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Identification of Common Cystic Fibrosis Mutations in African-Americans with Cystic Fibrosis Increases the Detection Rate to 75%

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Summary

Cystic fibrosis (CF)—an autosomal recessive disorder caused by mutations in CF transmembrane conductance regulator (CFTR) and characterized by abnormal chloride conduction across epithelial membranes, leading to chronic lung and exocrine pancreatic disease—is less common in African-Americans than in Caucasians. No large-scale studies of mutation identification and screening in African-American CF patients have been reported, to date. In this study, the entire coding and flanking intronic sequence of the CFTR gene was analyzed by denaturing gradient-gel electrophoresis and sequencing in an index group of 82 African-American CF chromosomes to identify mutations. One novel mutation, 3120+1G→A, occurred with a frequency of 12.3% and was also detected in a native African patient. To establish frequencies, an additional group of 66 African-American CF chromosomes were screened for mutations identified in two or more African-American patients. Screening for 16 "common Caucasian" mutations identified 52% of CF alleles in African-Americans, while screening for 8 "common African" mutations accounted for an additional 23%. The combined detection rate of 75% was comparable to the sensitivity of mutation analysis in Caucasian CF patients. These results indicate that African-Americans have their own set of "common" CF mutations that originate from the native African population. Inclusion of these "common" mutations substantially improves CF mutation detection rates in African-Americans.

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Introduction

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disorder in Caucasians. Classically affected patients manifest chronic sinopulmonary disease with *pseudomonas* infection, pancreatic exocrine insufficiency, elevated sweat chloride concentrations, and male infertility due to bilateral absence of the vas deferens (Welsh et al. 1995; Cutting 1997). The reported incidence in Caucasians has varied between 1/2,000 to 1/3,000 live births, depending on the population sampled and the detection method used (Welsh et al. 1995). The disease is caused by alterations in the CF transmembrane conductance regulator (CFTR), which functions as a chloride channel and regulator of other channels in epithelial cells (Welsh et al. 1995). The most common mutation in CFTR is a deletion of 3 nt that lead to the omission of phenylalanine at position 508 (ΔF508) and is present on 67% of Caucasian CF chromosomes worldwide (Cystic Fibrosis Genetic Analysis Consortium 1994).

CF occurs in non-Caucasian populations but is much less common than in the Caucasian population (Cutting 1997). Native Africans and native Asians with no known Caucasian ancestors have been reported with the classic form of the disease, indicating that CF alleles are present in all racial groups (Levin et al. 1967; Wang et al. 1968). The incidence of CF in African and Asian populations is higher where considerable mixing has occurred with Caucasians. For example, ~1/15,300 individuals of African descent living in the United States is born with CF (Hamosh et al., in press). The identification of CF alleles in African-Americans that are common in Caucasians indicates that the increased incidence of CF is the consequence of genetic admixture with Caucasians (Cutting et al. 1992; Ober et al. 1992). However, admixture alone does not account for the occurrence of CF in African-Americans. Haplotype studies and limited mutation analysis suggest that the distribution of CF mutations differs among African-Americans and U.S. Caucasians (Cutting et al. 1989, 1990*b*, 1992). Furthermore, African-Americans carry CF mutations that have

never been identified in Caucasian CF patients (Cutting et al. 1992). It appears that the combination of Caucasian CF alleles introduced by admixture and the presence of CF alleles from the African population accounts for the incidence of CF in African-Americans. Therefore, analysis of this group of patients provides a powerful tool to identify CF alleles derived from a population where the disease is rare. To this end, a sensitive mutation-detection technique was used to identify the vast majority of CF mutations in an index group of African-American CF patients. Additional patients were then screened to establish frequencies of alleles observed more than once in African-American patients. We have discovered that African-Americans have their own subset of "common" mutations that appear to be of African origin. It is interesting that one mutation (3120+1A→G) accounts for about half of the African CF alleles. These results further our understanding of the distribution of CF alleles among human populations and facilitate the development of more sensitive tests for CF mutation analysis in African-Americans.

