Three Novel Mutations in Turkish Children with Cystic Fibrosis: Case Report

Kistik Fibrozisli Türk Çocuklarında Üç Yeni Mutasyon

Ayfer ULGENALP, MD,[a]
Nevin UZUNER, MD,[b]
Duygu ÖLMEZ, MD,[b]
Arzu BABAYİĞİT, MD,[b]
Elçin BORA, MD,[a]
Özlem GİRAY, MD,[a]
Claude FEREC, MD,[c]
Özkan KARAMAN, MD,[b]
Derya ERCAL, MD[a]

[a]Department of Pediatrics, Division of Genetics,
[b]Department of Pediatrics, Division of Allergy,
Dokuz Eylul University Hospital, İzmir,
[c]Génétique Moléculaire et Génétique Epidémiologique, Brest, France

Geliş Tarihi/*Received:* 24.06.2008
Kabul Tarihi/*Accepted:* 17.09.2008

Yazışma Adresi/*Correspondence:*
Nevin UZUNER, MD
Dokuz Eylul University Faculty of Medicine, Department of Pediatrics, Division of Allergy, Izmir, TÜRKİYE/TURKEY
nuzuner@deu.edu.tr

ABSTRACT Cystic fibrosis is an autosomal recessive disease caused by mutations in the cystic fibrosis transmembrane conductance regulator gene that results in abnormal viscous mucoid secretions in multiple organs. It mainly occurs in European derived populations. The main clinical features of the disease are pancreatic insufficiency and chronic endobronchial infection. There are currently more than 1500 mutations, of which F508del is the most common. Here we reported three Turkish children who were diagnosed with cystic fibrosis based on characteristic manifestations of the disease and pathological sweat test results. Genetic analysis revealed three novel mutations, p.Leu812X, 3608insG ve p.Ile853Cys in the children, which have not been reported yet in cystic fibrosis patients. In the first case, a missense mutation R347P was identified in a compound heterozygote state with p.Leu812X.

Key Words: Cystic fibrosis transmembrane conductance regulator, mutation

ÖZET Kistik fibrozis, kistik fibrozis transmembran iletim regülatör genindeki mutasyonlara bağlı gelişen ve çok sayıda organda anormal visköz mukoid sekresyonlara neden olan otozomal resesif bir hastalıktır. Özellikle Avrupa toplumlarında görülür. Hastalığın başlıca klinik özellikleri pankreas yetmezliği ve kronik endobronşiyal enfeksiyondur. Günümüzde hastalığa ait 1500'den fazla mutasyon bildirilmiştir. En sık görüleni F508del mutasyonudur. Bu çalışmada, patolojik ter testi sonuçları ve kistik fibrozise ait karakteristik bulguları ile kistik fibrozis tanısı alan üç Türk çocuğunu sunduk. Genetik analiz sonucu çocuklarda, daha önce kistik fibrozis olgularında bildirilmemiş olan p.Leu812X, 3608insG ve p.Ile853Cys mutasyonları saptandı. İlk olguda missense mutasyon R347P ile p.Leu812X kompound heterozigot olarak saptandı.

Anahtar Kelimeler: Kistik fibrozis transmembran regülatör proteini, mutasyon

Turkiye Klinikleri J Med Sci 2009;29(6):1754-8

Cystic fibrosis (CF) is an autosomal recessive disease, most commonly encountered in Caucasian populations. CF prevalence is 1/2500-3000 in Caucasians and according to a limited number of studies 1/3000 in our population.[1] It is clinically characterized by obstructive lung disease, exocrine pancreatic insufficiency, elevated sweat electrolytes and male infertility.[2] CF is caused by mutations in both copies of the CF transmembrane conductance regulator (CFTR), a 230-kb gene located at chromosome 7q31.[3] CFTR encodes a 1,480-amino acid protein that acts as a cyclic adenosine monophosphate dependent chloride channel in the apical membrane of cells lining the lungs, sinuses, pancreas, intestines, vas deferens and sweat ducts.[4] Mutations in the CFTR gene can result in defects in synthesis, traf-

ficking, stability, function, and/or activation or regulation of the CFTR protein.[5] There are more than 1500 mutations listed on CFTR mutation database.[6]

The known (or predicted) functional consequences of the various mutations in CFTR have been categorized in six classes.[7]

