GENETIC TESTING AND MOLECULAR BIOMARKERS
Volume 15, Number 1-2, 2011

Pp. 1–4
DOI: 10.1089/gtmb.2010.0091

ORIGINAL ARTICLE

Spectrum of *CFTR* Gene Mutations in Iranian Azeri Turkish Patients with Cystic Fibrosis

Mortaza Bonyadi,[1,2] Omid Omrani,[1,2] Mandana Rafeey,[3] and Nemat Bilan[3]

Aims: Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the CF transmembrane conductance regulator (*CFTR*) gene. In the present study, for the first time, we determined the spectrum of *CFTR* gene mutations in 100 patients with CF originated from the Iranian Azeri Turkish ethnic group. *Results:* Here, we report identification of 17 previously known and one novel mutation, namely K1302X, in this cohort. The frequency of deltaF508 mutation was found to be 23%. *Conclusions:* Low frequency of deltaF508 mutation and detection of one novel and 16 known mutations reflect a heterogeneous spectrum of the mutations in this ethnic group.

Introduction

Cystic fibrosis (CF) is one of the most common autosomal recessive disorders characterized by chronic bronchopulmonary infection, gastrointestinal problems, growth failure, and male infertility (Minasian *et al.*, 2005). The CF transmembrane conductance regulator (*CFTR*) gene containing 27 exons on chromosome 7q31.2 encodes a 1480-amino acid protein that acts as a cyclic adenosine monophosphate (cAMP)-regulated chloride channel in the apical membrane of epithelial cells (Sheppard and Welsh, 1999). Since identification of the *CFTR* gene in 1989, more than 1500 mutations have been identified; and with the exception of the more common 3-base pair deletion in exon 10 (DF508), the vast majority of mutations are rare or restricted to certain populations (Kerem *et al.*, 1998).

Accurate knowledge of CF mutations in a specific population provides information for CF prevention programs applicable through heterozygote screening and prenatal diagnosis. Iran is a large country with different ethnic groups including Persian (51%), Azeri Turk (24%), Kurd (7%), Arab (3%), and other minorities such as Armenians. The 15–20 million Azeri Turks living in northwestern Iran are ethnically identical to Azeris and closely related to Turks. This is the first report regarding the mutation spectrum of the *CFTR* gene in Iranian Azeri Turkish patients with CF disease.

Subjects and Methods

Patients

A total of 100 unrelated families corresponding to 200 independent alleles with CF from the Iranian Azeri Turkish ethnic group were enrolled in this study. Diagnostic criteria involved positive sweat tests and typical clinical findings of pulmonary and gastrointestinal disease. Consanguinity among parents was proved in 73% of cases.

Mutation analysis

Informed consent was obtained from all subjects or in some cases from their parents. Genomic DNA was extracted from peripheral blood leukocytes according to standard protocols (Miller *et al.*, 1988).

Eight common regions (exons 4, 7, 10, 11, 13AB, 14b, 19, and 21) and their flanking intron sequences of the *CFTR* gene reported to be hot spots in the Turkish population (Bobadilla *et al.*, 2002) were amplified by PCR using specific oligonucleotide primers. The general procedure and the specific nucleotide primers used for the amplification of regions have been previously published (Zielenski *et al.*, 1991; Liechti-Gallati *et al.*, 1999). Samples were amplified by PCR and products were screened using single-stranded conformational polymorphism/heteroduplex analysis (SSCP/HD). SSCP/heteroduplex analysis technique has the ability to detect at least 97% of all point mutations (Liechti-Gallati *et al.*, 1999). For each exon, a control (non-CF) DNA sample was run in an adjacent lane; and a third lane was a mixture of the control and the sample DNA to detect heteroduplexes caused by homozygous changes.

Samples exhibiting shifts on SSCP gels relative to normal samples were subjected to automated DNA sequencing with forward and reverse primers. Some common mutations were confirmed by different methods such as restriction fragment

[1]Faculty of Natural Sciences, Center of Excellence for Biodiversity, University of Tabriz, Tabriz, Iran.
[2]Liver and Gastrointestinal Disease Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.
[3]Department of Pediatrics, Faculty of Medicine, Tabriz University of Medical Sciences, Tabriz, Iran.

