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Genotype-phenotype relationship in Iranian patients with cystic fibrosis

LIVER

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Original Article

ABSTRACT

Background/Aims: Cystic fibrosis (CF), the most common hereditary, life-threatening disease, is caused by a mutation in the *CFTR* gene. Because different mutations can affect clinical manifestations of patients, this study was conducted to investigate the possible genotype-phenotype relationship in a group of Iranian patients with CF.

Materials and Methods: This case-series study was conducted in 30 patients with CF who were referred to a tertiary pediatric hospital in Tehran. In this study, the DNA of the patients was evaluated for delta F508 mutation, whereas some parameters such as the age at diagnosis, the sweat chloride level, and clinical manifestations related to pancreatic insufficiency and pulmonary involvement were also assessed.

Results: Among all the studied patients, 16.6% had a delta F508 mutation, either homozygote or heterozygote. The mean age at diagnosis was lower in patients with the delta F508 mutation, but the sweat chloride level tended to be higher in these patients. All the patients with the delta F508 mutation had exocrine pancreatic insufficiency, which tended to be higher than 84% in those without this mutation. In addition, all of these patients had pulmonary involvement, which tended to be higher than 64% in those with negative delta F508 mutation.

Conclusion: According to the results of this study, the frequency of delta F508 mutations in Iranian patients appears to be much lower than what is seen in American and the European patients. In those with the delta F508 mutation, pulmonary involvement and pancreatic insufficiency are more common; the sweat chloride level tended to be higher, but the age at diagnosis was lower, all of which resemble a more severe form of disease.

Keywords: Cystic fibrosis, mutation, pulmonary involvement, pancreatic insufficiency

INTRODUCTION

Cystic fibrosis (CF) is a hereditary, multi-system disease, which is considered as the most common cause of pancreatic insufficiency in children and one of the most important causes of chronic lung disease. A variety of other clinical manifestations have also been described in CF patients, including failure to thrive, pancreatitis, gall stones, hepatic cirrhosis, insulin-dependent diabetes mellitus, nasal polyposis, sinusitis, and rectal prolapse (1).

Cystic fibrosis is an autosomal recessives disease, which is caused by a mutation of the *CFTR* gene, which encodes the *CFTR* protein, a chloride channel, which has regulatory function (1). About 2,000 mutations have already been detected in the *CFTR* gene till date (2). These mutations are classified into six groups: Mutations in classes 1 to 3 induce classic forms of disease, but classes 4 to 6 may have normal chloride level in the sweat test and be pancreatic sufficient (3,4); therefore, genotypes seem to affect the phenotype of affected patients.

The most common *CFTR* mutation is delta F508. This study was conducted to investigate the delta F508 mutation in patients with CF and to assess its possible relationship with clinical presentations.

MATERIALS AND METHODS

This study included 30 patients (16 men and 14 women) with CF who were referred to the Children's Medical Center Hospital, the Pediatrics Center of Excellence in

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Tehran, Iran from March 2012 to February 2013. All the patients with a confirmed diagnosis of CF who referred to this center during this period were enrolled and no patient was excluded. Patients with a confirmed diagnosis of CF who had elevated sweat chloride levels (Cl>60 mEq/L) at least twice on separate days were included in this study. This study was approved by the Ethics Committee of Tehran University of Medical Sciences.

Different items were asked, including age at diagnosis onset, sex, pulmonary involvement, exocrine pancreatic insufficiency, and sweat chloride level, and the delta F508 mutation was investigated in these patients.

Pulmonary involvement was defined according to the history of admission for pulmonary disease under the observation of a pediatric pulmonologist. Exocrine pancreatic insufficiency stated as stool trypsin activity <1/96, fecal elastase 1<200 U/Mg, and >100 fat droplet in Sudan stain III staining.

Mutation of delta F508 was investigated in exon 10 of chromosome 7 in all patients.

The data are presented as mean±standard deviation. Fishers exact and Mann-Whitney probability tests were used; a p value of less than 0.05 was considered statistically significant.

Statistical analyses were performed using the SPSS software (Statistical Package for the Social Sciences, version 17.0, SPSS Inc., Chicago, USA).

RESULTS

The mean age at diagnosis of CF was 12.3±11.9 months. This mean age at diagnosis for patients with the delta F508 mutation was 6.8±4.2 months, which tended to be lower than 13.1±12.9 months in those without this mutation. However, this difference was not statistically significant.

Three patients had a history of ileus meconium in the neonate period, two of whom had a homozygous delta F508 mutation and one had a heterozygous mutation (Table 1).

The age at diagnosis in patients with and without pancreatic insufficiency was 8.9±6.8 months and 31.5±22 months, respectively (p=0.03).

