

## Mutation Analysis of Exons 10 and 17a of CFTR Gene in Patients with Cystic Fibrosis in Kermanshah Province, Western Iran

Abbas Sahami<sup>1</sup>, Reza Alibakhshi<sup>2\*</sup>, Keyghobad Ghadiri<sup>3</sup>, Hamid Sadeghi<sup>1</sup>

1- Department of Biology, Jahrom Branch, Islamic Azad University, Jahrom, Iran

2- Nano Drug Delivery Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran

3- Nosocomial Infectious Disease Research Center, Kermanshah University of Medical Sciences, Kermanshah, Iran

### Abstract

**Background:** Cystic fibrosis (CF) is the most common genetic disorder with autosomal recessive inheritance among Caucasian populations. So far, more than 1950 different mutations were identified in cystic fibrosis transmembrane conductance regulator (CFTR) gene. CFTR gene has 27 exons. The type and distribution of mutations vary widely among different countries and/or ethnic groups. Therefore, a comprehensive analysis was performed on exon10 and exon17a of CFTR gene in CF patients in the Kermanshah province, western Iran.

**Methods:** We tested 27 patients admitted to the medical genetics laboratory of Kermanshah University of Medical Sciences. The patients were from different cities of Kermanshah province. All the patients had the clinical signals and two positive sweat tests. After filling agreement forms and questionnaire, the peripheral blood sampling and DNA extraction were done. DNA samples were extracted. PCR and sequencing special PCR were done. Finally analysis of the results with DNA sequencing analysis version 5.2 software was performed.

**Results:** CFTR mutations analysis identified 4 different mutations in our CF patients. The disease-causing mutations were p.F508del ( $\Delta F508$ ) (14.81%), p.S466X (1.85%), and p.T1036I (1.85%). M470V polymorphism with frequency of 74.1% was found in 23 patients (17 homozygous and 6 heterozygous).

**Conclusion:** Three disease-causing mutations in CF patients in the present study account for approximately 18.51% of mutations. The frequency of p.F508del, the most common mutation was 16–18.1% in Iranian population. The results of the present study can be applied for genetic counseling, population screening and prenatal diagnosis.

**Keywords:**  $\Delta F508$ , Cystic fibrosis, Direct sequencing, Iran, Kermanshah, M470V, S466X, T1036I.

**To cite this article:** Sahami A, Alibakhshi R, Ghadiri Kh, Sadeghi H. Mutation Analysis of Exons 10 and 17a of CFTR Gene in Patients with Cystic Fibrosis in Kermanshah Province, Western Iran. *J Reprod Infertil.* 2014;15(1):49-56.

\* Corresponding Author:  
Reza Alibakhshi, Nano Drug Delivery Research Centre, Kermanshah University of Medical Sciences, Kermanshah, Iran  
E-mail:  
ralibakhshi@yahoo.com  
ralibakhshi@kums.ac.ir

Received: Jul. 24, 2013

Accepted: Dec. 9, 2013

### Introduction

Cystic fibrosis (CF; MIM# 219700) is the most common lethal autosomal recessive disorder in Caucasian population; it affects 1 in 2500 newborns among Caucasians (1, 2). Cystic fibrosis (CF) is caused by mutations in cystic fibrosis transmembrane conductance regulator

(CFTR) gene, which encodes a cAMP- dependent chloride channel that is found at the apical membrane of epithelial cells, including respiratory epithelia and submucosal glands, exocrine pancreas, liver, sweat ducts, and the reproductive tract. CFTR is a member of the ATP-binding cassette

(ABC) membrane transporter superfamily that includes proteins such as the multiple drug resistance protein (MDR) and bacterial periplasmic permeases (1, 3). CFTR gene is localized on the long arm of chromosome 7 (7q21-34), spanning approximately 190 kb of genomic DNA. The gene consists of 27 exons and encodes a mature mRNA transcript of 6.5 kb that is translated into a 1480 amino acid protein (3). To date, more than 1950 mutations (4) have been identified in cystic fibrosis transmembrane conductance regulator (CFTR) gene (4). Many of these mutations are rare in the population and based on the reports only a few affected individuals. In addition to ΔF508 mutation, these mutations vary greatly in their frequency and distribution, but most of them are very rare. Only four mutations (p.G542X, p.N1303K, p.G551D, and p.W1282X) have overall frequencies greater than 1% (5). CF remains as a life-threatening autosomal recessive condition affecting Caucasians (5). In the US, each year 1000 new cases are diagnosed.