Patients, Material, and Methods

Patients

Blood samples for CFTR genotyping were collected from 71 unrelated African-American patients. Six unrelated mixed-race patients (one African-American parent and one Caucasian parent) were also studied. The index group consisted of 39 African-American and 4 mixed-race patients from 11 CF centers. Subsequently, two independent groups were collected to screen for the frequency of identified recurrent mutations. One group (17 African-Americans and 1 mixed-race patient) was obtained from six CF Foundation–accredited centers, and the other group (15 African-Americans and 1 mixed-race patient) was collected through the University of North Carolina at Chapel Hill. One DNA sample from a native African CF patient from Cameroon was available for study (Demay et al. 1984).

Mutation Analysis

All patients were screened for the ΔF508 mutation and 15 common Caucasian CF mutations using a reverse dot strip hybridization system (Kawasaki et al. 1993) (R117H, 621+1G→T, R334W, R347P, A455E, Δ1507, 1717-1G→A, G542X, S549N, G551D, R553X, R560T, 3849+10kbC→T, W1282X, and N1303K) (Welsh et al. 1995) and a deep intron 11 splice-site mutation, 1811+1.6kbA→G (Chillón et al. 1995). In the index group, samples without identified mutations were analyzed by denaturing gradient-gel electrophoresis (DGGE) of the coding and flanking intronic sequences of the CFTR gene as described elsewhere (Macek et al. 1997). Each electrophoretic variant detected by DGGE was sequenced to identify specific mutations. Exon 9 of CFTR was analyzed be sequencing only. Family studies were performed for patients carrying novel mutations to confirm independent assortment of the novel allele. Parental genotypes were analyzed for mixed-race patients to determine which allele was inherited from the African-American parent.

In the two independent African-American groups, samples were screened for eight mutations that have been identified in two or more African-American CF patients, including 405+3A→C (present study), 444delA (White et al. 1991), G480C (Smit et al. 1995), R553X (Cutting et al. 1990*b*), A559T (Cutting et al. 1990*b*), 2307insA (Smit et al. 1993), 3120+1G→A (present study), and S1255X (Cutting et al. 1990*a*). Screening protocols for mutations 444delA, G480C, 2307insA, and S1255X were previously reported in the references cited in the preceding sentence. The A559T mutation creates a unique *Mse*I restriction site. Procedures for allele-specific oligonucleotide hybridization (ASO) screening for the 405+3A→C and 3120+1G→A mutations are described below.

A panel of 94 African-American individuals with no family history of CF (188 normal CF chromosomes) and 13 African-American parents of a CF patient (13 non-CF chromosomes) were used as a control group for ASO screening of novel missense and splice-site mutations. An additional panel of 80 Caucasian CF patients (160 CF chromosomes), who were subjected to DGGE analysis simultaneously with the index group, were used to verify the racial derivation of novel African-American CF alleles. ASO screening was performed as described by Cutting et al. (1992). Primer sequences and final wash temperatures for each ASO are indicated in table 1. The 3120+1G→A mutation can be detected by a PCR-mediated site-directed mutagenesis assay (Friedman et al. 1991) by use of primer 3120+1G→A REV/MIS: 5′- CTTAACGGTACTTATTTTTACAGA-3′ and primer 16i-5′ (Zielenski et al. 1991*b*) annealing at 51°C. Digestion with *Bgl*II generates DNA fragments of 341 and 26 bp when the 3120+1G→A mutation is present.

