Cystic Fibrosis Carrier Testing in an Ethnically Diverse US Population

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BACKGROUND: The incidence of cystic fibrosis (CF) and the frequency of specific disease-causing mutations vary among populations. Affected individuals experience a range of serious clinical consequences, notably lung and pancreatic disease, which are only partially dependent on genotype.

METHODS: An allele-specific primer-extension reaction, liquid-phase hybridization to a bead array, and subsequent fluorescence detection were used in testing for carriers of 98 *CFTR* [cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)] mutations among 364 890 referred individuals with no family history of CF.

RESULTS: One in 38 individuals carried one of the 98 *CFTR* mutations included in this panel. Of the 87 different mutations detected, 18 were limited to a single ethnic group. African American, Hispanic, and Asian individuals accounted for 33% of the individuals tested. The mutation frequency distribution of Caucasians was significantly different from that of each of these ethnic groups ($P < 1 \times 10^{-10}$).

CONCLUSIONS: Carrier testing using a broad mutation panel detects differences in the distribution of mutations among ethnic groups in the US.

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Cystic fibrosis (CF)[2] is a panethnic autosomal recessive disease characterized by obstructive lung disease with microbial colonization, exocrine pancreatic insufficiency, diabetes, liver disease, and congenital bilateral absence of the vas deferens (CBAVD) in affected males *(1, 2)*. Advancing medical care has improved the quality of life for patients with CF, yet life expectancy is limited, with a mean life expectancy of 37.4 years in the US *(3)*. The age of onset and clinical expression of CF is variable and only partially dependent on *CFTR* [cystic fibrosis transmembrane conductance regulator (ATP-binding cassette sub-family C, member 7)] genotype, owing to the modification of the phenotype by other genes and environmental factors *(4–6)*. The prevalence of CF is highest in Caucasians (1 in 2500) and Ashkenazi Jews (1 in 2300) *(7)*, but CF is also present in a large number of Native Americans (1 in 10 900), African Americans (1 in 15 000), and Asians (1 in 35 000) *(7, 8)*. Estimates of the prevalence of CF in Hispanic Americans vary from 1 in 9200 to 1 in 13 500 *(7, 8)*. This variation is likely due to the imprecise classification of ethnicity when self-reported, as well as to regional variation in the ancestry of Hispanic populations *(9)*. Overall, the prevalence of CF in the US is approximately 1 in 3900 *(8)*.

In addition to the variable prevalence of CF disease in US populations, many of the approximately 1600 mutations that have been identified in the *CFTR* gene to date occur in multiple populations at varying frequencies. Notably, the frequency of p.F508del among CF patients is 72% in US Caucasians, 31%–44% in African Americans, and 18% in Iranians *(7, 10–13)*. Even among Caucasian CF patients worldwide, the p.F508del mutation frequency varies widely: from 88% in Danes to 30% in Ashkenazi Jews *(14, 15)*.

Other mutations are clearly more common in certain ethnic groups, suggesting an ancestral origin. For example, the frequency of c.2988+1G>A is 8.8%–14% in African American patients, 0.16%–2.2% in Hispanic patients, and 0.08%–0.1% in Caucasian CF patients *(7, 10–12)*. Mutations limited to specific ethnic groups have also been reported. For example, c.3744delA accounts for 2.2%–5.7% of CF chromosomes in Hispanics *(11, 16, 17)*, and p.Y1092X accounts for 37.5% of CF chromosomes in Iraqi Jews *(18)*. Identification of these apparently ethnicity-limited mutations is an important aspect of addressing comprehensive CF testing.

The American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) have published guidelines for CF screening for a panel of 23 mutations with a prevalence of at least 0.1% in all US CF patients (19–21). The 2001 guidelines stated that additional mutations may be added to improve the analytical sensitivity for other ethnic groups, and the 2004 revised guidelines provided a list of such mutations, although their relationship to clinical presentation was not assessed. The recommended 23-mutation panel provides a high detection rate for Caucasians (88.4%) and Ashkenazi Jews (94.1%), the 2 groups for whom testing was initially recommended (7, 10). In 2005, ACOG recommended that information about screening be made available to all couples regardless of race or ethnicity (22).