The CF incidence is estimated to be 1.2500 to 1.90,000 live births (1). In the case of CFTR, different patterns of migration and settlement have led to worldwide variation of the mutations CF is rare and less documented among Asians (1, 2). The exact incidence of CF is not known but the predictions show the variation of 1.10,000 to 1.350,000 of estimated frequencies. Minimal information is available concerning the prevalence of CF in Iran. Many children with CF in these populations probably remain undiagnosed due to lack of clinical suspicion and proper diagnostic facilities. The objective of this study was to determine the presence of CFTR gene mutations (exons 10 and 17a) among 27 CF patients in the population of Kermanshah province with respiratory and gastrointestinal manifestations similar to those reported in CF patients.

**The importance of study:** Adequate and accurate information of CF mutations in particular populations provides information for CF prevention programs applicable via heterozygote screening and prenatal diagnosis (6). The identification of muta-

tions and their frequencies are thus critically important for designing gene probes for effective diagnosis of CF in a given population. Further, different mutations are associated with varying severity and prognosis management (7). Among different provinces of Iran, high genetic heterogeneity has been observed (8, 9). Additionally, CFTR mutations naturally reveal considerable heterogeneity. So, the molecular characterization of CF in Kermanshah province, as a diverse and mainly Kurdish populated area in Iran, seems a necessity.

**Methods**

This study has been done in Medical Genetics Laboratory of Kermanshah University of Medical Sciences in Iran. The study population included patients attending the pediatric outpatient clinics (pediatric chest and genetics clinics) and those who were admitted in the pediatric medicine or surgery wards with a suspected diagnosis of CF.

**Patients:** From 2011 to 2012, 27 unrelated families who had an affected child with CF were referred to the Medical Genetics Lab, Kermanshah University of Medical Science, Kermanshah, Iran. Peripheral blood samples of CF patients (sweat chloride >60 mmol/l diagnostic of CF and clinical symptoms characteristic of CF) were collected in EDTA tubes. Many patients had pulmonary complications and pancreatic insufficiency. The patients (27) were from many different regions of Kermanshah province. All patients were fully informed that their blood would be used for molecular investigation of CFTR gene mutations, and consent was obtained from each patient or his/ her guardians. Genomic DNA was collected from peripheral whole blood using the "salting out" procedure (12).

**Sweat test:** Sweat samples were gathered by the Macroduct sweat collection system (Wescor, Logan, UT). Localized sweating was stimulated by the iontophoresis of pilocarpine into the skin of the flexor surface of the forearm or thigh. Sweat was then collected in micro bore tubing (Macroduct), the amount of sweat was quantitated, and

**Table 1.** Primers and PCR conditions

Cystic fibrosis primer name	Exon amplified	Tm	Primer sequence (5'- 3')
CF10-F	10	57	5'-TTGGAGGCAAGTGAATCC- 3'
CF10-R	10	57	5'-CGATTGAATATGGAGCC- 3'
CF17a-F	17a	55	5'-TAAATCACTGACACACTTTGTCCA- 3'
CF17a-R	17a	55	5'-GTACACCAACTGTGGTAAGA- 3'

