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Mutation Analysis of CFTR Gene in 70 Iranian Cystic Fibrosis Patients

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ABSTRACT

Cystic fibrosis (CF) is the most common inherited disorder in Caucasian populations, with over 1400 cystic fibrosis transmembrane conductance regulator (CFTR) mutations. The type of mutations and their distributions varies widely between different countries and/or ethnic groups. Seventy Iranian cystic fibrosis patients were screened for the CFTR gene mutation using ARMS/PCR (amplification refractory mutation system) for the following mutations: ΔF508, N1303K, G542X, 1717-1G>A, R553X, W1282X, G551D, 621+1G>T, ΔI507 and R560T. Single strand conformation polymorphism (SSCP) analysis of exons 3, 7, 10, 11 and 17b, including both the exon/intron junctions, of the CFTR gene was performed in patients in whom no mutation could be identified on one or both CFTR genes. As a result of this screening, only three mutations were found: ΔF508 mutation was found in 25 (17.8%) alleles, N1303K in six (4.3%) alleles and G542X in five (3.6%) alleles. Thus, a total of 3 mutations cover 25.7% of CF alleles. These finding will be used for planning future screening and appropriate genetic counseling programs in Iranian CF patients.

Key words: Cystic fibrosis; Cystic fibrosis transmembrane conductance regulator; Mutations; Iran; Single-stranded conformational polymorphism

INTRODUCTION

Cystic fibrosis (CF; MIM # 219700) is resulted in by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR; #602421)*, which was cloned in 1989 and encodes a chloride channel.[1-3] Cystic fibrosis (CF) is the most common severe autosomal recessive disorder among white populations. The CFTR gene is the seventh member of the C subfamily of ATP-binding cassette (ABC) transporter gene superfamily (also called ABCC7).[4]

The ABCC7 spans approximately 190 kb of genomic DNA[5] at chromosomal location 7q31.2, with its coding sequence distributed among 27 exons.[6] The diagnosis of CF was routinely carried out by clinical features consistent with a CF phenotype together with an elevated sweat chloride concentration. CF is characterized by a defect in epithelial cells of exocrine tissues. Transport of electrolytes, water, and other solutes, across the cellular membranes is defective and leads to several symptoms, including a progressive decline in pulmonary function secondary to chronic lung disease, pancreatic exocrine insufficiency, infertility in males and elevated concentrations of

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chloride in sweat.[7,8] Cystic fibrosis is relatively infrequent in the Asian population.[9,10]

To date, more than 1400 mutations in the CFTR gene causing or non-causing disease have been reported to the Cystic Fibrosis Genetic Analysis Consortium.[11] The frequency of these mutations varies among different populations according to the geographical and ethnic origin of patients.[12,13] Most of them are rare and have been detected in only one family.

A few mutations (i.e., ΔF508, N1303Kand G542X) are frequent worldwide. Deleterious mutations in the ABCC7 gene can disrupt CFTR function by various mechanisms based on their nature and on the domain in which these alterations occur.[14,15] There is a correlation between phenotype and the genotype among patients sharing the same mutations.[16,17]

The main aim of this study was to characterize the CF-causing mutations and genotypes in Iranian population. Therefore, we screened 70 well characterized CF patients by ARMS/PCR and PCR/SSCP methods and identified three common mutations in over 25% of CF patients alleles.

MATERIALS AND METHODS

Patients and Sampling

From 2002 to 2005, seventy unrelated families who had an affected child with CF had referred to division of Medical Genetics at Children Medical Centre (CMC) hospital in Tehran; detailed questionnaires, including clinical and family history, were collected. The patients including 39 males and 31 females originated from Iran (at least three generations). They represented all major ethnic groups in Iran (such as Persian, Azari, Gilaki, Kurd, Arab and Lur) and different geographic areas in the Centre, North, Northeast, Northwest, West, Southwest, South and East of Islamic Republic of Iran (44 cities). Diagnostic criteria were based on repeated positive sweat chloride tests (>60mmol/l) and on typical findings of pulmonary /gastrointestinal disorder (except five of them in whom sweat test was borderline).

