

## Prevalence of Cystic Fibrosis Trans-membrane Conductance Regulator Gene common mutations in children with cystic fibrosis in Isfahan, Iran

Mohsen Reisi<sup>1</sup>, Mahdiyeh Behnam<sup>2</sup>, Seyed Javad Sayedi<sup>3</sup>, Farzaneh Salimi<sup>2</sup>, Pegah Kargar<sup>2</sup>, Mansoor Salehi<sup>4</sup>, \*Hossein Saneian<sup>1</sup>, Iman Kashani<sup>5</sup>, Roya Kelishadi<sup>1</sup>

<sup>1</sup>Pediatric Department, Child Growth and Developmental Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran. <sup>2</sup>Medical Genetics Laboratory of Genome, Isfahan, Iran. <sup>3</sup>Neonatal Research Center, Akbar Hospital, Mashhad University of Medical Sciences, Mashhad, Iran. <sup>4</sup>Department of Genetics and Molecular Biology, Medical School, Isfahan University of Medical Sciences, Isfahan, Iran. <sup>5</sup>Department of Pediatric Surgery, Dr. Sheikh Hospital, Mashhad University of Medical Science, Mashhad, Iran.

### Abstract

**Background:** Cystic fibrosis (CF) is the most common lethal genetic disorder of Cystic Fibrosis Trans-membrane Conductance (CFTR) Regulator gene mutations. We aimed to investigate common mutations in CF patients and to assess its possible relationship with clinical presentations.

**Materials and Methods:** This cross sectional study was conducted on 36 CF patients who were referred to a tertiary pediatric hospital in Isfahan, Iran. They were evaluated for 34 common mutations in CFTR gene by using reverse dot blot strip assay. Other parameters such as the age of diagnosis, the sweat chloride level, and clinical manifestations due to lung involvement and pancreatic insufficiency were also assessed. According to genotype mutations, children were divided in three groups:  $\Delta F508$  mutation (group 1), non- $\Delta F508$  mutation (group 2), without current mutations (group 3). Finally, genotype, and phenotype relationship were reported.

**Results:** The mean age of patients was 8.1+2.3 months, and 23 of them (63%) were male. CFTR mutations were found in fourteen patients (38.8%).  $\Delta F508$  mutation has the highest prevalence in the studied samples with allele frequency of 15.27%, and c. 2183 AA>G was in the second standing. Furthermore, p.R553X, p.G542X, C.1766+1, p.N1303K mutated alleles also were obtained in lower level. Mean age at the diagnosis time of CF, sweat chloride level and pancreatic insufficiency were not different between groups but lung complications were significant in children with common mutations.

**Conclusion:** Our findings showed that commercial kit designed to identify 34 common CFTR mutations failed to detect 61.2% of alleles of our patients. This necessitates designing local diagnostic kits for proper diagnosis of CF in Iranian children.

**Key Words:** Children, Cystic fibrosis, Mutations, Prevalence, Sweat test.

\*Please cite this article as: Reisi M, Behnam M, Sayedi SJ, Salimi F, Kargar P, Salehi M, et al. Prevalence of Cystic Fibrosis Trans-membrane Conductance Regulator Gene common mutations in children with cystic fibrosis in Isfahan, Iran. Int J Pediatr 2019; 7(4): 9333-39. DOI: [10.22038/ijp.2018.36309.3168](https://doi.org/10.22038/ijp.2018.36309.3168)

### \*Corresponding Author:

Hossein Saneian (M.D), Pediatric Department, Child Growth and Developmental Research Center, Research Institute for Primordial Prevention of Non-Communicable Disease, Isfahan University of Medical Sciences, Isfahan, Iran.

Email: [Saneian@med.mui.ac.ir](mailto:Saneian@med.mui.ac.ir)

Received date: Jan.12, 2018; Accepted date: Dec.22, 2018

## 1- INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive, multi-organ disorder which is the leading cause of lung disease and pancreatic insufficiency in children (1-2). A variety of clinical manifestations such as chronic bronchitis, bronchiectasis, sinusitis, nasal polyps, failure to thrive, cirrhosis and diabetes mellitus have been described in CF patients (3). Approximately one in 2,000 to 3,000 newborns in populations of European ancestry are affected, but in other ethnic groups such as Asian Americans the incidence is lower (4, 5). Up to now, more than 2,000 CFTR mutations have been identified. These mutations are classified into six groups. Classes 1 to 3 of mutations induce severe forms of disease, but classes 4 to 6 induce milder disease and may be associated with pancreatic sufficiency (6, 7); therefore, genotypes seem to influence the phenotype of patients.