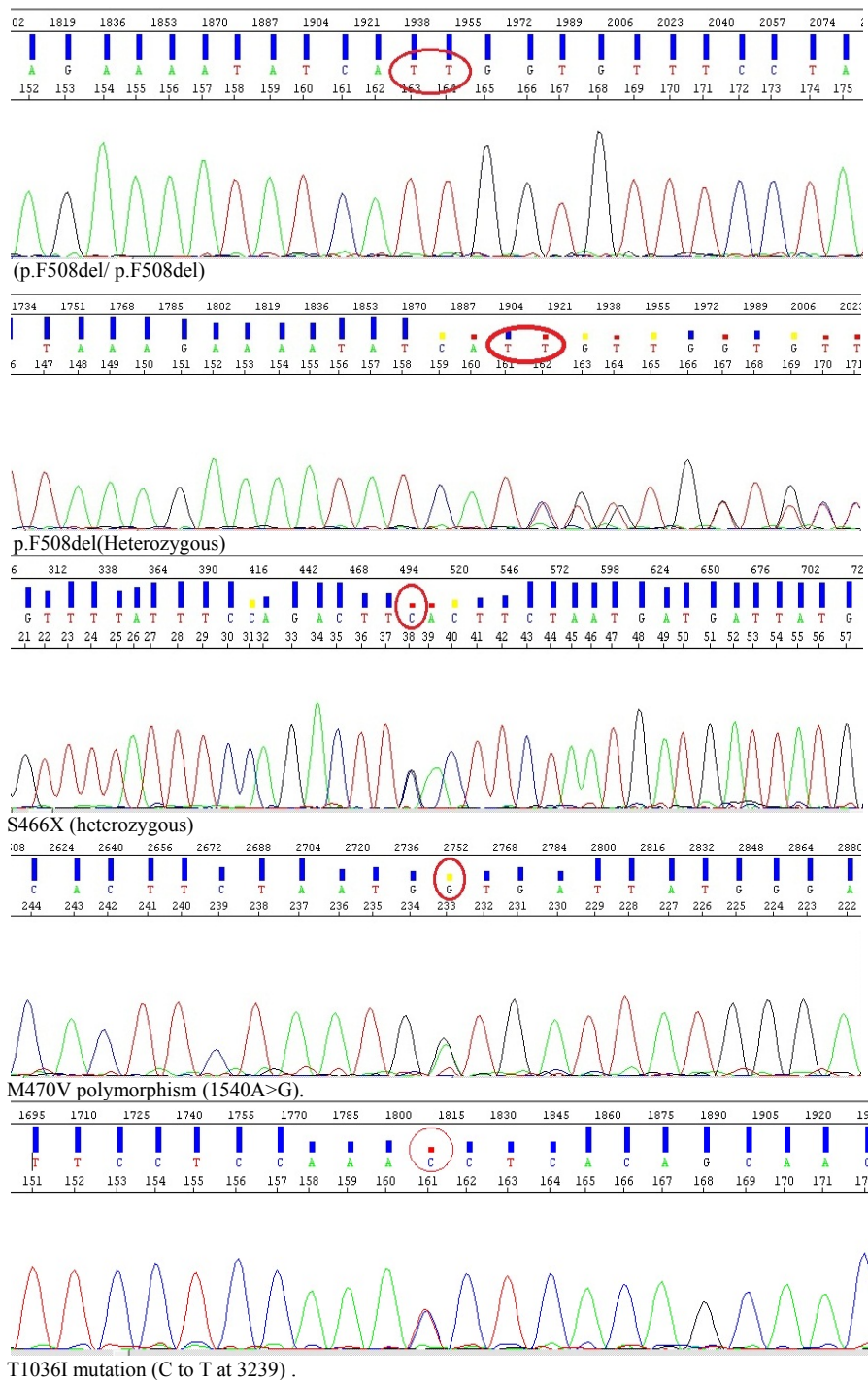


Figure 1. Nucleotide alterations identified by sequencing

the sample was then analyzed for chloride concentration, using the standard ISE method.

**Mutation analysis:** PCR amplification of exon10 and 17a of CFTR gene was performed using Gene Amp PCR System 9700 (Applied Biosystems, USA). The primers used in this study were synthesized by Operon (Metabion, Germany). The se-

quences of primers, listed in 5'-3' direction are shown in the (Table 1). All samples were sequenced in both the forward and reverse direction using the same primers used in the PCR reactions. PCR products were purified using QIAquick PCR purification kit. For sequencing analysis, samples were analyzed by direct sequencing of exon10 and

17a of CFTR gene and their flanking introns in an ABI-3130 DNA analyzer (Applied Biosystems, USA). The sequences were compared with the wild-type CFTR nucleotide sequence using Seqscape software (Applied Biosystems). All identified sequence changes were confirmed by direct DNA sequencing in the reverse direction.

**Results**

**Clinical profile and laboratory findings:** A total of 27 unrelated patients (13 males and 14 females) aged two months to 19 years originating from Kermanshah province were analyzed in this study. 66.7% of the patients were from consanguineous marriage (mostly between first cousins).

In our investigation, nearly 80% of the patients had malnutrition, abnormal stools and failure to thrive, 22 cases had been diagnosed with meconium ileus and intestinal obstruction. All patients had either persistent or acute symptoms of respiratory. There are numerous studies which have reported similar results.

