



Genotype-Phenotype Correlation for Cystic Fibrosis According to Registry Center of Cystic Fibrosis

Mandana Rafeey¹, Morteza Jabarpour-Bonyadi², Leila Vahedi^{3*} 

Abstract

Objectives: The present study aimed to investigate the correlation of genotype-phenotype in patients with cystic fibrosis (CF) in the Azeri-Turkish population, Iran.

Materials and Methods: In this descriptive-analytical study which was conducted according to Registry Center of Cystic Fibrosis, 206 patients with CF were investigated from 2001 to 2017. The data included clinical, laboratory, and genetic results. Descriptive statistics, chi-square test, and independent t test were applied using SPSS version 21.0. The odds ratio with 95% confidence interval and $P < 0.05$ were considered significant.

Results: Thirty-one variants and 47 genotypes were observed. The $\Delta F508$ genotype (the most common genotype), especially homozygous and compound heterozygous genotypes were significantly different from other genotypes for chronic sinopulmonary disease, gastrointestinal and nutritional abnormalities, and salt loss syndromes, with a higher sweat test measures, higher mortality rate, and complications.

Conclusions: Except for $\Delta F508$, the rest of mutations were the same, and milder clinical course, and most mutations belonged to this group. The challenge in cystic fibrosis consists of no detected mutations and high heterogeneity of cystic fibrosis transmembrane conductance regulator (CFTR) mutations.

Keywords: Cystic fibrosis, Genotype, Phenotype, Mutation, Correlation, Comparison

Introduction

Cystic fibrosis (CF) is a recessive genetic disease with different clinical phenotypes (1,2), including recurrent respiratory infections, digestive complications, abnormal sweat test measures, and male infertility (3). The incidence of CF in Asian countries has been estimated 1 in 10000-350000 live births (3). Mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene located in the long arm of chromosome 7 (7q31.2) lead to disorders in the CFTR protein (4,5). Till now, more than 2000 mutations and 200 polymorphisms have been reported for CF (5), the frequency of which varies between ethnic groups; however, the most common mutation have been $\Delta F508$ (6). There is a decreasing trend in the frequency of $\Delta F508$ mutation from east to west (7). In a review study conducted by Singh et al, the approximate frequency of $\Delta F508$ was reported 31% in India, 15% in Saudi Arabia, 36% in Lebanon, and 31% in Turkey (8). In another study carried out on 272 patients with CF from European and North African countries, $\Delta F508$ (66%) was the most common mutation (9). Besides the genotype, several factors such as moderating genes and environmental factors can affect the final phenotype (10,11). Depending on the location of the mutations on

the CFTR gene, phenotypes associate with the severe form to milder CF (6). Generally, CF patients who are $\Delta F508$ homozygotes have greater organ damage (6). In a study conducted on 45 CF patients, 29 common CFTR gene mutations were examined, in which $\Delta F508/\Delta F508$ was the most common genotype and undefined genotype was 60% (12). In another study, the complications such as meconium ileus and liver disorder was related to homozygous $\Delta F508$ (13).

In the current study, we aimed to assess the correlation between the phenotype (clinical and laboratory results, and complications) and genotype among different genotypes in patients with CF in the Azeri-Turkish population of Iran.

In this report, we described the results of an expanded study, in which many patients were evaluated in terms of the associations between clinical phenotypes of CF and the $\Delta F508$ mutation. In addition to their pancreatic function, we examined clinical indexes such as pulmonary function, growth measurements, the presence or absence of meconium ileus, and sweat chloride levels.

Materials and Methods

This analytical descriptive study was conducted in Tabriz

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¹Department of Gastroenterology, Tabriz Children's Hospital, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. ²Department of Medical Genetics, Faculty of Natural Sciences, Center of Excellence for Biodiversity, University of Tabriz, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran. ³Department of Medical Genetics, Liver and Gastrointestinal Diseases Research Center, Tabriz University of Medical Sciences, Tabriz, Iran.

*Corresponding Author: Leila Vahedi, Tel: 09141042900, Email: vahedi.l49@gmail.com



University of Medical Sciences, Iran, according to data of Iranian Registry Center of Cystic Fibrosis, from 2001 to 2017. Iranian Registry Center of Cystic Fibrosis was established in Tabriz Liver and Gastrointestinal Diseases Research Center by the Ministry of Health and Medical Education, Iran, in 2017, in order to collect and analyze the data of CF patients to improve their quality of life and provide better services for them (14). Almost 90% of patients were Azeri who comprise the biggest ethnic group in Iran (10).

