Novel CFTR Mutations in Two Iranian Families with Severe Cystic Fibrosis

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Received 15 August 2015; revised 14 September 2015; accepted 7 October 2015

ABSTRACT

Background: Cystic fibrosis (CF) is a common autosomal recessive disorder that affects many body systems and is produced by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. CF is also the most frequently inherited disorder in the West. The aim of this study was to detect the mutations in the CFTR gene in two Iranian families with CF. Methods: After DNA extraction using the salting out method, a mutation panel consisting of 35 common mutations was tested by PCR, followed by reverse hybridization Strip Assay. To confirm the mutations, we have also performed Sanger sequencing for all 27 exons, intronic flanking regions, and 5’ and 3’ UTRs of the CFTR gene. Results: Carrier testing in a spouse revealed a novel nonsense mutation in the CFTR gene (c.2777 T>A (p.L926X)) in exon 17 for husband and a previously described heterozygous splice site pathogenic mutation (c.1393-1G>A) in his wife. The other novel compound heterozygous missense mutation (c.3119 T>A (p.L1040H)), which was previously reported as nonsense c.3484C>T (p.R1162X) mutation, was found in exon 19 in patient screening. Conclusion: Two novel CFTR mutations in exons 17 and 19 are responsible for CF with severe phenotypes in two Iranian families. These two mutations supplement the mutation spectrum of CFTR and may contribute to a better understanding of CFTR protein function. DOI: 10.7508/ibj.2016.04.003

Keywords: Cystic fibrosis, Cystic fibrosis transmembrane conductance regulator protein, Mutation, Sequence analysis, Iran

INTRODUCTION

Cystic fibrosis (CF) (MIM 219700) is a common autosomal recessive disorder that affects many different organs. The leading cause of morbidity and mortality is the progressive decline in pulmonary function resulting from airway damage caused by thickened secretions complicated by chronic microbial infection. Moreover, the other clinical symptoms of the CF patients include insufficiency of the exocrine pancreas in about 85% of CF patients, meconium ileus in nearly 15%, diabetes mellitus in 15% and severe liver disease in about 5%. Furthermore, 99% of CF males are infertile because of congenital bilateral absence of the vas deferens.

CF is common among Caucasians of Northern European descent, with about 1/2500 affected and a carrier rate of about 1/25. However, other ethnic and racial groups are less commonly affected. For example, the prevalence of CF among African-Americans is approximately 1/17,000, which corresponds to a carrier rate of 1/68. Few reports
have described the distribution and abundance of cystic fibrosis transmembrane conductance regulator (CFTR) gene (MIM 602421) mutations in Iranian patients\textsuperscript{[8-12]}. A study on 37 Iranian CF patients in 2004 detected six mutations, including p.F508del, p.W1282X, p.G542X, p.R117H, p.R347H and p.A120T\textsuperscript{[11]}. Another study on 69 Iranian CF patients identified 37 mutations, of which the p.F508del was the most frequent mutation\textsuperscript{[12]}. In a recent study performed on a northern Iranian population, the p.F508del mutation was also the most frequent\textsuperscript{[13]}.

At the molecular level, a defective CFTR protein leads to inadequate transport of chloride ions between the intra- and extra-cellular environments of epithelial cells in affected organs. In pancreatic ducts, the same defect leads to inspissated secretions, which blocks the duct and prevents the transport of pancreatic enzymes into the digestive tract. The biliary tree, vas deferens duct and prevents atypical CF with equivocal sweat test results and a single CF symptom.

**Genetic analysis**

After genetic counseling, a blood sample (10 mL) was collected from each patient, and genomic DNA was extracted\textsuperscript{[24]}. This was followed by PCR and reverse hybridization using the CF StripAssay (ViennaLab Diagnostics, Vienna, Austria) to detect the following 35 common mutations: CFTRdel2,3 (21 Kb) (c.54-5940_273+10250del); 1507del(·ATC) (c.1519_1521delATC); F508del (·CTT) (c.1521_1523delCTT); 1717-1G>A (c.1585-1G>A); G542X (c.1624G>T); G551D (c.1632G>A); R553X (c.1657C>T); R560T (c.1679G>C); 2184delA (c.2052delA); 2184insA (c.2052_2053insA); 2789+5G>A (c.2657+5G>A); R1162X (c.3484C>T); 3659delC (c.3528delC); 3905insT (c.3773dupT); W1282X (c.3846G>A); N1303K (c.3909C>G); G85E (c.254G>A); 394delTT (c.262_263delTT); R117H (c.350G>A); Y122X (c.366T>A); 621+1G>T (c.489+1G>T); 711+1G>T (c.579+1G>T); 1078delT (c.948delT); R334W (c.1000C>T); R347H (c.1040G>A); R347P (c.1040G>C); A455E (c.1364C>A); 1898+1G>A (c.1766+1G>A); 3120+1G>A (c.2988+1G>A); 3272-26A>G (c.3140-26A>G); Y1092X (c.3276C>A); 3849+10KbC>T (c.3718-2477C>T).