The most common mutation in CFTR gene is  $\Delta F508$  which accounts for approximately 70% of CFTR gene alleles in European CF patients (8-10). The prevalence of CF mutations and the relationship between genotypes and phenotypes is not well clarified in Iranian population (11). So this study was conducted to investigate common mutations in CF patients and to assess its possible relationship with clinical presentations.

## 2- MATERIALS AND METHODS

### 2-1. Study design and population

We conducted a cross sectional study to investigate common mutations in some Iranian CF children. The study was performed at Department of Pediatrics, Imam Hossein hospital, Isfahan University of Medical Sciences for duration of 16 months between October 2014 and April 2016. All children who had fulfilled the diagnostic criteria for CF defined by the

International CF Consortium were enrolled in the study. This study was approved by the ethical committee of Isfahan University of Medical Sciences. The written informed consent was signed by the patients or their parents.

### 2-2. Methods

Genomic DNA was extracted from blood samples by the proteinase-K extraction method. Analysis of 34 common mutations in CFTR gene was done by using Reverse Dot Blot (RDB) procedure by CF strip Assay Kit (Vienna Lab, Austria). Other parameters such as the age of diagnosis, the sweat chloride level, and clinical manifestations due to lung involvement and pancreatic insufficiency were also assessed. According to genotype mutations, children were divided in three groups:  $\Delta F508$  mutation (group 1), non- $\Delta F508$  mutation (group 2), without current mutations (group 3). Finally genotype and phenotype relationship were reported.

### 2-3. Laboratory measurements

Analysis of 34 common mutations in CFTR gene was performed using Reverse Dot Blot (RDB) procedure by CF strip Assay Kit (Vienna Lab, Austria). This method is a simple, rapid and reliable method which allows simultaneous detection of different mutations in a single hybridization assay. The studied mutations are: CFTR del 2,3(21Kb), 1507 del(-ATC), F508 del, 1717-1 G>A G542X, G551D, R560T, R553X, 2143delT, 2183AA>G, 2184delA, 2184insA, 2789+5G>A, R1162X, 3659delC, 3905insT, N1303K, W1282X, G85E, 394delTT, R117H, Y122X, 621+1G>T, 711+1G>T, 1078delT, R334W, R347H, R347P, A455E, 1898+1G>A, 3120+1G>A, 3272-26A>G, Y1092X, 3849+10Kb C>T along with IVS85T/7T/9T polymorphism covered on two different strips. The pancreatic insufficiency is defined as >100 fat droplets in Sudan black staining and fecal elastase 1<200 U/Mg (7). Pulmonary

involvement was defined according to the history of chronic wet cough or the evidence of bronchiectasis in lung CT-scan.

#### 2-4. Ethical consideration

This study was approved by local ethics committee of Isfahan University of Medical Sciences Ethics committee ID-number: IR.MUI.REC.193047.

#### 2-5. Inclusion and exclusion criteria

All children who had elevated sweat chloride values ( $>60$  mEq/L) on two separate days, were enrolled and no one was excluded. The patients fulfilled the diagnostic criteria for CF defined by the International CF Consortium.

#### 2-7. Data Analyses

The data are presented as mean  $\pm$  standard deviation (SD). Mann-Whitney probability and Fisher exact tests were used; P-value  $< 0.05$  was considered statistically significant. Statistical analyses were performed by using the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS Inc., Chicago, USA).