Table 1. Spectrum and Frequencies of Mutations in the Cystic Fibrosis Transmembrane Conductance Regulator Gene Identified in 100 Iranian Azeri Turkish Alleles

Mutation	*Exon/intron*	*No. of alleles*	*Frequency (%)*
Delta F508 (c.1520_1522delTCT, p.Phe508del)	E10	46	23
1677delTA (c.1545_1546delTA, p.Tyr515X)	E10	9	4.5
2789+5G>A (c.2657+5G>A)	I14b	6	3
2183AA->G (c.2051A>G, p.Lys684Arg)	E13AB	5	2.5
G542X (c.1624G>T, p.Gly542X)	E11	5	2.5
A120T (c.358G>A, p.Ala120Thr)	E4	3	1.5
I148T (c.443T>C, p.Ile148Thr)	E4	3	1.5
S466X(TAG) (c.1397C>G, p.Ser466X)	E10	2	1
2043delG (c.1911delG, p.Gln637Hisfs*26)	E13AB	2	1
2184insA (c.2052_2053insA, p.Gln685Thrfs*4)	E13AB	2	1
R1158X (c.3472C>T, p.Arg1158X)	E19	2	1
K1302X[a] (c.3904A>T, Lys1302Stop)	E21	2	1
1525-1 G>A (c.1393-1G>A)	I9	1	0.5
1548delG (c.1418delG, p.Gly473Glufs*54)	E10	1	0.5
406-6T>C (c.274-6T>C)	I3	1	0.5
R117H (c.350G>A, p.Arg117His)	E4	1	0.5
R334W (c.1000C>T, p.Arg334Trp)	E7	1	0.5
3849+5G>A (c.3717+5G>A)	I19	1	0.5
Number of alleles identified		93	46.5

[a]New mutation.

length polymorphism and amplification refractory mutation system analysis, where this was available (Ferrie *et al.*, 1992).

Results

Eighteen different mutations were detected in 93 of the 200 mutant alleles (diagnostic efficiency of 46.5%). These included four missense mutations, four splice mutations, five frameshift mutations, four nonsense mutations, and one deletion (Table 1).

Of the 100 unrelated families studied, 4 showed compound heterozygous mutant alleles, 29 were homozygotes, 27 heterozygotes (only one mutant allele identified), and no CF-causing mutations were detected in the remaining 40 families (40%). Genotypes of 100 patients with CF from this cohort are shown in Table 2. DeltaF508 was the most common mutation observed in our population (23%) followed by 1677delTA (4.5%).

Novel mutation and phenotypic feature

The 3-month-old male child born to a consanguineous marriage had a homozygous K1302X genotype and presented with recurrent respiratory infections and loose stools since 1 month of age. His sweat chloride level was 135mEq/L. During the workup of the infant in the ward, his respiratory tract showed colonization of *Staphylococcus aureos*. The elder sibling also had respiratory complaints and died of pneumonia (Figs. 1 and 2).

Discussion

We characterized the molecular defects in 93 out of 200 CF alleles in Iranian Azeri Turkish patients. We first identified two of the mutations by amplification refractory mutation system (delta F508 & G542X) and one by restriction digestion (2789+5G>A) and later identified by SSCP and direct sequencing 14 known (1677delTA, 2183AA>G, A120T, I148T, S466X, 2043delG, 2184insA, R1158X, 1525-1 G>A, 1548delG, 406-6T>C, R117H, R334W, and 3849+5G>A) and one previously unreported mutations (K1302X).

The frequency of the ΔF508 mutation, the most common mutation in Caucasians, was only 23% of the analyzed and 50.5% of the identified CF alleles. This is in contrast with the

Table 2. Genotypes of 100 Patients with Cystic Fibrosis from Iranian Azeri Turkish Ethnic

Genotype	*No. of patients*
Delta F508/delta F508	16
1677delTA/1677delTA	3
2789+5G>A/2789+5G>A	2
2183AA>G/2183AA>G	2
G542X/G542X	2
S446X/S446X	1
2043delG/2043delG	1
2184insA/2184insA	1
K1302X/K1302X	1
Delta F508/1548delG	1
Delta F508/R334W	1
Delta F508/1677delTA	1
1677delTA/R1158X	1
Delta F508/U	11
A120T/U	3
I148T/U	3
2789+5G>A/U	2
1677delTA/U	1
1525-1G>A/U	1
406-6 T>C/U	1
R117H/U	1
2183AA>G/U	1
R1158X/U	1
3849+5G>A/U	1
G542X/U	1
U/U	40

U, unidentified.