Patients

Three hundred and twenty patients with CF were enrolled into this study using the census method. The clinical diagnosis of CF was done according to the Guidelines for Diagnosis of CF (Cystic Fibrosis Foundation Consensus Report) (1,11). Ultimately, 206 cases with complete genetic documents based on the agreement of 3 expert appraisers were included. The patients with mutations were identified if there were no genetic results in electronic records. The staff of the Medical Genetics Laboratory carried out the study of mutants after obtaining informed consent.

Analysis of Mutations

DNA was extracted from the whole blood using a standard method (15). Ten hot spots (exons of 3, 4, 8, 11, 12, 14, 16, 20, 22, and 24) and their adjacent introns in the *CFTR* gene were amplified according to the hot spots in the Turkish population using specific oligonucleotide primers with polymerase chain reaction (PCR) (15). The PCR processing was performed as recommended in previously published studies (16). The products were assessed using the Sanger sequencing method which is a gold standard method (17).

Data Analysis

The mutations, clinical manifestation at onset and at lifespan, sweat test, age at onset, and mortality were considered as variables. Descriptive statistics such as mean, standard deviation, median, frequency, percentage, maximum, and minimum were analyzed using SPSS version 21. The chi-square test and Fisher exact test were used for the comparison of qualitative data; and the independent samples *t* test was used for the comparison of quantitative and qualitative data. In this study, odds ratio (OR) with 95% confidence interval and $P < 0.05$ were considered as statistically significant.

Results

In this study, 10 hot spots (exons of 3, 4, 8, 11, 12, 14, 16, 20, 22, and 24) and their adjacent introns in the *CFTR* gene were examined for 206 CF patients, for which 31 variants and 47 genotypes were finally observed. $\Delta F508$ (35.5%), 1540G-A (14.7%), and $\Delta F508/\Delta F508$ (34.1%) were the common mutation, polymorphism, and genotype, respectively.

First Phase

The frequency of genotypes, genders, clinical manifestations, outcome, and the mean sweat test were calculated for CF patients with stratified genotypes (i.e., homozygous, heterozygous, and compound heterozygous) whose results are shown in Table S1 (See online Supplementary file 1). The results of remaining genotypes were not mentioned due to their low frequency; however, they have been considered in total calculations.

According to Table S1, the most frequent respiratory, digestive, and salt loss symptoms at the onset related to the genotypes of $\Delta F508$, 1540G-A, and M470V, respectively. The highest level of sweat test was related to the genotypes of compound heterozygote $\Delta F508$, heterozygote $\Delta F508$, and homozygote $\Delta F508$, respectively. The $\Delta F508$ homozygous and G542X/ G542X patients with CF had the highest rate of mortality.

Second Phase

The distribution was studied for the common genotypes (10 genotypes) whose results are shown in Table 1. In addition, the results of remaining genotypes were not mentioned due to their low frequency. As seen in Table 1, the genotypes of $\Delta F508$, especially homozygous types were related to the highest frequency of bronchiectasis, finger clubbing, meconium ileus, fatty liver, intestinal obstruction, and renal failure. Additionally, urogenital abnormalities were only observed in the CF patients with M470V variants.

Third Phase

The variables in Table S1 and Table 1 were compared for the common genotypes (10 genotypes), along with the data of subjects with statistical significance (Table 2). According to Table 2, there was a significant difference between 13 subjects, especially between $\Delta F508$ homozygous patients regarding clinical presentations and outcome.

Discussion

In the present study, 31 variants and 47 genotypes were recognized. The most common mutation and genotype for CF were the $\Delta F508$ mutation and the $\Delta F508$ genotype, respectively.

The highest levels of sweat test, mortality, and side effects such as bronchiectasis, meconium ileus, and fatty liver were related to the $\Delta F508/\Delta F508$ genotype.

