

Frequency of Genotype With $\Delta F508$ Mutation in CFTR Gene Among Iranian Cystic Fibrosis Patients With Pancreatic Insufficiency

Ahmad Khodadad,^{1,2,*} Elaheh Elahi,³ Setareh Sadat Bani Hassani,¹ Pejman Rouhani,¹ Bamdad Sadeghi,⁴ and Nima Rezaei^{4,5,6}

¹Children's Medical Center, Pediatric Center of Excellence, Tehran University of Medical Sciences, Tehran, IR Iran

²Department of Pediatrics, Tehran University of Medical Sciences, Tehran, IR Iran

³School of Biology, College of Science, University of Tehran, Tehran, IR Iran

⁴Research Center for Immunodeficiencies, Children's Medical Center, Tehran University of Medical Sciences, Tehran, IR Iran

⁵Department of Immunology, School of Medicine, Tehran University of Medical Sciences, Tehran, IR Iran

⁶Universal Scientific Education and Research Network, Tehran, IR Iran

*Corresponding author: Ahmad Khodadad, Children's Medical Center, Pediatric Center of Excellence, Tehran University of Medical Sciences, Tehran, IR Iran. Tel: +98-2166929234, Fax: +98-2166929235, E-mail: khodadadm@yahoo.com

Received 2015 July 5; Accepted 2015 August 22

Abstract

Background: Cystic fibrosis (CF) is the most prevalent lethal autosomal recessive disease with a broad spectrum of phenotypes. Mutation of $\Delta F508$ in the CFTR gene is the most important and lethal mutation in CF, which contains 70% of all predisposing mutations for CF worldwide.

Objectives: Determining frequency of genotypes with $\Delta F508$ mutation in CFTR gene, and evaluation of correlation between genotype and phenotype of Iranian patients with CF.

Patients and Methods: Thirty six patients were included in this cross sectional study. $\Delta F508$ mutations in both alleles of the CFTR gene were checked.

Results: Among 36 pediatric patients, $\Delta F508$ mutation was detected in 9 (25%) patients; 2 patients were heterozygous, and 7 patients homozygous for this mutation. From overall 72 tracked alleles, 11 (15.2%) were found to have $\Delta F508$ mutations. Differences in prevalence of dyspnea and bronchiectasis were significant in homozygote group, compared with non-mutated group for $\Delta F508$.

Conclusions: It seems that more $\Delta F508$ mutated alleles lead to more severe symptoms of CF.

Keywords: Cystic Fibrosis, $\Delta F508$ Mutation, Genotype, Phenotype

1. Background

Cystic fibrosis (CF) is the most prevalent severe genetic disorder in western countries, with an incidence rate of 1 in 2,000 to 4,000 births (1). CF is a heterogeneous disease with variable signs and symptoms (2-5). Variation and distribution of mutations in the CFTR (cystic fibrosis transmembrane conducting regulator) gene along with environmental factors are effective in multiple organs damage. It seems that $\Delta F508$ is the most frequent mutation, which occurs in 70% of CF patient chromosomes worldwide (6). $\Delta F508$ causes the most severe and lethal form CF disease.

2. Objectives

Determining frequency of genotypes with $\Delta F508$ mutation in CFTR gene, and evaluation of correlation between genotype and phenotype of Iranian patients with CF.

3. Patients and Methods

In this cross sectional study, 36 documented CF patients (17 males and 19 females), aged 2 to 42 months, with domi-

nant phenotype of pancreas insufficiency (PI) who regularly attended Children's Medical Center in Tehran were investigated. The inclusion criteria for Pancreas Insufficiency (PI) were defined as two positive results of sweat test and history of steatorrhea confirmed by detecting fat drops in stool examinations. Demographic data, along with clinical, paraclinical and radiologic features which represent the spectrum of phenotypes were obtained by hospital documents and questionnaires designed for the study. Blood samples of 1-2 cc volumes were provided from each patient and DNA was extracted and examined for genetic assays to detect $\Delta F508$ mutation of each allele of CFTR.

