IBBJ
Spring 2016, Vol 2, No 2

Original Article

Mutation and Rare Polymorphisms Insight in Exons 7 and 20 of *CFTR* Gene in Non-Caucasian Cystic Fibrosis Patients

Reza Tabaripour[1], Haleh Akhavan-Niaki[2,3*], Mohammad Reza Esmaeili Dooki[4]
Tahereh Dadkhah[3,5], Ali Mohammad Shirafkan[6], Elham Ghadami[3]

1. Department of Cellular and Molecular Biology, Islamic Azad University, Babol Branch, Iran.

2. Genetic laboratory of Amirkola children's hospital, Babol University of Medical Sciences, Babol, Iran.

3. Department of Genetics, Faculty of Medicine, Babol University of Medical Sciences, Babol, Iran.

4. Non-Communicable Pediatric Diseases Research Center, Babol University of Medical Sciences, Babol, Iran.

5. Cellular and Molecular Biology Research Center, Babol University of Medical Sciences, Babol, Iran.

6. Islamic Azad University, Damghan Branch, Iran.

Submitted 01 Oct 2016; Accepted 15 Nov 2016; Published 05 Dec 2016

Cystic fibrosis (CF) is the most common severe autosomal recessive disorder caused by a wide spectrum of mutations in the gene encoding for the cystic fibrosis transmembrane conductance regulator (CFTR) protein. The frequencies, types and distributions of mutations vary widely between different populations and ethnic groups. The aim of this study was to perform a comprehensive analysis of the *CFTR* gene in an Iranian heterogeneous population. 20 CF patients diagnosed according to clinical evaluation and elevated sweat chloride value and presenting no common *CFTR* mutation, were analyzed for mutations and polymorphisms in exons 7, 20 and 21 and parts of introns 6, 7, 19, 20 and 21 of *CFTR* gene using PCR-sequencing. Sequencing of exon 7 revealed the presence of two variations c.864G>A (rs766189605) and c.910C>T (rs121909011) with the frequencies of 10% and 2.5%, respectively. c.864G>A is a synonymous variant that happens in amino acid valine 318 (GTG/GTA) and c.910C>T is a pathogenic missense variant that occurs at amino acid 334 (R334W) of CFTR protein leading to the change of arginine to tryptophan. Two variations c.3780A>G (rs1800130) and c.3783+117T>G (rs10155917) were also found in exon 20 and intron 20 with the frequencies of 7.5% and 5%, respectively. No mutation or polymorphism was found in exon 21. Ascertainment of *CFTR* mutation carrier frequencies and CF incidence among heterogeneous Iranian populations seems to be a necessity.

Key words: Cystic fibrosis, polymorphism, non-Caucasian, R334W

Cystic fibrosis is the most common severe autosomal recessive disorder affecting 1 in 2500 newborns among Caucasians (1, 2) though its frequency may vary in specific groups (3). The major clinical characteristics of CF are pancreatic insufficiency and progressive lung disease, caused

*Correspondence: Genetic laboratory of Amirkola children's hospital, Babol University of Medical Sciences, Babol, Iran.
E-mail: halehakhavan@yahoo.com

[Downloaded from ibbj.org on 2026-09-09]

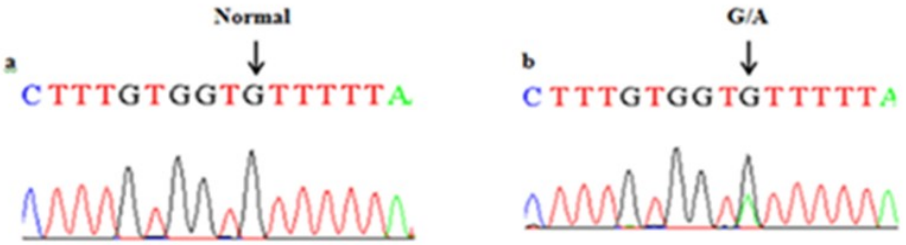

Figure 1. c.864G>A (rs766189605) variation in exon 7 of *CFTR*. a: normal sequence; b: heterozygous sequence with rs766189605 variant.

