ORIGINAL RESEARCH ARTICLE | Genetics inMedicine

Cystic fibrosis carrier screening in a North American population

Val V. Zvereff, MD,PhD[1], Hawazin Faruki, DrPh[1], Marcia Edwards, BA,BS[1] and Kenneth J. Friedman, PhD[1]

Purpose: The aim of this study was to compare the mutation frequency distribution for a 32-mutation panel and a 69-mutation panel used for cystic fibrosis carrier screening. Further aims of the study were to examine the race-specific detection rates provided by both panels and to assess the performance of extended panels in large-scale, population-based cystic fibrosis carrier screening. Although genetic screening for the most common *CFTR* mutations allows detection of nearly 90% of cystic fibrosis carriers, the large number of other mutations, and their distribution within different ethnic groups, limits the utility of general population screening.

Methods: Patients referred for cystic fibrosis screening from January 2005 through December 2010 were tested using either a 32-mutation panel (n = 1,601,308 individuals) or a 69-mutation panel (n = 109,830).

Results: The carrier frequencies observed for the 69-mutation panel study population (1/36) and Caucasian (1/27) and African-American individuals (1/79) agree well with published cystic fibrosis carrier frequencies; however, a higher carrier frequency was observed for Hispanic-American individuals (1/48) using the 69-mutation panel as compared with the 32-mutation panel (1/69). The 69-mutation panel detected ~20% more mutations than the 32-mutation panel for both African-American and Hispanic-American individuals.

Conclusion: Expanded panels using race-specific variants can improve cystic fibrosis carrier detection rates within specific populations. However, it is important that the pathogenicity and the relative frequency of these variants are confirmed.

Genet Med advance online publication 19 December 2013

Key Words: carrier detection; cystic fibrosis; race specific; variants

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene.[1] To date, more than 1,900 unique mutations have been described in the *CFTR* gene.[2] The observed phenotypic heterogeneity of CF is due to the variable impact of the underlying mutations, in combination with the action of genetic modifiers and environmental factors.[3] The phenotypic spectrum of CF ranges from a mild disease, found in ~15% of CF patients, to a progressive, multisystem disease primarily involving the pulmonary, pancreatic, and gastrointestinal systems.[1,4] Individuals with a mild form of the disease currently have a median survival of 56 years, whereas those with classic CF have a median survival of ~37 years, with the most common cause of death being respiratory failure.[1]

Although CF is most prevalent in Caucasians (1 in 2,500), it is a panethnic disease in North America, found in all races, including African-American (1 in 15,000), Hispanic-American (1 in 13,500), and Asian-American (1 in 35,000) individuals.[5,6] This reflects the heterogeneity of the North American population. The incidence of CF in the North American population is primarily due to the higher-frequency alleles present mainly in the Caucasian population. Alleles found in relatively high frequency in other races are less likely to be found in high frequency in the general population due to the lower prevalence of CF in these populations.[4,7]

In 2001, the American College of Medical Genetics and Genomics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) recommended a 25-mutation panel for CF carrier screening, which was later refined to one with 23 mutations.[8] Selection of CF variants was based on pathogenicity and an allele frequency of ≥0.1% of the general affected US population.[6] The panel did include variants predicted to be panethnic. This panel has performed well in the past decade and is predicted to have detected close to 90% of CF carriers in the white or Ashkenazi Jewish populations.[4] However, the panel does not detect CF carriers equally for all races.[4,7] Although the detection rate for non-Hispanic whites is 88%, the detection rates for Hispanic-American (72%), African-American (64%), and Asian-American (49%) CF carriers are significantly lower.[4] In 2011, the ACOG recognized the fact that assigning a single ethnicity to individuals with CF had become increasingly difficult and expanded its 2001 recommendations to recommend offering CF carrier screening to all women regardless of ethnicity.[4] A number of studies have identified apparent race-specific alleles in affected individuals. Those alleles in highest frequency have been recommended as additions to the current 23-mutation panel as a means to increase CF carrier detection rates in ethnic populations to levels similar to that of non-Hispanic whites.[9–13] Several commercial reference laboratories now offer extended panels consisting of all variants from the 23-mutation panel, as well as an increasing number of race- or region-specific alleles.[9,14] An analysis

[1]Department of Molecular Genetics, Center for Molecular Biology and Pathology, Laboratory Corporation of America, Research Triangle Park, North Carolina, USA. Correspondence: Val V. Zvereff (zverefv@labcorp.com)

Submitted 8 August 2013; accepted 31 October 2013; advance online publication 19 December 2013. doi:10.1038/gim.2013.188

of the performance of these extended panels has been difficult due to the paucity of published data. Heim et al.[14] examined a total of 5,840 chromosomes from a variety of races and demonstrated that a detection rate in the range of 70–95% could be achieved for all races, except Asians, with a panel of 50–70 *CFTR* mutations. Rohlfs et al.[9] reported on the use of a 97-mutation panel among 364,890 individuals referred for CF carrier screening. They confirmed that the mutation frequency distribution for Caucasians was significantly different from that observed for other races. The purpose of the current study was to compare the mutation frequency distribution observed with a 32-mutation panel versus a 69-mutation panel used for CF carrier screening. The CF carrier detection rate of each panel for different ethnic groups was also determined, and the implications of these data to the composition of future screening panels are discussed.

