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CFTR mutation distribution among U.S. Hispanic and African American individuals: Evaluation in cystic fibrosis patient and carrier screening populations

Elaine A. Sugarman, MS[1], Elizabeth M. Rohlfs, PhD[1], Lawrence M. Silverman, PhD[2], and Bernice A. Allitto, PhD[1]

Purpose: We reviewed *CFTR* mutation distribution among Hispanic and African American individuals referred for CF carrier screening and compared mutation frequencies to those derived from CF patient samples. **Methods:** Results from *CFTR* mutation analyses received from January 2001 through September 2003, were analyzed for four populations: Hispanic individuals with a CF diagnosis ($n = 159$) or carrier screening indication ($n = 15{,}333$) and African American individuals with a CF diagnosis ($n = 108$) or carrier screening indication ($n = 8{,}973$). All samples were tested for the same 87 mutation panel. **Results:** In the Hispanic population, 42 mutations were identified: 30 in the patient population (77.5% detection rate) and 33 among carrier screening referrals. Five mutations not included in the ACMG/ACOG carrier screening panel (3876delA, W1089X, R1066C, S549N, 1949del84) accounted for 7.55% detection in patients and 5.58% among carriers. Among African American referrals, 33 different mutations were identified: 21 in the patient population (74.4% detection) and 23 in the carrier screening population. Together, A559T and 711+5G>A were observed at a detection rate of 3.71% in CF patients and 6.38% in carriers. The mutation distribution seen in both the carrier screening populations reflected an increased frequency of mutations with variable expression such as D1152H, R117H, and L206W. **Conclusions:** A detailed analysis of *CFTR* mutation distribution in the Hispanic and African American patient and carrier screening populations demonstrates that a diverse group of mutations is most appropriate for diagnostic and carrier screening in these populations. To best serve the increasingly diverse U.S. population, ethnic-specific mutations should be included in mutation panels. ***Genet Med* 2004:6(5):392–399.**

Key Words: *cystic fibrosis, Hispanic, African American,* CFTR *mutations, carrier screening*

Classic cystic fibrosis presents with chronic pulmonary disease, pancreatic insufficiency, male infertility, and elevated sweat electrolyte concentrations.[1] Although clinical expression of cystic fibrosis (CF) may vary, most affected individuals experience substantial morbidity and require lifelong care.[1,2] With an incidence of 1:3,200,[3] CF is frequently cited as being one of the most common autosomal recessive disorders among Caucasians, yet its incidence among other ethnic groups is appreciable as well. One in 9,200 individuals of Hispanic descent and 1 in 15,000 African Americans are affected with CF.[3]

In 2001, the American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) issued a joint statement recommending that CF carrier screening be offered to individuals of Ashkenazi Jewish or Caucasian descent and be made available to individuals of other ethnic and racial groups.[4,5] The challenges cited in designing, and now in implementing, a general population screening program for CF include the variable prevalence of *CFTR* mutations among different ethnic and racial groups, the ethnic heterogeneity and increasing admixture of the U.S. population, and the difficulty in accurately assessing an individual's ethnic background for inclusion or exclusion from screening criteria.[4] The ethnic heterogeneity and diversity of the U.S. population continues to grow. From 1970 to 2002, the White non-Hispanic population, decreased from 83% to 69% of the total U.S. population.[6] In 2002, the Hispanic population comprising 13.5%[7] of the total United States population became the largest minority in the U.S. followed by the Black population with almost 13%.[8] In addition, 11.4% of the population is foreign born and 52.2% of the foreign born are from Latin America.[9]

Although the ACMG/ACOG recommended CF carrier screening panel was designed to primarily serve the recommended Caucasian and Ashkenazi Jewish screening populations, the possible need for inclusion of ethnic-specific mutations to serve regional populations has been recognized by the ACMG[10,11] and recommended by others[12,13] as necessary to

From the [1]Genzyme Corporation, Genzyme Genetics, Molecular Diagnostic Laboratory, Westborough, Massachusetts; and [2]University of Virginia, Department of Pathology, Charlottesville, Virginia.

Elaine A. Sugarman, MS, Genzyme Genetics, Molecular Diagnostic Laboratory, 3400 Computer Drive, Westborough, MA 01581.