High heterogeneity of *CFTR* mutations were reported in the population of the Middle East (8,18,19), and the $\Delta F508$ mutation is still the most common mutation with different frequencies worldwide (7-9). In a study conducted by Mirtajani on 32 CF patients in Tunisia, the most common mutations were $\Delta F508$ (50.7%) and E1104X (16.18%) followed by G542X, W1282X, and N1303K in the Mediterranean regions (20). The frequency of the $\Delta F508$ mutation in this study was almost similar to the results of Turkey and Lebanon (8), considering that

Table 1. Complications of Common Genotypes in Patients With Cystic Fibrosis

	A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P
Homozygote ΔF508	6 (272)	-	2 (100)	1 (100)	3 (37.5)	1 (50)	8 (61.5)	19 (17.1)	1 (20)	1 (20)	1 (20)	1 (33.3)	1 (20)	-	1 (50)	1 (7.7)
Heterozygote ΔF508	3 (13.6)	-	-	-	-	-	-	18 (16.2)	1 (20)	1 (40)	-	1 (33.3)	2 (20)	2 (40)	1 (50)	5 (38.5)
Compound heterozygote ΔF508	-	-	-	-	-	-	-	1 (0.9)	-	-	-	-	-	-	-	1 (7.7)
All types of 1677delTA	-	-	-	-	-	-	-	3 (2.7)	1 (20)	-	-	1 (33.3)	-	-	-	-
All types of G542X	-	1 (100)	-	-	1 (12.5)	-	-	3 (2.7)	-	-	-	-	-	-	-	-
All types of 2183AA-G	1 (4.5)	-	-	-	-	1 (50)	2 (15.4)	4 (3.6)	-	1 (20)	-	-	-	-	-	-
+Homozygote1540C-A	-	-	-	-	-	-	-	4 (3.6)	-	-	1 (20)	-	-	1 (20)	-	-
+Heterozygote 1540G-A	8 (36.3)	-	-	-	-	-	1 (7.7)	18 (16.2)	1 (20)	-	-	-	-	-	-	3 (23.1)
+Homozygote M470V	1 (4.5)	-	-	-	1 (12.5)	-	-	6 (5.4)	1 (20)	1 (20)	2 (40)	-	3 (60)	-	-	3 (23.1)
+Homozygote1525-61A-G	-	-	-	-	1 (12.5)	-	2 (15.4)	11 (9.9)	-	-	-	-	-	2 (40)	-	-
Total	19 (100)	1 (100)	2 (100)	1 (100)	8 (100)	2 (100)	13 (100)	84 (100)	5 (100)	4 (100)	4 (100)	3 (100)	6 (100)	5 (100)	2 (100)	13 (100)

Abbreviations: A, Bronchiectasis; B, Pulmonary embolism; C, Pulmonary fibrosis; D, Pulmonary fibrosis; Clubbing fingers; E, Nasal polyps; G, Sinusitis; H, Failure to thrive; I, Meconium ileus; J, Fatty liver; K, Intestinal obstruction; L, Intestinal atresia; M, Gall stones; N, Kidney stones; O, Renal failure; P, Metabolic disorders.

- It is noted that mutations 3849+5G-A, 406-6T-C, 4096-283T-C, E92K, R334W, 2789+5G-A, D110H, P111L, R1158X, I148T, K1177X, K1302X, 3130del A, 1393-1G-A, 1717-2A-G, F1052V, 1248+ 1G-A, 1716+1G-A, 2043delG, 2181nsA, 2789G-A, 3601-65C-A, A120T and S466X not to mention because of being few in number.

+These variants as disease-causing mutations have been reported, and the geneticists agreed not (referred to discussion).

Table 2. Comparison of Clinical Presentations and Outcome Among Different Genotypes in Patients With Cystic Fibrosis

Genotypes	Variables	OR	95% CI	P Value
F508ΔHeterozygote / F508Δ Homozygote	Outcome (Dead/Alive)	3	0.94-9.54	0.05
F508/1677delITΔHomozygote	Outcome (Dead/Alive)	0.55	0.42-0.72	0.01
Homozygote ΔF508/ Homozygote 1540G-A	R (Positive/Negative)	5.14	1.17-22.6	0.02
Homozygote ΔF508/ Heterozygote 1540G-A	R (Positive/Negative)	4.11	1.35-12.48	0.01
Homozygote ΔF508/M470V	R (Positive/Negative)	4.5	1.22-16.47	0.01
Homozygote ΔF508/1525-61A-G	R (Positive/Negative)	4	1.11-14.34	0.02
	Outcome (Dead/Alive)	5.54	1.12-27.42	0.02
M470V/F508Δ Heterozygote	G (Positive/Negative)	1.36	1-1.85	0.01
F508ΔCompound heterozygote / F508Δ Homozygote	Failure to thrive*	-	-	<0.001
F508/1677delITΔHomozygote	Failure to thrive	-	-	0.001
G542X/ F508Δ Homozygote	Failure to thrive	-	-	0.001
2183AA>G / F508Δ Homozygote	Failure to thrive	-	-	0.002
Homozygote 1540G>A / F508Δ Homozygote	Failure to thrive	-	-	0.002
Homozygote M470V / F508Δ Homozygote	Failure to thrive	-	-	0.009

It is noted that the significant subjects have been mentioned.

