

Ethnic Heterogeneity and Cystic Fibrosis Transmembrane Regulator (*CFTR*) Mutation Frequencies in Chicago-Area CF Families

Carole Ober,^{*†} Lucille A. Lester,[‡] Carol Mott,^{*} Chris Billstrand,^{*} Amy Lemke,^{*} Katrin van der Ven,^{*} Seth Marcus,^{||} Jerry Kraut,^{||} John Lloyd-Still,[§] and Carol Booth^{||}

^{*}Department of Obstetrics and Gynecology, [†]Committee on Evolutionary Biology, and [‡]Department of Pediatrics, University of Chicago; and [§]Children's Memorial Hospital, Northwestern University Medical School, Chicago; and ^{||}Department of Pediatrics, Lutheran General Hospital, Park Ridge, IL

Summary

The identification of a common mutation, $\Delta F508$, in the *CFTR* gene allowed, for the first time, the detection of cystic fibrosis (CF) carriers in the general population. Further genetic studies revealed >100 additional disease-causing mutations in this gene, few of which occur on >1% of CF chromosomes in any ethnic group. Prior to establishing counseling guidelines and carrier risk assessments, we sought to establish the frequencies of the *CFTR* mutations that are present in CF families living in the Chicago area, a region notable for its ethnic heterogeneity. Our sample included 283 unrelated CF carriers, with the following ethnic composition: 78% non-Ashkenazi Caucasians, 5% Ashkenazi, 9% African-American, 3% Mexican, 0.3% Native American, and 5% mixed ancestry. When a panel of 10 mutations ($\Delta F508$, $\Delta I507$, G542X, G551D, R553X, S549N, R1162X, W1282X, N1303K, and 1717-1G→A) was used, detection rates ranged from 75% in non-Ashkenazi Caucasians to 40% in African-Americans. These data suggest that the goal of screening for 90%–95% of CF mutations may be unrealistic in this and other, similar U.S. populations.

Introduction

Cystic fibrosis (CF) is the most common autosomal recessive disease in Caucasians. The recent identification and cloning of the *CFTR* gene (Riordan et al. 1989; Rommens et al. 1989) revealed a common 3-bp deletion at amino acid position 508, called " $\Delta F508$." The identification of a mutation accounting for 70%–75% of CF mutations (Kerem et al. 1989a; Lemna et al. 1990) enabled, for the first time, the detection of carriers among the general population. However, because 25%–30% of carriers may have mutations other than $\Delta F508$, national advisory boards recommended that population screening for CF carrier status not be offered until 90%–95% of mutations could be detected, and pilot programs were initiated to evaluate

strategies for implementing CF screening programs (Caskey et al. 1990; Statement from the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene 1990).

Subsequent genetic investigations have revealed >100 additional disease-causing mutations in the *CFTR* gene, with few accounting for >5% and many accounting for <1% of CF mutations. Furthermore, the frequency of the $\Delta F508$ mutation and other *CFTR* mutations varies among racial groups, as well as among groups of different European ancestries (Cystic Fibrosis Genetic Analysis Consortium 1990, and in press; Romeo and Devoto 1990). These complexities may be particularly problematic in the United States, a country characterized by striking ethnic diversity and regional differences in prevalent European ancestries. Therefore, the goal of screening for 95% of mutations may be unrealistic in the United States, and the panel of non- $\Delta F508$ mutations with the highest sensitivity may vary regionally. Thus, the implementation of effective screening programs may require novel strategies in this country.

Received February 14, 1992; revision received July 7, 1992.

Address for correspondence and reprints: Carole Ober, Ph.D., Department of Obstetrics and Gynecology, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637.

© 1992 by The American Society of Human Genetics. All rights reserved.
0002-9297/92/5106-0016\$02.00

We undertook this study to determine the frequency of $\Delta F508$ and to identify other mutations present in CF families living in the greater Chicago area, a region notable for its ethnic heterogeneity. The main objective of this study was to establish counseling guidelines and risk assessments derived from local population-based mutation frequencies, prior to implementing pilot screening programs for CF carriers.