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best serve the panethnic U.S. population. Selection of ethnic specific mutations for inclusion in mutation panels requires collection and assessment of data from these populations.

Limited carrier screening data exists for *CFTR* mutation frequency among African American and Hispanic populations.[14,15] Given our experience in receiving specimens from over 15,000 Hispanic individuals and almost 9,000 African American individuals for CF carrier screening, we sought to provide data regarding *CFTR* mutation frequencies in these populations and to compare the *CFTR* mutation frequency data from these carrier screening populations with that of CF patient samples analyzed for the same 87 mutations. In this study, we contribute to the data on *CFTR* mutation frequency among the two largest minorities in the U.S. and provide information useful in designing mutation panels to best serve the increasingly diverse U.S. population.

MATERIALS AND METHODS

Patient samples

Among individuals referred for *CFTR* mutation analysis from January 2001 through September 2003, results from four patient populations were analyzed: Hispanic individuals with either a clinical diagnosis of CF ($n = 159$) or referred for carrier screening ($n = 15{,}333$) and African American individuals with a clinical CF diagnosis ($n = 108$) or referred for carrier screening ($n = 8{,}973$). Patient ethnic background and indication for testing were provided by the referring physician. Carrier screening refers to carrier tests performed for individuals with a general population carrier risk and excludes individuals with increased risk factors such as family history of CF or echogenic bowel. The CF patient population is derived from individuals referred with an indication of "known affected" and excludes individuals referred with a "suspected diagnosis" indication. Affected siblings were removed from the CF patient analyses.

Among Hispanic patients with a clinical diagnosis of CF, the age at testing ranged from 1 month to 45 years with 1 year of age being the most frequent age for referral, whereas the average age was 10.1 years. In the affected African American population, the age at testing had a similar range to that of Hispanics (1 month to 46 years), but the most frequent age for referral was later, 8 years, with a population average of 13.8 years.

Geographic distribution varied between affected and carrier screening referrals. Among Hispanic affected patients, samples were referred from 25 states across the U.S. with ≈40% from California, followed by New York (9.3%), Texas (8.7%) and Florida (7.4%). In contrast, carrier screening referrals came from 42 different states with New York and Florida being the most frequent origin of referrals (34% and 24%). Among African American individuals, New York was the most frequent site of referral for both groups; affected individuals were referred from 26 different states, whereas carrier screening referrals came from 42 different states.

Mutations

All individuals were initially tested for the same panel of 87 mutations. Specimens positive for D1270N (see later) or I148T in the absence of 3199del6 were reclassified as negative for the purpose of this analysis. Specimens identified as I148T positive before the start of 3199del6 reflex testing are included in the analysis because their disease causing status is unknown.

87 mutation panel

The following mutations were included in the panel: ΔF508, ΔF311, ΔI507, A455E, A559T, C524X, D1152H, D1270N, E60X, G178R, G330X, G480C, G542X, G551D, G85E, G91R, I148T, K710X, L206W, M1101K, N1303K, P574H, Q1238X, Q359K/T360K, Q493X, Q552X, Q890X, R1066C, R1158X, R1162X, R117C, R117H, R1283M, R334W, R347H, R347P, R352Q, R553X, R560T, S1196X, S1251N, S1255X, S364P, S549I, S549N, S549R, T338I, V520F, W1089X, W1282X, Y1092X, Y563D, 1078delT, 1161delC, 1609delCA, 1677delTA, 1717−1G>A, 1812−1G>A, 1898+1G>A, 1898+5G>T, 1949del84, 2043delG, 2143delT, 2183delAA>G, 2184delA, 2307insA, 2789+5G>A, 2869insG, 3120+1G>A, 3120G>A, 3659delC, 3662delA, 3791delC, 3821delT, 3849+10kbC>T, 3849+4A>G, 3905insT, 394delTT, 405+1G>A, 405+3A>C, 444delA, 574delA, 621+1G>T, 711+1G>T, 711+5G>A, 712−1G>T, 3876delA

CFTR mutation analysis

Genomic DNA was extracted from peripheral blood lymphocytes, buccal cell swabs, or bloodspots by Qiagen QIAmp 96 DNA Blood Kit. Specimens were tested for 87 mutations by a pooled allele-specific oligonucleotide (ASO) hybridization method as previously described.[16,17] Two multiplex chain reactions (PCR) were used to amplify 19 regions of the *CFTR* gene. The amplified PCR products were immobilized on nylon positively charged membrane and hybridized with groups of radioactive probes. Individual mutation identification of pool-positive samples was made by individual ASO hybridization to normal or mutant alleles.