In this study, we report the frequency of 98 different CF mutations in 364 890 individuals referred for carrier testing and demonstrate the diversity of specific mutations among ethnic groups constituting the pan-ethnic US population.

Materials and Methods

PATIENT SAMPLES

From September 2005 through May 2008, 371 389 peripheral blood, blood spot, or mouthwash samples were received in our reference laboratory for *CFTR* mutation analysis to determine individual carrier status. Samples were received from all 50 states in the US. It is standard for the referring physician to obtain informed consent. Ethics approval was not required because testing was performed for clinical purposes. The referring physician or laboratory noted the clinical indications for testing on the test requisitions. The predominant clinical indication (98.3%) was carrier testing with no known family history of CF. To assess mutation frequencies among individuals tested for screening purposes only, we excluded all individuals younger than 10 years of age from this analysis. Individuals between 10 and 14 years of age were included only if they were female and pregnancy was recorded on the test requisition. Individuals ≥15 years of age were included. After assessment for age and pregnancy status, 364 890 individuals referred for carrier testing with no family history of CF were included in this analysis. The majority of the samples (93.1%) were from females; 5.6% were from males. The individual's sex was not provided in 1.3% of the cases. The remaining 1.7% (n = 6499) of the referrals were excluded from further analysis because their clinical indications were consistent with an individual carrier risk greater than that of the general population. Such indications included abnormal ultrasound findings in a fetus, a family history or suspected family history of CF, and follow-up testing of a parent after a positive result in the child's newborn-screening test.

The ethnicity or country of origin, which was noted on 85% of the test requisitions, represented over 1400 different ethnicities, combinations of ethnicities, or countries of origin. When a country of origin was provided, the individual's ethnicity was categorized as follows. The "Hispanic" category included individuals from South and Central American countries, Cuba, Puerto Rico, the Dominican Republic, Jamaica, and Mexico. The "Caucasian" category included individuals from European countries, Russia, Canada, Australia, and New Zealand, unless another ethnicity was specified. The "Asian" category included individuals from Japan, China, Thailand, Indonesia, and neighboring countries. "Southern Asians" included individuals from India, Pakistan, Nepal, Afghanistan, and neighboring countries. The "Middle Eastern" category included individuals from Lebanon, Egypt, Iraq, Iran, Kuwait, and neighboring countries. The "Jewish" category included individuals whose ethnicity was provided as "Sephardic Jewish" or "Jewish" and did not include individuals whose ethnicity was provided as "Ashkenazi Jewish." Individuals from countries that could not be categorized as described above were grouped as "Other." Individuals with multiple ethnicities were categorized according to whether they were part African American, part Caucasian, part Hispanic, or other ethnicities exclusive of these.

MUTATION PANEL

All samples were tested for a panel of 98 mutations selected on the basis of their reported frequency in the literature, their association with CF, and their predicted effect on the CFTR protein. The mutations included in the panel are listed in Table 1 in the Data Supplement that accompanies the online version of this article at http://www.clinchem.org/content/vol57/issue6. Mutations are named according to GenBank mRNA reference sequence NM_000492.3 and the Human Genome Variation Society's recommended convention of specifying the A of the ATG initiation codon as nucleotide number 1 (23). The mutation analysis discriminated between p.F508del and the benign polymorphisms p.F508C, p.I506V, and p.I507V.