**Genotype analysis:** Mutation screening of CFTR gene in 54 alleles by sequencing reaction for all

common mutations (exon 10 and exon 17a) showed that 10 alleles were ΔF508 (14.81%), S466X (1.85%) and T1036I (1.85%) and also showed 40 alleles (74.1%) with M470V polymorphism. Five patients were heterozygous for ΔF508, one patient was homozygous for ΔF508 and one patient was a compound heterozygous (ΔF508/S466X) (Table 2, Figure 1). All had respiratory difficulties and failure to thrive. In this research, M470V polymorphism frequency of 74.1% was observed in 23 patients. M470V polymorphism was 63% in homozygote genotype patients and it was 11.1% for the heterozygote genotypes (Figure1).

**Discussion**

Different ethnic groups and tribes live in Iran and ethnic/genetic heterogeneity has resulted in a high number of different mutations that account for CF. Comparison between different provinces showed that the mutation spectrum differs substantially in types and frequencies (8–10).

To evaluate and analyze our results, there is a need for some general facts about the ethnicity of the people who were investigated in this study.

**Table 2.** CFTR gene mutations identified as a result of the study (exon 10 and exon 17a)

Gene Location	Nucleotide change	Mutation type	No. of Patients		Global distribution
			Homozygote	Heterozygote	
Exon 10	Deletion of CTT from 16533	p.F508del	1	6	Global
Exon 10	C to G at 1529	p.S466X	-	1	Germany-Iran
Exon17a	C to T at 3239	p.T1036I	-	1	Iran

**Table 3.** Comparison of the frequency of common CFTR mutations (%) in the present study, west Asia, north Africa and Indian subcontinent

Region or Country	Frequency of CF alleles (%)		
	F508del	S466x	T1036I
This study	14.81	1.85	1.85
Lebanon	34-37	-	-
Palestine	23.5	-	-
Jordan	7.4-12	-	-
Syria	1 patient	-	-
Saudi Arabia, United Arab Emirates, Oman, Qatar, Kuwait	12	-	-
Saudi Arabia	13-15	-	-
Algeria	16.7	-	-
Bahrain	7.7	-	-
Turkey	24-27	*	-
Pakistan	17-56	-	-
Tunisia	18	-	-
Indian	19-40	*	-

\* Some reports about this mutation (S466X) in Italy's northeast, France's northwest, Turkey, Greece and India

**Table 4.** Comparison of the frequency of common CFTR mutations,  $\Delta F508$  and S466X in Iran

	Frequency $\Delta F508$ (%)	Frequency S466X (%)
Alibakhshi et al. (2006)	18.1	5.8
Elahi et al. (2006)	16	1.66
The present study (2012)	14.81	1.85

This is the first time that such a study is done for Kurdish people in Islamic Republic of Iran. The Kurdish people or Kurds are part of the Iranian people native to the Middle East, which includes adjacent parts of Iran, Iraq, Syria, and Turkey (11).

Kermanshah province has the most diverse Kurdish population. It even extends to some other neighboring ethnics such as Lorr, Turk and Persians. This province is located in neighbor of the provinces: Hamadan, Lorestan, Ilam and Kurdistan. The west part of this province is along Iraqi borders. Due to these unique geographical, ethnical and lingual properties of this region, "screening" of Kurdish people for this illness is quite important.

**$\Delta F508$  mutations:** Only ten disease-causing mutations were identified in this study.  $\Delta F508$  was present on 8 (14.81%) of all 54 CF chromosomes analyzed (11.11% heterozygote and 3.7% homozygote). Although the frequency of  $\Delta F508$  among these patients was lower (14.81%) than its frequency in European countries (Table 3), it was still the most frequent mutation among those reported to date in Iran (8–10). Previous studies representing overall Iranian CF patients reported a frequency of 16–18.1% for this mutation (9, 10).  $\Delta F508$  is the most common mutation in the world and this mutation accounts for ~70% of worldwide (7) mutated alleles of CF chromosomes but with great variation of frequency from 100% (Faroe Islands) (11) to 18% (Tunisia). Most of the mentioned chromosomes share the same but rare haplotype for a one occurrence mutation in a normal population.  $\Delta F508$  mutation distribution shows a decreasing frequency in Europe. In the case of Middle East, ethnic background and consanguinity account for the variation. The preliminary reports on the mutation spectrum in Iranian CF children indicate that p.F508del is the most common mutation, accounting for about 16–25% of the mutations. Identification of the mutations is of great importance for further evaluation, patient counseling and prenatal diagnosis.