Ascertainment bias among CF patient population

As described previously,[17] our laboratory experiences ascertainment bias among CF patient referrals. This bias is the result of a portion of patients being tested in a tiered approach where initial mutation analysis is performed at another laboratory and then patients who are not fully informative for the common mutations are sent to our laboratory for expanded mutation analysis. An example of this practice is described by Kharrazzi et al.[18] The use of this tiered approach by some physicians results in our CF patient population having a lower frequency of the common ΔF508 mutation and a resulting lower overall detection rate. To approximate the detection rate, it is necessary to correct for the lower ΔF508 frequency and this was done by comparison with frequencies reported for populations presumed to be free of ascertainment bias.[19,20] Conversely, such referral practices predict an over-ascertain-

ment of less common mutations though attempts to correct for this demonstrate differences between our population and an unbiased comparison population to be minimal. Ascertainment bias is not expected or observed among the carrier screening populations.

D1270N/R74W analysis

A subset of 192 D1270N-positive samples derived from this sample set as well as samples received for a variety of indications and ethnicities were analyzed for the R74W sequence change using LightCycler (Roche) amplification and melting curve analysis.

The 10-μL amplification reaction contained ≈100ng genomic DNA, 10× LightCycler DNA Master Hybridization Probes mix, 5 pmol of each primer, 2 pmol of each fluorescent probe and 4 mmol/L of magnesium chloride. One probe (5'-AGAGCTGGCTTCAAAGAAAAATCCTAAACTC-3') was labeled on the 3' end with fluorescein and the other probe (5'-TAATGCCCTTCGGCGATGT-3'), which spanned nucleotide 352, was labeled on the 5' end with LC-Red640. Samples were amplified by an initial 30-second incubation at 95°C followed by 45 cycles of repeated denaturation (0 seconds at 95°C), annealing (10 seconds at 55°C), and extension (5 seconds at 72°C). Amplification was monitored by the measurement of emitted fluorescence at the end of each annealing step. Melting curve analysis was performed by an increase to 95°C for 30 seconds followed by 1 minute at 40°C then heating to 80°C during which time the melting curve was recorded. The melting curves allowed for differentiation between the C or T nucleotide at position 352.

Statistical analysis

The differences between the ΔF508 frequency in the Hispanic carriers from this study and the ΔF508 frequency in the California registry[20] and the detection rates were tested using the Pearson Chi-square statistic. Exact inference was performed using StatXact (Cytel Software Corporation) with a significance level of 5%.

Comparisons between observed carrier frequencies and expected frequencies used the one-sample test of proportions as implemented in StatXact (Cytel Software Corporation).

RESULTS

From January 2001 through July 2003, 74% of CF carrier screening referrals to our laboratory were for individuals whose ethnicity was identified as Ashkenazi Jewish or Caucasian while the remaining 26% reported an ethnicity of Hispanic (9%), African American (5%), Asian (4%), or Mixed/Other/Unknown ethnicity (8%). Individuals indicating more than one race/ethnicity (e.g., both African American and Caucasian) or an ethnicity not included in the classifications (e.g., Filipino) or not providing information regarding ethnicity were combined in the Mixed/Other/Unknown category.

Hispanic CF patient population

The distribution of the 42 different mutations identified in the Hispanic population is shown in Table 1. Among 318 CF patient chromosomes, 30 mutations were identified with ΔF508, G542X, R334W, 3120+1G>A, W1089X, 3876delA, and R1066C representing 52.52% of the total. An additional 7 mutations were identified 2 to 3 times and 16 mutations were found once. Of the 30 different mutations identified in this group, 18 are included in the ACMG/ACOG recommended screening panel and 12 are not (Table 3). The 12 non-ACMG/ACOG panel mutations accounted for 9.72% detection among CF patient chromosomes.

Overall, the 30 mutations resulted in a detection rate of 62.20%. The ΔF508 mutation was identified on 118 of 318 (37.11%) Hispanic CF patient chromosomes. This is lower than the 52.46% (447/852 chromosomes) observed among Hispanic patients in the California Cystic Fibrosis Patient Registry.[20] When the overall detection rate is corrected using the California registry as a source of unbiased ΔF508 frequency, the corrected detection rate is 77.55%.