Class I includes mostly nonsense, frameshift or missense mutations that result in defective protein biosynthesis. Class II mutations produce a misfolded functional CFTR protein, preventing trafficking to the apical surface of the cell. Class III mutations affect channel activation by preventing binding and hydrolysis of adenosine triphosphate at one of the two nucleotide binding domains. Class IV mutations produce a protein with impaired function because of abnormal anion conduction. Class V mutations result in a reduced number of normally functioning CFTR molecules on the apical surface. Class VI mutations result from truncation of the C-terminus of CFTR and produce a functional protein, which is unstable at the apical membrane surface.[8,9]

The most common mutation, a base-pair deletion in exon 10, which encodes the first nucleotide binding domain of the CFTR protein, results in a deletion of phenylalanine at position 508.[10] F508del is the most common mutation, with a frequency of 66% worldwide.[6] The R347P (exon 7) is a common missense mutation located within the first membrane spanning domain of the CFTR protein. This mutation occurs with an overall worldwide frequency of about 0.2%. p.Leu812X (exon 13), 3608insG (exon19) and p.Ile853Cys (exon14) mutations have not been reported yet in patients with CF.

Herein we reported three Turkish children with novel mutations who received CF diagnosis based on characteristic manifestations and pathological sweat test results.

CASE REPORTS

CASE 1

A Turkish girl, who was born to non-consanguineous parents, presented with pneumonia at the age of 3 months. She was first hospitalized at three years of age because of dehydratation, metabolic alkalosis, hyponatraemia, and hypochloraemia. At that time, the diagnosis of CF was verified by two pathological sweat test results (122-121 meq/L). Deoxyribonucleic acid mutation analysis was negative for F508del. Pancreatic sufficiency was evidenced by normal weight and bowel habits, and negative analysis of fecal fat excretion. Pulmonary colonization with *Pseudomonas aeruginosa* was observed at five years of age. The second and final pneumonia developed at the same age. At the age of ten, pulmonary function tests included FVC 83% predicted, FEV_1 80% predicted, FEV_1/FVC 97%, PEF 55%, and FEF_{25-75} 60% predicted. Two areas of bronchiectasis were demonstrated in the middle and the lower lobe of the right lung on high-resolution computed tomography (HRCT). A recent genetic analysis revealed R347P mutation in a compound heterozygote state with a novel mutation, p.Leu812X, which has not been reported elsewhere before. After the genotype of our patient was determined, the mother had a second pregnancy. The fetus was also compound heterozygote for the same CFTR mutation genotype and the patient underwent therapeutic abortion. Later the patient had a healthy 2-year-old brother who was born after prenatal evaluation. The characteristic features of the three patients including Case 1 were given in Table 1.

CASE 2

An 8-month-old Turkish boy, who was born to consanguineous parents, presented with failure to thrive, vomiting, steatorrhea, and recurrent severe lower respiratory tract infections. The disease had had its onset at two months of age. CF was diagnosed at eight months based on clinical manifestations and positive sweat tests (111-116 meq/L). Chest radiograph revealed pulmonary infiltrations during the infection periods; however thorax computerized tomography (CT) could not be available. Pulmonary function tests could not be performed because of lack of cooperation. He was given pancreatic enzyme supplementation because of high fecal fat excretion. The patient was lost to follow-up after the three months visit. The patient was identified with 3608insG/unknown

TABLE 1: Characteristic features of the patients.

	Sweat test	Pulmonary symptoms	Pancreatic insufficiency	Mutations
Case 1	121-122 meq/L	Moderate	No	R347P/p.Leu812X
Case 2	111-116 meq/L	Severe	Yes	3608insG/unknown
Case 3	105-110 meq/L	Mild	No	p.Ile853Cys/unknown

mutation. The new mutation was detected in heterozygous form. Thus, the patient was considered compound heterozygote state as he had CF-related phenotype.

CASE 3

An 8 year-old boy, who was born to non-consanguineous parents, was admitted to our hospital because of recurrent bronchiolitis starting from four months of age. He also had pneumonia that did not require hospitalization at four years of age. CF was verified with two pathological sweat test results (105-110 meq/L) in infancy. Other conditions with high values of sweat test results such as adrenal gland problems, hypothyroidism and kidney failure were excluded. No abnormality was seen on chest radiograph and CT of thorax. Pulmonary function tests included FVC 90% predicted, FEV_1 95% predicted, PEF 92%, and $FEF_{25–75}$ 80% predicted. The respiratory symptoms alleviated with increasing age and he became symptom free after 6 years old. He did not show gastrointestinal manifestations of CF and had normal weight and height measurements. Pancreatic sufficiency was evidenced by negative fecal fat excretion. Genetic analysis revealed p.Ile853Cys mutation in heterozygous form (p.Ile853Cys/ unknown). The patient was considered compound heterozygote state as he had two positive sweat test results.