The mean sweat chloride level in all patients was 84.1±16 mEq/L. The mean sweat chloride level in patients with and without the delta F508 mutation was 91±13.9 mEq/L and 82.7±17 mEq/L, respectively, which was not statistically significant. Although no statistically significant difference was found between exocrine pancreatic insufficiency and pancreatic sufficiency, the chloride concentration in the sweat test of those with a history of ileus meconium was significantly higher than that of other patients (100 mEq/L vs. 82 mEq/L, p=0.048).

All girls were pancreatic insufficient, whereas only 12 of 16 boys had pancreatic insufficiency. Meanwhile, pulmonary involvement in boys tended to be higher than that in girls (13 vs. eight patients); however, it was not significant.

In this study, five patients had the delta F508 mutation, which was homozygous in two patients and heterozygous in three.

Pancreatic insufficiency frequency between patients with the delta F508 mutation and those without this mutation was 100% (n=5) and 84% (n=21), respectively. However, this difference was not statistically significant. All the patients with the delta F508 mutation had pulmonary involvement, whereas 64% of patients (n=16) without the delta F508 mutation pulmonary involvement.

DISCUSSION

The relationship between the phenotype and genotype is a challenging issue in patients with CF (1). Severe mutations appear to be almost always associated with exocrine pancreatic insufficiency. In addition, they are related with rapidly progressive pulmonary disease (1). This study was conducted to check the possible association between the genotype (delta F508 mutation) and phenotype in CF patients.

In our study, 16.6% of patients have heterozygote or homozygote forms of the delta F508 mutation, which is much lower than that in other regions; in northern Europe, 50% of CF patients appear to have a homozygote delta F508 mutation and at least 80% of patients have a mutation of this allele (1). As a matter of fact, delta F508 is the most common CF mutation worldwide (3). The frequency of this mutation in previous stud-

Table 1. Characteristics of cystic fibrosis patients with and without the delta F508 mutation

	delta F508 negative (n=25)	delta F508 positive (n=5)	Total (n=30)
Sex	Male: 13, Female: 12	Male: 3, Female: 2	Male: 16, Female:14
Mean age at diagnosis	12.9 months	6.8 months	11.93 months
Pulmonary involvement	16	5	21
Pancreatic involvement	21	5	26
Mean sweat chloride	82.7 mEq/L	91.2 mEq/L	84.1 mEq/L
Ileus meconium	0	3	3

ies in Iran was reported to be about 16–23% (5-9), which shows a lower frequency of this mutation, compared with the Western countries.

The age at the onset of diagnosis appears to be directly related to the severity of disease in CF patients. The average age at diagnosis in our study was 11.9 months. CF patients are usually diagnosed in the first year of life (3). In our previous study on CF patients, the mean age at diagnosis was 5 months (10). In this study, the age at diagnosis in patients with the delta F508 mutation was lower than that in other patients (6.8 months compared with 12.0 months), which is compatible with a previous study by Kerem et al. (11) that showed that all patients who were homozygous for delta F508 had a lower age at diagnosis.

Although all patients with delta F508 had exocrine pancreatic insufficiency, this was not statistically significant. In two other studies by Kerem et al. (11) and Campbell et al. (12), 92% and 100% of patients, respectively, who were homozygous for the delta F508 mutation had pancreatic insufficiency. In the study on 39 CF patients by Fekete et al. (13), exocrine pancreatic insufficiency was seen more in patients with the delta F508 mutation. It appears that there is a direct relationship between the delta F508 mutation and pancreatic insufficiency in CF patients; however, the genotype is not fully compatible with normal pancreatic function. In contrast, in the study on 396 CF patients, 10 patients who were homozygote for the delta F508 mutation had normal pancreatic function (4).

In our study, all patients with the delta F508 mutation had pulmonary involvement. However, 64% of patients without this mutation had pulmonary involvement as well. The study by Borgo et al. (14) showed that there is no direct relationship between the delta F508 mutation and pulmonary involvement, which is in contrast with some other studies (3,13). In the study by Campbell et al. (12), patients who were not homozygous for the delta F508 mutation had a much lower pulmonary involvement.

The mean sweat chloride level was 84.1 mEq/L in our study, which was higher in patients with the delta F508 mutation but the difference was not statistically significant. In the study on 293 CF patients, the sweat chloride level in patients who were not homozygous for the delta F508 mutation was much lower than that in the other group (11). On the other hand, a higher sweat chloride level was associated with exocrine pancreatic insufficiency (4). There may be a relationship between higher sweat chloride levels as a marker of severity of CF disease and the delta F508 mutation; however, it is necessary to study more in this regard.

This study has some limitations. The sample size is small and the design is cross-sectional. To make a better conclusion on the genotype–phenotype correlation in Iranian patients with CF, larger analytical studies are recommended.

The relationship between the genotype and phenotype in CF patients has been investigated in different studies, and delta F508 is the most common mutation worldwide. There is a documented relationship between this mutation and pancreatic insufficiency; however, to hypothesize about the relationship between this mutation and some other aspects of CF (for example, pulmonary involvement, the sweat chloride level, and the age at diagnosis), further studies are warranted with a higher number of patients.

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