The samples were further analyzed by Sanger sequencing for all 27 exons, intronic flanking regions, and 5’ and 3’ UTRs of the CFTR gene using the ABI PRISM™ BigDye Terminator Cycle Sequencing kit and the ABI PRISM™ 3130-Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). Alleles were discriminated using CodonCode Aligner software version 6.0.2. The pathogenicity of novel variants was predicted using bioinformatics software such as PolyPhen, Conseq, Sift and MutationTaster. The study was approved by the University of Social Welfare and Rehabilitation Sciences Institutional Ethics Committee for Research Protocols.

**MATERIALS AND METHODS**

We investigated two previously diagnosed Iranian families with a history of CF, who were referred to the Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran for molecular diagnostic and carrier testing. The first family had a 5-month-old child, who was affected with CF and had passed away; therefore, his parents were referred to this center for carrier detection (Family I). The patient in the other family had CF symptoms with a strong suspicion of CF disease (Family II). The patient in Family I had a clinical diagnosis of CF according to both the clinical presentation and the results of repeated sweat tests (quantitative pilocarpine iontophoresis\textsuperscript{[23]}) and was hence defined as a “sweat test confirmed” CF patient. The patient in Family II was suspected of having atypical CF with equivocal sweat test results and a single CF symptom.

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MATERIALS AND METHODS

We investigated two previously diagnosed Iranian families with a history of CF, who were referred to the
RESULTS

Family I
A healthy and unrelated couple, a 36-year-old male and a 27-year-old female, both with Persian ancestry, was referred to our center by a gastroenterologist and gynecologist for diagnostic testing. They were referred as a result of CF diagnosis in their first child who died at the age of five months and who was the only affected subject in this family. His first symptom was steatorrhea at 17 days of age, and he presented pulmonary symptoms such as cough. Ultrasound investigations revealed posterior urethral valves and hernia, and so he was diagnosed with CF early after birth.

In molecular assessment of father, we found a new heterozygous nonsense mutation in exon 17 of the CFTR gene, which changed T to A at position 2777 defined as (c.2777 T>A (p.L926X)). To predict the pathogenicity of this mutation, in silico analysis software such as MutationTaster, PolyPhen-2 and Sift was used. MutationTaster showed that the mutation was disease causing and might affect protein features, and the result was confirmed by PolyPhen-2 software (Fig. 1). Furthermore, a heterozygous splice site mutation in the CFTR gene, defined as c.1393-1G>A and described previously as a pathogenic mutation, was found in the mother by direct sequencing.

Family II
The patient, a 32-month-old male, was the first child of non-consanguineous Persian parents, who referred to our center for CFTR DNA analysis. This family had only one affected individual with CF symptoms. The molecular finding in this patient showed a compound heterozygous mutation in exon 19 of the CFTR gene, defined as one novel missense mutation changing T to A at position 3119 (c.3119T>A), which caused the substitution of leucine to histidine at position 1040 (c.3119T>A (p.L1040H)) and also one previously reported nonsense mutation c.3484C>T (p.R1162X) in exon 22 of CFTR gene.

To confirm the novel mutation c.3119T>A, we used in silico analysis tools. The results predicted that this mutation could be a causative mutation (Fig. 2), which may confirm the clinical diagnosis for this patient.

DISCUSSION
In this study, we report two novel mutations, one nonsense in a healthy adult male (having an infant died at five months) who was referred for carrier detection, and one missense in a 2.5-year-old child with a definite clinical length diagnosis of CF. To date, over 2000 mutations have been identified in the CFTR gene;
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Fig. 2. Characterization of a novel missense variant identified in CFTR gene. (A) MutationTaster, Sift and PolyPhen results showing c.3119T>A to be a disease causing mutation. (B) Confirmation of a compound heterozygous mutation by direct sequencing; a novel thymine-to-adenine substitution occurs at nucleotide 3119 in exon 19 (c.3119T>A) and a previously reported cytosine-to-thymine substitution at nucleotide 3884 in exon 22 (c.3484C>T).