Results

Analysis of the entire coding region identified putative disease-producing mutations in 79 (96%) of the 82 African-American CFTR genes in our index group. The common mutation in Caucasians, ΔF508, was the most frequently observed mutation in African-Americans, accounting for 36 (44%) of the alleles. We discovered another common mutation, 3120+1G→A, in 10 patients, (12%) which was also detected in the native African, suggesting it originated in that population. Four other mutations were observed more than once in the index group (405+3A→C [2], R553X [3], A559T [2], and S1255X [2]). Twelve novel mutations were identified in one patient each: W19C, 621G→A, 1002-3T→G,

Table 1

Novel CFTR Mutations Identified in This Study, in African-American CF Patients

Mutation	Nucleotide Change	Exon/Intron	Consequence	Location	Screening Method
1 W19C	G→T at 189	Exon 2	Trp→Cys at 19	...	Loss of *Ava*II; ASO
2 405+3A→C	A→C at 405+3	Intron 3	Splice mutation	TM 1	ASO
3 621G→A	G→A at 621	Exon 4	Splice mutation	...	ASO
4 1002-3T→G	T→G at 1002-3	Intron 6B	Splice mutation	...	ASO
5 1119delA	delA at 1119	Exon 7	Frameshift	...	Direct sequencing
6 G330X	G→T at 1120	Exon 7	Gly→Stop at 330	...	Direct sequencing
7 S364P	T→C at 1222	Exon 7	Ser→Pro at 364	...	Loss of *Hin*fI; ASO
8 1504delG	delG at 1504	Exon 9	Frameshift	NBF I	Loss of *Bam*HI
9 Y563D	T→G at 1819	Exon 12	Tyr→Asp at 563	NBF I	Loss of *Xca*I; ASO
10 I618T	T→C at 1985	Exon 13	Ile→Thr at 618	R	Loss of *Vsp*I or *Asu*I; ASO
11 R764X	C→T at 2422	Exon 13	Arg→Stop at 764	R	Direct sequencing
12 2734delG/insAT	delG/insAT at 2734	Exon 14A	Frameshift	TM 7	Creates *Vsp*I or *Pac*I
13 3120+1G→A	G→A at 3120+1	Intron 16	Splice mutation	TM 9	Loss of *Bst*NI
14 3791delC	delC at 3791	Exon 19	Frameshift	...	Direct sequencing

NOTE.—TM indicates transmembrane segment; NBF denotes nucleotide binding fold; and R indicates regulatory domain of CFTR. Oligonucleotides for allele-specific oligonucleotide (ASO) hybridization screening of novel African-American CF mutations are as follows: W19C, 5′-TTT TAG CTG TAC CAG ACC A-3′ (final wash [FW] at 51°C); 405+3 A→C, 5′-ATT TAG GGG TCA GGA TCT-3′ (FW at 53°C); 621 G→A, 5′-TTG ATT TAT AAG AAA GTA ATA CTT-3′ (FW at 54°C); 1002-3 T→G, 5′-GTT CTG TTC TAT AAA AAA CAA-3′ (FW at 53°C); S364P, 5′-GTA TGA CCC TCT TGG-3′ (FW at 45°C); Y563D, 5′-TCA TCT TTG TCT ACT GAG AG-3′ (FW at 51°C); and I618T, 5′-CAA AAT ATT AAC TTT GCA TGA A-3′ (FW at 52°C).

1119delA, G330X, S364P, 1504delG, Y563D, I618T, R764X, 2734delG/insAT, and 3791delC (table 1). Of these, only W19C occurred in a pancreatic-sufficient patient. DNA from normal individuals was screened to evaluate whether any novel mutation was a neutral variant. The 405+3A→C mutation was observed once in the panel of normal African-American CF chromosomes from individuals without a family history of CF. Finally, 13 mutations found in one patient each had been previously reported in Caucasian patients (Q98R, R352Q, V520F, 1812-1G→A, G542X, S549N, and Y913C) (Romey et al. 1995; Welsh et al. 1995) or in African-American patients (444delA, G480C, 1342-2delAG [originally reported as 1342-1G→C], 2307insA, 3662delA, and W1316X) (Cutting et al. 1990*b*; White et al. 1991; Zielenski et al. 1991*a*; Smit et al. 1993, 1995).