MATERIALS AND METHODS

Patients

In this study, we performed testing of samples from 1,711,138 individuals referred to LabCorp's Center for Molecular Biology and Pathology for CF screening from January 2005 through December 2010. Samples were tested using either a 32- or 69-mutation *CFTR* panel. The CF32 panel, which includes all 23 mutations recommended by the ACMG and the ACOG, was used to test 1,601,308 cases. A total of 109,830 individuals were tested using the expanded CF69 mutation panel, which also includes all 23 mutations recommended by the ACMG/ACOG. The majority of samples for CF screening (95%) were submitted by obstetricians/gynecologists who followed the ACMG/ACOG guidelines. The remainder of the samples came chiefly from in vitro fertilization clinics, family practitioners, internists, urologists, pulmonologists, and gastroenterologists. Samples were received from all 50 US states. Institutional review board approval was not required because testing was performed for clinical purposes. Patient ethnic background was provided by the referring physicians in 40% of cases. Ethnicity was categorized as Native American, Asian, African American, Caucasian, or Hispanic. Because the indication for testing was not routinely captured, we presume the majority of cases were referred for population-based carrier screening. However, we realize that similar to Strom et al.,[7] in some cases we could have tested known carriers, symptomatic individuals, or patients' relatives. We excluded all cases wherein two mutations were identified. A paired sample *z*-test was used to test the difference in detection rates within different ethnic groups.

Molecular testing

DNA was extracted from peripheral blood and/or buccal swabs using standard nonorganic protocols. DNA samples were screened for *CFTR* gene mutations using the 32-mutation panel (oligonucleotide ligation assay; Celera Diagnostics, Alameda, CA) or the 69-mutation (Universal Array Platform, Luminex, Toronto, Ontario) panel approved by the US Food and Drug Administration. The 69-mutation panel was a combined panel that included variants approved by the Food and Drug Administration with 10 additional variants (R117C, R352Q, S364P, 3120G>A, 2869insG, G480C, 405+3A>C, 1812-1G>A, 444delA, and F311del) added on the basis of their published frequencies and relevancy to CF.

Oligonucleotide ligation assay

The oligonucleotide ligation assay was performed according to manufacturer recommendations (Celera Diagnostics). In brief, genomic DNA was amplified in a multiplex PCR. Next, the multiplex oligonucleotide ligation was performed on *CFTR* amplicons. The final oligonucleotide ligation assay products were size fractionated using an ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). The multiplex signal was analyzed with Gene Mapper Software (Applied Biosystems).

Luminex Universal Array Platform

Patient DNA was amplified with multiplex PCR, then allele-specific primer extension was used to discriminate among the alleles. The allele-specific primer extension products, 5′-tagged with 24-mer sequences selected from the Tm 100 Universal Sequence Set (Luminex), were captured by their tag complements, which were chemically coupled to polystyrene beads (Luminex). The xMAP instrument (Luminex Corporation, Austin TX) was used to examine the beads for both normal and mutation sequences. The data were analyzed by TDAS software (Luminex).

RESULTS

The 32-mutation panel is composed of the 23 variants from the ACMG/ACOG screening panel combined with 9 additional mutations, namely, 3876delA, R347H, S549N, 3905insT, 1078delT, V520F, 394delTT, S549R, and 2183AA>G. **Table 1** gives a frequency distribution of the mutations detected by the 32-mutation panel. Thirty of the 32 mutations on the panel were detected with a frequency that exceeded the 0.1% ACMG/ACOG prevalence threshold among affected individuals. Even the 1078delT mutation that was removed from the ACMG/ACOG screening panel achieved 0.1% prevalence.[8] Only the S549R variant was observed at a lower frequency (0.07%). The frequency of the 2183AA>G mutation could not be established because this variant was added to the 32-mutation panel less than a year before the data collection end point. The argument can be made that the original ACMG/ACOG panel's 0.1% prevalence threshold was derived from the panethnic cohort of the affected individuals, not the carriers routinely discussed in the literature and in the current study. We would like to emphasize that in our study we consistently compared allelic frequencies of the mutations detected by the 32- and 69-mutation panels with frequencies of ACMG/ACOG–selected variants detected in the carrier population.

