Diagnostic Testing by *CFTR* Gene Mutation Analysis in a Large Group of Hispanics

Novel Mutations and Assessment of a Population-Specific Mutation Spectrum

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Characterization of *CFTR* mutations in the U.S. Hispanic population is vital to early diagnosis, genetic counseling, patient-specific treatment, and the understanding of cystic fibrosis (CF) pathogenesis. The mutation spectrum in Hispanics, however, remains poorly defined. A group of 257 self-identified Hispanics with clinical manifestations consistent with CF were studied by temporal temperature gradient electrophoresis and/or DNA sequencing. A total of 183 mutations were identified, including 14 different amino acid-changing novel variants. A significant proportion (78/85) of the different mutations identified would not have been detected by the ACMG/ACOG-recommended 25-mutation screening panel. Over one third of the mutations (27/85) occurred with a relative frequency >1%, which illustrates that the identified mutations are not all rare. This is supported by a comparison with other large *CFTR* studies. These results underscore the disparity in mutation identification between Caucasians and Hispanics and show utility for comprehensive diagnostic *CFTR* mutation analysis in this population. (*J Mol Diagn* 2005, 7:289–299)

Cystic fibrosis (CF) is a common and generally severe autosomal recessive disorder, caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*) on chromosome 7q31. *CFTR* encodes a protein that forms a chloride channel and regulates transport pathways.^{1,2} It is primarily expressed in the apical membrane of exocrine epithelial cells. CF may present

with meconium ileus, rectal prolapse, failure to thrive, recurrent respiratory infections, or obstructive jaundice. Later in life, bacterial endobronchitis and progressive deterioration of lung function can result in respiratory failure. Exocrine pancreatic dysfunction is present in the vast majority of affected individuals, and the prevalence of CF-related diabetes mellitus increases to approximately 30% with age.^{3,4} The phenotype, however, is a continuum that can range from very mild manifestations with isolated features such as congenital bilateral absence of the vas deferens (CBAVD) or chronic sinusitis, to a fulminant course resulting in death within the first year of life.⁵ Such variation results from differences in underlying molecular defects, modifying genes, and occurrence of complications.^{6,7}

Since the cloning of the *CFTR* gene,^{8,9} more than 1300 sequence variants have been reported to the CF mutation database.¹⁰ Caucasians and Ashkenazi Jews have the highest incidence of CF (1: 2500 and 1: 2270, respectively) and are the most thoroughly studied populations.^{11,12} The incidence in individuals of other ethnic backgrounds is lower and knowledge of their mutation spectra is limited. In the U.S., classifying or excluding patients based on ethnicity is difficult, considering the marked and growing ethnic admixture. The American College of Medical Genetics (ACMG) and the American College of Obstetricians and Gynecologists (ACOG) in 2001 proposed a new standard of care with universal CF carrier screening, including discussion of this option with couples from all ethnic and racial groups (Ref. 12, recently modified, Ref. 13). This recommended panel was envisioned for prenatal carrier screening rather than diagnostic testing and specifically developed for the non-Hispanic Caucasian population.¹² The recommendation was to offer testing to the non-Hispanic Caucasian and Ashkenazi Jewish populations and to make it available to the other racial/ethnic groups. ACMG/ACOG recommended testing only for mutations with an allele frequency of at least 0.1% among the general U.S. popula-

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tion for such population-based screening. Most of the more than 1300 described CFTR mutations are rare, or prevalent in specific ethnic subgroups only, so this requirement was met by only 25 mutations. With this panel, the detection rate among Hispanic CFTR mutation carriers is merely ~52% compared to ~72 to 97% in all other Caucasians grouped together, and Ashkenazi Jews.¹³ The Hispanic carrier frequency of 1/46, however, is only modestly lower than that in Caucasians (1/29).¹² In the year 2004 Hispanics comprise 14% (an estimated 41 million people) of the general U.S. population and 34% of the population in California.¹⁴ Absolute and relative growth of this subpopulation in the U.S. continues at a rapid pace, with Hispanics representing one out of every two children born in California today.¹⁵

Diagnostic delay in children of ethnic minorities¹⁶ prevents early intervention and adequate treatment, thus possibly affecting long-term prognosis. Lack of information about the scope and prevalence of mutations that contribute to CF in Hispanic individuals, further limits the sensitivity of carrier screening and appropriate genetic counseling in this population. Full characterization of the prevalent CFTR sequence changes in Hispanics is pivotal to addressing these issues. We performed comprehensive CFTR analysis in a large group of U.S. Hispanics with suspected CF and report here a summary of this experience. Fourteen novel amino acid changing variants were identified in a total of 183 CFTR sequence changes (excluding the common M470V and I506V polymorphism and 5T/7T/9T variants in intron 8). The identified mutations were compared to those described in the literature and to those in our Caucasian control group. In addition, relative mutation frequencies were determined in conjunction with other comprehensive CFTR studies of Hispanic individuals.

Materials and Methods

Subjects

A total of 257 Hispanic individuals, all with clinical manifestations consistent with the spectrum of CF are reported. Diagnostic DNA studies were conducted as part of the clinical evaluation. Some of the subjects presented with an atypical clinical history, such as mild lung disease, chronic sinusitis, isolated male infertility, and pancreatitis. All efforts were made to only include proband DNA and to exclude any relatives. No persons undergoing carrier screening or evaluation solely because of a positive family history are included in this series.