Hispanic CF carrier screening population

In the Hispanic carrier screening population (n = 15,333), 33 different mutations were found among the 287 carriers (1/53) identified (Table 1). The most prevalent mutations were as follows: ΔF508, D1152H, R117H, G542X, L206W, I148T (3199del6 status unknown), ΔI507, R1066C, R553X, 3849+10kbC>T, and R334W representing 83.72% of the total identified. An additional 10 mutations were identified 2 to 5 times, and 12 others were identified once. Of the 33 different mutations identified among carriers, 16 are included in the ACMG/ACOG-recommended screening panel and 18 are not included (Table 3).

The ΔF508 frequency observed in the carrier screening population (47.39%) is not statistically significantly different (P = 0.15) from the 52.5% observed in the California patient registry. This supports the contention that the screening population is not influenced by ascertainment bias. Assuming ≈77% detection for the mutations analyzed and an observed carrier frequency of 1/53, if one corrects to a 100% detection, then the observed carrier frequency would be 1/43, which is significantly different from the 1/48 predicted by disease incidence (P = 0.0052).[3] This difference may be due to sample size and the increased number of variable mutations identified among carriers.

The mutation distribution among carriers varied from that seen in the patient population. ΔF508 was the most common mutation in both groups. With the exception of W1089X, the next 6 most frequent mutations in the patient population (G542X, R334W, 3120+1G>A, 3876delA, W1089X, and R1066C) were all seen in the carrier population at frequencies of 1.4% to 4.2%. The W1089X mutation, which accounted for 2.2% detection among CF patients, was not seen in the carrier population. In the carrier screening group, 4 mutations, D1152H, R117H, ΔI507, and L206W, had frequencies of 3.8%

Table 1
CFTR mutation distribution among Hispanic CF patients and carrier screening referrals

	CF Patients		Carrier Screening Referrals	
CFTR Mutation Identified	# of CF Chromosomes	% Detection	# of Carrier Screen Referrals	% of Positive Carriers
ΔF508[a]	118	37.11[c]	136	47.39
G542X[a]	11	3.46	12	4.18
R334W[a]	11	3.46	6	2.09
3120 + 1G > A[a]	7	2.20	5	1.74
3876delA[b]	7	2.20	4	1.39
W1089X[b]	7	2.20		
R1066C[b]	6	1.89	9	3.14
3849 + 10kbC > T[a]	3	0.94	6	2.09
R1162X[a]	2	0.63	5	1.74
G85E[a]	2	0.63	3	1.05
S549N[b]	2	0.63	2	0.70
711 + 1G > T[a]	2	0.63	1	0.35
2789 + 5G > A[a]	2	0.63	1	0.35
1949del84[b]	2	0.63	1	0.35
R117H[a]	1	0.31	14	4.88
ΔI507[a]	1	0.31	11	3.83
R553X[a]	1	0.31	7	2.44
ΔF311[b]	1	0.31	1	0.35
1078delT[a]	1	0.31	1	0.35
621 + 1G > T[a]	1	0.31	1	0.35
3659delC[a]	1	0.31	1	0.35
Q890X[b]	1	0.31	1	0.35
G551D[a]	1	0.31		
1812 − 1G > A[b]	1	0.31		
I148T + 3199del6[a]	1	0.31		
A559T[b]	1	0.31		
1717 − 1G > A[a]	1	0.31		
3905insT[b]	1	0.31		
3821delT[b]	1	0.31		
G178R[b]	1	0.31		
D1152H[b]			18	6.27
L206W[b]			11	3.83
I148T (3199del6 status unknown)[a]			10	3.48
N1303K[a]			4	1.39
W1282X[a]			4	1.39
R117C[b]			4	1.39
R352Q[b]			2	0.70
712 − 1G > T[b]			2	0.70
Y1092X[b]			1	0.35
444delA[b]			1	0.35
S549R[b]			1	0.35
1609delCA[b]			1	0.35
Negative for mutations analyzed	120	37.74	15046	
Total	318	62.20[d]	15333	100.00