DISCUSSION

Previous studies have reported a low frequency of F508del in Turkey, indicating a high genetic heterogenity of the disease in this population.[11,12] In a study reported by Onay et al[13] from Turkey, 122 unrelated chromosomes from 73 Turkish CF families were analyzed for the entire coding sequence of the CFTR gene. This extensive screening study revealed that 18 mutations accounted for 52.5% of the disease genes in the Turkish CF population. The frequency of the most common mutation, F508del, was 18.8% in that study population which confirmed the considerable molecular heterogeneity of CF among Turks. Onay et al[14] recently presented an updated report of mutations found in the Turkish population, which revealed 27 different mutations accounting for 60% of the disease genes in the Turkish population. Dayangaç et al[15] investigated congenital bilateral absence of the vas deferens as a primarily genital form of CF in 52 Turkish males and identified 27 different mutations on 72.5% of the investigated alleles. Kılınç et al[16] analyzed the CFTR locus in 83 Turkish CF patients to identify mutations, haplotypes and the carrier frequency in the population. They detected 36 different mutations in 125 (75%) of the total 166 CF chromosomes and suggested that the Turkish population had the highest genetic heterogeneity at the CFTR locus reported so far.

Dean et al[17] first described R347P mutation with a mild pulmonary manifestation of CF and pancreatic sufficiency as in Case 1. The patients originally described with this mutation were compound heterozygotes with the F508del mutation and had a very mild course of CF, suggesting that R347P, similar to other missense mutations affecting the MSD1 domain, caused a mild phenotype. The phenotype for R347P has been associated with mild disease in other reports too.[18,19] In contrast, Varon et al[20] reported a group of 19 CF patients with the R347P mutation of German, Bulgarian, Czech, and Slovak origin, including two homozygotes. Most patients in the mentioned study presented with early disease onset, pancreas insufficiency and early pulmonary involvement,

suggesting that this mutation can lead to a severe course of CF. The possibility of recurrent mutation at codon 347 may explain the diversity of the disease severity. There are several nucleotide positions that are susceptible to mutation. Mutations affecting these positions might be associated with more than one haplotype. Mutation R347P results from a G ® C change in a CpG dinucletide.[21] The analysis with markers flanking this mutation suggests that it has arisen more than once.[22] The analysis has indicated that different haplotypes are unlikely to have arisen by double recombination, recombination and slippage, or double slippage events. Therefore, recurrence of this mutation seems to be the most plausible explanation.[21]

In Case 1, the second child of the family was compound heterozygote for the same CFTR mutation that our patient had, thus therapeutic abortion was performed. In France, physicians had difficulty managing detected compound heterozygotes with CFTR variant (R117H), whose implication in CF remained unclear. Thus, R117H was withdrawn from the panels of CFTR mutations used in CF newborn screening because prenatal diagnosis of this variant was very common leading to frequent therapeutic abortion in that specific area.[23] In our Case 1, although the therapeutic abortion might not be recommended, the parents could not take the risk of a child with CF.

The 3608insG is a frameshift mutation leading to a truncated protein, clearly deleterious. It is probably correct to state that 3608insG is probably a Class 1 mutation. However, the mutation p.Ile853Cys is located between the R-domain and MSD2. This aminoacid substitution may be a benign sequence variant or a disease-causing missense mutant. Thus, there is insufficient evidence to support p.Ile853Cys being a Class 2 mutation, especially in the absence of sequence data to corroborate the findings.

In conclusion, we reported three children diagnosed with CF with three novel mutations, which have not been reported elsewhere before. In Case 1, we presented a patient who had R347P/p.Leu812X genotype. The combination of p.Leu812X on one chromosome and R347P on the other resulted in a mild phenotype in our case. As long as the second mutation is unknown, a novel CFTR sequence variant cannot be classified as a disease-causing lesion. However, 3608insG mutation may be a severe one as in Case 2, leading to early disease onset, failure to thrive, pancreatic insufficiency and severe pulmonary involvement. Case 3 presented with mild pulmonary symptoms and pancreatic sufficiency suggesting that p.Ile853Cys mutation may lead to a mild course of CF. Extensive mutation scanning is required to identify the second mutations in our cases.

