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Investigating CFTR gene variations in patient groups with positive newborn screening test results and preliminary clinical diagnosis of cystic fibrosis in the eastern anatolia region of Turkey

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Abstract

Cystic fibrosis (CF, OMIM: #219700), caused by biallelic pathogenic variations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, is the most common monogenic disease. The present study aimed to investigate CFTR gene variations in patients with a positive screening test result for CF and those with a clinical suspicion of CF. Overall, 443 patients (190 females/253 males) were retrospectively included. Of these, a positive neonatal screening test result for CF was reported in 124 patients (58 females/66 males) and a preliminary clinical diagnosis of CF based on recurrent lung infection and/or delayed weight gain was reported in 327 patients (134 females/193 males). All patients were evaluated based on clinical findings, sweat test (ST) results, and CFTR gene sequence analysis results. In the group of 116 patients having a positive neonatal screening test result for CF, heterozygous variations were observed in 21 (18%) patients, compound heterozygous variations in 9 (8%) patients, and homozygous variations in 5 (4%) patients. In the group of 327 patients with a clinical suspicion of CF, heterozygous variations were observed in 52 (16%) patients, compound heterozygous variations in 26 (8%) patients, and homozygous variations in 11 (3%) patients. When the two groups were cumulatively evaluated, the most common mutant alleles were Pro1013Leu (10.4%), Tyr515* (9.6%), and Phe508del (4.8%). In the total study sample of 443 patients, 51 different variants were detected. To the best of our knowledge, this is the first comprehensive study that demonstrated CFTR gene variation distribution in the Eastern Anatolia Region of Turkey. In this study, mutation distribution was highly heterogeneous, and we believe that investigation of the entire CFTR gene is necessary and would improve the diagnostic rates for CF in the Turkish population.

Keywords: CFTR, newborn screening, immunoreactive trypsinogen, sweat test

Introduction

Cystic fibrosis (CF, OMIM: #219700) is caused by biallelic pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene and is the most common monogenic disease in the Caucasian population [1]. Its prevalence is 1/2000–1/4000 in the Caucasian population, 1/40,000–1/100,000 in the Indian population, and 1/350,000–1/1,000,000 in the Japanese population [2]. CF phenotype is characterized by lung disease (recurrent respiratory infections, bronchiectasis, and progressive lung disease), exocrine pancreatic insufficiency, developmental delay, and male infertility [3]. CFTR is responsible for electrolyte and chloride ion transport from the apical membrane of epithelial cells of organs such as the respiratory tract, intestines, pancreas, kidneys, and salivary glands [4].

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When a mutant CFTR protein is present, thick and viscous mucus accumulation is observed owing to chloride ion accumulation in mucus-producing cells. The accumulated mucus causes clinical manifestations such as airway obstruction, bacterial lung infections, pancreatic failure, and infertility [5]. The clinical presentation of CF is quite heterogeneous. It shows clinical variability even in patients with the same genotype. Its clinical presentation is considered to be affected by environmental factors and modifier genes [4].

The relationship of the gene CFTR with CF was discovered in 1989 by Tsui, Riordan, and Collins [5-7]. CFTR is located in chromosome 7q31.2, is composed of 27 exons, and encodes the protein comprising 1480 amino acids [6]. To date, >2000 CFTR gene variants have been reported, and the c.1521_1523delCTT (p.Phe508del) variant is most commonly associated with the disease [8]. With the increasing number of variants, it has become increasingly difficult to predict the relationship of each rare variant with the clinical severity of the disease. Reportedly, some rare variants cause CFTR-related disorders, whereas others do not

affect the clinical presentation [9]. The Clinical and Functional Translation of CFTR project (CFTR2) website provides information on clinical findings (pancreatic insufficiency or lung disease) and severity of the disease for the most common 322 variants (November 2020). The CFTR gene variants are divided into 5 categories according to their functional effect: Class I, II, and III mutations (production, processing, and gating mutations) are associated with a more severe phenotype wherein CFTR protein is minimally present or absent. Class IV and V mutations are characterized by low sweat chloride levels and pancreatic insufficiency wherein the protein is partially functional [12].