Mutation Detection

Genomic DNA was extracted from peripheral blood leukocytes using the salting out precipitation method. The mutations were detected based on the following procedures. In a first step, all subjects were analyzed, using the amplification refractory mutation system (ARMS-PCR) technique as described by Ferrie et al,[18] for the following mutations: ΔF508, N1303K, G542X, 1717-1G>A, R553X, W1282X, G551D, 621+1G>T, ΔI507 and R560T. Twenty nine cycles of PCR for six mutations and 30 cycles of PCR for four mutations with denaturation at 95ºC for 30s, annealing at 58ºC for 45s and elongation at 72ºC for 45s were performed. The reaction was initiated by 5 min incubation at 94ºC and terminated by 10 min incubation at 72ºC. The PCR products were then visualized with ethidium bromide stain and UV transillumination. As a result of this screening, only three mutations were found: ΔF508 mutation was found in 25 (17.8%) alleles, N1303K in six (4.3%) alleles and G542X in five (3.6%) alleles (Table1 and Figure 1).

In a second step, single strand conformation polymorphism (SSCP) analysis of exons 3, 7, 10, 11 and 17b, including both the exon/intron junctions, of the CFTR gene was performed in patients in whom no mutation could be identified on one or both CFTR genes in the first phase.[19] The 5 exons were amplified by the polymerase chain reaction (PCR). PCR amplifications carried out in 50 µl reaction volumes containing approximately 200ng of genomic DNA,10mM dNTPs, 10 mM Tris (pH 8.3), 50 Mm KCl, 1.5 mM MgCl2, 2.5 unit Taq polymerase. Thirty one cycles of PCR with denaturing at 94ºC for 30s, annealing at 57 ºC (exons 10 and 11) or 53 ºC (exons 3, 7 and 17b) for 30s and elongation at 72ºC for 45s were performed. PCR products (3 µl) were mixed with 3 µl SSCP loading buffer, denatured at 95 ºC for 10 min and analysed on polyacrylamide gels followed by visualization of the DNA using silver stain (Figures 2 and 3).

Table 1. Frequencies of CFTR mutations identified in 70 Iranian cystic fibrosis patients.

Mutation	E/I[1]	Nucleotide change	Consequence	Chromosomes No.
ΔF508	E10	deletion of CTT from1653	deletion of Phe at 508	25
G542X	E11	G to A at 1756	Gly to stop at 542	5
N1303K	E21	C to G at 4041	Asn to Lys at 1303	6