by thick and dehydrated airway mucus frequently infected with bacteria like pseudomonas and staphylococcus leading to respiratory failure and CF mortality (4, 5). Other CF characteristics include males infertility due to congenital bilateral absence of the vas deferens, bile duct obstruction, high sweat chloride, intestinal obstruction, nasal polyp formation, liver disease and diabetes (1, 2, 6). The gene responsible for the disease is cystic fibrosis transmembrane conductance regulator (*CFTR*) that comprises 27 coding exons spanning over 250 kb on chromosome 7q31.2 that encode a protein with 1480 amino acids and acts as ATP-binding cassette (ABC) chloride transporter channel in apical membrane of exocrine epithelial cells (7). Although over 2000 sequence variations (mutations and polymorphisms) have been characterized along the entire *CFTR* gene (http://www .genet.sickkids .on.ca/cftr/), the frequencies, types and distributions of mutations vary widely between different populations (8, 9). The most common *CFTR* gene mutation, F508del (c.1521-1523delCTT) is found in nearly 70% of CF chromosomes worldwide. However, its frequency varies greatly among different ethnic groups. The prevalence of this mutation in Europe shows decreasing gradient from northwest to southeast (10-15) and its frequencies in Arab (16, 17), Turkish (18), Indian (19) and Iranian (20, 21) populations varies between 13% and 44%. Four mutations (p.G542X, p.N1303K, p.G551D and p.W1282X) have overall frequencies greater than 1% (12) and all the other mutations are mostly rare. Direct mutation analysis in Iranian populations with high heterogeneity of *CFTR* mutations is not easy and previous reports of Iranian *CFTR* mutations showed a different pattern and distribution of *CFTR* mutations from Caucasians (22). In this study the exon 7 which encodes for the transmembrane domain and exons 20 and 21 of CFTR protein which encode for the topological domain that corresponds to the subcellular compartment where each non-membrane region of a membrane-spanning protein is found, were investigated in patients who were previously screened by sequencing and reverse dot blot (RDB) assay for common mutations present in Caucasians (23).

Table 1. Demographic characteristics of studied population

Primer	Sequence 5'⟶3'	Amplicon size (bp)
E7F **E7R**	ACTACAAGCAAAACACTGGT ACCATTTGCAACCTCTGGCC	969
E20F **E20R**	CATTGGTCAGGATTGAAAGTG CAATTCCACTACCTCATGATTC	720
E21F **E21R**	GAATGATACAAAGCAGACATGA GAAGTATGCTCTCACGAGACA	769

[Downloaded from ibbj.org on 2026-09-09]

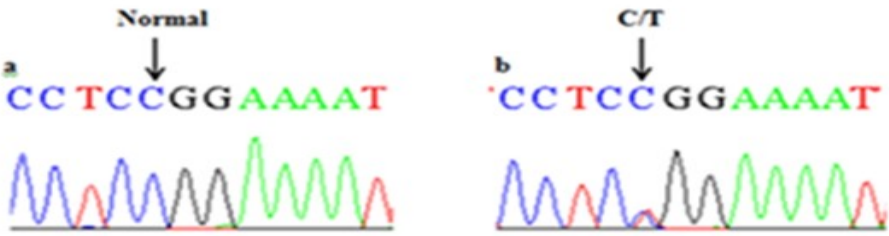

Figure 2. c.910C>T (rs121909011) variation in exon 7 of *CFTR*. a: normal sequence; b: heterozygous sequence with rs121909011 variant.

Materials and methods

Patients

In this study, 20 CF patients diagnosed based on clinical evaluation and elevated sweat chloride value (> 60 mEq/L) were investigated. The age of patients were between six months and fourteen years. All patients were from the north of Iran and were referred by the pediatric hospital of Babol University of Medical Sciences. All patients' parents gave their informed consent to participate in this study which was approved by the ethical committee of Babol university of medical sciences.