Material and Methods

Sample Composition

Between October 1, 1990 and March 1, 1991, free mutation testing was offered to all individuals with CF who were living in the greater Chicago area and to parents of deceased CF children living in the Chicago area. Participants provided one blood sample for mutation studies, as well as a family history with particular regard to ethnic origin or ancestry. Informed consent was obtained from each adult participant or from a parent of participants who were minors. Clinical histories were obtained on all individuals who attended one of the three CF centers (Wyer Children's Hospital, University of Chicago; Lutheran General Hospital; or Children's Memorial Hospital, Northwestern University Medical School). Data regarding clinical history and disease severity are presented elsewhere (Lester et al., submitted). After genetic studies were completed, an additional blood sample was requested from one parent of CF probands who were heterozygous for any one mutation *and* whose parents differed with respect to ethnic group. Results and interpretations of genetic studies were reported back to the referring physician or geneticist. If the subject was self-referred, results were discussed directly with him or her by one of us (C.O. or A.L.). In addition to DNA from volunteers for free testing, DNA from members of 26 CF families living in the Chicago area that was already stored in our laboratory was studied. Previously these families had requested CF prenatal or carrier studies.

Our sample included 141 individuals with CF (including two sib pairs and one first-cousin pair) and 16 parents of 14 additional CF children who were either deceased or unavailable for study. Mutation studies were performed on 295 unrelated CF chromosomes, of which 220 were non-Ashkenazi Caucasian, 14 were Ashkenazi, 28 were African-American, 10 were Mexican, and 1 was Native American (Oneida) in origin. Twenty-two chromosomes were derived from carriers

with mixed ethnicity (European/Ashkenazi, 2; European/Mexican, 4; European/Native American, 12; African-American/Native American, 1; African-American/Mexican, 2; and European/African-American/Native American, 1).

Mutation Analysis

DNA from each subject was screened for the following mutations: $\Delta F508$ (Kerem et al. 1989a), G542X (Kerem et al. 1990), G551D (Cutting et al. 1990), R553X (Cutting et al. 1990), S549N (Cutting et al. 1990), R1162X (Gasparini et al. 1991), W1282X (Vidaud et al. 1990), N1303K (Osborne et al. 1991), and 1717-1G→A (Guillermit et al. 1990; Kerem et al. 1990). The following protocols were used to detect each mutation:

$\Delta F508$.—DNA (200–400 ng) was subjected to amplification by PCR using primers C16B (Kerem et al. 1989b) and C16D (Kerem et al. 1989a). DNA was amplified according to published protocol (Kerem et al. 1989a), except that 0.8 pmol of primer C16B was end-labeled with [γ - 32 P]ATP. Two microliters of the PCR reaction in 4 μ l loading buffer A (0.1% xylene cyanol, 0.1% bromophenol blue, 10 mM EDTA in formamide) was electrophoresed in 5% acrylamide in 1 \times TBE at 75 W for 90 min. The dried gel was exposed against radiographic film for 1 h. The normal sequence was visualized as a 98-bp band, the mutation as a 95-bp band. This protocol does not distinguish between the 3-bp deletions at positions 508 ($\Delta F508$) and 507 ($\Delta I507$). However, because the $\Delta I507$ mutation is detected so infrequently, we have assumed that all mutations detected by this method were $\Delta F508$.

G542X.—DNA (200–400 ng) was subjected to PCR amplification in duplicate by using primer 11i-5 (Kerem et al. 1990) and either primer 542M (5'-AGTGTGATTCCACCTTCTCA-3'), which is complementary to the mutant sequence, or primer 542N (5'-AGTGTGATTCCACCTTCTCC-3'), which is complementary to the normal sequence. DNA was amplified and the mutation visualized according to the same protocol described above for $\Delta F508$.

G551D, R553X, and S549N.—DNA (0.5–1 μ g) was subjected to amplification by PCR using primers 11i-5 and 11i-3, according to published protocol (Kerem et al. 1990). Mutations were visualized according to protocols described by Cutting et al. (1990).

R1162X.—DNA (0.5–1 μ g) was subjected to amplification by PCR using primers 19i-5 and 19i-3 (Kerem et al. 1990), according to published protocols (Kerem et al. 1990). The PCR product was digested with *DdeI*

and electrophoresed in 3% NuSieve agar (FMC Bio Products, Rockland, MD).