### 3- RESULTS

Thirty-six children with cystic fibrosis (23 males and 13 females), were enrolled this study. The mean age of patients was  $8.1 \pm 2.3$  months. 91.7% of them were issued from consanguineous marriage (mostly first cousins), and 19.4% of the patients have positive family history (more than one affected patient in a pedigree). Fourteen patients (38.8%) had 25 mutated alleles. Five patients had homozygote  $\Delta F508$  mutation and one of them was heterozygote for  $\Delta F508$  mutation. c. 2183 AA>G homozygote mutation was observed in 3 patients. Two patients were heterozygote for p.R553X (c.1637C>T) mutation. In addition, p.G542X, C.1766+1 and p.N1303K mutations were obtained in homozygote status, each in one patient. In

this way among 72 alleles, 16(22.22%) were  $\Delta F508$ , 6(8.33%) were c. 2183 AA>G, and frequency of each of the p.R553X, p.G542X, C.1766+1, p.N1303K alleles was 2(2.72%). Current mutations were not found in 22 patients. Clinical presentations of CF patients with CFTR mutations are shown in **Table.1**.

The mean age at diagnosis of CF for patients with the  $\Delta F508$  mutation (group 1) was  $4 \pm 2.5$  months, in patients with non  $\Delta F508$  mutations (group 2) was  $2.5 \pm 1.7$  months, and in those without current mutations (group 3) was  $8.8 \pm 6.2$  months. The difference was not statistically significant ( $P > 0.05$ ). The mean sweat chloride level in three groups was  $94.83 \pm 14.2$  mEq/L,  $110 \pm 19.6$  mEq/L, and  $85 \pm 16.1$  mEq/L, respectively, which was not statistically significant ( $P > 0.05$ ). 100% of patients in the  $\Delta F508$  mutation ( $n=6$ ), and non  $\Delta F508$  mutations ( $n=8$ ) group had exocrine pancreatic insufficiency, but only 76% ( $n=16$ ) of other patients (group 3) had pancreatic insufficiency.

However, this difference was not statistically significant ( $P > 0.05$ ). Five patients in group 1 (71%), and 5 patients in group 2 (62%) had pulmonary involvement. The difference was not significant ( $P > 0.05$ ). Only 6 patients in group 3 (28%) had lung involvement. This difference was statistically significant ( $P < 0.05$ ). Ten patients had a history of meconium ileus in the neonatal period, 4 of whom had delta F508 mutation, and 6 had other mutations. The difference was not significant ( $P > 0.05$ ).

History of meconium ileus was not found in patients without current mutations. This difference was statistically significant ( $P < 0.05$ ). Characteristics of cystic fibrosis patients with and without the delta F508 mutation are shown in **Table.2**. **Figure 1 and 2** showed banding pattern of PCR product of mix A/ B and examples of strip assay respectively.

**Table-1:** Clinical presentations of CF patients with CFTR mutations.

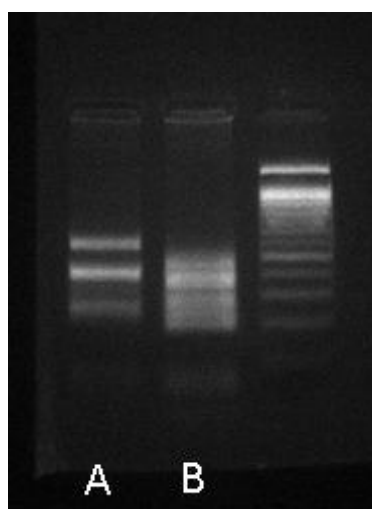
No	Sex	Current age, year	Age at diagnosis (months)	Sweat chloride (mEq/lit)	Cause of diagnosis	Consanguinity of parents	Family history	Mutation
1	F	10	8	130	Cough, respiratory infection	Second cousin	+	p.R553X(c.1657C>T)/U rs74597325
2	M	5	3	80	Steatorrhea, vomiting	No	-	ΔF508(c.16543delCTT)/U Rs74597325
3	F	6	2	125	Cough/respiratory infection	Second cousin	+	2183AA>G(c.2051-2052delAA ins G)/2183AA>G(c.2051-2052delAA ins G) Rs 121908799
4	F	8	6	135	FTT, meconium ileus	First cousin	+	ΔF508(c.16543delCTT)/ΔF508(c.16543delCTT Rs74597325
5	M	4	4	87	Respiratory infections, meconium ileus	First cousin	-	2183AA>G(c.2051-2052delAA ins G)/2183AA>G(c.2051-2052delAA ins G) Rs 121908799
6	M	5	At birth	126	FTT, steatorrhea, vomiting	-	-	p.R553X(c.1657C>T)/U rs74597325
7	F	4	At birth	80	Skin rash, edema, vomiting	First cousin	-	p.G542X(c.1624G>T) rs113993959
8	M	6	9	85	Wheezing, cough, FTT	First cousin	-	ΔF508(c.16543delCTT)/ΔF508(c.16543delCTT Rs74597325
9	M	4	At birth	135	FTT, Intestinal obstruction	First cousin	-	c.1766+1G>A(1898+1G>A
10	M	3	At birth	100	Intestinal obstruction, edema	Second cousin	-	p.N1303K(c.3909C>G) rs rs80034486
11	M	5	3	90	Meconium ileus, wheezing, cough	First cousin	-	ΔF508(c.16543delCTT)/ΔF508(c.16543delCTT Rs74597325
12	F	3	2	90	FTT, meconium ileus	First cousin	+	ΔF508(c.16543delCTT)/ΔF508(c.16543delCTT Rs74597325
13	M	14	4	100	Respiratory infection, wheezing, fever	First cousin	-	2183AA>G(c.2051-2052delAA ins G)/2183AA>G(c.2051-2052delAA ins G) Rs 121908799
14	F	6	1	89	FTT, steatorrhea	Second cousin	-	ΔF508(c.16543delCTT)/ΔF508(c.16543delCTT Rs74597325