"Hispanic" ethnicity was based on self-identification. The majority of our subjects identified their ethnic background as strictly Hispanic and only 49/257 (19%) are of mixed background. The term Hispanic in this report refers to Hispanic individuals mostly from the U.S., and includes individuals of Spanish or Portuguese European extraction, and from Mexican, Central American, Southern American, and Caribbean heritage.

The eight index individuals from Stanford were examined at the Stanford Cystic Fibrosis Clinic. Consanguinity

was denied in all families. The study was approved by the Institutional Review Board and peripheral blood samples were obtained from the probands under informed consent. In addition, 249 consecutive Hispanic patients referred to Ambry Genetics for testing over the period from July 2002 through July 2004 were added.

Results from a Caucasian control group, referred to Ambry Genetics for diagnostic CF testing, were compiled as a comparison group. This group included 257 patients analyzed from July 2002-August 2003. Informed consent for research use of the DNA had been obtained for all samples.

DNA Isolation

Genomic DNA (gDNA) was isolated from blood leukocytes according to standard procedures at both laboratories. gDNA isolated at the Ambry lab with GFX genomic blood isolation kit (Amersham Pharmacia Biotech) was assessed for quality and quantity by agarose gel electrophoresis.

Stanford Sequencing Analysis

Before sequencing at the Stanford lab, gDNA CFTR (OMIM # *602421¹⁷) amplification was carried out with Amplitaq Gold DNA polymerase 250 Units, 5 U/uL (Perkin Elmer) and the four 2'-deoxynucleotide 5'-triphosphates (Amersham Pharmacia). Primer pairs from intronic sequences that flank individual CFTR exons were used as described.¹⁸ For exon 1, however, a new forward primer was designed (5'-CCAGAGTAGTAGGCTTTGGC -3') to facilitate successful amplification. The presence and size of amplified products were confirmed by agarose-gel electrophoresis, and products were processed by PCR purification using QIAquick PCR purification or QIAquick gel extraction (Qiagen). Purified samples were sequenced directly with fluorescent di-deoxy terminators (ABI) on an ABI 310 or 377 sequencing instrument (Applied Biosystems). Sequence analysis was facilitated by the Accelrys SeqWeb programs (Accelrys, Inc). All identified mutations were confirmed by direct DNA sequencing in the opposite direction.

Ambry Analysis

The majority (249/257) of the patients in this study were analyzed with the Ambry Test for CF. The Ambry Test for CF is a full mutation scan of the CFTR gene by temporal temperature gradient electrophoresis analysis (TTGE) followed by dye terminator DNA sequencing of suspect regions. The Ambry Test covers all CFTR exons, plus at least 20 bases 5' and 3' into each intervening sequence, and select deep intronic mutations.

All CFTR exons as well as relevant intronic regions were amplified using polymerase chain reaction (PCR) and proprietary primers. Standard PCR amplification was performed using HotStarTaq Master Mix (Qiagen) with 100–150 ng input gDNA per reaction. Typical PCR conditions were 1 cycle: 95°C/15 minutes, 35 cycles: 94°C/30 sec-

onds, 54°C/30 seconds, 72°C/30 seconds, 1 cycle: 72°C/10 minutes. Annealing temperatures differ depending on primer pair. Before Ambry gel analysis the PCR products were denatured and slowly cooled to allow for maximal heteroduplex formation. For a subset of CFTR regions, DNA was mixed with known wild-type DNA to facilitate detection of homozygous mutations. PCR products were then processed for TTGE on DCode gels (BioRad) in adherence with the Ambry Test technology. Polyacrylamide gels were analyzed for the presence of mutations following staining in ethidium bromide (EtBr) and image capture under UV using the Gel Doc 1000 system (BioRad). Gel analysis was performed by two individuals and fragments were scored against known controls.

Regions indicating the presence of a mutation by TTGE were then processed for sequencing. Apparently affected exons were first amplified with a unique primer set using TaqPCR Master Mix (Qiagen, Valencia CA). Typical PCR conditions were 1 cycle: 95°C/5 minutes, 35 cycles: 94°C/30 seconds, 54°C/30 seconds, 72°C/30 seconds, 1 cycle: 72°C/10 minutes. Annealing temperatures differ depending on the primer pair. PCR products were analyzed via agarose gel electrophoresis, followed by treatment with ExoSAP-It (USB) according to manufacturer recommendations. Standard dye terminator cycle sequencing DTCS (Beckman Coulter) was conducted followed by loading onto a CEQ2000 sequencer. Exons were always sequenced in both sense and antisense directions. In rare instances, however, only one direction may give adequate sequence due to repeat regions such as the poly-T site upstream of CFTR exon 9. All reported mutations follow the numbering of GenBank entry NM 000492.¹⁹

Results

A total of 183 mutant alleles were identified in 257 study subjects, or 36% of the 514 total CFTR alleles studied (Table 1). No mutant CFTR alleles were identifiable in 147 (57%) subjects, and 45 subjects appeared heterozygous with only one mutant allele identified. Of the 45 with only a single mutation detected, 7 (16%) were novel mutations.

