program utilizes the IRT enzyme test, which is performed on dried blood spots derived from newborn heel sticks, as the primary triaging test. In children with an elevated result, however, this initial step is followed by a second IRT test, molecular testing for one or more *CFTR* variants, or both (**Table 2** and **Figure 1**).

Every laboratory test has strengths and limitations. The IRT test, which is highly sensitive overall, can occasionally produce

Table 3 Number of variants detected by ethnic ancestry and screening test in the genotyped CF population of 2013

p.Phe508del	**White only**		**Hispanic only**		**Black–any**		**Asian–any**		**Native–any**	
Variants detected	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2	11,133	50	532	27	234	19	33	21	82	48
1	8,775	40	835	43	522	43	60	38	60	35
0	2,298	10	588	30	458	38	63	40	29	17
Total	22,206	100%	1,955	100%	1,214	100%	156	~100%	171	100%
P value[a]	Reference group		<0.0001		<0.0001		<0.0001		0.005	
Hologic InPlex CF molecular test[b]	**White only**		**Hispanic only**		**Black–any**		**Asian–any**		**Native–any**	
Variants detected	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2	16,782	76	933	48	454	37	48	31	116	68
1	4,775	22	712	36	536	44	64	41	42	25
0	649	3	310	16	224	18	44	28	13	8
Total	22,206	~100%	1,955	100%	1,214	~100%	156	100%	171	~100%
P value[a]	Reference group		<0.0001		<0.0001		<0.0001		0.0003	
Hologic CF InPlex card	**White only**		**Hispanic only**		**Black–any**		**Asian–any**		**Native–any**	
Variants detected	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2	17,082	77	997	51	477	39	53	34	121	71
1	4,502	20	678	35	528	43	61	39	38	22
0	622	3	280	14	209	17	42	27	12	7
Total	22,206	100%	1,955	100%	1,214	~100%	156	100%	171	100%
P value[a]	Reference group		<0.0001		<0.0001		<0.0001		0.001	
Luminex xTAG CF39 kit v2	**White only**		**Hispanic only**		**Black–any**		**Asian–any**		**Native–any**	
Variants detected	*N*	%	*N*	%	*N*	%	*N*	%	*N*	%
2	17,195	77	1,024	52	554	46	57	37	121	71
1	4,433	20	682	35	481	40	63	40	38	22
0	578	3	249	13	179	15	36	23	12	7
Total	22,206	100%	1,955	100%	1,214	~100%	156	100%	171	100%
P value[a]	Reference group		<0.0001		<0.0001		<0.0001		0.0003	

Percentages are rounded and may not add up to exactly 100%, indicated, where applicable, by "~100%." Shaded rows indicate the percentages of each ethnic ancestry group that would not be identified by the given testing method.

[a]Carrying 0 mutations of the selected mutation panel. [b]Panel composition corresponds to the 23-variant carrier screening panel recommended by the American College of Medical Genetics and Genomics (ACMG).

false-negative results in the presence of meconium ileus. As is typical for a screening test, CFNBS emphasizes sensitivity more than specificity; hence, false-positive results are common and expected. Algorithms based on single (IRT) or sequential (IRT+IRT) hypertrypsinogenemia testing have a reported sensitivity of 85–90%, with a considerable proportion (~94%) of false-positive results for single-tier IRT tests.[12,19,22,23] These can result from, for example, clinical conditions, such as perinatal stress, low Apgar scores, and premature birth, as well as ethnic background, such as African ancestry.[24] Given that IRT levels in infants without CF decrease with age, a follow-up IRT test, as applied in 18 of 51 programs in the United States (35.3%), improves specificity (**Table 2**).[8] Of these 18 programs, 10 also incorporate molecular testing but 8 do not. When a CFNBS algorithm does not include a molecular component, however, the opportunities for clinical and etiologic confirmation, prognostic assessment, and specific genetic counseling are missed, or at the least postponed.