The frequency of this mutation in western Asia and north of Africa is less than Europe. These frequencies were compared in table 3 (13–27).

$\Delta F508$  mutation frequency is decreasing from northwest of Europe to its southeast, in such a way that this frequency in Denmark is 88% (28), in England is 74% (29), in Germany is 71% (30), in Swiss 71%, in France 69% (30), in Bulgaria 60%, in Spain 53% (31), in Sweden 52% (30), in Greece 52% (32) and in Italy 45% (30). Because of decreasing mutation frequency from northwest to southeast of Europe, it is obvious that it is about 24–27% in Turkey (33) and also it is clear that Iran's mutation frequency is similar to Turkey's.

**S466X mutation:** This mutation is the most widespread one among Iran's proportional mutations and in contrast to other reported mutations has remarkable percentage in the country. In previous studies, the percentage of homozygote of this mutation has reached to 5.8%. In the present study, this mutation is observed for the first time in Iran as compound heterozygous and this should be noted that heterozygote genotype along with mutation of  $\Delta F508$  (1.85%) were found in one of the patients. This patient was not born out of consanguinity marriage and this patient showed the symbols of malnutrition, respiratory problems and meconium ileus. In previous studies, in the common wealth of independent states (CIS), the mutation of S466X with  $\Delta F508$  in the form of compound heterozygous was determined. This mutation is usually connected with pancreas deficiency (Table 4). This deficiency was observed in our study with the same condition. S466X mutation with homozygote genotype was previously reported in Tehran, Khorasan, Hamedan and Markazi provinces. It seems that this mutation exists among many families in different regions of Iran and it seems relatively ubiquitous in Iran. Nonetheless, this mutation is very rare worldwide; for example, the frequency of 0.5% has been reported in Serbia and Montenegro. There are also some reports about this mutation in Italy's northeast, France's northwest, Turkey, Greece and India (16, 17, 30, 32–34).

**T1036I mutations:** In exon 17a, T1036I mutation is reported as a heterozygote for the second time in the world. This mutation was reported for the first time in 2006, in Iran (8). This mutation may be connected to low frequency of studied patients and the common tribal marriages in Iran and west

Asian cultures. However, this mutation has been reported once and was originated from Iran. The frequency of T1036I mutation (C to T at 3239) was 1.85% in this study and it was involved with just one patient (Figure 1 and Table 2). The patients had the disorders of respiratory-digestive and meconium ileus and the parents of the patient had no genetic relationship (1).

**M470V polymorphism:** Polymorphism of M470V is a silent polymorphism which changes one nucleotide and converts the methionine amino acid to valine in the place of 42376223. This polymorphism has little or no effect on the protein function. However, M470V is not strongly neutral because a study showed that this polymorphism leads to degradation of CL transitions through the cell's membrane. It has also been showed that whenever M470V polymorphism comes along with another polymorphism mutation that leads to less activation of CFTR protein function (8, 35). This polymorphism is also effective in exon 9 process and causes genetic faulting. There are some reports worldwide which show M470V polymorphism with T5 and T7 allele (35) from Tn polymorph placement cause illness in combination (T repetitions are studied in CF patients and men's infertility) (36). Some reports also evaluated the potential connection of men's prostate cancer with this polymorphism (37, 38). The effectiveness of this allele in the illness phenotype is not completely clear yet and it deserves more investigations.

The incidence of cystic fibrosis is high in case of Europeans. Medical community's lack of knowledge of the disease, poor access to medical facilities and health care for CF patients, confounding diagnosis, a high infant mortality rate, and low life expectancy in general could account for the unexplained low CF incidence in some countries with a large proportion of Caucasians of European origin. In some cases, intermediate sweat chloride levels may exist in some patients, and this may lead to misdiagnosis of them.