The 69-mutation panel incorporated all variants from the 32-mutation panel along with additional pathogenic variants present with relatively high frequency in Caucasian, Hispanic,

Table 1 *CFTR* allele frequency identified by the CF32 mutation panel

Variant[a]		Number of detected alleles	Mutation (%)
Legacy nomenclature	HGVS nomenclature		
F508del[b]	p.F508del	31,142	68.69
R117H[b]	p.R117H	5,198	11.46
G542X[b]	p.G542X	1,162	2.56
G551D[b]	p.G551D	989	2.18
W1282X[b]	p.W1282X	824	1.82
3120+1G>A[b]	c.2988+1G>A	706	1.56
N1303K[b]	p.N1303K	648	1.43
R553X[b]	p.R553X	487	1.07
3849+10kbC>T[b]	c.3717+12191C>T	436	0.96
621+1G>T[b]	c.489+1G>T	410	0.90
1717-1G>A[b]	c.1585-1G>A	388	0.86
2789+5G>A[b]	c.2657+5G>A	382	0.84
I507del[b]	p.I507del	258	0.57
R334W[b]	p.R334W	257	0.57
R1162X[b]	p.R1162X	211	0.47
G85E[b]	p.G85E	199	0.44
1898+1G>A[b]	c.1766+1G>A	170	0.37
R347H[c]	p.R347H	160	0.35
3659delC[b]	c.3528delC	155	0.34
3876delA[c]	c.3744delA	153	0.34
R560T[b]	p.R560T	132	0.29
S549N[c]	p.S549N	125	0.28
3905insT[c]	c.3773dupT	121	0.27
R347P[b]	p.R347P	117	0.26
2184delA[b]	c.2052delA	107	0.24
A455E[b]	p.A455E	106	0.23
711+1G>T[b]	c.579+1G>T	65	0.14
394delTT[c]	c.262_263delTT	56	0.12
V520F[c]	p.V520F	54	0.12
1078delT[c]	c.948delT	52	0.11
2183AA>G[a,c]	c.2051_2052delAAinsG	37	0.08
S549R[c]	p.S549R	31	0.07
	Total	45,338	100

[a]2183AA>G variant was added to the panel in 2010. [b]Variants from ACMG/ACOG CF screening panel. [c]Classified as a CF-causing mutation by the CFTR2 Database.

ACMG, American College of Medical Genetics and Genomics; ACOG, American College of Obstetricians and Gynecologists; CF, cystic fibrosis; HGVS, Human Genome Variation Society.

and African-American populations. Genotyping data presented in **Table 2** demonstrate that the frequency rate of the F508del variant detected by the 69-mutation panel is lower (60.5%) as compared with the rate obtained with the 32-mutation panel (68.7%; $P < 0.01$). This may be the result of population stratification because usage of the 69-mutation panel appears to be biased for testing among non-Caucasian individuals.

The R117H variant was the second most common variant identified by both panels (**Tables 1** and **2**). Among 5,198 carriers of the R117H variant detected by the 32-mutation panel, two copies of 5T alleles were detected in only 23 cases (0.44%). In 4,708 cases (90.56%), the 5T allele was not detected (**Table 3**). The 5T status of the R117H variant could not be determined in 468 cases (9%) for carriers of 5T/7T or 5T/9T alleles. The 69-mutation panel identified two cases with two copies of the 5T allele (0.73%) among 274 R117H carriers, whereas the majority of carriers (89.41%) did not carry a 5T allele (**Table 4**). In 27 occurrences (9.86%) the 5T background of the R117H variant could not be identified.

The variants D1152H (4.0%) and L206W (2.4%), each associated with a variable CF phenotype, were the most common variants not present on the ACMG/ACOG 23-mutation panel. The Hispanic-specific variant 3876delA (1.0%) and the panethnic variant F311del (0.8%) were the next most common variants from the extended panel.

Overall, 53 mutations were detected by this panel with a frequency that met or exceeded the ACMG-proposed threshold of 0.1% (**Table 2**). This threshold could not be reached