[a]Mutation included in the ACMG/ACOG Recommended Core Mutation Panel for general population CF carrier screening.[4,5]
[b]Mutation not included in the ACMG/ACOG Recommended Core Mutation Panel for general population CF carrier screening.
[c]Referral bias contributes to a lower than expected ΔF508 frequency. See Discussion.
[d]Corrected overall detection rate ≈77%. Referral bias contributes to a lower than expected ΔF508 frequency. See Discussion.

to 6.2%, whereas the same mutations were either observed only once or not at all in the CF patient population. With the exception of ΔI507, each of these mutations is associated with variable phenotypic expression[21–26] and when paired with ΔF508 has been reported in individuals with cystic fibrosis as well as CAVD.[24]

African American CF patient population

Table 2 depicts the distribution of 33 *CFTR* mutations identified in the African American population. Among 216 African American CF patient chromosomes, 21 different mutations were identified. The most frequent mutations (ΔF508, 3120+1G>A, 2307insA, and A559T) have been previously reported to be frequent in the African American population[19] and represent 46.77% of the total. Eleven of the 21 mutations identified are included in the ACMG/ACOG-recommended panel. The 10 non-ACMG/ACOG mutations identified accounted for 7.41% detection among the African American CF patient chromosomes (Table 3).

In total, 21 mutations led to a detection rate of 57.41%. This is lower than the 75% detection observed by Macek et al.[19] in a presumably unbiased African American sample of 148 chromosomes. This underestimate is once again explained by the lower frequency of ΔF508 chromosomes: 31% ΔF508 frequency observed compared to 48%[19] expected. Using a 48% ΔF508 frequency to approximate an unbiased population, the detection rate for the mutations analyzed in this population would be 74.4%. This corrected detection rate is not significantly different from the 81%[17] previously derived in 202 African American chromosomes analyzed in our laboratory ($P = 0.1259$).

After ΔF508, the next most-frequent mutations were 3120+1G>A (8.8%), 2307insA (4.17%), A559T (2.78%), and R553X (1.39%). The 3120+1G>A frequency observed in this study was lower than the 13.8% we had observed in our earlier dataset; however, the difference is not significant ($P = 0.1214$). An additional 4 mutations (G551D, 1717−1G>A, G542X, 711+5G>A) were detected twice (0.93% each), whereas 12 other mutations were identified on one chromosome each. Five of the twelve mutations identified once are considered to be "African American" mutations (3791delC, G330X, G480C, 444delA, and S1255X).[19]

African American CF carrier screening population

Among the 8,973 African American individuals referred for carrier screening, 23 different mutations were identified among 94 (1/95) carriers (Table 2). The most frequent mutations were ΔF508 (52.1%), 3120+1G>A (9.6%), A559T (6.38%), and R117H (5.32%). The 2307insA mutation, which had a frequency of > 4% among CF patients, was not identified among carriers. Twelve mutations identified in the carrier screening population were not identified among the CF patient population. These include mutations known to be associated with variable phenotypic expression such as R117H,[21,22] D1152H,[23–25] and 3849+10kbC>T.[27,28]

Assuming ≈74% detection for the mutations analyzed and an observed carrier frequency of 1/95, an adjustment to 100% detection would result in a carrier frequency of 1/76, which is not significantly different than the expected 1/61[3] based on the disease incidence ($P = 0.1779$).

Reclassification of specimens positive for the D1270N sequence change

Mutation analysis for all samples included D1270N. Comparison of D1270N chromosomes between African American CF patients and carrier screening referrals revealed a > 100-fold increase among carriers (2 CF patient chromosomes: 228 CF carrier alleles). To investigate a potential modifying effect of R74W on the D1270N phenotype, we analyzed 192 D1270N-positive individuals for the presence of R74W.[29] Patients were of varying ethnicities (46.4% African American, 29.2% Hispanic, 11.9% Caucasian, 12.5% Other/Mix/Not Provided) and indications (Table 4). Two individuals with the indication of carrier testing were homozygous for both D1270N and R74W. In addition, a 27-year-old Hispanic male with recurrent respiratory infections had the same genotype. Six individuals carried the genotype of a severe CF mutation (i.e., ΔF508), one copy of D1270N and one copy of R74W. Two of these individuals had the indication of carrier testing, two were affected with CF, one was suspected of having CF, and one was referred in followup to newborn screening. Among those samples ($n = 167$) received for carrier testing, 169 D1270N alleles and 159 R74W alleles were detected. While we were unable to determine phase, overall 94% of individuals with a D1270N allele also carried an R74W allele. In this data set there is no apparent correlation between D1270N, R74W, and phenotype. The D1270N sequence change was therefore not included in analysis of data for the larger study and has subsequently been removed from our CF test.