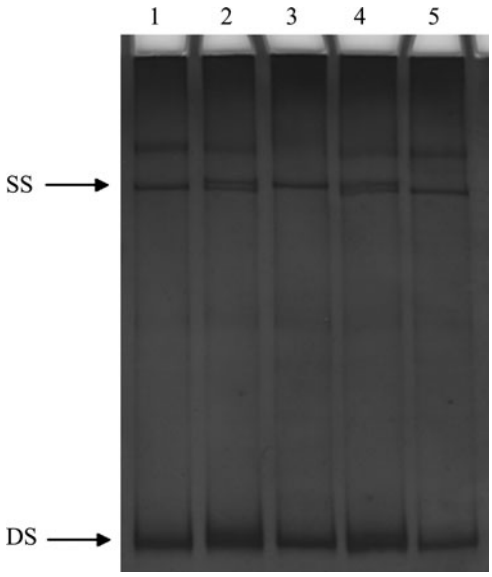

FIG. 1. SS conformational polymorphism mutation screening in exon 21 of the cystic fibrosis transmembrane conductance regulator gene. Slots 1 and 5 refer to control persons presenting wild type sequences that have normal band (lower band) in SS. Slot 3 demonstrates PCR product from our patient that has mutant band (upper band) in SS. Slots 2 and 4 demonstrate PCR products from heterozygous parents of the case that have two bands (normal and mutant bands). SS, single strand; DS, double strand).

high frequency of ΔF508 mutation in European and other populations where the frequency of the ΔF508 is more than 50%. The geographical distribution of the ΔF508 shows a decreasing frequency of this mutation from the Northwest to the Southeast, in the European population (Guilloud-Bataille *et al.*, 2000; Teder *et al.*, 2000; Scotet *et al.*, 2002; Wald *et al.*, 2003).

The ΔF508 mutation was identified in 30 Azeri Turkish patients with CF, of which 16 were homozygous and 3 were compound heterozygous for deltaF508/1548delG, deltaF508/R334W, and deltaF508/1677delTA and 11 patients had deltaF508/Unidentified genotype.

Mutation 1677delTA, the second most prevalent mutation (4.5%) in this cohort, was also reported to have a similar frequency in the neighboring Turkish population (Bobadilla *et al.*, 2002). Three of the patients carried this mutation in homozygous state, two patients were compound heterozygous for 1677delTA/deltaF508 and 1677delTA/R1158X, and one patient had 1677delTA/Unidentified genotype.

DeltaF508 and 1677delTA mutations account for 62% of the identified mutant alleles in this cohort. These mutations are located in exon 10 of *CFTR* gene; and to achieve an efficient detection strategy, specimens will be tested first for these two mutations and the DNA testing will be considered complete if two mutations in compound heterozygous state are identified.

The rest of mutations are found to be uncommon in our ethnic group (Table 1). These mutations have also been reported to have low frequencies in some other ethnics including neighboring Turkish population (Bobadilla *et al.*, 2002).