CFTR MUTATION ANALYSIS

Genomic DNA was extracted from peripheral blood lymphocytes or buccal cells (obtained from mouthwash samples) by using a minor modification to reagent volumes of the procedure for the QIAmp 96 DNA Blood Kit (Qiagen). Similar modification of the procedure for the Qiagen QIAmp DNA Micro Kit was used to extract genomic DNA from blood spot samples

(http://www.qiagen.com). Regions of the *CFTR* gene were amplified and subjected to multiplex allele-specific primer extension with subsequent hybridization to a Luminex xMAP® bead array and fluorescence detection with the Luminex 100™ (Luminex Corporation; http://www.luminexcorp.com). The median fluorescent intensity was determined, and the presence or absence of mutant and wild-type alleles was evaluated from the ratio of the mutant signal to the wild-type signal for the following mutations: c.1155_1156dupTA, c.2657+5G>A, c.3717+12191C>T, p.A455E, p.D1152H, p.F508del, p.G542X, p.G551D, p.I507del, p.L206W, p.N1303K, p.R117H, p.W1282X, and c.54-5940_273+10250del21kb. Some mutations were detected by determining the absolute median fluorescent intensity, with zygosity determined by bidirectional sequence analysis using the BigDye® Terminator v3.1 Cycle Sequencing Kit followed by capillary electrophoresis (Applied Biosystems; http://www.appliedbiosystems.com). The remaining mutations were detected with beads designed to hybridize to PCR products generated from >1 mutant allele. In these instances, specific alleles were identified by bidirectional sequence analysis, as described above. For QC and quality-assurance purposes, 2 types of controls were tested with each batch of 768 patient samples. The controls included DNA extracted from individuals with known mutations and synthetic controls amplified from 100-mer oligonucleotides representing every mutation. In addition, all primer and bead lots were verified with these controls before their use for patient testing.

CFTR intron 8 poly(T) variant analysis was performed for all samples positive for p.R117H. PCR products containing the portion of the *CFTR* gene spanning the poly(T) tract were immobilized on a positively charged nylon membrane and hybridized with radioactively labeled allele-specific oligonucleotides corresponding to the 5T, 7T, or 9T alleles. Poly(T) genotypes were determined after exposure of the nylon membrane to x-ray film.

STATISTICAL ANALYSIS

The differences between corrected carrier frequencies and carrier frequencies calculated from CF incidence data *(7)* were tested with a 2-population Z-test. In particular, the observed number of carriers for a specific ethnic group was corrected by dividing the number of carrier samples by the panel detection rate. The resulting binomial distribution was approximated by the corresponding normal distribution. The second normal population was derived from the birth prevalence estimates [see Table 5 in *(7)*]. The logarithm of the birth prevalence SD was calculated from the log-transformed 95% confidence limits under the assumption of normality. The carrier rate SD was estimated by multiplying the logarithm of the birth prevalence SD by the first derivative of the transformation function at the mean where the transformation function was defined as $f(x) = 2 \times \exp{(x/2)}$, with x being the logarithm of the birth prevalence. The Z-test P values were calculated for the difference in the means between the 2 distributions. The calculations were performed in Microsoft Excel.

The P value for the Pearson χ^2 statistic was used to test the differences between mutation frequencies for the different ethnic groups. The difference in the carrier frequencies between the overall and "Not Provided" groups were tested with a nonadjusted χ^2 statistic. Statistical inference was performed with SYSTAT 12 (Systat Software).

Results

CARRIER TESTING IN INDIVIDUALS WITH NO FAMILY HISTORY OF CF

Among 364 890 individuals with no family history of CF who were tested for the 98 mutations, the frequency of detected mutations was 1 in 38, and 87 unique mutations were identified (see Table 1 in the online Data Supplement). Eleven of the 98 mutations were not detected in this sample set; however, 8 of the mutations were identified in sample sets from other time periods and for clinical indications other than carrier testing (i.e., patient known to be affected with CF, patient suspected to have CF, family history of CF; data not shown). Mutations not included in the ACMG/ACOG mutation panel accounted for 13% of all mutations identified. The least frequently identified ACMG/ACOG mutation was c.2052delA (0.09%).

p.F508del accounted for 57.7% of all mutations, followed by p.R117H (8.9%). Of the 841 individuals who carried a single p.R117H mutation, 3 females carried 2 copies of the 5T allele, whereas the majority (n = 757) did not carry a 5T allele in cis with the p.R117H mutation on the basis of the poly(T) results of 7T/7T, 7T/9T, or 7T/11T. The poly(T) background of the p.R117H mutation could not be determined for the remaining 81 individuals who carried 5T/7T or 5T/9T.