W1282X.—DNA (0.5–1 µg) was subjected to amplification by PCR using primers 20i-5 and 20i-3 according to published protocols (Kerem et al. 1990). The PCR product was digested with *MnII* and electrophoresed in 3% NuSieve agar (Shoshani et al. 1992).

N1303K and 1717-1G→A.—DNA (0.5–1 µg) was subjected to amplification by PCR using primers and PCR conditions described by Friedman et al. (1991). Amplified DNA was digested with *DdeI* and electrophoresed in 10% acrylamide (Friedman et al. 1991).

Results

The ethnic composition and mutation frequencies for Chicago-area CF carriers are shown in table 1.

Discussion

When a panel of 10 mutations was used, only 75% of *CFTR* mutations were detected in our sample of non-Ashkenazi Caucasian CF carriers. The fact that only approximately 60% of our Caucasian sample was of northern European ancestry may account for the relatively low frequency (.60) of $\Delta F508$ in Chicago-area non-Ashkenazi Caucasians.

The frequencies of the next two most common mutations, G542X and G551D, are also consistent with the ethnic composition of our sample. The G542X mutation is fairly common throughout Europe, particularly in southern Europe (Nunes et al. 1991). The presence of this mutation in two carriers with Mexican ancestry and two carriers with Native American (one Cherokee and one unspecified) ancestry may reflect southern European (i.e., Spanish) admixture in these populations. The G551D mutation is believed to be of Celtic origin (Macek et al. 1991); eight of the nine carriers in our sample were of Irish or English ancestry. The frequency of the R553X mutation, which was detected in one Caucasian carrier and in one African-American carrier, was lower than frequencies reported by other U.S. laboratories (Cystic Fibrosis Genetic Analysis Consortium, in press).

The R1162X mutation is the third most common mutation in southern Europe (frequency .036), where all carriers can be traced to the same northeastern Italian region, Veneto (Nunes et al. 1991). The two R1162X carriers in our sample were unaware of any Italian ancestry. One was half African-American and half Native American (Cherokee), and one was German, French, and Native American (tribal affiliation

not specified). The W1282X mutation is the most common Ashkenazi mutation in Israel (Shoshani et al. 1992). Among Ashkenazi carriers in Chicago, this mutation was less frequent than $\Delta F508$; however, our sample was too small to draw a conclusion regarding the frequency of this mutation. Nevertheless, testing for this mutation significantly increases the detection rate among Ashkenazi, although it is relatively infrequent among non-Ashkenazi Caucasians.

The N1303K mutation, which is fairly common throughout Europe, was detected in five carriers who were all of English, Irish, or German ancestry. The 1717-1G→A was detected in seven carriers of varied ethnicity. One carrier was African-American, one was Mexican, and three were of mixed Caucasian (mostly northern European) ancestry.

Approximately 5% of non-Ashkenazi Caucasian carriers in our sample had Native American ancestors, most of whom were members of eastern and midwestern tribes. If the CF gene in these carriers was inherited from the Native American ancestor, and if the mutations present in these ancestors differed from the common mutations in Europeans, then the mutation detection rate in carriers with Native American ancestry would be lower than the mutation detection rate in carriers without Native American ancestry. Contrary to this expectation, the detection rate in carriers with Native American ancestry was 92% (table 1). Combining data from all non-Ashkenazi Caucasian carriers, including data from those with Native American ancestry, yields a 76% detection rate, which is not different from the mutation detection rate in carriers without Native American admixture (table 1). This suggests that the mutations present in carriers with Native American ancestry are a result of European admixture. This is further supported by the fact that one $\Delta F508$ carrier in our sample is a Native American (Oneida). These data differ from data presented by Grebe et al. (1991) for southwestern Native American CF subjects. Among 20 Pueblo and Navajo CF chromosomes, only one mutation (G542X) was detected by a panel of six mutations ($\Delta F508$, G542X, G551D, R553X, G542X, and N1303K). Grebe et al. suggest that unique CF mutations are present in these groups. Alternatively, the mutations present in these groups may be from admixture with Europeans from countries with lower mutation detection rates, such as Spain (Nunes et al. 1991).