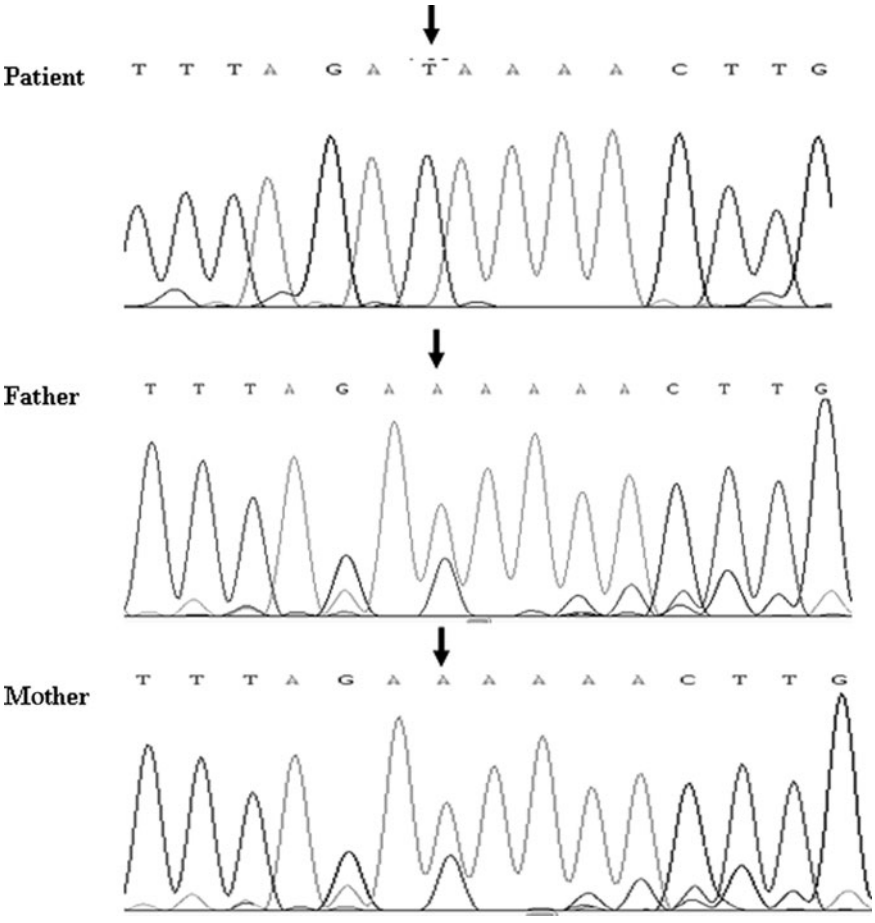

FIG. 2. Sequencing analysis of fragment including exon and intron 21 of the cystic fibrosis transmembrane conductance regulator gene in the patient and his parents.

Here, we also report one rare novel mutation identified in a patient from this ethnic group. This novel mutation [K1302X (c.3904A>T) (Lys1302Stop)] located in exon 21 (second nuclear binding domain [NBD2]) was found in homozygous state. Most of the mutations identified in CF occur in the NBD1, whereas very few have been reported to occur in NBD2. NBD2 domain is a highly conserved motif predicted to bind and hydrolyze ATP (Sheppard and Welsh, 1999). The mutation in our patient creates a stop codon that might result in mRNA decay or lead to the deletion of 178 *C*-terminus amino acids of the CFTR protein. This mutation is classified in the most severe group of *CFTR* mutations leading to premature termination of mRNA and virtual absence of functional CFTR protein. Early and severe presentation of the patient carrying K1302X mutation could be caused by the absence of functional CFTR protein. Both parents of the index patient were, therefore, examined for the presence of and were found to be heterozygous carriers of this mutation. To avoid overestimation of the pathological significance of this rare mutation, 100 chromosomes from the normal population were analyzed for the mutation, and it was not found in any of the normal chromosomes tested.

The Mediterranean area has the highest CFTR heterogeneity, making genetic diagnosis by mutation analysis especially difficult (Chillon *et al.*, 1994; Bonizzato *et al.*, 1995; Kanavakis *et al.*, 1995). For Spanish patients with CF, 73 different mutations have been identified, accounting for 87% of CF alleles with only 10 of them having a frequency higher than 1%; and for the French population, 105 mutations account for 86% of CF chromosomes (Chevalier-Porst *et al.*, 1994; Casals *et al.*, 1996). Studies of North-eastern Italian patients with CF identified 62 mutations, accounting for 73.8% of detectable CF alleles; whereas for the Celtic population from Brittany in France, 19 mutations account for 98% of CF alleles (Ferec *et al.*, 1992; Gasparini *et al.*, 1992).

This study revealed the presence of 18 different mutations covering 46.5% of CF alleles, proving that the Iranian Azeri Turkish population has also one of the highest amounts of CFTR mutation heterogeneity. This heterogeneity could be the result of local history and demography.

Identification of population-specific mutations for inherited disorders such as CF is of particular importance in populations that appear to have limited and distinct ancestry.