There was one patient in whom neither mutation could be identified: a pancreatic-sufficient 27-year-old male with nasal polyposis, mild pulmonary disease (forced expiratory volume in 1 min 92% predicted) and an elevated sweat chloride concentration (88 mM and 112 mM, on two occasions). Although he was considerably older at the time of diagnosis than most patients (8 years 11 mo), his clinical findings are entirely consistent with the pancreatic-sufficient form of the disease. The third unidentified mutation occurred in a 15-year-old boy with the classic form of the disease (pancreatic insufficiency and elevated sweat chloride concentration). He carried the frameshift mutation 3662delA in his other gene.

To further establish the distribution of CF mutations in African-Americans, we analyzed two additional groups of patients. One group (15 African-Americans and 1 mixed-race patient) attended the CF Clinic at the University of North Carolina, and the other (17 African-Americans and 1 mixed-race patient) was referred by CF clinics across the United States. Each individual was screened for 16 "common Caucasian" mutations and 8 mutations that had been observed in two or more African-American patients (see Patients, Material, and Methods). The distribution of mutations in the two screening groups was similar to our index group. The ΔF508 mutation was the most frequent (53%), followed by 3120+1G→A (12%) with "common Caucasian" mutations and "common African-American" mutations accounting for 6% each. Altogether, 77% of mutations were identified in these two groups of African-American CF patients. When the index and two screening groups were combined, screening for the 16 "common Caucasian" and 8 "common African-American" mutations identified 111 (75%) of the 148 African-American CFTR genes studied (table 2). This detection rate is comparable to the efficiency of screening Caucasian patients for common CF mutations (79%).

Discussion

This is the most comprehensive analysis of CF mutations in African-Americans, to date. As suggested by earlier studies, the overall distribution of mutations differed from U.S. Caucasians. The most common muta-

Table 2

Distribution of CF Mutations in African-American and U.S.-Caucasian CF Patients

Mutation	African-American (n = 148)	%	U.S. Caucasian[a] (n = 8,714)	%
Caucasian mutations:				
ΔF508	71	48	5,769	66.2
R117H	0	0	47	.5
621+1 G→T	0	0	68	.8
R334W	1	.7	7	.1
R347P	0	0	24	.3
A455E	0	0	5	.1
ΔI507	1	.7	10	.1
1717-1 G→A	1	.7	39	.5
G542X	1	.7	204	2.3
S549N	1	.7	4	.1
G551D	1	.7	173	2.0
R553X (Caucasian)[b]	0	0	87	1.0
R560T	0	0	16	.2
3849+10kb C→T	0	0	51	.6
W1282X	0	0	235	2.7
N1303K	0	0	116	1.3
Subtotal	77	52	6,855	78.7
African-American mutations:				
405+3 A→C	2	1.4	...	...
444delA	1	.7	...	...
G480C	2	1.4	...	...
R553X (African)[b]	3	2.0	...	...
A559T	3	2.0	...	...
2307insA	3	2.0	...	...
3120+1 G→A	18	12.2	...	...
S1255X	2	1.4	...	...
Subtotal	34	23	...	...
Total	111	75.0	6,855	78.7

NOTE.—Percentages are rounded. "n" refers to the number of chromosomes analyzed.

[a] U.S. Caucasian data were derived from data reported to the CF Genetic Analysis Consortium (1994).

[b] The R553X mutation was included in each group of common mutations, since it has arisen independently in the Caucasian and African populations on different chromosome backgrounds (Reiss et al. 1991).

tion in Caucasians, ΔF508 (66%), was also the most common in African-Americans (48%). This observation was consistent with smaller studies of African-American patients in the southeastern United States (50%) and the Chicago area (25%) (Ober et al. 1992; Phillips et al. 1995). Several mutations belonging to a subset of CF alleles that occur in common among Caucasians were discovered in the African-American patients. Like ΔF508, the presence of these mutations in African-Americans was likely the result of Caucasian admixture. This subset accounts for ~13% of Caucasian CF alleles but was responsible for a much smaller fraction of African-American CF mutations (4%).

