Novel CFTR Mutations in Two Iranian Families with Severe Cystic Fibrosis

Marzieh Mohseni1,2, Mohammad Razzaghmanesh1, Elham Parsi Mehr1, Hanieh Zare1, Maryam Beheshtian1,2 and Hossein Najmabadi*1,2

1Kariminejad-Najmabadi Pathology and Genetics Center, Tehran, Iran; 2Genetics Research Center, University of Social Welfare and Rehabilitation Sciences, Tehran, Iran

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.1,2 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.3 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.4

CF is common among Caucasians of Northern European descent, with about 1/2500 affected and a carrier rate of about 1/25.5,6 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 1/65.7 Few reports

Iran. Biomed. J. 20 (4): 201-206
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 and sweat ducts are likewise compromised. CF is generated by mutations in the CFTR gene. In 1989, in a collaborative study, Kerem and colleagues identified the gene responsible for CF\textsuperscript{[14]} and found that, in the majority of CF patients, the gene was missing three nucleotides, which resulted in the in-frame deletion of a phenylalanine residue at position 508 of the polypeptide chain (ΔF508)\textsuperscript{[15]}

The CFTR gene is situated at the location 7q31.2 and contains 27 exons. The CFTR protein output is located in the cell membrane and functions as an ion channel. Mutations in the CFTR gene affect the protein function and cause CF symptoms\textsuperscript{[5-10]}

CF is characterized by substantial allelic heterogeneity, with more than 2000 different mutations (Cystic Fibrosis Mutation Database, www.genet.sickkids.on.ca/cftr/) reported within the CFTR gene\textsuperscript{[1]}. Approximately 50% of Caucasian CF patients are homozygous for the ΔF508 mutation, which results in complete loss of CFTR function and classic, severe manifestations of the disease. About 40% of CF patients have ΔF508 on one chromosome and another less common mutation on the other chromosome. The remaining ~10% has two rare mutations\textsuperscript{[20,21]}

Previous research in the Iranian population indicated that ΔF508 is the most common mutation in the Iranian population and in other populations, and all known mutations have a high heterogenic frequency in Iran\textsuperscript{[22]}. Therefore, the current study aimed at defining the molecular aspects of CF in Iran.

**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.

**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 (21Kb) (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); 2134delT (c.2012delT); 2183AA>G (c.2051_2052delAAinsG); 2184delA (c.2052delA); 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); 1078delE (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\textsuperscript{TM} BigDye Terminator Cycle Sequencing kit and the ABI PRISM\textsuperscript{TM} 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.

**Novel Mutations in CFTR Gene**

Mohseni et al.

Iran. Biomed. J. 20 (4): 201-206
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;
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 fraction 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). Bioinformatic 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 pathogenic 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.Leu926AlafsX48 (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. 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. This mutation has also been reported from Shiraz city, Fars Province in the south of Iran. 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.

REFERENCES

1. Yang H, Ma T. F508del-cystic fibrosis transmembrane regulator correctors for treatment of cystic fibrosis: a patent review. Expert opinion therapeutic patents 2015; 25(9): 991-1002.
2. Drum M, Ziad AG, Davis PB. Genetic variation and clinical heterogeneity in cystic fibrosis. Annual review of pathology 2012; 7: 267-282.
3. De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, Sinaasappel M on behalf of the Diagnostic Working Group. Cystic fibrosis: terminology and diagnostic algorithms. Thorax 2006; 61(7): 627-635.
4. Laboratory testing for mutations associated with cystic fibrosis. University of North Carolina Hospitals Molecular Genetics Laboratory. www.uncmedicalexcenter.org/app/files/public/572/pdfs-mclendon-labs.
5. Lim RM, Silver AJ, Silver MJ, Borrot C, Spurrier B, Petrossian TC, Larson JL, Silver LM. Targeted mutation screening panels expose systematic population bias in detection of cystic fibrosis risk. Genetics in medicine 2016; 18(2): 174-179.
6. Smyth AR, Bell SC, Bojcin S, Bryon M, Duff A, Flume P, Kashiwaya N, Munck A, Ratjen F, Schwarzenberg SJ, Sermet-Gaudelus I, Southern KW, Taccetti G, Ullrich G, Wolfe S, European Cystic Fibrosis Society. European Cystic Fibrosis Society. standards of care: best practice guidelines. Journal of cystic fibrosis 2014;
Novel Mutations in CFTR Gene

Mohseni et al.

13(Suppl 1): S23-S42.

7. Bonyadi M, Omrani O, Rafeey M, Bilan N. Spectrum of CFTR gene mutations in Iranian Azeri Turkish patients with cystic fibrosis. Genetic testing and molecular biomarkers 2011; 15(1-2): 89-92.

8. Davis PB. Molecular and cell biology of cystic fibrosis. Journal of applied physiology (1985) 1991; 70(5): 2331-2333.

9. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, Zielenski J, Lok S, Plavsic N, Chou JL, Drumml LM, Lannuzzi, MC, Collins FS, Tsui LC. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989; 245(4922): 1066-1073.

10. Qadri YJ, Cornet-Boyaka E, Rooj AK, Lee W, Parpura V, Fuller CM, Berdiev BK. Low temperature and chemical rescue affect molecular mobility of DeltaF508-cystic fibrosis transmembrane conductance regulator (CFTR) and epithelial sodium channel (ENaC). The journal of biological chemistry 2012; 287(20): 16781-16790.