DISCUSSION

The term Hispanic has been used to describe individuals originating from Latin America yet having diverse racial and ethnic backgrounds.[30] Because Hispanic individuals may be of any race, the designation of Hispanic for purposes of genetic analyses has presented challenges and been the subject of discussion in the genetics literature.[31,10] Although specific race or country of origin data are not available for the majority of patients referred for testing, there remains utility to making observations for the Hispanic population as a whole. Subdividing patients based on country of origin or race would result in an insufficient number of CF chromosomes from which to make observations. We have previously reported the identification of numerous mutations that are not considered Hispanic-specific, among CF chromosomes from Hispanic CF patients.[17] The data from this current study support the similar conclusion from Heim et al.,[17] that to best serve the genetically heterogeneous Hispanic population, a panethnic mutation panel that includes mutations traditionally considered to be "Hispanic," "African American" as well as "Caucasian" is op-

Table 2
CFTR mutation distribution among African-American CF patients and carrier screening referrals

	CF Patients		Carrier Screening Referrals	
CFTR Mutation Identified	# CF Chromosomes	% Detection	# Carrier Screen Referrals	% of Positive Carriers
ΔF508[a]	67	31.02[c]	49	52.13
3120 + 1G > A[a]	19	8.80	9	9.57
2307insA[a]	9	4.17		
A559T[b]	6	2.78	6	6.38
R553X[a]	3	1.39	2	2.13
G551D[a]	2	0.93	1	1.06
1717 − 1G > A[a]	2	0.93	1	1.06
G542X[a]	2	0.93	1	1.06
711 + 5G > A[b]	2	0.93		
3791delC[b]	1	0.46	2	2.13
1812 − 1G > A[b]	1	0.46	1	1.06
G330X[b]	1	0.46	1	1.06
G480C[b]	1	0.46	1	1.06
444delA[b]	1	0.46		
S1255X[b]	1	0.46		
R1162X[a]	1	0.46		
R334W[a]	1	0.46		
E60X[b]	1	0.46		
S549R[b]	1	0.46		
N1303K[a]	1	0.46		
R560T[a]	1	0.46		
R117H[a]			5	5.32
D1152H[b]			3	3.19
3849 + 10kbC > T[a]			2	2.13
ΔF311[b]			2	2.13
R1158X[b]			1	1.06
R1066C[b]			1	1.06
ΔI507[a]			1	1.06
2789 + 5kbG > A[a]			1	1.06
S364P[b]			1	1.06
L206W[b]			1	1.06
405 + 3A > C[b]			1	1.06
S1255X[a]			1	1.06
Negative for mutations analyzed	92	42.59	8879	
Total	216	57.41[d]	8973	100.00

[a]Mutation included in the ACMG/ACOG Recommended Core Mutation Panel for general population CF carrier screening.[4,5]
[b]Mutation not included in the ACMG/ACOG Recommended Core Mutation Panel for general population CF carrier screening.
[c]Referral bias contributes to a lower than expected ΔF508 frequency. See Discussion.
[d]Corrected overall detection rate ≈74%. Referral bias contributes to a lower than expected ΔF508 frequency. See Discussion.

Table 3
Mutation identificaiton relative to ACMG/ACOG recommended screening panel

	Total No. of Different Mutations Identified	No. of ACMG/ACOG Mutations Detected	No. of Other Mutations Detected	Detection Rate for Other Mutations
Hispanic				
CF patients	30	18	12	9.72%[a]
CF carriers	33	16	17	N/A
Combined[c]	42	21	21	
African American				
CF patients	21	11	10	7.41%[b]
CF carriers	23	11	12	N/A
Combined[c]	33	17	16	

[a]Refer to Table 1.
[b]Refer to Table 2.
[c]Some mutations were identified in both CF patients and carriers, whereas others did not overlap.

timal. In the current study, 42 different mutations were identified among the Hispanic individuals (patients and carriers) tested and the most common mutations included those previously reported to be common among Hispanics, 3876delA,[32] W1089X,[17] as well as mutations considered frequent in African Americans (3120+1G>A)[19] and panethnic (e.g., G542X, ΔI507) populations.[33] Although regional variation in overall detection rates may occur, these data provide general guidance when developing a panethnic mutation panel and information useful for genetic counseling purposes.