In conclusion, the mutation detection rate in an ethnically heterogeneous U.S. Caucasian population is only 75%, when a panel of 10 mutations is used. Reaching a 90% mutation detection rate in this popu-

Table 1

CFTR Mutation Frequencies and Detection Rates, by Ethnic Group

ETHNIC GROUP	No. of Mutations Detected/No. of Chromosomes Screened (frequency)										MUTATION DETECTION RATE
	ΔF508 ^a	G542X	G551D	R553X	R1162X	W1282X	N1303K	1717-1G→A			
Non-Ashkenazi Caucasians ^b	127/211 (.602)	13/211 (.062)	8/210 (.038)	1/210 (.005)	0/210 (0)	1/197 (.005)	4/203 (.020)	4/191 (.021)			.753
Non-Ashkenazi Caucasians, including Native American ^c ...	8/12 (.667)	2/12 (.167)	0/11 (0)	0/11 (0)	1/11 (.090)	0/11 (0)	0/11 (0)	0/11 (0)			.924
Native American	1/1 (1)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)	0/1 (0)			1.0
Ashkenazi	5/14 (.357)	1/14 (.071)	0/14 (0)	0/14 (0)	0/14 (0)	3/14 (.214)	0/14 (0)	0/13 (0)			.642
African-American	7/28 (.250)	1/28 (.036)	1/28 (.036)	1/28 (.036)	0/27 (0)	0/23 (0)	0/21 (0)	1/22 (.045)			.403
Mexican	3/10 (.300)	2/10 (.200)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	0/10 (0)	1/6 (.167)			.667
Total ^d	165/295 (.559)	22/295 (.075)	9/292 (.031)	2/288 (.007)	2/292 (.007)	4/275 (.015)	5/275 (.018)	6/261 (.023)			.735

NOTE. — The S549N mutation was not detected on 288 CF chromosomes and was not included in the table.

^a Includes ΔI507.

^b Twenty-eight percent from the British Isles, primarily Ireland; 31% from northern Europe, primarily Germany; 9% from southern Europe, primarily Italy; 13% from eastern Europe, primarily Poland; 11% from more than one European region; 1% French-Canadian; and 7% who did not know the nationalities of their ancestors.

^c Fifty percent Cherokee, 8% Blackfoot, 8% Choctow, and 34% unspecified tribal affiliation.

^d Includes 19 CF chromosomes not included in ethnic group categories listed above, either because the carrier was of mixed ancestry (N = 10) or because parental origin of chromosome could not be assigned in heterozygous CF proband (N = 9).

lation would require testing for 15–20 additional low-frequency (i.e., <1%) mutations, albeit the cost-effectiveness of such a strategy is questionable. As a result, the goal of screening for 90%–95% of mutations may never be achieved in this and other, similar heterogeneous populations. Because regional differences in ethnic composition will influence both cost-benefit analyses and risk assessments, uniform policies regarding population screening for CF carriers may not be appropriate in the United States.

Acknowledgments

We thank Dr. James Bowman for his comments regarding the manuscript, and we thank the following genetic counselors for referring patients and obtaining pedigrees: Teresa Hadro (University of Chicago), Ellyn Hauselman (University of Illinois), Gina Morley and Nancy Williamson (Rockford Memorial Hospital), Charlene Schulz (Humana Hospital—Michael Reese), and Monica Barth and Maureen Deichman-Smith (Northwestern Memorial Hospital). We also thank Ken Friedman (University of North Carolina, Chapel Hill) for providing technical consultations. This research was supported by Mothers' Aid of the Chicago Lying-In Hospital (University of Chicago). C.O. is supported in part by grant HD21244.