As expected, the most common mutation observed was Δ F508, which was detected in 53/183 positive alleles, thus representing 29% of the identified mutations, but an overall relative allelic frequency of only 10%. Δ F508 was observed in homozygosity 6 times (11%), but with another ACMG/ACOG defined common mutation only (R334W) once (2%). Δ F508 was the only mutation identified in 12 cases (23%) and occurred in combination with non-ACMG/ACOG mutations 28 times (53%) of which 6 were novel variants (11%). Other than Δ F508 itself and I1027T, typically occurring in *cis*-, only two individual mutations, W1204X and L206W, were observed with Δ F508 more than once (twice each) in this study.

An additional 82 distinct mutations make up the remainder of the total 183 mutations observed. Of these additional 82 mutations 15 were novel, but one of these occurred twice (E588V). Overall, of the 85 separate mutations seen, 27 (32%) were observed in more than one individual (Table 1). Apart from Δ F508, only three other

ACMG/ACOG mutations were observed multiple times. G542X, which is a common allele of European origin, occurred a total of 7 times (1%), including once in homozygosity, while R334W and R553X occurred twice each. In contrast, among the 78 non-ACMG/ACOG mutations observed in our study group, 24 were noted two or more times, including six instances of the splice site mutation 406-1G>A, and four instances of W1204X. While Δ F508 was homozygous in six subjects, seven other less common alleles (G542X, W1204X, R75X, V232D, E116K, T501A, 3272-26 A>G) were also seen in the homozygous state.

Novel Mutations

Fifteen (14 different) novel amino acid changing mutations were identified in fifteen individuals (Table 2).

1429del7bp

A one-year-old Hispanic female has a novel 1429del7bp in combination with the well-established G542X mutation (exon 11). She presented with an ileus in the newborn period, however sweat chloride testing was unsuccessful. She continues to manifest symptoms consistent with CF. This novel mutation, located in exon 9, results in a stop codon at amino acid 441. This would result in premature termination of translation, which is known to result in CF, presumably as a result of nonsense-mediated mRNA decay or dysfunctionally shortened protein products.²⁰ Promising effects of gentamicin treatment on CFTR expression may enhance targeted treatment in patients with these mutations.²¹

S573C

A 9-year-old boy with pancreatitis has a C>G transversion at nucleotide position 1850. No additional clinical history was available. This mutation in exon 12 substitutes a cysteine for serine at amino acid position 573. Missense mutations at this residue have not been described. However, both adjacent amino acids have been affected previously. D572N was identified in a Russian patient²² and P574H was identified in two patients with pancreatic sufficiency.²³ The serine residue at position 573 is highly conserved across species, as are at least seven residues on either side in mammals, and two in amphibians and fish.²⁴ A second mutation in this subject was not identified.

Y913X

A male newborn of European/Brazilian ancestry, with meconium ileus at birth and severe pancreatic insufficiency was found to have Δ F508 and two additional mutations, including the novel Y913X in exon 15. He has had no significant pulmonary disease, and sweat chloride testing was reported as borderline. The novel Y913X results in a premature termination codon resulting from a T>A change at nucleotide 2872, while the third mutation is a known

Table 1. Spectrum of CFTR Sequence Variants in 257 Hispanic Patients Who Underwent Diagnostic DNA Testing for CF

Mutations in 257 patients	Allele counts of each mutation	% of variant alleles (183)	% of all alleles tested (514)
ACMG/ACOG recommended 25 mutation panel*			
<i>DeltaF508</i>	53	28.96	10.31
<i>G542X</i>	7	3.83	1.36
<i>R334W</i>	2	1.09	0.39
<i>R553X</i>	2	1.09	0.39
<i>DeltaI507</i>	1	0.55	0.19
1717 – 1 G>A	1	0.55	0.19
3120 + 1 G>A	1	0.55	0.19
7 different mutations	67	36.61	13.04
All mutations included ACMG/ACOG			
1248 + 1 G>A	1	0.55	0.19
1249 – 29delAT	1	0.55	0.19
1288insTA	1	0.55	0.19
1341 + 80 G>A	1	0.55	0.19
1429del7	1	0.55	0.19
1525 – 42 G>A	1	0.55	0.19
1717 – 1 G>A	1	0.55	0.19
1717 – 8 G>A	2	1.09	0.39
1811 + 1 G>A	1	0.55	0.19
2055del9->A	3	1.64	0.58
2105–2117del13insAGAAA	1	0.55	0.19
2215insG	1	0.55	0.19
2585delT	1	0.55	0.19
2752 – 6 T>C	1	0.55	0.19
296 + 28 A>G	1	0.55	0.19
3120 + 1 G>A	1	0.55	0.19
3271 + 8 A>G	1	0.55	0.19
3271delGG	1	0.55	0.19
3272 – 26 A>G	2	1.09	0.39
3876delA	2	1.09	0.39
4016insT	1	0.55	0.19
406 – 1 G>A	6	3.28	1.17
406 – 6 T>C	1	0.55	0.19
4374 + 13 A >G	1	0.55	0.19
663delT	1	0.55	0.19
874insTACA	1	0.55	0.19
A1009T	2	1.09	0.39
A559T	1	0.55	0.19
D1152H	1	0.55	0.19
D1270N	3	1.64	0.58
D1445N	2	1.09	0.39
D836Y	1	0.55	0.19
DeltaF311	1	0.55	0.19
<i>DeltaF508</i>	53	28.96	10.31
<i>DeltaI507</i>	1	0.55	0.19
E116K	2	1.09	0.39
E585X	1	0.55	0.19
E588V	2	1.09	0.39
E831X	1	0.55	0.19
F311L	1	0.55	0.19
F693L	1	0.55	0.19
G1244E	1	0.55	0.19
<i>G542X</i>	7	3.83	1.36
G576A	1	0.55	0.19
H199Y	3	1.64	0.58
I1027T	3	1.64	0.58
I285F	1	0.55	0.19
L206W	3	1.64	0.58
L320V	1	0.55	0.19
L967S	1	0.55	0.19
L997F	3	1.64	0.58
P1372L	1	0.55	0.19
P205S	1	0.55	0.19
P439S	1	0.55	0.19
Q1313X	1	0.55	0.19
Q890X	2	1.09	0.39
Q98R	1	0.55	0.19
R1066C	1	0.55	0.19
R1066H	1	0.55	0.19