By directly comparing *CFTR* mutation distribution between CF chromosomes identified via affected patients and carrier screening, we have made a number of interesting observations. Although the overall carrier frequency observed in the carrier screening population did not differ significantly from that predicted by disease incidence in the African American population, it did differ in the Hispanic population. In addition, the distribution of the mutations identified differed among CF patient and carrier screening populations for both groups. In both the Hispanic and African American populations, mutations associated with a variable clinical phenotype such as R117H, D1152H, and L206W were more common in the carrier screening population than the affected population. Similar findings of discrepant frequencies for these variable mutations are well known in the Caucasian population and are now confirmed among these minority populations as well. Each of these variable mutations has been reported in the literature in individuals with a diagnosis of cystic fibrosis, CAVD, and in unaffected carriers of 2 mutations.[15,24,25,34] Geographic differences in the origin of the carrier versus patient referrals may also contribute to the variation in mutation distributions between the groups. The lack of detection in the carrier screening population of mutations identified among patients (e.g., 2307delA, W1089X) is not unexpected given the inherent variability in estimates of low-frequency mutations.

Table 4
Indications for 192 D1270N-positive patient samples

Indication	No. of Specimens
Carrier testing–no family history	153
Carrier testing–family history	9
Carrier testing–abnormal fetal ultrasound findings	5
Known affected–CF diagnosis	3
Infertility	5
Suspected diagnosis (CF or pancreatitis)	15
Indication not provided	2

From direct comparison of patient and carrier screening populations tested for the same mutation panel, we also identified a > 100-fold increase in the number of D1270N chromosomes among African American carriers as compared with African American CF patients. D1270N has been identified in individuals with cystic fibrosis[24,33,35,36] as well as in men with congenital absence of the vas deferens.[24,37] Similar discrepancies in mutation frequency between carrier and patient populations are now well known for the R117H[15] and I148T[38,39] sequence changes, each of which has a *cis*-acting modifier influencing phenotype (5T[21] and 3199del6,[40] respectively). There have been similar reports that R74W may be a potential modifier for the clinical phenotype of D1270N.[29] The ΔF508/D1270N-R74W genotype has been reported in a girl with CF symptoms,[41] in 2 CF patients, 3 CBAVD patients,[24] and a 27-year-old man with CBAVD, elevated sweat chlorides, recurrent respiratory infection, and rhinitis.[35] Our study of 192 D1270N-positive specimens was not suggestive of a role for R74W as a modifying allele, and a definitive explanation for the variable D1270N frequencies remains unknown.

In the Hispanic population, our findings support the inclusion, minimally, of 3876delA, R1066C, S549N, and 1949del84 in a mutation panel. Although not yet identified among our carrier screening population, the W1089X mutation with a 2.2% frequency among CF patients would also be valuable. Together these 5 mutations accounted for 7.55% detection for the CF patient population and 5.58% among carriers. Similar recommendations have been made by other groups to add mutations that would increase detection in the Hispanic populatin.[13,18,32] In the African American population, the A559T mutation was the 4th most common mutation among CF patient chromosomes (2.78%) and the third most frequent among CF carriers (6.38%) and as such would warrant inclusion, with 711+5G>A (0.93%), in any screening program serving an African American population. As with our previous dataset,[17] we conclude that the populations benefited from being tested with a panethnic mutation panel that was not limited to mutations frequent in a given ethnic group. This is important, not only for the Hispanic population where individuals may be of numerous ethnic or racial backgrounds, but also for all panethnic populations given the increasing admixture and ethnic diversity of our society.[6,13,42]

With 14% of our carrier screening referrals being from Hispanic and African American individuals, and another 12% from individuals with ethnicities other than the ACMG/ACOG recommended Caucasian or Ashkenazi Jewish, it is evident that clinicians are offering or making CF carrier screening available to a diverse population, many of whom choose testing. To best serve these individuals, a *CFTR* mutation panel that reflects diversity is necessary.

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