Table 2 *CFTR* allele frequency identified by the CF69 mutation panel

Variant[a]			
Legacy nomenclature	**HGVS nomenclature**	**Allele frequency**	**Mutation (%)**
F508del[b]	p.F508del	1,868	60.49
R117H[b]	p.R117H	274	8.87
D1152H[c]	p.D1152H	125	4.05
G542X[b]	p.G542X	98	3.17
L206W[d]	p.L206W	73	2.36
3120+1G>A[b]	c.2988+1G>A	65	2.10
G551D[b]	p.G551D	47	1.52
N1303K[b]	p.N1303K	42	1.36
W1282X[b]	p.W1282X	38	1.23
3849+10kbC>T[b]	c.3717+12191C>T	28	0.91
3876delA[d]	c.3744delA	28	0.91
F311del[e]	p.F312del	24	0.78
I507del[b]	p.I507del	24	0.78
R553X[b]	p.R553X	24	0.78
R117C[d]	p.R117C	22	0.71
621+1G>T[b]	c.489+1G>T	21	0.68
1717-1G>A[b]	c.1585-1G>A	18	0.58
S549N[d]	p.S549N	18	0.58
R334W[b]	p.R334W	17	0.55
2789+5G>A[b]	c.2657+5G>A	16	0.52
G85E[b]	p.G85E	14	0.45
3199del6[e]	c.3067_3072delATAGTG	12	0.39
R1066C[d]	p.R1066C	11	0.36
1898+1G>A[b]	c.1766+1G>A	10	0.32
R347H[d]	p.R347H	10	0.32
R1162 X[b]	p.R1162X	9	0.29
W1089X[d]	p.W1089X	9	0.29
2184delA[b]	c.2052delA	8	0.26
2307insA[d]	c.2175dupA	8	0.26
1078delT[d]	c.948delT	7	0.23
R75X[d]	p.R75X	7	0.23
3120G>A[d]	c.2988 G>A	6	0.19
3659delC[b]	c.3528delC	6	0.19
Q493X[d]	p.Q493X	6	0.19
R1158X[d]	p.R1158X	6	0.19
R560T[b]	p.R560T	6	0.19
1812-1G>A[d]	c.1680-1G>A	5	0.16
2055del9>A[d]	c.1923_1931del9insA	5	0.16
406-1G>A[d]	c.274-1G>A	5	0.16
A559T[d]	p.A559T	5	0.16
R347P[b]	p.R347P	5	0.16
S1255X[d]	p.S1255X	5	0.16
1677delTA[d]	c.1545_1546delTA	4	0.13
711+1G>T[b]	c.579+1G>T	4	0.13
E60X[d]	p.E60X	4	0.13
R352Q[d]	p.R352Q	4	0.13
Y1092X[d]	p.Y1092X	4	0.13
2183AA>G[d]	c.2051_2052delAAinsG	3	0.10
3791delC[d]	c.3659delC	3	0.10
3905insT[d]	c.3773dupT	3	0.10

Table 2 Continued on next page

Table 2 (Continued)

Variant[a]			
Legacy nomenclature	**HGVS nomenclature**	**Allele frequency**	**Mutation (%)**
394delTT[d]	c.262_263delTT	3	0.10
G178R[d]	p.G178R	3	0.10
V520F[d]	p.V520F	3	0.10
2143delT[d]	c.2012delT	2	0.06
935delA[e]	c.803delA	2	0.06
A455E[b]	p.A455E	2	0.06
Q890X[d]	p.Q890X	2	0.06
S549R[d]	p.S549R	2	0.06
2869insG[d]	c.2737insG	1	0.03
405+3A>C[e]	c.273+3A>C	1	0.03
G480C[e]	p.G480C	1	0.03
M1101K[d]	p.M1101K	1	0.03
Y122X[d]	p.Y122X	1	0.03
Total		3,088	100

[a]1898+5G>T[e], 444delA, G330X, S364P[e], K710X, and S1196X mutations were not detected in the target population. [b]Variants from the ACMG/ACOG CF screening panel. [c]Classified as a mutation of variable clinical consequence by the CFTR2 Database. [d]Classified as a CF-causing mutation by the CFTR2 Database. [e]Variant not characterized by the CFTR2 Database.

ACMG, American College of Medical Genetics and Genomics; ACOG, American College of Obstetricians and Gynecologists; CF, cystic fibrosis; HGVS, Human Genome Variation Society.

Table 3 Frequency of 5T/7T/9T genotypes as a result of R117H reflex testing

	Number of detected alleles (%)	
Poly-T alleles	**CF32 panel**	**CF69 panel**
5T/5T	23 (0.44)	2 (0.73)
5T/7T	430 (8.27)	26 (9.49)
5T/9T	38 (0.73)	1 (0.37)
7T/7T	4,103 (78.93)	219 (79.92)
7T/9T	604 (11.61)	26 (9.49)
9T/9T	1 (0.02)	0
Total	5,198 (100)	274 (100)