(Table continues)

Table 1. *Continued*

Mutations in 257 patients	Allele counts of each mutation	% of variant alleles (183)	% of all alleles tested (514)
R1070W	1	0.55	0.19
R1158X	1	0.55	0.19
R1438W	1	0.55	0.19
R334W	2	1.09	0.39
R352W	1	0.55	0.19
R553X	2	1.09	0.39
R668C	2	1.09	0.39
R74W	3	1.64	0.58
R75X	3	1.64	0.58
S1235R	2	1.09	0.39
S492F	2	1.09	0.39
S549N	1	0.55	0.19
S573C	1	0.55	0.19
S945L	1	0.55	0.19
T351S	1	0.55	0.19
T501A	2	1.09	0.39
T604S	1	0.55	0.19
V11I	1	0.55	0.19
V201 mol/L	1	0.55	0.19
V232D	2	1.09	0.39
V754 mol/L	1	0.55	0.19
W1089X	2	1.09	0.39
W1098C	1	0.55	0.19
W1204X	4	2.19	0.78
Y563N	1	0.55	0.19
Y913X	1	0.55	0.19
85 different mutations	183	100.00	35.60

Novel variants are in boldface, mutations on the ACMG/ACOG panel are italicized.

*A recent report by Palomaki and colleagues¹¹ found higher mutation frequencies within the ACOG/ACMG recommended testing panel. This may be due to a degree of second-tier bias in this study.

missense variant, I1027T (3212T>C), in exon 17a.²⁵ Family studies have not been performed to identify which allele carries two mutations. However, I1027T has been reported in *cis* with the Δ F508 mutation in the Human Gene Mutation Database²⁶ and has been observed in this phase in several family studies at Ambry, as well. Moreover, if I1027T is in *cis* with Δ F508, little additional effect would be expected as Δ F508 is a "severe" allele, whereas if it is in *cis* with the novel

Y913X, the premature termination of translation would occur before reaching I1027T, and no additional effect might result.

E588V

A 5-month-old female of Northwestern European and Hispanic ancestry with a positive newborn screening by

Table 2. Novel Variants Detected in 257 Hispanic Patients

Patient	Novel variant 1	Other variants	Age and symptoms
1	1429del7bp	G542X	Newborn with intestinal blockage
2	S573C	None	9 years old, pancreatitis, limited clinical history
3	Y913X	deltaF508/I1027T	1 month old, vomiting, weight loss, diarrhea
4	E588V	deltaF508/R1438W	Identified one time in a family, family studies revealed deltaF508 and R1438W are in <i>cis</i>
5	E588V	G542X	Newborn with pneumonia and sweat chloride of 59 mmol/L
6	P439S	R668C	10 years old with mild CF symptoms; another patient with CBAVD has P439S/R334W
7	T604S	deltaF508	1 month old
8	874insTACA	deltaF508	Newborn with meconium ileus and IUGR
9	2585delT	deltaF508/I1027T	13 years old with CF
10	1811 + 1 G to A	None	44 years old with positive sweat chloride; also seen in 5-year-old CF patient with 3821delT mutation
11	I285F	None	1 year old with chronic respiratory problems, also carries a silent mutation at A455
12	P1372L	None	1 month old, rule out CF
13	3271 + 8 A to G	None	16 years old, borderline sweat test
14	1341 + 80 G to A	None	Recurrent sinusitis
15	1525 - 42 G to A	None	Two patients, one 9 years old with FTT, and one 18 months old with chronic lung disease, pulmonary hypotension, hypoxia

CBAVD, congenital bilateral absence of the vas deference; IUGR, intrauterine growth retardation.