Thirty-six of the tested individuals carried 2 mutations. Twenty-one of these individuals carried at least 1 copy of p.R117H (Table 1). Twenty of the 21 individuals carried either 7T/7T or 7T/9T, and 1 individual, a 32-year-old woman, carried 5T/9T and p.R117H/p.F508del. Fifteen individuals (i.e., 1 in 24 326) carried at least 1 mutation that has been reported to be associated with variable phenotypes in some patients *(24–28)*. Of these mutations, p.R117H, c.3717+12191C>T, and p.R347P are included in the ACMG panel, a finding consistent with that panel's inclusion

Table 1. Genotypes of carriers with 2 mutations.

CFTR mutations[a]	Individuals, n
p.F508del/p.R117H	16
5T/9T	1
7T/9T	15
p.F508del/p.D1152H	3
p.R117H/p.R117H, 7T/7T	2
p.D1152H/p.D1152H	2
p.W1282X/p.D1152H	2
p.D1152H/p.G551D	1
c.3717+12191C>T/p.R352Q	1
c.3717+12191C>T/c.3717+12191C>T	1
p.F508del/c.3717+12191C>T	1
p.F508del/p.L206W	1
p.F508del/p.R117C	1
p.F508del/p.R347H	1
p.F508del/p.R347P	1
p.R117H/p.W1282X, 7T/7T	1
p.R117H/p.G551D, 7T/7T	1
p.R117H/p.G542X, 7T/9T	1

[a] Human Genome Variation Society nomenclature [Ogino et al. (23)]. See Table 1 in the online Data Supplement for the corresponding legacy nomenclature.

of mutations associated with mild or severe disease (19).

When the test was updated in 2005, 21 of the 98 mutations in the panel were added to include additional clinically relevant mutations, including those expected to increase the detection rate for Hispanic and African American individuals. Fourteen of the 21 mutations were detected in 168 individuals and accounted for 1.7% of all mutations identified. c.54-5940_273+10250del21kb was identified in Ashkenazi Jewish, Caucasian, and Hispanic individuals and accounted for 0.45% of all mutations. Four of the newly added mutations were identified in African Americans, and of these mutations, p.R764X accounted for 0.63% of all mutations detected in this group. Ten of the newly added mutations were identified in Hispanic individuals, and of these mutations, c.803delA accounted for 1.15% of all mutations in Hispanics.

ETHNICITY-SPECIFIC CARRIER FREQUENCY

Among individuals with no family history of CF, the most common ethnicity was Caucasian (43%), and the least common was Native American (0.1%) (Table 2). All 7 of the Native Americans who carried a mutation carried p.F508del. Observed carrier frequencies based on the 98 mutations tested ranged from 1 in 28 in Caucasians to 1 in 242 in Asians. The observed carrier frequency in those for whom ethnicity or country of origin was not provided was 1 in 37, which was not significantly different from the overall observed carrier frequency of 1 in 38 ($P = 0.69$). Among individuals reporting multiple ethnicities (2.3%), the observed carrier frequency of 1 in 34 fell within the range of the carrier frequencies of the predominant US ethnic groups tested (i.e., Caucasian, African American, and Hispanic).

Together, the Asian, South Asian, and Middle Eastern individuals represented 6.5% of the individuals tested. Testing of 19 334 Asians identified 81 mutations for an observed carrier frequency of 1 in 242. The corrected carrier frequency in Asians (1 in 176) was not significantly different ($P = 0.11$) from the carrier frequency calculated from the CF incidence (1 in 94); this assessment was based on the wide 95% CI (1 in 59 to 1 in 179) estimated from Palomaki et al.(7) (Table 2). The observed carrier frequencies for South Asian and Middle Eastern individuals were 1 in 118 and 1 in 91, respectively. The fact that mutations targeting these ethnic groups were not specifically selected when the mutation panel was defined suggests that these data likely reflect an underestimate of the carrier frequency in these groups. Alternatively, the results may be due to an appropriate carrier frequency reflecting the lower CF incidence in these groups.