M: male; F: female; FTT: failure to thrive.

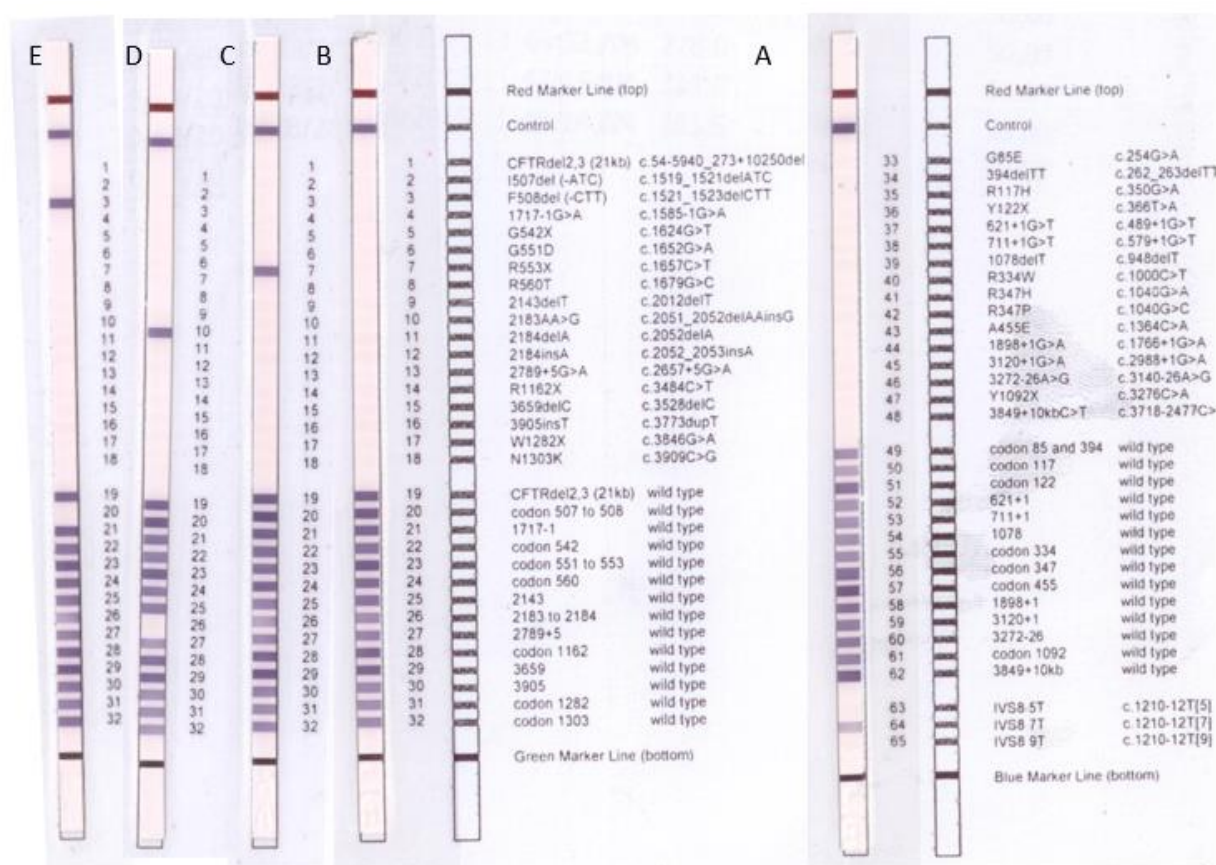
**Table-2:** Characteristics of cystic fibrosis patients with and without the delta F508 mutation, (n= 36).