11. Jalalirad M, Houshmard M, Mirafkhrnia R, Goharbari MH, Mirzajaniz F. First study of CF mutations in the CFTR gene of Iranian patients: detection of DeltaF508, G542X, W1282X, A120T, R117H, and R347H mutations. Journal of tropical pediatrics 2004; 50(6): 359-361.

12. Alibakhshir K, Kianishirazi R, Cassiman JJ, Zamani M, Cuppens H. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. Journal of cystic fibrosis 2008; 7(2):102-109.

13. Kholghi Osaeoei V, Esmaeli Dooki MR, Tabaripour R, Mirzajani S, Pourbughr R, Akhavan-Niahi H. CFTR haplotypes in northern Iranian population. Gene 2013; 512(1): 55-60.

14. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, Buchwald M, Tsui LC. Identification of the cystic fibrosis gene: genetic analysis. Science 1989; 245(4922); 1073-1080.

15. Teem JL, Berger HA, Ostedgaard LS, Rich DP, Tsui LC. Welsh MJ. Identification of revertants for the cystic fibrosis delta F508 mutation using STE6-CFTR chimeras in yeast. Cell 1993; 73(2): 335-346.

16. Lucarelli M, Bruno SM, Pierandrei S, Ferraguti G, Stamato A, Narzi F, Amato A, Cimino G, Bertasi S, Quattrucci S, Strom R. A genotypic-oriented view of CFTR genetics highlights specific mutational patterns underlying clinical macrocategories of cystic fibrosis. Molecular medicine 2015; 21(1): 257-275.

17. Davies JC, Alton EW, Bush A. Cystic fibrosis. BMJ 2007; 335(7632): 1255-1259.

18. O’Sullivan BP, Freedman SD. Cystic fibrosis. Lancet 2009; 373(9678): 1891-1904.

19. Knowles MR, Durie PR. What is cystic fibrosis? The new England journal of medicine 2002; 347(6): 439-442.

20. Kopp BT, Nicholson L, Paul G, Tobias J, Ramananath C, Hayes D Jr. Geographic variations in cystic fibrosis: An analysis of the U.S. CF Foundation Registry. Pediatrics pulmonology 2015; 50(8): 754-762.

21. Schwarz C, Staab D. Cystic fibrosis and associated complications. Internist (Berl) 2015; 56(3): 263-274.

22. Najafi M, Alimadadi H, Rouhani P, Kiani MA, Khodadad A, Motamed F, Moraveji A, Hooshmand M, Haghji Ashhtiani MT, Rezaei N. Genotype-phenotype relationship in Iranian patients with cystic fibrosis. The Turk journal of gastroenterology 2015; 26(3): 241-243.

23. LeGrys VA, Yankaskas JR, Quittell LM, Marshall BC, Mogayzel PJ Jr. Cystic Fibrosis Foundation. Diagnostic sweat testing: the Cystic Fibrosis Foundation guidelines. Journal of pediatrics 2007; 151(1): 85-89.

24. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic acids research 1988; 16(3):1215.

25. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993; 73(7): 1251-1254.

26. Wilshanski M, Kerem E. New drugs for cystic fibrosis. Expert opinion on investigational drugs 2011; 20(9):1285-1292.

27. Zielearni S, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. Annual review of genetics 1995; 29: 777-807.

28. Haardt M, Benharouga M, Lecherdeur D, Kartner N, Lukacs GL. C-terminal truncations destabilize the cystic fibrosis transmembrane conductance regulator without impairing its biogenesis. A novel class of mutation. The journal of biological chemistry 1999; 274(31): 21873-21877.

29. Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, Tullis E, Assael BM, Bombieri C, Brown A, Casals T, Claustres M, Cutting GR, Dequeker E, Dodge J, Doull I, Farrell P, Ferec C, Giordon E, Johannesson M, Kerem B, Knowles M, Munck A, Pignatti PF, Radojkovic D, Rizzotti P, Schwarz M, Stuhrmann M, Tetzis M, Zielearni S, Elborn JS. Consensus on the use and interpretation of cystic fibrosis mutation analysis in clinical practice. Journal of cystic fibrosis 2008; 7(3): 179-196.

30. Reddy MM, Quinton PM. Functional interaction of CFTR and ENaC in sweat glands. Pflugers archive 2004; 455(4): 499-503.

31. Essawi O, Farraj M, De Leeneer K, Steyaert W, De Pauw K, De Paepe A, Claes K, Essawi T, Coucke PJ, Pauw K, De Paepe A, Claes K, Essawi T, Coucke PJ. Next generation sequencing to determine the cystic fibrosis mutation spectrum in Palestinian population. Diseases markers 2015; 2015: Article ID 458653.

32. Watson MS, Cutting GR, Desnick RJ, Driscoll DA, Klinger K, Mennuti M, Palomaki GE, Popovich BW, Pratt VM, Rohlfes EM, Strom CM, Richards CS, Witt DR, Grody WW. Cystic fibrosis population carrier screening: 2004 revision of American College of Medical Genetics mutation panel. Genetics in medicine 2004; 6(5): 387-391.

33. Farjadian S, Moghtaderi M, Kashef S, Alyasin S, Najib K, Saki F. Clinical and genetic features in patients with cystic fibrosis in southwestern Iran. Iranian journal of pediatrics 2013; 23(2): 212-215.