Molecular analyzes

Genomic DNA was prepared from peripheral blood leukocytes using alkaline lysis method. DNA was amplified using specific primers for exons 7, 20 and 21 (Table 1). PCR was carried out in 50 µl reaction volume using approximately 250 µM dNTPs, 2 mM MgCl2, 200 nM each forward and reverse primers and 1.5 units Taq DNA polymerase. Thermo-cycling conditions for amplification of these exons were 94 ˚C for 4 min followed by 35 cycles of 94 ˚C for 1 min, 59 ˚C for 30 s and 72 ˚C for 45 s with a final extension at 72 ˚C for 7 min. PCR products were detected on 2% agarose gel after

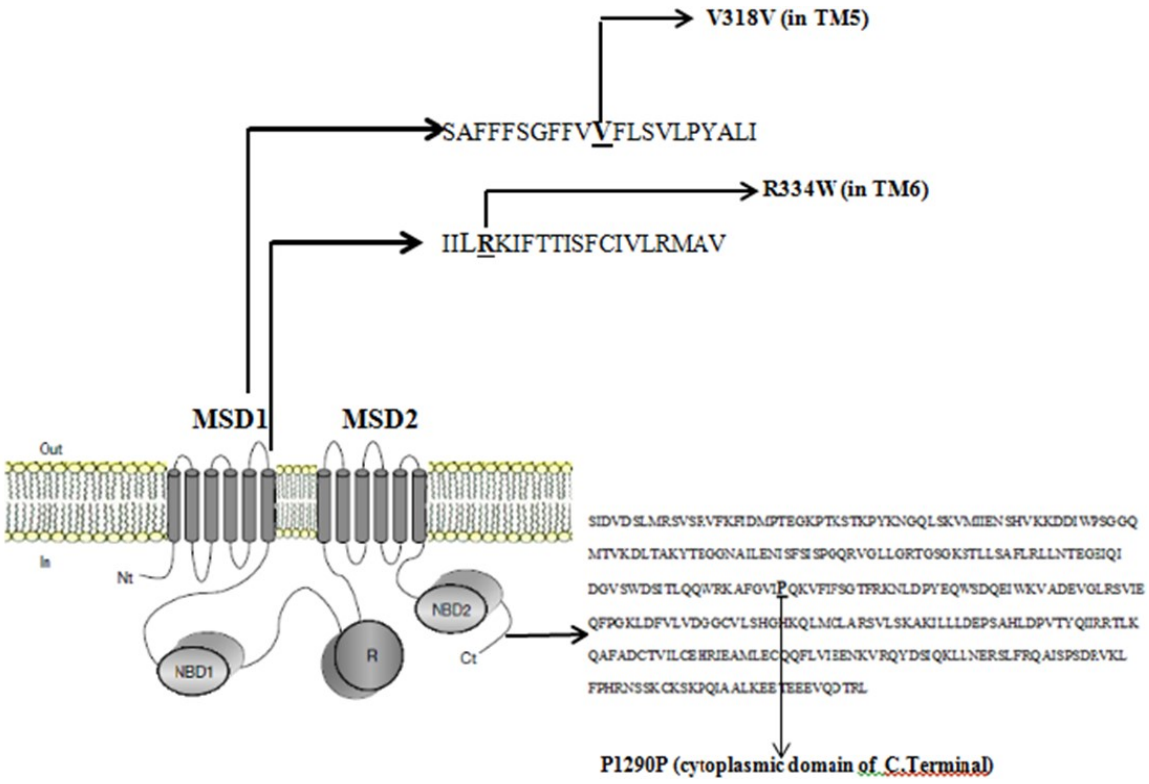

Figure 3. Position of different variants and mutations in CFTR protein. c.864G>A (V318V) and c.910C>T (R334W) variations are located in TM5 and TM6 of membrane spanning domain 1 (MSD1) in CFTR protein and c.3780A>G (P1290P) is located in cytoplasmic domain of C terminal region of CFTR protein.