IRT had one previously identified $\Delta F508$ allele. This child remained asymptomatic as of the time of testing. Comprehensive analysis identified two additional amino acid substitutions. One of these is novel, whereas the other has very recently been reported in the CF mutation database.¹⁰ R1438W is caused by a C>T nucleotide substitution in position 4444 in the final exon (#24) of the CFTR gene. It encodes the poorly conserved carboxy-terminus past the second nuclear binding domain. Although the change from a charged polar residue to a bulky non-polar amino acid appears drastic, functional changes cannot be determined without expression studies. The results of family studies confirm that the $\Delta F508$ mutation and R1438W sequence variant are in *cis* in our study subject. In the CF mutation database, this variant also occurred in combination with other sequence variants: S977F in *cis*, and $\Delta F508$ in *trans*.¹⁰ The E588V variant, which is in *trans* in our subject, is caused by an A>T nucleotide substitution in position 1895 in exon 12. This mutation has also been detected in another patient in this series of Hispanic subjects. This was a newborn with pneumonia and a borderline sweat chloride result of 59 mmol/L, who also carried a G542X mutation.

P439S

A 10-year-old Hispanic boy with somewhat atypical features of CF has a novel mutation, P439S (1447C>T), in exon 9. He has a sweat chloride level of 59 mmol/L, pancreatic insufficiency, and repeated infections with *Staphylococcus*. However, overall his pulmonary disease is mild, and he is without clubbing. The affected proline residue is conserved in monkeys and rodents²⁴ but not further conserved across species. The other mutation in this child is R668C in exon 13. Previous reports of this second mutation were in two patients with only CBAVD and a 7T/9T constellation, despite combination with $\Delta F508$.²⁷ Because these patients did not have other clinical manifestations of cystic fibrosis, that mutation may be presumed to be mild, and may account for the relatively mild disease in this patient.

T604S

A male newborn identified as affected at a large CF center has a novel C>G substitution at nucleotide position 1943. This changes a conserved threonine to a serine (T604S) within exon 13 of the CFTR gene. The patient also carries a $\Delta F508$ mutation. No additional clinical information was available.

874insTACA

A female newborn with meconium ileus and intrauterine growth retardation (IUGR) has a novel insertion of 4 bp (TACA) at nucleotide position 874 in exon 6a, resulting in a shift of the reading frame and a premature stopcodon in exon 6b at amino acid position 257. Other reported frameshift mutations are typically deleterious. She also carries a $\Delta F508$ mutation.

2585delT

A 13-year-old male of mixed Caucasian and Hispanic ancestry with CF had a novel deletion of a single thymine residue at nucleotide position 2585 in exon 13, resulting in a frameshift with premature termination of translation at amino acid position 520, also in exon 13. While not previously described in the literature, we have observed this 2585delT in several other affected non-Hispanic individuals. This patient carries two additional mutations including a $\Delta F508$ mutation and I1027T. As previously indicated these two mutations have been described to occur in *cis* in other families (Ref. 26 and personal observation).

1811 + 1 G>A

A 44-year-old female with reportedly positive sweat chloride levels has a single bp substitution of G to A, one nucleotide into intron 11. No other mutations were identified. A G>C substitution, resulting in a mRNA splicing defect, has previously been reported at the same site.¹⁰ We have also just recently identified this mutation in a 5-year-old Hispanic CF affected child (data obtained after selection of this data series) who also carries a 3821delT mutation.

I285F

A 1-year-old male of Hispanic and African American ancestry with chronic respiratory problems had a novel A>T nucleotide substitution at position 985. No additional clinical information was provided. This mutation changes an isoleucine to a phenylalanine within exon 6b. A second mutation was not identified. However, this patient also carries a novel single nucleotide polymorphism (SNP), 1497G>A, within exon 9 of the CFTR gene. While this nucleotide substitution does not result in an amino acid change, theoretically, such SNP's may result in changes in mRNA splicing and expression level. Thus, whereas 1497G>A may contribute to the clinical findings in this child, a clinical correlation is not possible at this time.

P1372L

A newborn female referred to "rule out CF" had a novel C>T substitution at nucleotide position 4247, which changes a proline to a leucine within exon 22. No other mutations were identified.

3271 + 8A>G

A 16-year-old Chilean male with a borderline sweat chloride level has a point mutation of an A>G, eight nucleotides into intron 17a. No other variants were identified. This sequence change may result in alternative splicing, but mRNA studies were not performed.

1341 + 80G>A

A 13-year-old female with recurrent sinusitis has a G>A nucleotide substitution of unknown clinical significance, 80 bp into intron 8. No other mutations were identified.

1525 – 42G>A

An 18-month-old male with chronic lung disease, hypoxia, and pulmonary hypertension has a single change of a G>A, 42 nucleotides into intron 9. Other sequence variants were not identified. The novel variant was identified subsequently in two additional subjects (not included in the study population). One is a 9-year-old with failure to thrive, the other a 6-month-old child with clinical suspicion for CF. No additional clinical information is available.

W1204X*

A boy with classic CF, has a W1204X mutation in exon 19, as well as a Δ F508 mutation as the other allele. Although this amino acid change has been reported previously,²⁸ the nucleotide change in our subject is novel, occurring at 3744 (TGG>TGA) which is adjacent to the previously reported 3743 (TGG>TAG).