4. Results

Assessment of 36 patients with mutant primer showed that 9 were identified to have deletion mutations of $\Delta F508$. Then, all samples were investigated for homozygosity with normal primer in which from all patients, 34 had one normal allele. 7 were heterozygotes, and 2 were

homozygous for $\Delta F508$ mutation. At last, from 72 studied alleles, 11 were found affected by $\Delta F508$ mutation. Patients were classified into 3 groups by genotypes: homozygous for $\Delta F508$ (5.5%), heterozygotes (19.4%) and non-mutated by $\Delta F508$ (75%). In this study, all patients had FTT (Fail to Thrive) with a growth percentile curve of fewer than 3% or depletion of growth curve more than 2 curves in growth percentile chart. In patients with positive pharyngeal culture, organisms were as follow: *Pseudomonas aeruginosa*, streptococcus group A, *Staphylococcus aureus*, *Klebsiella* and *Candida*.

The frequency of various clinical presentations categorized by genotypes is shown for all patients (except in one with incomplete medical document) in Table 1. Dyspnea was one of the clinical symptoms with different correlations in different genotype. In homozygous patients for $\Delta F508$ mutations compared with those not affected by

$\Delta F508$, dyspnea was significantly more prominent ($P = 0.049$). Frequency of bronchiectasis in homozygotes group was also significantly higher than in non-mutated subjects ($P = 0.015$), while this rate was significantly higher in heterozygotes in comparison with non-mutated group ($P = 0.043$). Quantitative comparison of sweat chloride concentration in homozygotes group with non-mutated group showed significant difference ($P = 0.012$), but this significance was not the same between homozygotes and heterozygotes group for $\Delta F508$ mutation ($P = 0.1$), and between heterozygotes and non-mutated groups ($P = 0.213$). Fatty droplets in stool exam were significantly more numerous in homozygotes group for $\Delta F508$ than others ($P = 0.022$), but there were no significant difference of droplets number between homozygotes and heterozygotes group, and heterozygotes with non-mutated $\Delta F508$ ($P = 0.088$ and $P = 0.516$, respectively).

Table 1. Clinical Manifestations of All Patients and Respected to $\Delta f508$ Mutation Genotypes

Clinical Manifestations	Presence of signs/symptoms			Without $\Delta F508$ Mutation			Heterozygous for $\Delta F508$			Homozygote for $\Delta F508$		
	Yes	No	Unknown	Present ^a	Absent ^a	Unknown	Present ^a	Absent ^a	Unknown	Present ^a	Absent ^a	Unknown
Cough	32	3	1	24 (92.3)	2 (7.7)	1	7 (100)	0	0	1 (50)	1 (50)	0
Dyspnea	15	20	1	8 (30.8)	18 (69.2)	1	5 (71.4)	2 (28.6)	0	2 (100)	0	0
Bronchitis	2	33	1	1 (3.8)	25 (96.2)	1	1 (14.3)	6 (85.7)		2 (100)	0	0
Bronchiectasia	4	31	1	1 (3.8)	25 (96.2)	1	2 (28.6)	5 (71.4)	0	1 (50)	1 (50)	0
Bronchiolitis	2	33	1	0	26 (100)	1	2 (28.6)	5 (71.4)	0	0	2 (100)	0
Pneumonia	22	13	1	14 (53.8)	12 (46.3)	1	7 (100)	0	0	1 (50)	1 (50)	0
Diarrhea	7	28	1	4 (15.4)	22 (84.6)	1	2 (28.6)	5 (71.4)	0	1 (50)	1 (50)	0
Edema	7	28	1	5 (19.2)	21 (80.8)	1	1 (14.3)	6 (85.7)	0	1 (50)	1 (50)	0
Anemia	10	12	14	9 (56.2)	7 (43.8)	11	1 (20)	4 (80)	2	0	1 (100)	1
Total Hyperbilirubinemia	3	28	5	2 (9.1)	20 (90.9)	5	0	7 (100)	0	1 (50)	1 (50)	0
Direct Hyperbilirubinemia	3	28	5	2 (9.1)	20 (90.9)	5	0	7 (100)	0	1 (50)	1 (50)	0
Salt kiss	11	5	20	10 (71.4)	4 (28.6)	13	1 (50)	1 (50)	5	0	0	2
Ascitis	1	29	6	1 (4.5)	21 (95.5)	5	0	6 (100)	1	0	2 (100)	0
Reflux	10	19	7	6 (30)	14 (70)	7	3 (42.9)	4 (57.1)	0	1 (50)	1 (50)	0
Hyperinflation in CXR	16	14	6	10 (47.6)	11 (52.4)	6	4 (42.9)	3 (57.1)	0	2 (100)	0	0
Vomiting	12	20	4	13 (53.5)	10 (43.5)	4	2 (28.6)	5 (71.4)	0	0	2 (100)	0
Hypokalemia	13	19	4	11 (40.7)	12 (52.2)	4	5 (71.4)	2 (28.6)	0	0	2 (100)	0
Hypernatremia	13	19	4	11 (47.8)	12 (52.2)	4	2 (28.6)	5 (71.4)	0	0	2 (100)	0
Metabolic alkalosis	14	18	4	12 (44.4)	11 (40.7)	4	2 (28.6)	5 (71.4)	0	0	2 (100)	0
Organomegaly	8	24	4	7 (30.4)	16 (69.6)	4	0	7 (100)	0	1 (50)	1 (50)	0