Carrier screening of populations provides the frequency of specific CFTR alleles in healthy populations for comparison with that in CF patient populations. For example, although p.I148T was initially identified in >0.1% of affected patients, it was subsequently reclassified as a benign allele after a frequency higher than expected was observed in the carrier-screening population (29, 30). To rule out the presence of similarly unexpected benign alleles in the panel, we compared our observed carrier frequency (corrected with published detection rates to approximate 100% detection) with the carrier frequency calculated from the CF incidence (7, 10, 11, 15). When the observed Caucasian carrier frequency was corrected with the detection rate of 91.56% [Table 1, Cystic Fibrosis Foundation data excluding p.I148T, in (10)], the carrier frequency in Caucasians increased from 1 in 28 to 1 in 25. This carrier frequency is equal to the carrier frequency calculated from the incidence of CF in Caucasians (7). Similar analyses show that the observed carrier frequency in African Americans, Ashkenazi Jews, Asians, and Hispanics is not significantly different from the carrier frequency calculated from CF incidence (Table 2).

Ethnic group	Individuals, n	Proportion of tested individuals, %	Observed carrier frequency	Corrected carrier frequency	Calculated from incidence[a]	P
Table 2. Ethnicity-specific data for individuals with no history of CF referred for carrier screening.						
African American	40 071	11.0	1 in 84	1 in 61[b]; 1 in 63[c]	1 in 61	0.88; 0.57
Ashkenazi Jewish	15 244	4.2	1 in 29	1 in 27[c]; 1 in 28[d]	1 in 24	0.12; 0.052
Asian	19 344	5.3	1 in 242	1 in 176[c]	1 in 94	0.11
Caucasian	158 658	43.5	1 in 28	1 in 25[e]	1 in 25	0.63
Hispanic	60 930	16.7	1 in 59	1 in 45[b]; 1 in 49[c]	1 in 58	0.11; 0.3
Jewish	1887	0.5	1 in 32			
Middle Eastern	1726	0.5	1 in 91			
Native American	491	0.1	1 in 70			
South Asian	2365	0.7	1 in 118			
Other ethnicity	1663	0.5	1 in 111			
>1 Ethnicity	8514	2.3	1 in 34			
Part African American	1238	0.3	1 in 56			
Part Caucasian	7510	2.1	1 in 32			
Part Hispanic	2380	0.7	1 in 51			
Not provided	53 997	14.8	1 in 37			
All individuals	364 890	100	1 in 38			

[a] Palomaki et al. (7).
[b] Carrier frequency corrected with detection rate reported by Sugarman et al. (11).
[c] Carrier frequency corrected with detection rate reported by Palomaki et al. (7) (Cystic Fibrosis Foundation data).
[d] Carrier frequency corrected with the detection rate reported by Abeliovich et al. (15).
[e] Carrier frequency corrected with the detection rate reported by Palomaki et al. (10) (Cystic Fibrosis Foundation data).

ETHNICITY-SPECIFIC DISTRIBUTION OF MUTATIONS

Among Caucasians, the 23 mutations in the ACMG/ACOG standard panel were detected, along with 44 additional mutations. The 44 additional mutations accounted for 9.3% of all detected alleles in Caucasians (Table 3). Eighteen different mutations were identified in Ashkenazi Jewish individuals; 5 non-ACMG mutations (p.D1152H, c.54-5940_273+10250del21kb, p.S549R, p.W1089X, p.[Q359K;T360K]) accounted for 13% of all alleles detected in this group. Non-ACMG/ACOG mutations accounted for a substantial proportion of the alleles identified in African Americans (22.7%), Hispanics (26.9%), and Asians (37.0%).