[1] Exon/ Intron

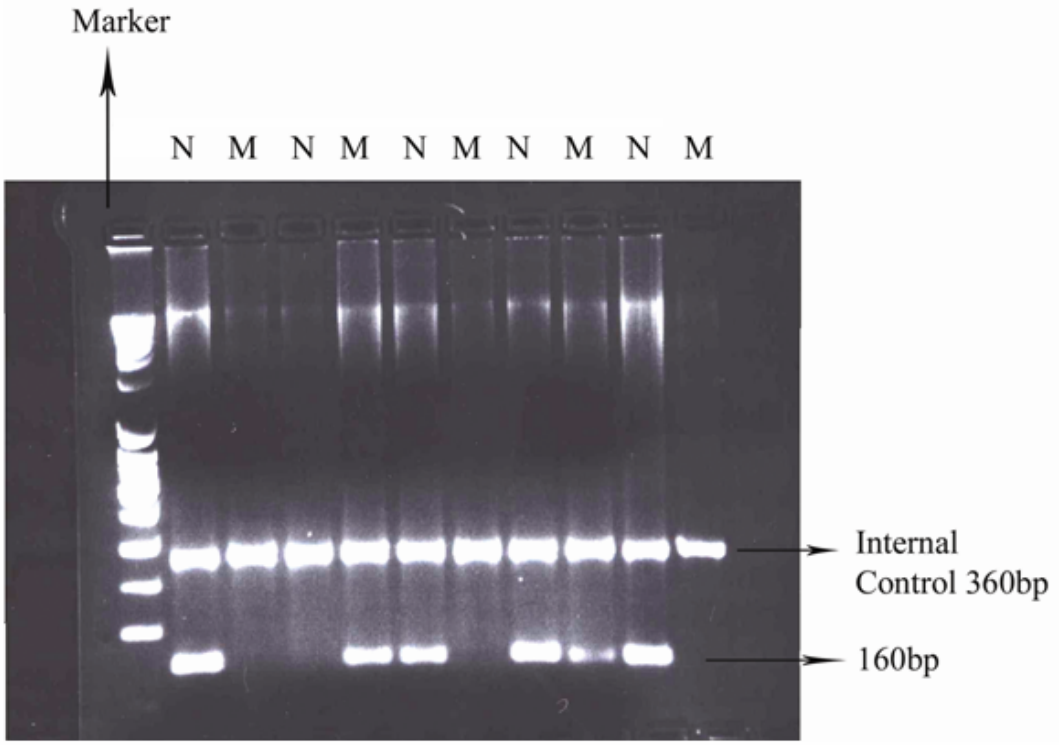

Figure. 1 Single ARMS tests. The results obtained for ΔF508 mutation. The first track in each pair is the product of the normal ARMS reaction, and the second is the product of the mutant ARMS reaction. Each test contains an additional control PCR product, and location of this band is indicated by an arrow. Lanes 1–10 are test samples; lane 11 is negative control.

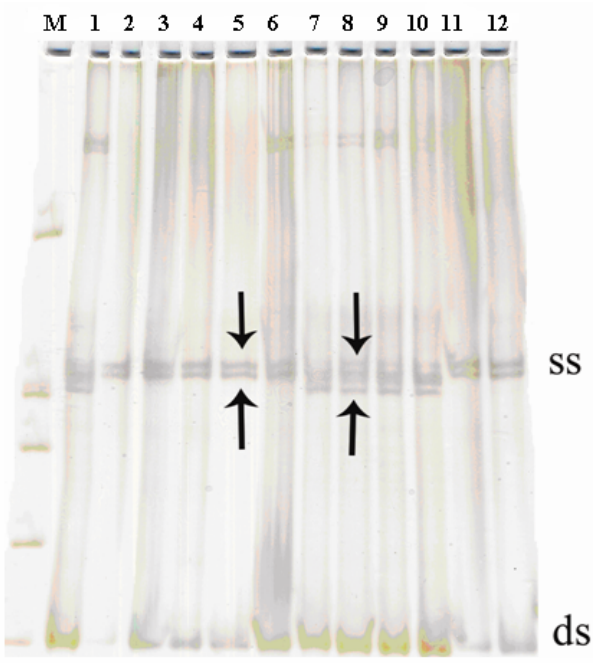

Figure 2. PAGE analysis and silver staining of the control person (lane 12) and cystic fibrosis patients (lanes 1-11) for the SSCP mutation screening in exon 3 of the CFTR gene. ss = Single strand DNA, ds = Duble strand DNA

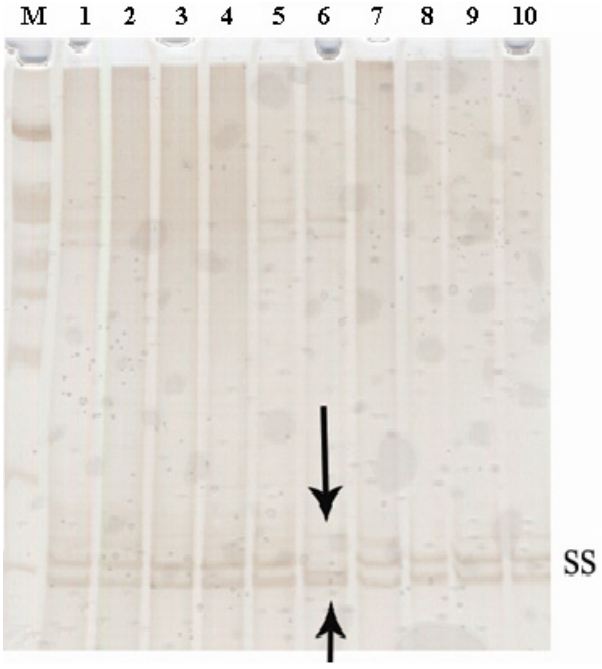

Figure 3. SSCP analysis of CF mutations in exon 11 of the CFTR gene. The slot 1 presents wild type, 2-10 present amplification products from CF patients. ss = single strand DNA

RESULTS

Mutation Frequencies

The study group included 70 patients that belonged to 70 unrelated families (39 male and 31 female; aged between 2 months and 15 years). All families were from Iran. In the present study 60% of patients are as a result of consanguine marriage. The degree of relationship between parents of these CF patients was mostly 3 (cousins). Diagnostic criteria for 65 subjects were based on repeated positive sweat chloride tests (>60 mmol/l) and on typical findings of pulmonary/ gastrointestinal disorders.