In 43 of 51 programs (84.3%), IRT testing is combined with DNA analysis in the following ways: IRT+DNA, IRT+IRT+DNA, IRT+DNA+DNA, or IRT+IRT+DNA+DNA (**Table 2**). In such programs, the newborn is referred for clinical evaluation if one or more *CFTR* variants are identified. Whereas enhanced specificity is intrinsic to a DNA testing component, one added advantage of combining IRT testing with DNA analysis is enhanced screening program sensitivity because a larger proportion of IRT-positive newborns (1.0–5.0 vs. 0.5–1.0%) is typically identified for second-tier testing.[25–27] Although this approach may result in a larger number of CF center referrals based on infants with an initial elevated IRT level and who carry a single *CFTR* variant, cost-effectiveness may prevail because of reductions in morbidity and hospitalizations of successfully identified patients.[28] To address loss of sensitivity due to a limited number of *CFTR* mutations on panels, many programs have implemented a "fail-safe" IRT procedure in which newborns with a very high IRT value (typically, at or above the 99.8th percentile) and no identified mutations are automatically referred for clinical evaluation.[26,27] However, such an approach is nonspecific and associated with disadvantages such as a very low positive predictive value (<1%).[29,30]

Clinical referral of infants who do not have CF can be further limited by including a second level of DNA testing. In California, for example, newborns with a single variant on the initial panel test receive sequencing of the *CFTR* gene and are referred to a CF center only when an additional reportable variant is identified. A screening approach that includes comprehensive sequence analysis, even though it cannot detect up to 2.4% of CF cases with gross deletions and insertions,[16] is expected to miss the smallest number of patients, given that some infants (at least 6% of cases) have inconclusive initial sweat chloride levels and symptoms that cannot solidify a clinical diagnosis of CF in the first months of life.[19,28,31]

In the ethnically diverse population of the United States, the selection of variants for molecular panel testing warrants special consideration. Splice-site variant 3120+1G>A (c.2988+1G>A) illustrates this well: with a prevalence of 10–12%, this variant is the second most common variant identified in black individuals with CF, although it is relatively rare in non-Hispanic whites.[18,32] This variant, as one of the 23 ACMG-recommended carrier screening variants, is included in most molecular CFNBS components (**Table 1**). In our original study of 3,496 nonwhite and 22,206 white CF patients, both the frequency and the spectrum of nonwhite *CFTR* variants were significantly different in comparison with whites.[16] When the 23-variant carrier screening panel, which is frequently selected for diagnostic testing, was assessed for its ability to identify variants across ethnic ancestry groups, CF patients of Hispanic, black, or Asian ancestry were less likely to carry two variants and more likely to have no detected *CFTR* variants ($P \leq 0.0001$). Although similar results were obtained for Native American patients, this group appeared to be the most genetically similar to the white population, probably because of historic admixture. These findings confirmed that the ACMG list of 23 variants that was specifically developed for population carrier screening is inadequate as a general diagnostic CF test. Although expanded variant panels are commercially available and claim to be enriched for variants with increased prevalence in nonwhite populations, these panels were typically designed before the mutation spectrum of nonwhite groups had been extensively studied. Therefore, such panels have inherently limited value for diverse populations in the context of carrier screening, diagnostic testing, and CFNBS.[16,33]

To minimize disparities resulting from population differences in the clinical sensitivity of neonatal screening algorithms, racial/ethnic *CFTR* variant diversity should be considered. This point is illustrated by a variant detection comparison of the panels most commonly used for CFNBS: the Hologic InPlex CF Molecular Test that reflects the 23 ACMG variants, the Hologic CF InPlex Card with these 23 variants plus 18 additional variants evaluated in this study, and the Luminex xTAG CF39 assay with the 23 variants plus 16 additional variants included in this investigation (**Tables 1** and **2**). These panels, together with the approach of evaluating only for p.Phe508del, account for 93.0% (40/43) of targeted variant test components in CFNBS algorithms. Each of these modalities, however, would detect significantly fewer variants in Hispanic, black, and Asian newborns as compared with whites ($P \leq 0.0001$). A significant decrease in detected variants would also be observed in Native Americans (P values ranging from 0.001 to 0.0003), but of all the nonwhite groups, Native Americans would be closest in percentage to whites (**Table 3**). Because the panels are composed differently (**Table 1**), their detection rates in various populations are not the same. When compared with the 23-variant Hologic InPlex CF Molecular Test, the Hologic CF InPlex Card assay demonstrated improved detection for Hispanic newborns expected to be identified with two variants ($P = 0.004$), whereas the Luminex xTAG CF39 assay demonstrated an advantage for the percentages of affected Hispanic and black newborns ($P \leq 0.0001$ each).