by 10 variants: the 2143delT, A455E, S549R, Y122X, and M1101K mutations, typically observed in Caucasians; 935delA, 2869insG, and Q890X in Hispanics; and 405+3A>C and G480C in the African-American population. In addition, six variants were not identified in our target population. These were 1898+5G>T, 444delA, G330X, S364P, K710X, and S1196X. The low frequency of the A455E mutation (0.07%) from the ACMG/ACOG panel may be explained by patient preselection through the use of the 32-mutation panel, or random drift. Although the relative frequencies of test populations screened using either the 32-mutation panel or the 69-mutation panel were generally similar (**Table 4**), there were more individuals with reported Caucasian ethnicity in the 32-mutation panel (27.4%) than in the 69-mutation panel (14.8%). The carrier frequencies detected in Caucasians by both panels were the same, 1 in 28 for the 32-mutation panel and 1 in 27 for the 69-mutation panel. There was higher representation of the Hispanic population in the 69-mutation panel (12.7%) as compared with the 32-mutation panel (5.5%). Consequently, the carrier frequency observed in Hispanics from the 69-mutation panel screening (1 in 48) was higher than that observed for Hispanics screened using the 32-mutation panel (1 in 69). By contrast, the carrier frequency detected by the 32-mutation panel for the Asian-American population (1 in 261) was higher than the frequency identified by the 69-mutation panel (1 in 313). The Native-American population demonstrated higher carrier frequency in a group tested by the 32-mutation panel (1 in 59) than in the group screened by the 69-mutation panel (1 in 67; **Table 4**). In general, the number of observations for the Asian-American and Native-American populations that underwent CF screening was low, especially for the group screened by the 69-mutation panel, and thus the significance of these observations is limited.

To evaluate the performance of the ACMG/ACOG panel, we analyzed groups of carriers from the 32- and 69-mutation panels using only 23 ACMG/ACOG variants. Then we compared carrier frequencies and detection rates in different ethnic groups using the 23 ACMG/ACOG variants versus the 32- and 69-mutation panels. Of the carriers detected by the 32-mutation panel, the ACMG/ACOG panel would identify 97.4% in the Native Americans, 95.5% in Asian Americans, 97.3% in African Americans, 98.9% in Caucasians, 94.4% in Hispanic Americans, and 98.3% in two groups without reported ethnicity that were screened using the 32-mutation panel (see **Supplementary Table S1** online).

The ACMG/ACOG panel detects only 78.3% of carriers in the African-American ethnic group identified by the 69-mutation panel ($P < 0.01$). The CF69 panel identified a further 21.7% of CF mutations (12 variants) that are not present on the ACMG/ACOG 23-mutation panel (see **Supplementary Table S2** online). Six of these variants were specific to African Americans (R75X, G480C, A559T, 2307insA, 3791delC, and S1255X). The

Table 4 Carrier frequencies by ethnic group identified by the 32- and 69-mutation panels

	Individuals, *n* (%)[a]		Carriers (*n*)		Carrier frequency	
Ethnic group	**CF32 panel**	**CF69 panel**	**CF32 panel**	**CF69 panel**	**CF32 panel**	**CF69 panel**
African American	152,584 (9.5%)	10,118 (9.2%)	1,451	128	1 in 105	1 in 79
Native American	2,300 (0.1%)	133 (0.1%)	39	2	1 in 59	1 in 67
Asian	20,356 (1.3%)	1,566 (1.4%)	78	5	1 in 261	1 in 313
Caucasian	438,026 (27.4%)	16,242 (14.8%)	15,718	596	1 in 28	1 in 27
Hispanic	88,331 (5.5%)	13,924 (12.7%)	1,287	292	1 in 69	1 in 48
Other	38,177 (2.4%)	3,324 (3.0%)	521	54	1 in 73	1 in 62
Not provided	861,534 (53.8%)	64,523 (58.7%)	26,244	2,011	1 in 33	1 in 32
All individuals	16,01,308 (100%)	109,830 (100%)	45,338	3.087	1 in 35	1 in 36

[a](%) Is the relative frequency for each ethnic group as a percentage of the total number of individuals tested.

69-mutation panel did not significantly improve the detection rate demonstrated by the 23 ACMG/ACOG mutations (93.5%) in the Caucasian population ($P = 0.123$), although an additional 16 variants were identified (see **Supplementary Table S2** online).

In Hispanic individuals, the ACMG/ACOG panel would have detected only 68.2% of *CFTR* mutations identified by the 69-mutation panel. The CF69 panel identified 15 additional variants (31.8%; $P < 0.01$; see **Supplementary Table S2** online). Two variants, L206W and D1152H, accounted for 19.1% of the mutant alleles detected in this ethnic group. There were two variants identified only in Native Americans (40%), and none were unique to the Asian-American group.