Five patients with a broad spectrum of respiratory diseases or undefined pancreatic and borderline (40-60 mmol/l) sweat chloride values, were analyzed. Among the identified mutations, the ΔF508 mutation was found to be the most common mutation, accounting for 17.8% of the CFTR mutations. Screening of CF samples for ten mutations, using ARMS/PCR assay, only revealed three mutations: ΔF508 mutation was found in 25 (17.8%) alleles, N1303K in six (4.3%) alleles and G542X in five (3.6%) alleles (Table1). Analysis of exons 3, 7, 10, 11 and 17b was performed by SSCP technique. In many samples, PCR products of some exons especially exon 10 showed a different mobility from those of the reference control. In the next step, the relevant PCR products will be analyzed by direct sequencing.

DISCUSSION

There are several ethnic groups and geographic areas in Iran, and the frequency of most common CFTR mutations varies among different ethnic groups and geographic areas.[20] The overall distribution of CFTR mutations among Iranian population considerably differs from those reported for Pakistan, India, Turkey and Arabian countries.[13,20-24] All of our subjects were Iranian origin and they were from different geographic areas in Iran.

There are only a few reports available describing frequency of CFTR gene mutations in Iran.[25,26] In the present study, we analyzed CFTR gene mutations by ARMS/PCR and SSCP methods.

The frequency of the ΔF508 mutation, the most common mutation in Caucasians, was 17.8% of the CFTR mutations in Iranian CF patients. This is in contrast with the high frequency of ΔF508 mutation in European and other populations where the frequency of the ΔF508 is more than 50%.[30,27-30] The geographical distribution of the ΔF508 shows a decreasing frequency of this mutation from the Northwest to the Southeast, in European population.

The frequency of this mutation differs in different counties: the Faeroe Islands (100%), Denmark (87.5%), United Kingdom (75.33%), Belgium (75.1%), Germany (71.8%), France (67.7%), Austria (62.9%), Poland (57.1%), Romania (36.6%) and Turkey (24.5%).[13,20,27,29,31,32] Islamic Republic of Iran located in the Southeast of Turkey and based on this gradient, we expected to observe the lower frequency of ΔF508 mutation in Iran compared to Turkey.

The ΔF508 mutation was found in 15 Iranian CF patients and 10 of them were homozygous and five patients were compound heterozygous for ΔF508 /T1036I, ΔF508 /dele9, ΔF508 /R1066C and ΔF508 /R1162X (two patients).The patients were from different parts and ethnic groups (Persian, Azari, Gilaki, Kurd, Arab and Lur) of Iran. Different haplotypes described for ΔF508, suggests a recurrent origin or rather, several recombinant events. In some reports it has been shown that the ΔF508 mutation arose in a population genetically distinct from the present European population.[33,34] This has been proposed that original founder ΔF508 mutation occurred in those inhabiting the Iranian plateau.[34] Based on the reasons mentioned; Iran could be one of the origins of the ΔF508 mutation.

The G542X mutation was reported as the most common mutation in the Mediterranean regions of Europe and Africa[20] and it accounts for 2.4%of CF mutations worldwide.[34] In this study, the frequency of this mutation was found on 3.6% of the CF chromosomes. Two of them were homozygous.

The fourth most common mutation in Iran was N1303K (4.3%). The N1303K mutation was found in different geographical areas and ethnic groups in Iranian population. It was detected as homozygous or compound heterozygous. N1303K was reported as a common mutation around the Mediterranean and it reaches its highest frequency (17.2%) in Tunisia.[35] The W1282X mutation is one of the most common CFTR mutations in the Mediterranean countries, while we did not find it in our samples.

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