Comparison with Caucasian Control Group

To assess whether the mutations identified in Hispanics differed from those seen in Caucasians, or appeared in different frequencies, we compared our results to a non-Hispanic Caucasian reference group consisting of 257 subjects studied at Ambry over the first year of this series. The group included 257 consecutive diagnostic samples, about half of which reported an atypical clinical history. Samples of mixed ethnic origin were excluded, and only self-identified Caucasians were selected. The greater prevalence of subjects with an atypical clinical course in the Caucasian group may be attributed to a difference in ascertainment, since prior testing with traditional mutation panels would be expected to detect a higher proportion of mutations present in non-Hispanic Caucasians with typical CF. Therefore, individuals in this comparison group are expected to have a lower relative frequency of the most frequently identified mutations and an increased frequency of rarer mutations. A similar effect should be observed in our Hispanic study group, but to a lesser extent.

The predicted bias is supported by the relative frequency of Δ F508 and the spectrum of mutations identified (data not shown). Mutation Δ F508 was observed in 90/514 (18%) of all examined Caucasian alleles. It occurred in 79 individuals, of which 68 were heterozygous and 11 were homozygous. The relative frequency compared to all positive alleles in this diagnostic control group was 51% (90/176). This is lower than the ~65% relative mutation frequency in Caucasian individuals.¹¹ In addition, the mutations that were identified in this control

group were largely rare, and only 13/61 (including I148T) different mutations are included in the 25 mutation screening panel recommended by ACMG/ACOG. Due to ascertainment bias, six mutations not included in the recommended panel occurred with a relative frequency greater than 1%: E60X, G576A, I1027T, P67L, R668C, and S1235R. These are not congruent with common mutations in our Hispanic study population.

Combination with Prior Studies

Four previous diagnostic studies focused on Hispanics in the United States.^{29–32} In these studies combined, 134 mutations were identified, 32 of which were different. When combined with the mutations identified in this report, 41/101 different mutations occurred more than once (Table 3). Δ F508 accounted for 38.8% of detected mutations, whereas mutation 3876delA was the second most frequent at 4.7%. Mutations G542X and 406–1G>A accounted for 3.8% and 2.5% respectively, and 3849 + 10kbC>T was present at 1.6%. Mutations R75X, 935delA, S549N, W1204X, and R334W were present at a relative frequency of 1.3%, and 12 additional mutations were each represented at a frequency of 1% of detected mutations (Table 3). All other mutations were less frequent. Of the 22 mutations present at a relative frequency of 1% or more, only eight are currently included in the standard 25 mutation panel recommended (I148T, R334W, Δ F508, G542X, R553X, 1717–1G>A, 3120 + 1G>A, and 3849 + 10kbC>T), although a recent ACMG revision will remove variant I148T.¹³ The California Department of Health Services is also tracking Hispanic mutations.¹⁵ However, these may duplicate some of those described in the other reports and therefore are not included in this analysis.

Three South American and European studies were included in our comparative analysis. Other studies were excluded due to the limited nature of the mutation analysis, or lack of explicit information as to the extent of CFTR analysis. In comprehensive non-U.S. studies from Brazil, Colombia, and Spain, 420 mutations were identified (231, 117, and 72, respectively).^{33–35} Only seven occurred with a relative frequency >1%: Δ F508 (67.4%), G542X (9%), N1303K (2.4%), R1162X (2.4%), R334W (2.1%), W1282X (1.2%), and S549R (1%). Interestingly, all but one of these (S549R) are part of the 25 mutation screening panel, which may reflect a greater Caucasian admixture.

Finally, because the majority of Hispanic individuals living in the U.S. are of Mexican origin, we grouped results of our study with those from the four previous studies in Hispanics from the U.S. and with those from a large and comprehensive Mexican study³⁶ (Table 4). Percentages were determined based on the total number of sequence variants identified: 183 from our report, 145 described in Orozco et al,³⁶ and 134 in the previous studies from the U.S. combined.^{29–32} When the 462 variants were combined, 13 variants occurred with a relative frequency above 1% (Table 4). Of these, less than half are included in the ACMG/ACOG 25 mutation panel.

Table 3. CFTR Sequence Variants Identified in Five Comprehensive CFTR Studies in US Hispanics

CFTR mutations	Alleles	Relative mutation frequency (%) (of 317)
deltaF508	123	38.80
3876delA	15	4.70
G542X	12	3.80
406 – 1G>A	8	2.50
3849 + 10kbC>T	5	1.60
R75X	4	1.30
935delA	4	1.30
S549N	4	1.30
W1204X	4	1.30
R334W	4	1.30
2055del9>A	3	1
R74W	3	1
H199Y	3	1
L206W	3	1
663delT	3	1
3120 + 1G>A	3	1
L997F	3	1
I1027T	3	1
R1066C	3	1
W1089X	3	1
D1270N	3	1
2105del13insAGAAA	3	1
Q98R	2	<1
E116K	2	<1
I148T	2	<1
R668C	2	<1
P205S	2	<1
V232D	2	<1
S492F	2	<1
T501A	2	<1
1949del84	2	<1
Q890X	2	<1
3271delGG	2	<1
3272 – 26A>G	2	<1
G1244E	2	<1
D1445N	2	<1
R553X	2	<1
E588V	2	<1
1717 – 8G>A	2	<1
A1009T	2	<1
S1235R	2	<1
G85E	1	<1
296 + 28A>G	1	<1
406 – 6T>C	1	<1
V11I	1	<1
Q179K	1	<1
V201 mol/L	1	<1
874insTACA	1	<1
I285F	1	<1
deltaF311	1	<1
F311L	1	<1
L320V	1	<1
T351S	1	<1
R352W	1	<1
1248 + 1G>A	1	<1
1249 – 29delAT	1	<1
1288insTA	1	<1
1341 + 80G>A	1	<1
1429del7	1	<1
1525 – 42G>A	1	<1
P439S	1	<1
1717 – 1G>A	1	<1
1811 + 1G>A	1	<1
deltaI507	1	<1
G551D	1	<1
A559T	1	<1
Y563N	1	<1