The CF frequency might be higher for some isolated populations due to consanguinity. In case of Turkey and Saudi Arabia, the same consideration can be mentioned. Hence, CF can be regarded as a prevalent illness even outside Europe and United State which may have different mutation spectrums. In this regard, ascertainment of CFTR mutation carrier frequencies and the CF incidence are necessary among Iranian populations.

### Conclusion

Frequency of  $\Delta F508$ , S466X and T1036I mutations in this study are quite comparable to similar studies in Iran and neighboring regions. The remaining mutations and their frequency identification call for a need to design some specific tests to achieve more precision in clinical diagnosis. Moreover determination of the full spectrum of mutations in CFTR gene in Kermanshah province entails the analysis of remaining exons in CF patients. Establishment of CF prevention programs by carrier screening and prenatal diagnosis seems to be an essential program in genetic counseling and prenatal care of carrier families.

### Acknowledgement

The authors are thankful of the patients and their families for their participation in this study. We also want to specially thank all the people in the Medical Genetics Laboratory at Kermanshah University of Medical Sciences for their great collaboration and kindness.

### Conflict of Interest

The authors declare no conflict of interest.

### References

1. Allen J, Panitch H, Rubenstein R, editors. Cystic fibrosis. New York: Informa Healthcare USA; c2010. 1-3 p. (Kreindler JL, Foskett JK, editors. Lung biology in health and disease series; vol. 242).
2. Geborek A, Hjelte L. Association between genotype and pulmonary phenotype in cystic fibrosis patients with severe mutations. *J Cyst Fibros.* 2011;10(3): 187-92.
3. Dorwart M, Thibodeau P, Thomas P. Cystic fibrosis: recent structural insights. *J Cyst Fibros.* 2004;3 Suppl 2:91-4.
4. Rommens JM. Cystic Fibrosis Mutation Database [Internet]. Toronto (CA): The Hospital for Sick Children; 1989 [updated 2011 Apr 25; cited 2013 Dec 20]. Available from: <http://www.genet.sickkids.on.ca/Home.html>.
5. 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(2):135-54.
6. Dawson KP, Frossard PM. The geographic distribution of cystic fibrosis mutations gives clues about population origins. *Eur J Pediatr.* 2000;159(7):496-9.