DISCUSSION

Universal CF carrier screening panels must balance detection of CF carriers in the highest-prevalence populations, Caucasians and Ashkenazi Jews, with the need to adequately screen for a panethnic autosomal recessive disease within a racially heterogeneous North American population. The introduction of the ACMG/ACOG panel in 2001 was a huge step forward in universal CF carrier screening. The 23-mutation panel has performed well over the years and has met the expectations of a minimum CF carrier screening panel.[5,15] However, the panel does not perform as well in regions with a higher percentage of African Americans, Hispanic Americans, or members of other ethnic groups.[9,13,14,16] The ACMG/ACOG panel was originally designed for the Caucasian and Ashkenazi Jewish population and excludes mutations that are important in other ethnic groups "that are significant components of the American mosaic."[16] One may reduce bias in CF carrier screening by using a screening panel containing *CFTR* variants representing the population to be screened. However, much of the CF carrier screening is performed at reference laboratories, where tailored CF screening panels are not tenable. Instead, extended CF carrier screening panels are used. These panels are composed of the ACMG/ACOG *CFTR* variants and additional variants that represent mutations found in high frequency in a number of racial or ethnic groups.[9,13]

The most common variants from the 32-mutation panel are high-frequency alleles found on the ACMG/ACOG panel (**Table 1**). The nine additional variants detected only an additional 0.9% of *CFTR* mutations. At least one *CFTR* mutant allele was identified for 63 of the 69 mutations (88.6%) making up the extended 69-mutation panel (**Table 2**). Forty variants not present on the 23-mutation panel accounted for 14.4% of all mutations, similar to the results reported by Rohlfs et al.[9] using a 97-mutation panel. These authors identified 64 mutations that were not part of the 23-mutation panel, accounting for 13% of the mutations identified.

Several alleles not found on the ACMG/ACOG panel were found at relatively high frequency (**Table 2**), including D1152H (4.0%), L206W (2.4%), c.3744delA (0.9%), F311del (0.8%), R117C (0.7%), and S549N (0.6%). Clearly, the variants found on the 23-mutation panel are not the only variants present at a relatively high frequency among CF carriers.

The disparity in our study between the proportions of Caucasian individuals in the populations screened using the 32-mutation panel (27.4%) and the 69-mutation panel (14.8%) probably results from a clinician bias toward utilization of expanded panels for non-Caucasians and a relaxation in ethnicity data collection as CF carrier screening test volumes increased and other ethnic groups began to be included in the screening. For example, Strom et al.[7] used the 32-mutation screening panel and observed ~60% Caucasians in their test population, whereas Rohlfs et al.,[9] using a 97-mutation panel, observed only 43% Caucasians in their test population.

The observed carrier frequencies for both the 32-mutation and the 69-mutation panel screening populations were very similar to those previously observed for Caucasians (1/28), individuals without ethnicity data provided (1/33), and individuals from large heterogeneous test populations (1/36).[7,9]

The carrier frequency observed for the test population as a whole is expected to be lower than that observed for Caucasians because the presence of ethnicities in which CF is less prevalent dilutes the overall carrier frequency. The similarity in the carrier frequencies between the overall test population and individuals for whom ethnicity data were not collected suggests an absence of bias in terms of ethnic makeup of the latter group, as reported by Rohlfs et al.[9]

The carrier frequency for Hispanics was greater in the population screened with the 69-mutation panel (1 in 48) than the frequencies obtained with either the 32-mutation panel (1 in 69) or the previously reported 23-mutation panel (1 in 58).[17]

This suggests greater mutation detection by the 69-mutation panel due to the addition of variants specific to the Hispanic population. In fact, eight non–ACMG/ACOG variants were observed only in Hispanics screened by the 69-mutation panel (see **Supplementary Table S2** online). These variants are 3876delA, S549N, 406-1G>A, 3199del6, W1089X, R1158X, R352Q, and 2183AA>G, and they account for 8.1% of the mutations detected in the Hispanic population. In addition, these variants include three of the five variants that Sugarman et al. (2004)[18] identified as accounting for 5.6% of *CFTR* mutations in Hispanic CF carriers and 7.6% of mutations in Hispanic CF patients. Three of these variants are also among the seven alleles recommended by Schrijver et al.[13] to increase carrier detection rates in Hispanics. These data also suggest that the CF carrier frequency in Hispanics may be greater than previously anticipated.

The carrier frequency for African Americans was higher in the 69-mutation panel population (1 in 79) relative to the 32-mutation panel (1 in 105) or the 23-mutation-panel-derived data (1 in 107). These data reflect greater mutation detection by the 69-mutation panel. The data for Native Americans and Asian Americans are difficult to interpret due to the low number of race-specific variants in the panel and the low prevalence of CF in these populations.