(Table continues)

Table 3. Continued

CFTR mutations	Alleles	Relative mutation frequency (%) (of 317)
G567A	1	<1
S573C	1	<1
E585X	1	<1
T604S	1	<1
F693L	1	<1
V754 mol/L	1	<1
2108delA	1	<1
2184delA	1	<1
2215insG	1	<1
2585delT	1	<1
2752 – 6T>C	1	<1
E831X	1	<1
D836Y	1	<1
Y913X	1	<1
S945L	1	<1
L967S	1	<1
3171delC	1	<1
3199del6	1	<1
3271 + 8A>G	1	<1
R1066H	1	<1
R1070W	1	<1
Y1092X	1	<1
W1098C	1	<1
3500 – 2A>T	1	<1
4016insT	1	<1
4374 + 13A>G	1	<1
D1152H	1	<1
R1158X	1	<1
R1162X	1	<1
W1282X	1	<1
N1303K	1	<1
Q1313X	1	<1
P1372L	1	<1
R1438W	1	<1
Total	317	100

Discussion

Classic cystic fibrosis is characterized by abnormal mucous secretions resulting in chronic pulmonary disease, failure to thrive, pancreatic insufficiency, elevated sweat electrolytes, late-stage diabetes and cardiac failure, and male infertility due to congenital bilateral absence of the vas deferens (CBAVD). Although the underlying genotypes have been identified in many patients with CF-associated phenotypes, the mutation spectrum in Hispanics has not been well-characterized. This may be due to the fact that CF is less prevalent in Hispanics (1 in ~13500¹¹) or to the relative discrepancy in access to health care. Only a limited number of comprehensive mutation analysis studies have been reported from the U.S. on Hispanic populations to date.^{29–32} In one series, the recently identified 3876del A mutation in exon 20 of the CFTR gene was present in 6% of unrelated Hispanic patients with CF.³⁰ This mutation has not been detected in any other ethnic group and may represent a significant proportion of the as yet unrecognized mutations in the Hispanic population. The identification of this mutation supports the notion that predominant mutations in Hispanics may occur, as has been observed in other populations.

Table 4. Comparison of Relative Frequencies of CFTR Sequence Variants in Comprehensive CFTR Studies in US and Mexican Hispanics

	This study %	Orozco 2000 %	US/ Mexican %
<i>deltaF508</i>	28.96	54.48	43.72
<i>G542X</i>	3.83	8.28	5.19
406 – 1G>A	3.28	2.07	2.38
W1204X	2.19	<1	1.08
R74W	1.64		<1
R75X	1.64	2.07	1.51
H199Y	1.64	<1	<1
L206W	1.64		<1
L997F	1.64		<1
I1027T	1.64		<1
2055del9>A	1.64	1.38	1.27
D1270N	1.64		<1
E116K	1.09		<1
V232D	1.09		<1
<i>R334W</i>	1.09		<1
S492F	1.09		<1
T501A	1.09		<1
<i>R553X</i>	1.09	<1	<1
E588V	1.09		<1
R668C	1.09		<1
Q890X	1.09		<1
W1089X	1.09		<1
S1235R	1.09		<1
D1445N	1.09		<1
3876delA	1.09		3.24
1717 – 8G>A	1.09		<1
3272 – 26A>G	1.09		<1
A1009T	1.09		<1
<i>deltaI507</i>	<1	3.45	1.30
S549N	<1	3.45	1.95
G567A	<1		<1
<i>I148T</i>		2.07	1.08
I506T		1.38	<1
<i>N1303K</i>		2.76	1.08
935delA		1.38	1.30
2183AA>G		1.38	<1
3199del6		1.38	<1
3849 + 10kbC>T		<1	1.30

ACMG/ACOG italicized.

In this study, we used temporal temperature gradient gel electrophoresis (TTGE) and direct DNA sequencing to increase the sensitivity of mutation detection in U.S. Hispanics, and to determine whether additional mutations are recurrent. TTGE is a comprehensive mutation screening method that has also been successfully used by others in the detection of mitochondrial and nuclear genes.³⁷ We successfully identified 183 affected alleles of the 514 (35.6%) tested in a study population of 257 Hispanics. Looser diagnostic criteria with inclusion of atypical presentations, or a relatively high prevalence of deletions, insertions, and other rearrangement mutations not routinely detected by our methodology may explain the still less than complete ascertainment of mutant alleles. Further analysis by complementary methods will be required to identify additional mutations, if any, in these individuals.