7. Shah U, Frossard P, Moatter T. Cystic fibrosis: defining a disease under-diagnosed in Pakistan. *Trop Med Int Health*. 2009;14(5):542-5.
8. Alibakhshi R, Kianishirazi R, Cassiman JJ, Zamani M, Cuppens H. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. *J Cyst Fibros*. 2008;7(2):102-9.
9. Elahi E, Khodadad A, Kupershmidt I, Ghasemi F, Alinasab B, Naghizadeh R, et al. A haplotype framework for cystic fibrosis mutations in Iran. *J Mol Diagn*. 2006;8(1):119-27.
10. Alibakhshi R, Zamani M. Mutation analysis of CFTR gene in 70 Iranian cystic fibrosis patients. *Iran J Allergy Asthma Immunol*. 2006;5(1):3-8.
11. Wikipedia, the free encyclopedia. Kurdish people [Internet]. Wikimedia Foundation, Inc; 2001 Jan 10 [updated 2013 Aug 14; cited 2013 Aug 20]. Available from: [http://en.wikipedia.org/wiki/Kurdish\\_people](http://en.wikipedia.org/wiki/Kurdish_people).
12. Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res*. 1988;16(3):1215.
13. Mateu E, Calafell F, Ramos MD, Casals T, Bertranpetit J. Can a place of origin of the main cystic fibrosis mutations be identified?. *Am J Hum Genet*. 2002;70(1):257-64.
14. Loumi O, Ferec C, Mercier B, Creff J, Fercot B, Denine R, et al. CFTR mutations in the Algerian population. *J Cyst Fibros*. 2008;7(1):54-9.
15. Bobadilla JL, Macek M Jr, Fine JP, Farrell PM. Cystic fibrosis: a worldwide analysis of CFTR mutations--correlation with incidence data and application to screening. *Hum Mutat*. 2002;19(6):575-606.
16. Sharma N, Singh M, Kaur G, Thapa BR, Prasad R. Identification and characterization of CFTR gene mutations in Indian CF patients. *Ann Hum Genet*. 2009;73(1):26-33.
17. Shastri SS, Kabra M, Kabra SK, Pandey RM, Menon PS. Characterisation of mutations and genotype-phenotype correlation in cystic fibrosis: experience from India. *J Cyst Fibros*. 2008;7(2):110-5.
18. Kilinc MO, Ninis VN, Dagli E, Demirkol M, Ozkinnay 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.
19. Rawashdeh M, Manal H. Cystic fibrosis in Arabs: a prototype from Jordan. *Ann Trop Paediatr*. 2000;20(4):283-6.
20. Wahab AA, Janahi IA, Hebi S, al-Hamed M, Kambouris M. Cystic fibrosis in a child from Syria. *Ann Trop Paediatr*. 2002;22(1):53-5.
21. Kambouris M, Banjar H, Moggari I, Nazer H, Al-Hamed M, Meyer BF. 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(5):303-9.
22. el-Harith EA, Dork T, Stuhmann M, Abu-Srair H, al-Shahri A, Keller KM, et al. Novel and characteristic CFTR mutations in Saudi Arab children with severe cystic fibrosis. 1997;34(12):996-9.
23. Banjar H. Geographic distribution of cystic fibrosis transmembrane regulator gene mutations in Saudi Arabia. *East Mediterr Health J*. 1999;5(6):1230-5.
24. Eskandarani HA. Cystic fibrosis transmembrane regulator gene mutations in Bahrain. *J Trop Pediatr*. 2002;48(6):348-50.
25. Lucotte G, Barre E, Berriche S. Frequency of the cystic fibrosis mutation delta F508 in Algeria. *Hum Genet*. 1991;87(6):759.
26. Messaoud T, Verlingue C, Denamur E, Pascaud O, Quere I, Fattoum S, et al. Distribution of CFTR mutations in cystic fibrosis patients of Tunisian origin: identification of two novel mutations. *Eur J Hum Genet*. 1996;4(1):20-4.
27. Saleheen D, Frossard PM. The cradle of the delta F508 mutation. *J Ayub Med Coll Abbottabad*. 2008;20(4):157-60.
28. Schwartz M, Johansen HK, Koch C, Brandt NJ. Frequency of the delta F508 mutation on cystic fibrosis chromosomes in Denmark. *Hum Genet*. 1990;85(4):427-8.
29. McIntosh I, Lorenzo ML, Brock DJ. Frequency of delta F508 mutation on cystic fibrosis chromosomes in UK. *Lancet*. 1989;2(8676):1404-5.
30. [No authors listed]. Gradient of distribution in Europe of the major CF mutation and of its associated haplotype. European Working Group on CF Genetics (EWGCFG). *Hum Genet*. 1990;85(4):436-45.
31. Casals T, Ramos MD, Gimenez J, Larriba S, Nunes V, Estivill X. High heterogeneity for cystic fibrosis in Spanish families: 75 mutations account for 90% of chromosomes. *Hum Genet*. 1997;101(3):365-70.
32. Tzetis M, Kanavakis E, Antoniadis T, Doudounakis S, Adam G, Kattamis C. Characterization of more than 85% of cystic fibrosis alleles in the Greek population, including five novel mutations. *Hum Genet*. 1997;99(1):121-5.
33. 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.
34. Castellani C, Cuppens H, Macek M Jr, Cassiman JJ, Kerem E, Durie P, et al. Consensus on the use

- and interpretation of cystic fibrosis mutation analysis in clinical practice. *J Cyst Fibros.* 2008;7(3):179-96.
35. Ciminelli BM, Bonizzato A, Bombieri C, Pompei F, Gabaldo M, Ciccacci C, et al. Highly preferential association of Non F508del CF mutations with the M470 allele. *J Cyst Fibros.* 2007;6(1):15-22.
  36. Cuppens H, Cassiman JJ. CFTR mutations and polymorphisms in male infertility. *Int J Androl.* 2004;27(5):251-6.
  37. Qiao D, Yi L, Hua L, Xu Z, Ding Y, Shi D, et al. Cystic fibrosis transmembrane conductance regulator (CFTR) gene 5T allele may protect against prostate cancer: a case-control study in Chinese Han population. *J Cyst Fibros.* 2008;7(3):210-4.
  38. O'Donnell DH, Ryan R, Hayes B, Fennelly D, Gibney RG. Hepatocellular carcinoma complicating cystic fibrosis related liver disease. *J Cyst Fibros.* 2009;8(4):288-90.