*The frequency of Failure to thrive has been compared between two genotypes (8-13) that the results were only based on p value.

R: Chronic sinopulmonary disease.

G: Gastrointestinal and nutritional abnormalities.

S: Salt loss syndromes.

the ΔF508 mutation is more frequent in the Europe and less abundant in the Far East (8,21).

Based on the results of different studies, the patients homozygous for the ΔF508 mutation typically produce the severe type, compared to other mutations. Santos and Steemburgo (22) and Farra et al (2) reported the higher sweat test, earlier onset, and higher mortality in CF patients homozygous for ΔF508 than CF patients with other variants in the *CFTR* gene. Moreover, the ΔF508 homozygous genotype was a common genotype among patients with CF. In another study conducted by Gökdemir et al in Turkey, out of 200 patients with CF, 35 patients had severe respiratory disorder which was associated with the ΔF508 mutation. However, the frequencies of genotypes were almost identical for both the homozygous and heterozygous patients with CF (23).

The noteworthy was related to heterozygous patients that had higher mortality in spite of CF is an AR disorder. In addition, patients with 1540GA, M470V, and 1525-61A-G variants had higher mortality in spite of they be considered as mild forms (24).

In a study done by Huang et al in China on the common *CFTR* haplotypes, the poly-T, TG-repeats, and M470V were the most common haplotypes, respectively (25). In some studies, a strong linkage has been reported between the pathogenic variants of *CFTR* gene and M470V (26, 27). It is likely that other unknown mutations have not been detected.

In the interpretation of clinical symptoms based on genotype, considering the following subjects is necessary: genotype is not predictive of phenotype, and different tissues require various ranges of protein expression for normal functioning (28,29). The severity of clinical

manifestations is normally related to the balance of mutations, especially in the presence of two different mutations (compound heterozygous) (3031). The modifier factors (10,32), the socioeconomic status of patients, nutrition, and infectious agents (e.g., bacteria and viruses) (32) are other factors that affect the phenotype of disease.

Finally, in European countries, mutations are not detected in 5%-10% of patients with CF, despite the use of advanced diagnostic techniques in genetics. Therefore, the symptoms of diseases manifest themselves in patients with a heterozygous genotype or the variants of 1540G-A, M470V, and 1525- 61A-G. *CFTR* gene is a large gene with high degree of genetic heterogeneity, so it is difficult to detect mutations associated with non-coding regions of the gene, promoter regions, or mutations located at places far from genes (33). On the other hand, in addition to the abovementioned problems, as well as the low frequency of ΔF508 as the most common mutation (about 20%) (33,34) in Iran, it is important to examine other mutations and other areas of the *CFTR* gene. Other mutations show a frequency of <2% (33,34), which requires the classification of *CFTR* mutations to different groups, or geographical areas, using national diagnostic kits (33).

Strengths

One strength of the current study was the appropriate sample size during a long period. Data were obtained from Disease Registry of Cystic Fibrosis in Tabriz University of Medical Sciences, Iran.

Conclusions

The genotypes of ΔF508 associated with a higher increased risk for sweat test measures, mortality rates, and

complications, especially the homozygote and compound genotypes. The remaining mutations were similar and milder in terms of clinical course. Moreover, the symptoms of diseases manifested themselves in patients with heterozygous genotype that needs to be examined further.

Considering high heterogeneity of CFTR mutations, low frequency of the $\Delta F508$ mutation in Iran than that in European regions based on the results of the present study, and a high incidence of undetected CFTR mutations in this region, it is necessary to use advanced techniques, and link with other expert countries, in order to use their experiences and regional commercial kits.

Conflict of Interests

Authors have no conflict of interests.

Ethical Issues

The protocol of the study was approved by the Ethics Committee of Tabriz University of Medical Sciences (Code of Ethics: 5/4/1775) and all data were kept confidential without stating the patients' names and addresses. It should be noted that the informed consent was obtained from the participants or parents.

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Supplementary Materials

Supplementary file 1 contains Table S1.

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