Characteristics	ΔF508 mutation	Other mutations	P- value	No mutation	P- value
Sex (Male/Female)	3/3	5/3	NS	12/10	NS
Mean age at diagnosis (months)	4±2.5	2.5±1.7	NS	8.8±6.2	NS
Sweat chloride level (mEq/L)	94.83±14.2	110±19.6	NS	85±16.1	NS
Pancreatic insufficiency	6/6(100%)	8/8(100%)	NS	16/22(72%)	NS
Lung involvement	5/6(83%)	5/8(62.5%)	NS	6/22(27.2%)	Significant
Meconium ileus	4/6(66.6%)	6/8(75%)	NS	0/22(0%)	Significant

NS: not significant.



**Fig. 1:** The results of PCR product. Mix A, mix B and 1 Kb ladder.



**Fig.2:** Examples of strip assay results were shown in figure 2.A and B represent normal patterns of different strips. Band patterns showed p.R553X, c. 2183 AA>G and  $\Delta$ F508 mutations in C, D and E, respectively. Two terminal bands in both sides of strips are used as control for accurate PCR amplification and staining procedure.

#### 4- DISCUSSION

This study was conducted to evaluate common mutations in CF patients and to assess its possible relationship with clinical presentations. In the present study we analyzed 36 CF patients from Isfahan province, central Iran, for 34 common mutations in CFTR gene by CF strip assay (Vienna Lab Diagnostics, Vienna, Austria). In this study, most patients were male (the female to male ratio was 1:1.76). In almost all of our patients, symptoms presented in the first six months of life and consanguineous marriages amount were quite significant. Although due to appropriate, modern enzymatic and antibiotic therapies in addition to nebulizer treatments, lifespan, and quality of life in CF patients are notably improved, during our study, 5 patients (13.88%) died after severe respiratory infections, and some pulmonary problems. 25 mutated alleles were detected in 14 patients. As expected  $\Delta F508$  mutation has the highest prevalence in the studied samples with frequency of 16.6% (6/36), emphasizing the previous studies on affected children from different provinces of Iran which reported that  $\Delta F508$  is the most frequent mutation accounting for about 16-18% (12-15). However, this frequency is much lower than this mutation frequency in European countries that is more than 50% (16-18).

The **c. 2183 AA>G** mutation is the second most frequent mutation detected in the current study and is found in 8.33% of alleles. This mutation is also mentioned as second mutation in southwestern Iranian CF patients with prevalence of 9% (19). Frequency of this mutation is 5.83% in southern Italian population as the second most frequent mutation, compatible with our study (20). In contrast, Alibakhshi et al. (2008) indicated p.N1303K as the second most frequent mutation with 6.5% in Iran (21). Our findings revealed 2.72% alleles for this mutation. Mutations spectrum varies by type and frequency

between different geographic areas and ethnic origins in Iran. Also, our findings showed that the commercial laboratory kit designed to identify common CFTR mutations, failed to detect 65% of the alleles of our Iranian patients. CFTR gene sequencing should be performed for still unidentified samples. Regarding low mutation sensitivity of current study and high population heterogeneity, investigation on CFTR gene mutations in order to detect the most frequent local mutations to design local diagnostic kits is necessary. This knowledge is applied to improve the clinical diagnosis and establish CF prevention programs in carrier screening and prenatal diagnosis.

##### 4-1. Limitations of the study

Few numbers of CF patients and single center study were the major limitations of our study.

#### 5- CONCLUSION

Our findings showed that commercial kit designed to identify 34 common CFTR mutations failed to detect 61.2% of the alleles of patients. According to these results, this necessitates designing local diagnostic kits for proper diagnosis of CF in Iranian children.

#### 6- CONFLICT OF INTEREST

#### 7- REFERENCES

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