^aData are presented as No. (%).

5. Discussion

This study was conducted regarding the necessity of detecting genetic mechanisms interacting in pathogenesis of CF, and description of phenotypes in relation to underlying genotypes. Frequency of $\Delta F508$ in our patients with PI was 11 (15.2%) from all 72 alleles studied. This value was comparable with data of previous reports on CF prevalence in Iran without considering special related phenotypes (7-9). In correlation of clinical phenotypes with the genotype groups, frequency of dyspnea in homozygotes for $\Delta F508$ was documented significantly higher than in heterozygotes and non-mutated groups for $\Delta F508$. Bronchiolitis was found more prevalent in heterozygote group than others; and bronchiectasis was also more significantly prevalent in heterozygous and homozygous patients for $\Delta F508$ mutations compared with non-mutated ones. The results of our research in agreement with other studies clarifies that the more $\Delta F508$ mutation affects alleles, the more respiratory complications are accompanied with genotype (10).

References

1. Morral N, Bertranpetit J, Estivill X, Nunes V, Casals T, Gimenez J, et al. The origin of the major cystic fibrosis mutation (delta F508) in European populations. *Nat Genet.* 1994;7(2):169-75. doi: 10.1038/ng0694-169. [PubMed: 7920636]
2. Jameson JL. *Principles of Molecular Medicine*. Humana Pr; 1998.
3. Farahmand F, Khalili M, Shahbaznejad L, Hirbod-Mobarakeh A, Najafi Sani M, Khodadad A, et al. Clinical presentation of cystic fibrosis at the time of diagnosis: a multicenter study in a region without newborn screening. *Turk J Gastroenterol.* 2013;24(6):541-5. [PubMed: 24623294]
4. Najafi M, Alimadadi H, Rouhani P, Kiani MA, Khodadad A, Motamed F, et al. Genotype-phenotype relationship in Iranian patients with cystic fibrosis. *Turk J Gastroenterol.* 2015;26(3):241-3. doi: 10.5152/tjg.2015.5945. [PubMed: 26006199]
5. Fallahi G, Najafi M, Farhmand F, Bazvand F, Ahmadi M, Ahmadi F, et al. The clinical and laboratory manifestations of Iranian patients with cystic fibrosis. *Turk J Pediatr.* 2010;52(2):132-8. [PubMed: 20560247]
6. Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. *Human Mut.* 1997;10(2):135-54. doi: 10.1002/(sici)1098-1004(1997)10:2<135::aid-humu6>3.3.co;2-p.
7. Elahi E, Khodadad A, Kupersmidt 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. doi: 10.2353/jmoldx.2006.050063. [PubMed: 16436643]
8. Jalalirad M, Houshmand M, Mirfakhraie R, Goharbari MH, Mirzajani F. First study of CF mutations in the CFTR gene of Iranian patients: detection of DeltaF508, G542X, W1282X, A120T, R117H, and R347H mutations. *J Trop Pediatr.* 2004;50(6):359-61. doi: 10.1093/tropej/50.6.359. [PubMed: 15537723]
9. 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. doi: 10.1016/j.jcf.2007.06.001. [PubMed: 17662673]
10. Eaton TE, Weiner Miller P, Garrett JE, Cutting GR. Cystic fibrosis transmembrane conductance regulator gene mutations: do they play a role in the aetiology of allergic bronchopulmonary aspergillosis? *Clin Exp Allergy.* 2002;32(5):756-61. doi: 10.1046/j.1365-2222.2002.01361.x. [PubMed: 11994102]