Numerous mutations were identified in only 1 ethnic group (see Tables 2 and 3 in the online Data Supplement). Four mutations (p.S1255X, p.G330X, c.313delA, p.S364P) were identified only in African Americans, 8 mutations (p.G178R, p.T338I, c.262_263delTT, p.M1101K, c.442delA, p.K710X, p.P574H, p.Q1238X) were identified only in Caucasians, and 3 mutations (c.580-1G>T, c.531delT, p.Q890X) were identified only in Hispanics. Consistent with the literature, an apparently ethnicity-limited mutation (p.Y1092X C>G) was found in 2 Jewish individuals (18). Overall, 68 of the 87 mutations were identified in >1 ethnic group or in 1

ethnic group and the "Not Provided" group. The mutation frequency distribution of Caucasians was significantly different ($P < 1 \times 10^{-10}$) from each of 4 ethnic groups (African Americans, Asians, Hispanics, and Ashkenazi Jews).

Discussion

The observed carrier frequency for 364 890 individuals tested with a 98-mutation panel was 1 in 38. This carrier frequency is lower than that for Ashkenazi Jewish or Caucasian individuals and reflects the contribution of Hispanic, African American, and Asian individuals, who represent 33% of the study population. Nearly 15% of the individuals tested did not provide ethnicity information; however, the data do not appear to show a bias due to nonreporting by a specific ethnic group, because the carrier frequency in this group was not significantly different from that of the total population.

This data set generated frequency data for 21 mutations that were added to the panel in 2005. Some mutations, such as c.54-5940_273+10250del21kb, were added because of their reported frequency in Caucasian CF patients (31). Three mutations (c.54-5940_273+10250del21kb, c.2052dupA, and p.R75X)

Ethnic group	Total individual mutations, n	Non-ACMG/ACOG mutations, n	ACMG/ACOG mutations, n	Total mutant alleles, n	Non-ACMG/ACOG alleles, n (%)
Table 3. *CFTR* mutations detected in individuals referred for carrier screening with no history of CF.					
African American	46	28	18	476	108 (22.7)
Ashkenazi Jewish	18	5	13	539	70 (13.0)
Asian	24	15	9	81	30 (37.0)
Caucasian	67	44	23	5763	536 (9.3)
Hispanic	53	34	19	1046	281 (26.9)
Jewish	11	3	8	59	14 (23.7)
Middle Eastern	9	3	6	19	4 (21.0)
Native American	1	0	1	7	0
Southern Asian	8	4	4	20	5 (25.0)
Other ethnicity	8	3	5	15	3 (20.0)
>1 Ethnicity	30	12	18	248	33 (13.3)
Part African American	10	4	6	22	4 (18.2)
Part Caucasian	29	11	18	236	30 (12.7)
Part Hispanic	14	4	10	47	8 (17.0)
Not provided	61	39	22	1454	180 (12.4)
All individuals	87	64	23	9727	1264 (13.0)

demonstrated a panethnic frequency consistent with that of mutations included in the ACMG/ACOG panel. An additional 3 mutations (c.803delA, p.R75X, and p.R709X) identified in Asians accounted for 8.6% of all alleles detected in this population. Ten of the 21 mutations were detected in >1 ethnic group and enhanced the overall panethnic mutation-detection rate, as well as that of Hispanic individuals (4.8% increase) and African American individuals (1.5% increase).

We identified individuals with 2 *CFTR* mutations and the clinical indication of carrier testing. Because of the origin of the patient population, we were not able to determine if any of the individuals had clinical symptoms of CF or CF-related disease; however, the identification of 2 mutation carriers during screening is consistent with the variable expression of *CFTR* mutations, which has been reported in other studies of carrier-testing populations (*32–34*). Ninety-four percent of the p.R117H-positive individuals with a second mutation did not carry a 5T allele. p.R117H in cis with 5T generally confers moderate to severe CF, whereas p.R117H in cis with 7T may confer a mild form of CF, a later CF onset, CBAVD, or no apparent disease (*35–37*). Similarly, other mutations identified in carriers of 2 mutations have been associated with variable phenotypic expression ranging from classic disease to CBAVD in adults or an asymptomatic phenotype when identified in the neonatal period (*24–28*). In general, the phenotypic variability in CF is substantial and is due to the *CFTR* genotype, as well as to other genetic

modifiers, environmental factors, and their interactions (*38, 39*). Recognizing the limited predictability of the CF clinical presentation from the *CFTR* genotype, the ACMG has recommended discussion of these limitations during genetic counseling and the informed-consent process (*21*).