References

- Caskey CT, Kaback MM, Beudet AL (1990) The American Society of Human Genetics statement on cystic fibrosis screening. *Am J Hum Genet* 46:393
- Cutting GR, Kasch LM, Rosenstein BJ, Zielenski J, Tsui L-C, Antonarakis SE, Kazazian HH (1990) A cluster of cystic fibrosis mutations in the first nucleotide-binding fold of the cystic fibrosis gene. *Nature* 346:366–369
- Cystic Fibrosis Genetic Analysis Consortium (1990) Worldwide survey of the $\Delta F508$ mutation—report from the Cystic Fibrosis Genetic Analysis Consortium. *Am J Hum Genet* 47:354–359
- . Worldwide survey of *CFTR* mutations—report from the Cystic Fibrosis Genetic Analysis Consortium. *Hum Mutat* (in press)
- Friedman KJ, Silverman LM, Perry TR, Highsmith WE (1991) Detection of twelve cystic fibrosis mutations using polymerase chain reaction-mediated site-directed mutagenesis (PSM). *Am J Hum Genet Suppl* 49:A187
- Gasparini P, Nunes V, Barcia A, Degnini M, Morral N, Gnona A, Bonizzato A, et al (1991) The four southern European cystic fibrosis mutations: identification of two new mutations, four variants, and intron sequences. *Genomics* 10:193–200
- Grebe T, Doane W, Clericuzio C, Goldberg B, Murphy S, Hernried L, McClure M, et al (1991) Cystic fibrosis in Native Americans of the Southwest. *Am J Hum Genet Suppl* 49:A470
- Guillermit H, Fanem P, Ferec C (1990) A 3' splice site consensus sequence mutation in the cystic fibrosis gene. *Hum Genet* 85:450–453
- Kerem B-S, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, et al (1989a) Identification of the cystic fibrosis gene: genetic analysis. *Science* 245:1073–1080
- . (1989b) Identification of the cystic fibrosis gene: genetic analysis (erratum). *Science* 245:1437
- Kerem B-S, Zielenski J, Markiewicz D, Bozon D, Gazit E, Yahaf J, Kennedy D, et al (1990) Identification of mutation in regions corresponding to the 2 putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc Natl Acad Sci USA* 87:8447–8451
- Lemna WK, Feldman GL, Kerem B-S, Fernbach SD, Zevcovich EP, O'Brien WE, Riordan JR, et al (1990) Mutation analysis for heterozygote detection and the prenatal diagnosis of cystic fibrosis. *N Engl J Med* 322:291–296
- Lester LA, Kraut J, Lloyd-Still J, Karrison T, Mott C, Billstrand C, Lemke A, et al. $\Delta F508$ genotype does not predict disease severity in an ethnically diverse cystic fibrosis population (submitted)
- Macek M Jr, Macek M, Serre J-L, Vavrova V, Burger J, Reis A, Schmidtke J, et al (1991) Population study of *CFTR* gene mutations in Bohemia and Moravia: hypothesis on the historical spread of G551D and $\Delta F508$ in Europe. *Am J Hum Genet Suppl* 49:A474
- Nunes V, Gasparini P, Novelli G, Gaona A, Bonizzato A, Sangiulo F, Balassopoulou A, et al (1991) Analysis of 14 cystic fibrosis mutations in five south European populations. *Hum Genet* 87:737–738
- Osborne L, Knight R, Santis G, Hodson M (1991) A mutation in the second nucleotide binding fold of the cystic fibrosis gene. *Am J Hum Genet* 48:608–612
- Riordan JR, Rommens JM, Kerem B-S, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, et al (1989) Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. *Science* 245:1066–1073
- Romeo G, Devoto M (eds) (1990) Population analysis of the major mutation in cystic fibrosis. *Hum Genet* 85:391–445
- Rommens JM, Iannuzzi MC, Kerem B, Drumm ML, Melmer G, Dean M, Rozmahel R, et al (1989) Identification of the cystic fibrosis gene: chromosome walking and jumping. *Science* 245:1059–1065
- Shoshani T, Augarten A, Gazit E, Bashan N, Yahav Y, Rivlin Y, Tal A, et al (1992) Association of a nonsense mutation (W1282X), the most common mutation in Ashkenazi Jewish cystic fibrosis patients in Israel, with presentation of severe disease. *Am J Hum Genet* 50:222–228
- Statement from the National Institutes of Health Workshop on Population Screening for the Cystic Fibrosis Gene (1990) *N Engl J Med* 323:70–71
- Vidaud M, Panen P, Martin J, Granem N, Nicolas S, Goossens M (1990) Three mutations in the *CFTR* gene in French cystic fibrosis patients: identification by denaturing gradient gel electrophoresis. *Hum Genet* 85:446–449