Overall, use of the ACMG/ACOG panel would have detected only 78.3% of mutations in the African-American ethnic group screened by the 69-mutation panel. The extended panel detected 21.7% more mutations ($P < 0.01$) using six additional race-specific (R75X, G480C, A559T, 2307insA, S1255X, and 3791delC) and seven panethnic variants (see **Supplementary Table S2** online). Among Hispanics, the 69-mutation panel detected 31.8% more pathogenic variants than the ACMG/ACOG panel ($P < 0.001$). Four ethnicity-specific variants (R352Q, 406-1G>A, 3199del6, and W1089X) and 12 panethnic variants were detected. Two variants, D1152H and L206W, account for 19.1% of all mutations identified in the Hispanic group and deserve special attention. In a combined African-American and Caucasian group, the relative frequencies for the two variants are only 1.6 and 3.2%, respectively (see **Supplementary Table S2** online). Their phenotypic variability has created controversy around their inclusion in extended CF panels. Strom et al.[7] advocate against adding these variants to CF panels, stating that detection of D1152H and L206W during carrier screening may increase the rate of pregnancy termination among parents who fear having a child with classic CF, not to mention inflating the mutation detection rate among Hispanic Americans.[7] It is acknowledged now in the literature that the D1152H variant belongs to both the CF-causing and CFTR-related disorder groups,[19–21] and, in conjunction with an established CF-causing mutation, it could still manifest as typical CF.[19,22] Burgel et al.[23] studied 42 patients with D1152H mutations and reported that the variant, in conjunction with a CF-causing mutation, can cause significant pulmonary disease, albeit with longer survival. One of the authors of this article observed similar results (K.J.F., unpublished data). Sosnay et al.[24] did not find enough evidence for the D1152H variant to meet clinical and functional criteria consistent with disease and hence categorized the variant as indeterminate. We agree with the authors that variants regarded by some as indeterminate warrant periodic review "to quantify the probability of causing or not causing disease,"[24] but their inclusion in conjunction with accurate counseling should not be summarily dismissed. Even without the D1152H variant, the use of the 69-mutation panel will improve the detection rate for the Hispanic population by 22.2% ($P < 0.04$).

The same disagreement exists with regard to the L206W variant. Clain et al.[25] analyzed 36 clinical cases of CF patients who were compound heterozygous for L206W and a severe CF mutation. Twelve of the cases were from the authors' own data, whereas the other 24 cases were obtained from the literature. Using clinical and functional studies, the group demonstrated that L206W is a disease-causing mutation with variable phenotype ranging from CF with pancreatic insufficiency to congenital bilateral absence of the vas deferens. Data from the CFTR2 project indicated that the L206W variant was observed in 82 CF patients worldwide and should be considered a disease-causing mutation.[26] As such, we support this conclusion.

Data from screening of two different populations for different sets of mutations allow for examining the role of screening population sample size on mutation detection. Screening a population of 1.6 million individuals with the 32-mutation panel detects a set of unique mutations for each ethnic group. As expected for mostly rare alleles, as population size decreases, the probability of detecting a set of mutations also declines. However, even with the population size remaining small, the number of mutations detected increases as the panel size increases. These are expected results, but they shed light on the relative improvement in race representation for a given panel. Among Caucasians, the increase in panel size across the same relatively small sample population reflects an observation of ACOG in its latest guidelines: "it is becoming increasingly difficult to assign a single ethnicity to affected individuals."[4] Thus, the number of mutations detected in Caucasians across the same population increases when an extended panel is enriched with other ethnicity-specific variants. Another factor may be that extended panels may be used preferentially by physicians for diagnostic testing. Admixture and uncertain ethnicity may also contribute to higher detection rates for extended panels.

Based on our data alone, there were no obvious advantages of using the 69-mutation panel instead of the 32-mutation panel for a purely Caucasian population. Rohlfs et al.[9], however, reported improved mutation detection with a 97-mutation panel as compared with the 23-mutation ACMG/ACOG panel in a well-defined Caucasian population. This discrepancy may arise from the contribution of 28 variants not included in the 69-mutation panel, especially 2184insA and CFTRdele2,3, which were the 15th and the 16th most common variants among Caucasians in the study by Rohlfs et al.[9] The apparent higher representation of Caucasians (43%) reported

by Rohlfs et al.[9] in their target population as compared with our Caucasian group screened for 69 mutations (14.8%) is also probably a contributing factor.

Recently, Sosnay et al.[24] extensively characterized 127 CF variants in a cohort of 36,696 mostly Caucasian CF patients (95%). According to the authors, use of those 127 variants for carrier screening would significantly increase sensitivity of testing for couples undergoing carrier screening, thus indicating possible advantages of this expanded panel in a primarily Caucasian population.

Our results demonstrate that extended panels have definable value for reference laboratories that analyze samples from many different regions of the country, with directors and genetic counselors well versed in results interpretation, and will benefit ethnic groups beyond the standard recommendation. There is also specific utility for the screening of partners of known CF carriers and equivocal diagnostic cases.

In conclusion, although the clinical utility of extended CF panels remains a topic of active discussion, their use improves the mutation detection rate for specific mutations within specific target populations.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

DISCLOSURE

V.V.Z. and M.E. are current full-time employees at Laboratory Corporation of America. The other authors declare no conflict of interest.