The panethnic composition of the US population poses a challenge to the development of mutation panels for most autosomal recessive diseases, because many of the disease-causing mutations have occurred as random events in specific populations. The prevalence of a particular mutation among populations is a function of its mobility and integration into other groups. In this study, we have shown that although the primary ethnic groups in the US share many mutations in common, there is a significant difference in the distribution of the mutations among the ethnic groups ($P < 1 \times 10^{-10}$). In addition, some mutations (such as p.S1255X, which accounted for >1% of the mutations identified in African Americans) appear to be limited to certain ethnic groups. In a panethnic population such as that of the US, the inclusion of both mutations found across several ethnic groups and mutations that appear to be limited to a particular group yet represent a substantial proportion of the group's mutations reduces the impact of uncertain ethnicity when interpreting test results.

CF carrier screening was not initially recommended for non-Caucasian and non–Ashkenazi Jewish individuals because of the lower disease incidence in such groups. In this study, however, fewer than 50% of

the individuals tested reported that they were Caucasian or Ashkenazi Jewish, indicating that other ethnic groups are offered testing and are accepting it. The estimated detection rates with the ACMG/ACOG panel are 64.0% for African Americans, 71.7% for Hispanic Caucasians, and 48.9% for Asians *(7)*. In these populations, both the incidence of CF and the total number of affected patients are lower than in Caucasians. Consequently, mutations that are more common in these ethnic groups may not reach the overall ACMG inclusion threshold of 0.1% in all CF patients. For example, c.2988+1G>A in this study represents 17% of the mutations detected in African American carriers but only 1.39% of the mutations overall. In addition, apparently ethnicity-limited mutations, such as c.2175dupA in African Americans and c.3744delA in Hispanics, make a substantial contribution to the mutation spectrum in these groups but not to the overall population. Consequently, the 22.7% and 26.9% of the *CFTR* alleles identified in this study in Hispanics and African American individuals, respectively, are not mutations that are included in the standard 23-mutation panel. Additionally, the *CFTR* carrier frequency in Hispanics and African American individuals is higher than the carrier frequency for other disorders (e.g., Fanconi anemia, Niemann–Pick disease, Bloom syndrome, and mucolipidosis type IV) in the Ashkenazi Jewish population, for which carrier testing is recommended *(40)*.

A recent publication by Strom et al. *(41)* reports the frequencies of 32 *CFTR* mutations. A comparison of their data and conclusions with ours is limited, however, because their study population was not defined by clinical indication but rather by result (i.e., the detection of a single *CFTR* mutation), ethnicity was not reported in 60% of the cases, and the 40% of cases with known ethnicity included significantly more Caucasians than we have reported ($P < 0.001$). Although the allele frequencies of mutations common to both panels are similar, the report of Strom et al. provides no information regarding 66 of the mutations included in our study. Additionally, in contrast to our analysis, Strom et al. do not include carriers of R117H plus 7T in their analysis, thereby lowering their overall carrier frequency. Furthermore, on the basis of a previous report of allele frequency by another group, Strom et al. state that dele2,3 (c.54-5940_273+10250del21kb) should not be included in CF mutation panels. In contrast, our data demonstrate an allele frequency not only higher (0.45%) than previously reported (0.2%) but also higher than the ACMG/ACOG inclusion threshold of 0.1%.

Overall, the 98-mutation panel identified a mutation in 1 in 38 individuals. Thirteen percent of the mutations, representing 1 carrier in 8, are not included in the standard panel. These data provide carrier and allele frequencies for a subset of *CFTR* mutations in the US populations currently receiving carrier testing and demonstrate that the mutation distribution in Caucasians is significantly different from that in Hispanics, African Americans, and Asians. These data may be considered when selecting or developing mutation panels for diverse populations.

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