This study does not represent a population-wide sampling across Hispanics living in the United States. In the 257 samples the $\Delta F508$ mutation occurred only 53 times (10% of analyzed alleles). This is well below the reported relative frequency of around 50% in Hispanics with CF.^{30,38,39} The observed frequency is most likely attrib-

utable to the ascertainment bias likely in this possibly prescreened population. Further bias may have resulted from the limited information available on referral documentation by referring physicians. The ascertainment here precludes extrapolation to population frequencies, and population based studies will be valuable to assess pathogenicity and allele frequencies of the sequence variants identified. However, this study is comprised of unrelated subjects only, and therefore the relative mutation frequencies are valuable for a determination of the general pattern of mutation distribution in Hispanics. The relative mutation frequencies in this study are compared to reported frequencies in U.S. and non-U.S. Hispanic series, as well as non-Hispanic Caucasian studies. When U.S. diagnostic studies are reviewed together, 22 mutations occurred with a relative frequency of 1% or more (Table 3). As most of the U.S. Hispanics are of Mexican origin, we also grouped these studies with a large and comprehensive Mexican study³⁶ (Table 4). The pooled data set demonstrates that the most frequently seen mutations are: $\Delta F508$, G542X, 406–1G>A, W1204X, R75X, 2055del9>A, 3876delA, $\Delta I507$, S549N, I148T, N1303K, 935delA, and 3849 + 10kbC>T. Of these 13 mutations,

which occur with a relative frequency above 1% in the pooled data set, only six ($\Delta F508$, G542X, $\Delta I507$, I148T, N1303K, and 3849 + 10kbC>T) were included in the ACMG/ACOG 25-mutation screening panel¹² and in the recent revision exclusion of I148T has been recommended.¹³ The most frequently seen mutations in the U.S. and Mexican studies combined ($n = 462$ identified mutations) include the 10 most frequent mutations observed in the Mexican study.³⁶ They also include all but one mutation (R334W) occurring with a relative frequency above 1% in the five combined studies performed in the U.S. In that group, only $\Delta I507$, N1303K and I148T were present at a relative frequency below 1%. As expected, the relative mutation frequencies between these studies differ somewhat.

Some of the disparity in mutation detection between Caucasians and Hispanics could be alleviated by adding at least seven additional mutations to the currently recommended ACOG/ACMG panel of 25 mutations: 406-1G>A, W1204X, R75X, 2055del9>A, 3876delA, S549N, and 935delA (Table 4). The addition of mutations particular to a specific population has been supported by ACMG.⁴⁰ In the five studies from the U.S. (Refs. 29-32 and this report) and the study from Mexico³⁶ combined, 103 different mutations were identified. Therefore, for optimal mutation detection in Hispanic individuals affected with CF, more comprehensive testing of the gene is expected to remain the most appropriate approach. This is further supported by the finding in our series, that 63% (116/183) of all mutations would not have been identified with the 25-mutation panel. This panel was designed for carrier screening rather than diagnostic testing, but in practice, is often used as a first approach to molecular diagnosis. Several expanded mutation panels are now available as well, and used for both carrier screening and diagnostic purposes. Yet, 52%-55% of all mutations would have been missed with one of these available expanded mutation panels (data not shown).

In the course of this study we identified 14 different novel mutations (Table 2). Although functional studies were not performed, the presence of clinical findings and projected molecular impact support the notion that these are, in fact, pathogenic. Four of the novel mutations (1429del7bp, Y913X, 874insTACA, and 2585delT) result in premature termination codons. Recent findings on promising effects of gentamicin treatment on CFTR expression in patients with premature stop codons, strongly support the value in further identification of similar rare mutations.²¹

The ethnic background of the population generally described as "Hispanic" is diverse, and may include individuals of predominantly Southern European or Native American extraction. The Hispanic population, as a whole, is possibly defined more by geography than by identical genetic background.⁴¹ Even within the U.S., geography related ethnic differences are evident: in the southwestern states, the vast majority of Hispanics are of Mexican origin, whereas in the eastern U.S., Puerto Rican, Cuban, and African admixture is observed.⁴² This relative absence of homogeneity, which is typical of the general U.S. population as well, underscores the difficul-

ties of defining broad population screening panels with optimal clinical utility. Only through a combination of several such panels or the application of more comprehensive testing, can these limitations be overcome.

In conclusion, although the carrier frequency of CFTR mutations in the Hispanic population is only moderately lower than that in Northern European Caucasians and Ashkenazi Jews, Hispanic CF patients and carriers are at a remarkable disadvantage for mutation detection with the currently available mutation panels. From our study, as well as our analysis of combined comprehensive U.S. and Mexican studies, it has emerged that the mutations most frequently seen in Caucasians are only partly congruent with those most prevalent in Hispanics. Considering the continuing relative paucity of data in the Hispanic population, we recommend that CF clinics and molecular genetic pathology laboratories serving Hispanic patients advocate a comprehensive mutation detection approach for CF diagnosis until population frequencies of CFTR mutations in Hispanics are fully characterized. For carrier screening of Hispanic patients, inclusion of additional mutations with significant frequency in the Hispanic CF population (especially 406-1G>A, W1204X, R75X, 2055del9>A, 3876delA, S549N, and 935delA) may be helpful to supplement the ACMG/ACOG panel. Accurate allele frequencies of the seven recommended additional mutations that emerged from this investigation should be determined in future large and unbiased studies of the Hispanic CF population.

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