Fourteen novel mutations discovered in this study are predicted to be disease producing. Six mutations introduce a premature termination codon either by frameshift or single nucleotide substitution. The deleterious consequence of this type of alteration on CFTR gene expression has been well documented (Hamosh et al. 1991; Smit et al. 1993; Will et al. 1995). Four mutations are predicted to alter RNA splicing. Three changes occur at nucleotides within the consensus sequence for splice-donor sites: the last nucleotide of exon 4 (621G→A) and in nucleotides at the +1 (3120+1G→A) and +3 (405+3A→C) positions of splice-donor sites in introns 16 and 3, respectively. Nucleotide substitutions at each of these positions have been shown to alter RNA processing of human genes (Krawczak et al. 1992). The 405+3 A→C mutation, found twice in CF patients, was also present in one CFTR gene from a healthy African-American. This finding might suggest that the 405+3 mutation is a neutral polymorphism. However, we believe that this mutation is deleterious for the following reasons. Analysis of splice-donor sites reveals that adenine is most common (60%) at the +3 position, followed by guanine (32%), while cytosine is the least common (3%) (Padgett et al. 1986). Second, mutation at this location has been shown to affect RNA splicing in a model system (Montell and Berk 1984). Finally, there are five reports of mutations at this location (G→T[2],

G→C [1], and A→G [2]) causing aberrant RNA splicing (Krawczak et al. 1992; Mertes et al. 1994; Brackett et al. 1995). Together, these observations indicate that it is likely that the 405+3 A→C mutation will affect CFTR mRNA splicing and suggest that the individual in the general population with this mutation is a carrier of a deleterious CF allele. The other splice-site mutation is a transversion at the −3 position of the splice-donor site; a location that is highly conserved and, when altered, is associated with aberrant splicing in a number of genes (Krawczak et al. 1992). The remaining four mutations are amino acid substitutions. None of these alterations were found on 180 normal or 13 non-CF chromosomes from African-Americans, indicating that these mutations are not polymorphisms.

Screening for mutations that had been observed in two or more patients in this study and for mutations identified in one patient that had been previously reported in African-American CF patients revealed that African-Americans have their own subset of "common" mutations. As a group, these mutations occur at a frequency comparable to the subset of "common Caucasian" mutations (excluding ΔF508). The "common African" mutations were not observed in any Caucasian chromosomes, suggesting that they are derived from the African population. It is interesting that the 3120+1G→A mutation is quite frequent among African-Americans. If Caucasian alleles were excluded, the 3120+1G→A mutation would account for 53% of African-American CF alleles. The high frequency of 3120+1G→A might be the result of a founder effect in the African-American population. However, a small study of native Africans living in South Africa discovered the 3120+1G→A mutation in four of six CFTR genes (Carles et al. 1996). Therefore, despite the apparent rarity of CF in Africa, the 3120+1G→A mutation may not be a rare allele in native Africans. It is plausible that CF may be underdiagnosed in native Africans, since failure to thrive and diarrhea due to CF can be difficult to distinguish from more common causes such as viral infection and malnutrition.

The discovery that a small number of mutations account for a significant fraction of CF alleles will simplify mutation screening in African-Americans. Similar findings have been made in geographically or ethnically distinct groups of Caucasians. The vast majority of CF alleles in Ashkenazi Jews can be detected by screening for five mutations (Abeliovich et al. 1992). Screening for ~20 mutations enables mutation sensitivity to exceed 90% for Celts living in Brittany, France, and for Belgian Caucasians (Férec et al. 1992; Mercier et al. 1993). Subsets of "common" CF alleles do occur in more diverse populations such as U.S. Caucasians but account for a smaller fraction of all CF alleles. Consequently, mutation screening for ΔF508 and the "common" alleles detects ~79% of CF mutations in U.S. Caucasians (Cystic Fibrosis Genetic Analysis Consortium 1994). This study indicates that similar rates of CF mutation detection can be achieved in African-Americans by including the "common African" CF alleles.

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