In addition, small differences between panels are observed in the percentages of affected nonwhite newborns who would be missed altogether because they had no variants identified. Among the three panels, these percentages range from 13 to 16% for Hispanics, 15 to 18% for blacks, 23 to 28% for Asians, and 7 to 8% for Native American newborns. Whereas the differences within each group are minor, an appreciable proportion of children with CF in nonwhite populations would be missed, as opposed to 3% of babies with white-only ancestry. Aside from the disproportionate identification of affected white versus nonwhite newborns inherent in most CFNBS algorithms because of panel variant selection, differences in the frequency of various types of mutations also exist. Whereas the majority of CF alleles in nonwhite patients affects single nucleotides, gene rearrangements by deletions and duplications account for up to 17% of unidentified CF alleles after sequencing.[16] As documented in the CF Foundation Patient Registry, *CFTR* gene rearrangements occur relatively frequently in nonwhite CF patients, with Asians appearing to have the highest overall frequency, accounting for 2.4% of all identified alleles.[16]

Given these findings, what would be a reasonable strategy to minimize disparities? The optimal CFNBS design would include appropriate variants that fully reflect the spectrum of ethnicities in each region. For example, expanded variant panels such as the Hologic CF InPlex Card and the Luminex xTAG CF39 assay provide more comprehensive genotyping (i.e., detection of two variants) for Hispanics and blacks than a smaller panel based on the 23 ACMG variants. Therefore, larger CFNBS panels may result in more urgent patient referral and earlier care for Hispanic and black patients based on two identified variants on the NBS report, potentially narrowing disparities in the health of nonwhites versus whites with CF. However, the use of panels is inherently inequitable and can be expected to limit parity in any state and to particularly affect programs that use a standard panel and serve a nonhomogeneous population. To improve the current algorithms, especially in states with ethnic diversity, several options could be considered: (i) DNA from IRT-positive newborns with known nonwhite ancestry could be sequenced, instead of tested by a panel of variants; (ii) DNA from IRT-positive newborns with known nonwhite ancestry could be tested with a variant panel

that is more inclusive of the constituency of a particular state or program, followed by a sequence-analysis step in the case of one identified variant; and (iii) DNA from all IRT-positive newborns could be sequenced instead of tested by a panel. With the rapid adoption of next-generation sequencing in various clinical settings, which overall can be more efficient and economical than Sanger sequencing,[34,35] these options may be within reach for CFNBS.[36–40] Each of these scenarios would need to be carefully weighed and, if adopted, monitored for sensitivity, specificity, cost, and anticipated outcomes.

At present, every program must conduct its own cost–benefit analysis regarding feasibility and overall practicality of offering inclusive molecular CFNBS testing of hypertrypsinogenemic infants. Inevitably, screening programs will fail to detect some individuals in order to be cost-effective. With careful evaluation of available algorithms and regional demographics, however, desirable sensitivities could be achieved for all populations and the differences between racial/ethnic groups could be minimized with the vital benefit of avoiding disproportionate disease burdens. In CF, a condition with considerable allelic heterogeneity and more than 2,000 CFTR variants described across populations (http://www.genet.sickkids.on.ca/cftr/StatisticsPage.html), a more comprehensive approach may well make sense.

In conclusion, this is the first large-scale and in-depth evaluation of the impact of CFNBS algorithm selection on nonwhite newborns with CF in the United States. Longitudinal analysis of detection rates and outcome data, together with the implementation of refined algorithms as new approaches become available, can facilitate optimization of CFNBS and benefit affected newborns, their families, and society at large.

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DISCLOSURE

The authors declare no conflict of interest.

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