almost all are point mutations or small (1–84 bp) deletions. Mutations in the CFTR gene can be categorized based on the disruption in CFTR protein function\(^{25,26}\). Mutations in Class I result in premature truncation of nascent CFTR polypeptide and lead to little or no protein expression and cause severe disease in the homozygous form or compound heterozygous form in combination with a Class II mutation. Class II mutations affect the synthesis of CFTR protein. The homozygous form of this mutation can lead to the development of severe disease (F508del)\(^{11}\). Class III mutations alter CFTR gating and result in lowered Cl\(^{-}\) transport, despite the expression of full protein at the apical plasma membrane of epithelial cells. Class IV mutations can cause reduced Cl\(^{-}\) permeability. Mutations in Class V induce decreased expression of CFTR protein, and with Class VI mutations, the protein has abnormally short residence time at the apical plasma membrane\(^{27,28}\). Individuals who carry Class IV–VI mutations often have milder disease. Although genotype-phenotype correlations in CF are imprecise\(^{29}\), a CF patient’s clinical phenotype will usually reflect either full loss of or some fractions of CFTR ion transport function if there is residual ion transport function afforded by one of the mutant CFTR alleles\(^{30}\).

In this study, we screened all exons and splicing sites in the CFTR gene in two families, and two novel mutations were identified. A substitution of leucine to stop codon at position 926 in the CFTR gene (p.L926X) occurred in the transmembrane domain of the CFTR gene in the first family. The replacement of
leucine to histidine at position 1040 (p.L1040H) of the 
*CFTR* gene occurred in the topological domain of the 
CFTR protein in the second family. We also found two 
previously reported mutations (c.1393-1G>A in Family 
I and c.3484C>T in Family II). Bioinformatics analysis 
showed that the two novel mutations were located in 
the transmembrane domains; these regions play a 
major role in the regulation of pore function in CFTR 
protein (CFTR admin database) so these mutations can 
damage CFTR protein function. Pathogenesis of these 
variants was evaluated by mutation classification, 
bioinformatic methods and also normal population 
study. Considering the clinical presentation and in 
silico software analyses such as dbSNP, Sift, 
PolyPhen, MutationTaster and our Iranian 
polymorphism database consisting of 400 normal 
ethnically adjusted samples (normal population study), 
the two novel mutations L926X and L1040H might not 
be polymorphisms, and they are presumably 
pathogenetic mutations (Figs. 1 and 2). Since these two 
novel mutations are located in the transmembrane 
domain, this could cause lowered Cl⁻ transport; 
therefore, these mutations could be considered to be a 
Class III mutation type.

Three changes have been reported at protein 
position 926: p.Leu926A1afsX48 (c.2775_2776delTT), 
p.Leu926CysfsX16 (c.2777delT) and p.Leu926Phe 
(c.2778G>T), of which two are frameshift mutations 
caused by thymine deletions. The only reported 
mutation at amino acid position 1040 is p.Leu1040Phe, 
which is a G to T substitution at location 3118 
(c.3118C>T).

The two previously reported mutations found in this 
study were c.1393-1G>A and c.3484C>T (p.R1162X) 
in two families. In Family I, c.1393-1G>A is a splice 
site variant in intron 10, which is not prevalent in 
the general population. In a recent study, the c.1393-1G>A 
has been shown to be one of the most frequent 
mutations in CF patients[31]. In Family II, R1162X is a 
nonsense mutation with substitution of arginine to stop 
codon at position 1162, and with a relative frequency 
of 0.3% in the general population. This mutation is 
among the panel of 10 core mutations that the ACMG 
CF Carrier Screening Working Group recommended to 
be screened during routine CF diagnostic testing and 
carrier screening in the general population[32]. This 
mutation has also been reported from Shiraz city, Fars 
Province in the south of Iran[33]. It has been reported 
that the R1162X transcript is stable and the truncated 
protein is probably misfolded; therefore, it is likely 
categorized in Class II. Our finding in Family II 
carrying two severe or classic mutations (Class II and 
III type) confirms the definitive and classic phenotype 
in this patient.

In Iran, complete genetic information is currently 
lacking to implement solid population-based *CFTR* 
screening programs that could enable adequate carrier 
detection of either typical or atypical CF patients or 
their family members. For national policies of CF 
prevention, it is acceptable to include only the most 
frequent mutations present in the population, which 
allows a 90% detection rate.

In conclusion, two novel *CFTR* mutations in the 
transmembrane domain and topological domain have 
been identified in CF families, which may extend the 
mutation spectrum of CF and contribute to better 
molecular understanding of the involvement of the 
*CFTR* gene. Additionally, this knowledge will help in 
developing new strategies to improve and extend the 
number of mutations screened for prenatal diagnosis 
and carrier screening.

ACKNOWLEDGMENTS

We are deeply grateful to the individuals who took 
part in this study, and we profoundly appreciate their 
collaboration that made this study possible.

CONFLICT OF INTEREST. None declared.

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