Acknowledgments

The authors would like to thank all participant patients and their relatives. This project was financially supported by Center of Excellence for Biodiversity (University of Tabriz) and Liver and Gastrointestinal Disease Research Center (Medical University of Tabriz).

Disclosure Statement

No competing financial interests exist.

References

Bobadilla JL, Macek M Jr., Fine JP, *et al.* (2002) Cysticfibrosis: a worldwide analysis of CFTR mutations-correlation with incidence data and application to screening. Hum Mutat 19: 506–575.

Bonizzato A, Bisceglia L, Marigo C, *et al.* (1995) Analysis of the complete coding region of the *CFTR* gene in a cohort of CF patients from north-eastern Italy: Identification of 90% of the mutations. Hum Genet 95:397–402.

Casals T, Gimenez J, Ramos M, *et al.* (1996) Prenatal diagnosis of cystic fibrosis in a highly heterogeneous population. Prenat Diagn 16:215–222.

Chevalier-Porst F, Bonardot AM, Gilly R, *et al.* (1994) Mutation analysis in 600 French cystic fibrosis patients. J Med Genet 31:541–544.

Chillon M, Casals T, Gimenez J, *et al.* (1994) Analysis of the *CFTR* gene confirms the high genetic heterogeneity of the Spanish population: 43 mutations account for only 78% of CF chromosomes. Hum Genet 93:447–451.

Ferec C, Audrezet MP, Mercier B, *et al.* (1992) Detection of over 98% cystic fibrosis mutations in a Celtic population. Nat Genet 1:188–191.

Ferrie RM, Schwarz MJ, Robertson NH, *et al.* (1992) Development, multiplexing, and application of ARMS tests for common mutations in the CFTR gene. Am J Hum Genet 51:251–262.

Gasparini P, Marigo C, Bisceglia G, *et al.* (1992) Screening of 62 mutations in a cohort of cystic fibrosis patients from north eastern Italy: their incidence and clinical features of defined genotypes. Hum Mutat 2:389–394.

Guilloud-Bataille M, De Crozes D, Rault G, *et al.* (2000) Cystic fibrosis mutations: report from the French Registry. The Clinical Centers of the CF. Hum Hered 50:142–5.

Kanavakis E, Tzetis M, Antoniadi T, *et al.* (1995) Mutation analysis of 10 exons of the *CFTR* gene in Greek cystic fibrosis patients: characterization of 74.5% of CF alleles including 1 novel mutation. Hum Genet 96:364–366.

Kerem B, Rommens JM, Buchanan JA, *et al.* (1998) Identification of the cystic fibrosis gene: genetic analysis. Science 245:1073–1080.

Liechti-Gallati S, Schneider V, Neeser D, *et al.* (1999) Two buffer PAGE system-based SSCP/HD analysis: a general protocol for rapid and sensitive mutation screening in cystic fibrosis and any other human genetic disease. Eur J Hum Genet 7:590–598.

Miller SA, Dykes DD, Polesky HF (1988) A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16:1215.

Minasian C, McCullagh A, Bush A (2005) Cystic fibrosis in neonates and infants. Early Hum Dev 81:997–1004.

Scotet V, Gillet D, Dugueperoux I, *et al.* (2002) Spatial and temporal distribution of cystic fibrosis and its mutations in Brittany, France: a retrospective study from 1960. Hum Genet 111:247–254.

Sheppard DN, Welsh MJ (1999) Structure and function of the CFTR chloride channel. Physiol Rev 79:S23–S45.

Teder M, Klaassen T, Oitmaa E, *et al.* (2000) Distribution of CFTR gene mutations in cystic fibrosis patients from Estonia. J Med Genet 37:1–4.

Wald NJ, Morris JK, Rodeck CH, *et al.* (2003) Cystic fibrosis: selecting the prenatal screening strategy of choice. Prenat Diagn 23:474–483.

Zielenski J, Rozmahel R, Bozon D, *et al.* (1991) Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Genomics 10:214–228.

Address correspondence to:
Mortaza Bonyadi, Ph.D.
Faculty of Natural Sciences
Center of Excellence for Biodiversity
University of Tabriz
University Ave.
Tabriz 51638-68639
Iran

E-mail: jabbarpour@tabrizu.ac.ir