[Downloaded from ibbj.org on 2026-09-09]

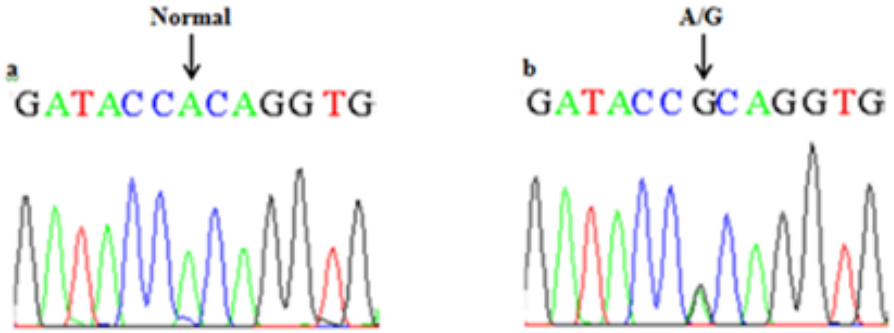

Figure 4. c.3780A>G (rs1800130) variation in exon 20 of *CFTR*. a: normal sequence; b: heterozygous sequence with rs1800130 variant.

electrophoresis under UV illumination and then amplicons were analyzed by direct sequencing (ABI-Genetic Analyzer 3730, Macrogen, Big Dye).

Results

PCR products of exons 7, 20 and 21 were analyzed for the presence of mutations or polymorphisms in 20 patients from the north of Iran. Sequencing of exon 7 revealed the presence of two variations c.864G>A (rs766189605) and c.910C>T (rs121909011) with the frequencies of 10% and 2.5%, respectively (figures 1 and 2). c.864G>A is a synonymous variant that happens in amino acid valine 318 (GTG/GTA), and c.910C>T is a pathogenic missense variant that occurs at amino acid 334 of CFTR protein leading to the change of arginine to tryptophan (CTC/TTC). Figure 3 shows the sequence of transmembrane domain 5 and 6 in membrane spanning domain 1 (MSD1) of CFTR protein where c.864G>A and c.910C>A variations occur, respectively. Screening of the samples for mutations or polymorphisms detection in the genomic region spanning the exon 20 revealed two variations, c.3780A>G (rs1800130) and c.3783+117T>G (rs10155917) in exon 20 and intron 20, respectively (figures 4 and 5). c.3780A>G is a synonymous mutation which occurs in amino acid proline 1290 (CCA/CCG) in the cytoplasmic domain of the C terminal part of CFTR protein (figure 3). This silent mutation was observed with the frequency of 7.5% in the studied patients. The frequency of c.3783+117T>G variation in intron 20 was 5%. No mutation or polymorphism was found found in exon 21.

Discussion

There are a few reports of spectrum, frequency and distribution of *CFTR* mutations among the Iranian CF patients (21-28) and the overall distribution of *CFTR* mutations in Iranian populations differ from the neighboring populations (8). It was commonly believed that CF incidence in Iranian populations was low, but investigations in the recent decade revealed relatively high incidence

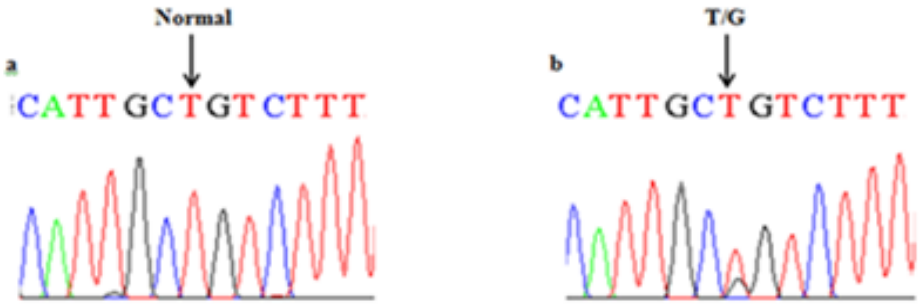

Figure 5. c.3783+117T>G (rs10155917) variation in intron 20 of *CFTR*. a: normal sequence; b: heterozygous sequence with rs10155917 variant.