REFERENCES

1. Aydemir O, Kiper N. [Cystic fibrosis disease in the world and Turkey]. Turkiye Klinikleri J Int Med Sci 2007;3(14 Suppl 1):1-3.
2. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993;73(7):1251-4.
3. Riordan JR, Rommens JM, Kerem B, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science 1989;245(4922):1066-73.
4. Gadsby DC, Vergani P, Csanády L. The ABC protein turned chloride channel whose failure causes cystic fibrosis. Nature 2006; 440(7083):477-83.
5. Welsh MJ, Smith AE. Molecular mechanisms of CFTR chloride channel dysfunction in cystic fibrosis. Cell 1993;73(7):1251-4.
6. Zielenski J, Tsui LC. Cystic fibrosis: genotypic and phenotypic variations. Annu Rev Gene 1995;29:777-807.
7. Zielenski J. Genotype and phenotype in cystic fibrosis. Respiration. 2000;67(2):117-33.
8. Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, et al. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. J Pediatr 1995;127(5):705-10.
9. Wilschanski M, Durie PR. Patterns of GI disease in adulthood associated with mutations in the CFTR gene. Gut 2007;56(8):1153-63.
10. Population variation of common cystic fibrosis mutations. The Cystic Fibrosis Genetic Analysis Consortium. Hum Mutat 1994;4(3):167-77.
11. Hundrieser J, Bremer S, Peinemann F, Stuhrmann M, Hoffknecht N, Wulf B, et al. Frequency of the F508 deletion in the CFTR gene in Turkish cystic fibrosis patients. Hum Genet 1990;85(4):409-10.
12. Yilmaz E, Erdem H, Ozgüç M, Coşkun T, Ozçelik U, Göçmen A, Ozalp I. Study of 12 mutations in Turkish cystic fibrosis patients. Hum Hered 1995;45(3):175-7.
13. Onay T, Topaloglu O, Zielenski J, Gokgoz N, Kayserili H, Camcioglu Y, et al. Analysis of the CFTR gene in Turkish cystic fibrosis patients: identification of three novel mutations (3172delAC, P1013L and M1028I) Hum Genet 1998;102(2):224–30.

14. Onay T, Zielenski J, Topaloglu O, Gokgoz N, Kayserili H, Apak MY, et al. Cystic fibrosis mutations and associated haplotypes in Turkish cystic fibrosis patients. Hum Biol 2001;73(2):191-203.
15. Dayangaç D, Erdem H, Yilmaz E, Sahin A, Sohn C, Ozgüç M, et al. Mutations of the CFTR gene in Turkish patients with congenital bilateral absence of the vas deferens. Hum Reprod 2004;19(5):1094-100.
16. Kilinç MO, Ninis VN, Dağli E, Demirkol M, Ozkinay F, Arikan Z, et al. Highest heterogeneity for cystic fibrosis: 36 mutations account for 75% of all CF chromosomes in Turkish patients. Am J Med Genet 2002;113(3):250-7.
17. Dean M, White MB, Amos J, Gerrard B, Stewart C, Khaw KT, et al. Multiple mutations in highly conserved residues are found in mildly affected cystic fibrosis patients. Cell 1990;61(5):863-70.
18. Wong LJ, Liang MH, Kwon H, Bai RK, Alper O, Gropman A. A cystic fibrosis patient with two novel mutations in mitochondrial DNA: mild disease led to delayed diagnosis of both disorders. Am J Med Genet 2002;113(1):59-64.
19. Sheppard DN, Rich DP, Ostedgaard LS, Gregory RJ, Smith AE, Welsh MJ. Mutations in CFTR associated with mild-disease-form Cl- channels with altered pore properties. Nature 1993;362(6416):160-4.
20. Varon R, Stuhrmann M, Macek M Jr, Kufardjieva A, Angelicheva D, Magdorf K. Pancreatic insufficiency and pulmonary disease in German and Slavic cystic fibrosis patients with the R347P mutation. Hum Mutat 1995;6(3):219-25.
21. Morral N, Llevadot R, Casals T, Gasparini P, Macek M Jr, Dörk T, et al. Independent origins of cystic fibrosis mutations R334W, R347P, R1162X, and 3849 + 10kbC-->T provide evidence of mutation recurrence in the CFTR gene. Am J Hum Genet 1994;55(5):890-8.
22. Morral N, Dörk T, Llevadot R, Dziadek V, Mercier B, Férec C, et al. Haplotype analysis of 94 cystic fibrosis mutations with seven polymorphic CFTR DNA markers. Hum Mutat 1996;8(2):149-59.
23. Scotet V, Audrézet MP, Roussey M, Rault G, Dirou-Prigent A, Journel H, et al. Immunoreactive trypsin/DNA newborn screening for cystic fibrosis: should the R117H variant be included in CFTR mutation panels? Pediatrics 2006;118(5):e1523-9.