REFERENCES

1. Moskowitz SM, Chmiel JF, Sternen DL, et al. Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med* 2008;10:851–868.
2. Ferec C, Cutting GR. Assessing the Disease-Liability of Mutations in CFTR. *Cold Spring Harb Perspect Med* 2012;2:a009480.
3. Schrijver I. Mutation distribution in expanded screening for cystic fibrosis: making up the balance in a context of ethnic diversity. *Clin Chem* 2011;57:799–801.
4. American College of Obstetricians and Gynecologists Committee on Genetics. ACOG Committee Opinion No. 486: update on carrier screening for cystic fibrosis. *Obstet Gynecol* 2011;117:1028–1031.
5. Grody WW, Cutting GR, Watson MS. The Cystic Fibrosis mutation "arms race": when less is more. *Genet Med* 2007;9:739–744.
6. Grody WW, Cutting GR, Klinger KW, Richards CS, Watson MS, Desnick RJ; Subcommittee on Cystic Fibrosis Screening, Accreditation of Genetic Services Committee, ACMG. American College of Medical Genetics. Laboratory standards and guidelines for population-based cystic fibrosis carrier screening. *Genet Med* 2001;3:149–154.
7. Strom CM, Crossley B, Buller-Buerkle A, et al. Cystic fibrosis testing 8 years on: lessons learned from carrier screening and sequencing analysis. *Genet Med* 2011;13:166–172.
8. Watson MS, Cutting GR, Desnick RJ, et al. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. *Genet Med* 2004;6:387–391.
9. Rohlfs EM, Zhou Z, Heim RA, et al. Cystic fibrosis carrier testing in an ethnically diverse US population. *Clin Chem* 2011;57:841–848.
10. Mercier B, Raguénès O, Estivill X, et al. Complete detection of mutations in cystic fibrosis patients of Native American origin. *Hum Genet* 1994;94:629–632.
11. Alper OM, Wong LJ, Young S, et al. Identification of novel and rare mutations in California Hispanic and African American cystic fibrosis patients. *Hum Mutat* 2004;24:353.
12. Macek M Jr, Mackova A, Hamosh A, et al. Identification of common cystic fibrosis mutations in African-Americans with cystic fibrosis increases the detection rate to 75%. *Am J Hum Genet* 1997;60:1122–1127.
13. Schrijver I, Ramalingam S, Sankaran R, et al. Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. *J Mol Diagn* 2005;7:289–299.
14. Heim RA, Sugarman EA, Allitto BA. Improved detection of cystic fibrosis mutations in the heterogeneous U.S. population using an expanded, pan-ethnic mutation panel. *Genet Med* 2001;3:168–176.
15. Palomaki GE. Prenatal screening for cystic fibrosis: an early report card. *Genet Med* 2004;6:115–116.
16. Gilbert F. Cystic fibrosis carrier screening: steps in the development of a mutation panel. *Genet Test* 2001;5:223–227.
17. Palomaki GE, FitzSimmons SC, Haddow JE. Clinical sensitivity of prenatal screening for cystic fibrosis via CFTR carrier testing in a United States panethnic population. *Genet Med* 2004;6:405–414.
18. Sugarman EA, Rohlfs EM, Silverman LM, Allitto BA. CFTR mutation distribution among U.S. Hispanic and African American individuals: evaluation in cystic fibrosis patient and carrier screening populations. *Genet Med* 2004;6:392–399.
19. Castellani C, Cuppens H, Macek M Jr, et al. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros* 2008;7:179–196.
20. Dequeker E, Stuhrmann M, Morris MA, et al. Best practice guidelines for molecular genetic diagnosis of cystic fibrosis and CFTR-related disorders–updated European recommendations. *Eur J Hum Genet* 2009;17:51–65.
21. Highsmith WE Jr, Friedman KJ, Burch LH, et al. A CFTR mutation (D1152H) in a family with mild lung disease and normal sweat chlorides. *Clin Genet* 2005;68:88–90.
22. Peleg L, Karpati M, Bronstein S, et al. The D1152H cystic fibrosis mutation in prenatal carrier screening, patients and prenatal diagnosis. *J Med Screen* 2011;18:169–172.
23. Burgel PR, Fajac I, Hubert D, et al. Non-classic cystic fibrosis associated with D1152H CFTR mutation. *Clin Genet* 2010;77:355–364.
24. Sosnay PR, Siklosi KR, Van Goor F, et al. Defining the disease liability of variants in the cystic fibrosis transmembrane conductance regulator gene. *Nat Genet* 2013;45:1160–1167.
25. Clain J, Lehmann-Che J, Duguépéroux I, et al. Misprocessing of the CFTR protein leads to mild cystic fibrosis phenotype. *Hum Mutat* 2005;25:360–371.
26. The Clinical and Functional Translation of CFTR (CFTR2), 2013. http://www.cftr2.org. Accessed 26 October 2013.