[Downloaded from ibbj.org on 2026-09-09]

of this disease in Iranian populations. In the present study we analyzed exons 7, 20 and 21 and parts of introns 6, 7, 19, 20 and 21 based on PCR sequencing. We have detected one mutation and three polymorphisms in northern Iranian CF patients. All of these variations were encountered in heterozygous state. c.910C>T (R334W) mutation is a pathogenic mutation which causes the substitution of arginine to tryptophan amino acid in CFTR protein. Tryptophan is a relatively huge amino acid and if settles on a protein instead of another amino acid, it can induce a major alteration in protein structure. The worldwide frequency of this mutation is 1% but its frequency in this sample of northern Iranian population which was screened negative for common *CFTR* mutations was 2.5%. In this study, three previously reported polymorphisms (rs766189605G>A, rs1800130 A>G and rs101559 17T>G) in exons 7 and 20 and intron 20, respe-ctively were also detected. For rs766189605G>A in exon 7 we found that the A allele had a frequency of 10%. The worldwide frequency of this polymorphism is very rare about 0.000824%. The frequency of rs1800130A>G polymorphism in exon 20 was 7.5% in the present study, but its frequency was reported 3% in a previous study in Iranian population (21). Allele G shows 4% frequency in northern and western European ancestry, 13% in African ancestry in southwest USA and a frequency of 2%, 7%, 1% and 2% in Italy, Mexican ancestry, Chinese and Indian populations, respectively (Ensembl variation-Data description). The rs10155917T>G in intron 20 have shown 5% frequency in the present study and its frequency in African ancestry in southwest USA, Mexican, Italian and Chinese populations is 9%, 3%, 2% and 1%, respectively (Ensembl variation-Data description). This and other studies demonstrated that the Iranian population is highly heterogeneous and *CFTR* alleles are highly polymorphic and are distinguished by different frequency of polymorphic alleles compared to other populations. The present study has some limitations, particularly regarding the sample size and lack of molecular analysis of healthy subjects. Investigation of larger number of CF patients and availability of mutations panel covering a large number of alleles can facilitate phenotype prediction in prenatal diagnosis or newborn screening programs.

Conflict of interest

The authors declared no conflict of interest.

References

1. Collins F S. Cystic fibrosis: molecular biology and therapeutic implications. Science. 1992;256: 774-9.

2. Welsh MJ, Tsui LC, Boat TF, et al. Cystic fibrosis; in Scriver CL, Beaudet AL, Sly WS, Valle D (eds): The Metabolic Basis of Inherited Disease. New York, McGraw-Hill, 1995;3799–3876.

3. Rowntree R K, Harris A. The phenotypic consequences of CFTR mutations. Ann Hum Genet. 2003;67:471-85.

4. Boucher R C. Cystic fibrosis: a disease of vulnerability to airway surface dehydration. Trends Mol Med. 2007;13:231-40.

5. Weiler C A, Drumm M L. Genetic influences on cystic fibrosis lung disease severity. Front Pharmacol. 2013;4:40.

6. Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration. 2000;67:117-33.

7. Riordan J R, Rommens J M, Kerem B, et al. Ident-fication of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245:1066-73.

8. Bobadilla J L, Macek M, Jr., Fine J P, et al. Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. Hum Mutat. 2002;19:575-606.

[Downloaded from ibbj.org on 2026-09-09]

9. Radivojević D, Lalić T, Djurišić M, et al. Analysis of extra-and intragenic marker haplotypes as part of molecular diagnosis of cystic fibrosis in patients from Serbia. Arch. Biol. Sci. 2008;60:5–10.
10. Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. The Biomed CF Mutation Analysis Consortium. Hum Mutat. 1997;10:135-54.
11. Kanavakis E, Efthymiadou A, Strofalis S, et al. Cystic fibrosis in Greece: molecular diagnosis, haplotypes, prenatal diagnosis and carrier identification amongst high-risk individuals. Clin Genet. 2003;63:400-9.
12. Morral N, Bertranpetit J, Estivill X, et al. The origin of the major cystic fibrosis mutation (delta F508) in European populations. Nat Genet. 1994;7:169-75.
13. Serre J L, Simon-Bouy B, Mornet E, et al. Studies of RFLP closely linked to the cystic fibrosis locus throughout Europe lead to new considerations in populations genetics. Hum Genet. 1990;84:449-54.
14. Yiallouros P K, Neocleous V, Zeniou M, et al. Cystic fibrosis mutational spectrum and genotypic/phenotypic features in Greek-Cypriots, with emphasis on dehydration as presenting symptom. Clin Genet. 2007;71:290-2.
15. Radivojevic D, Djurisic M, Lalic T, et al. Spectrum of cystic fibrosis mutations in Serbia and Montenegro and strategy for prenatal diagnosis. Genet Test. 2004;8:276-80.
16. Kambouris M, Banjar H, Moggari I, et al. Identification of novel mutations in Arabs with cystic fibrosis and their impact on the cystic fibrosis transmembrane regulator mutation detection rate in Arab populations. Eur J Pediatr. 2000;159:303-9.
17. Eskandarani H A. Cystic fibrosis transmembrane regulator gene mutations in Bahrain. J Trop Pediatr. 2002;48:348-50.
18. Yilmaz E, Erdem H, Ozguc M, et al. Study of 12 mutations in Turkish cystic fibrosis patients. Hum Hered. 1995;45:175-7.
19. Ashavaid T F, Kondkar A A, Dherai A J, et al. Application of multiplex ARMS and SSCP/HD analysis in molecular diagnosis of cystic fibrosis in Indian patients. Mol Diagn. 2005;9:59-66.
20. Jalalirad M, Houshmand M, Mirfakhraie R, et al. First study of CF mutations in the CFTR gene of Iranian patients: detection of DeltaF508, G542X, W1282X, A120T, R117H, and R347H mutations. J Trop Pediatr. 2004;50:359-61.
21. Elahi E, Khodadad A, Kupershmidt I, et al. A haplotype framework for cystic fibrosis mutations in Iran. J Mol Diagn. 2006;8:119-27.
22. Alibakhshi R, Kianishirazi R, Cassiman J J, et al. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. J Cyst Fibros. 2008;7:102-9.
23. Dooki M R, Akhavan-Niaki H, Juibary A G. Detecting Common CFTR Mutations by Reverse Dot Blot Hybridization Method in Cystic Fibrosis First Report from Northern Iran. Iran J Pediatr. 201;21:51-7.
24. Esmaeili Dooki M R, Tabaripour R, Rahimi R, et al. Mutation and new polymorphisms insight in introns 11 to 14a of CFTR gene of northern Iranian cystic fibrosis patients. Gene. 2015;564:193-6.
25. Tabaripour R, Niaki H A, Douki M R, et al. Poly thymidine polymorphism and cystic fibrosis in a non-Caucasian population. Dis Markers. 2012;32:241-6.
26. Kholghi Oskooei V, Esmaeili Dooki M R, Tabaripour R, et al. CFTR haplotypes in northern Iranian population. Gene. 2013;512:55-60.
27. Tabaripour R, Dooki M R E, Akhavan-Niaki H. Night Blindness and Diabetes in a Non-Caucasian Cystic Fibrosis Patient in Iran. World Appl Sci J. 2013;23:837-9.
28. Esmaeili Dooki M R, Akhavan-Niaki H, Shaba-

[Downloaded from ibbj.org on 2026-09-09]

ni S, et al. Molecular Screening of R117H (c.350G>A; p.Arg117His) Mutation in Non Caucasian Cystic Fibrosis Patients from North of Iran. Int Biol Biomed J. 2015;1:66-71.

[Downloaded from ibbj.org on 2026-09-09]