The newborn screening for CF was introduced in 1979 when Crossley et al. discovered the presence of high levels of immunoreactive trypsinogen (IRT) in the dry blood samples of newborns diagnosed with CF [13]. Although various protocols are implemented worldwide, the first line of screening in all diagnostic algorithms for CF is the measurement of IRT level measurement. In the case of an elevated IRT, measurement of pancreatitis-associated protein (PAP) level, a second measurement of the IRT level, and/or CFTR gene analysis is performed as the second-line of screening. The most common mutations that are known for each ethnic group are often screened in the CFTR gene analysis [14]. The frequent occurrence of false-positive results from screening tests based on IRT/PAP and IRT/IRT measurements renders a diagnostic sweat test (ST) necessary for verification purposes [15]. CF is diagnosed based on a positive ST (sweat chloride levels > 60 mmol/L) and the presence of two CFTR disease-causing variants. Patients with a positive newborn screening test and the presence of 0 or 1 CF variant and a borderline ST (30–60 mmol/L) or the presence of 2 CF variants with unclear clinical significance and a negative ST (<30 mmol/L) are referred to as patients with CFTR-related metabolic syndrome (CRMS) or CF screen positive, inconclusive diagnosis (CF-SPID). Studies have shown that 10%–44% of the patients with CF-SPID are diagnosed with CF in clinical follow-up examinations. Therefore, clinical follow-up examinations of these patients are important. With newborn screening programs for CF, the prognosis of the patients was observed to be better owing to early diagnosis [16]. Newborn screening for CF in Turkey was initiated on January 1, 2015, and the IRT/IRT algorithm has been used. Patients with an IRT value of ≥90 μg/L measured in the first 24–72 h after birth undergo the second IRT measurement between days 7 and 14. Patients with the second IRT value of ≥70 μg/L are referred to CF clinics for further evaluation in terms of CF diagnosis and ST.

The present study aimed to investigate the CFTR gene variants in patients with a positive newborn screening test for CF and those with a clinical suspicion of CF.

Materials and Methods

Patients

Overall, 443 patients (190 females/253 males) who were referred to Erzurum Regional Training and Research Hospital Medical Genetics clinic for CFTR gene analysis between January 2017 and September 2020 were retrospectively included in the study. A positive newborn screening test result for CF was reported in 116 patients (56 females/60 males) and a preliminary diagnosis of CF based on recurrent lung infection and/or delayed weight gain was reported in 327 patients (134 females/193 males). All patients were evaluated based on their clinical findings, family history, kinship status, and ST results. This study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (Approval Number: 2020/19-187).

Genetic Analysis

Genomic DNA was isolated from peripheral blood samples (200 μL) using the QIAamp DNA Blood Mini QIAcube Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The gene CFTR and all its exons, exon-intron boundaries, numerous selected deep intronic regions, and a part of the promoter region were amplified using the Agilent CFTR Master Dx Kit (Agilent, Santa Clara, CA, USA). Amplified polymerase chain reaction (PCR) products were sequenced using the Illumina MiSeq platform (Illumina, Inc, San Diego, CA, USA). CFTR gene deletion/duplication analysis was performed using the Multiplex Ligation-dependent Probe Amplification (MLPA) method (MRC Holland, Amsterdam, The Netherlands) if no pathogenic variant was detected or only one pathogenic variant was detected. The pathogenicity of the variations was evaluated using Clinvar, HGMD Professional, and CFTR2 (www.cftr2.org) databases. In-silico analysis programs (Mutation Taster, SIFT, and PolyPhen2), Varsome, and the American College of Medical Genetics and Genomics (ACMG) criteria were used for evaluating the pathogenicity of novel variants [17,18].

Results

The mean age of 443 patients (190 females/253 males) included in the study was 3.94 ± 6.81 years. The mean age of 116 patients (56 females/60 males) with a positive newborn screening test result for CF was 0.31 ± 0.41 years and that of 327 patients (134 females/193 males) referred with a preliminary clinical diagnosis of CF was 5.16 ± 7.56 years. ST was performed in 26 of the 116 patients having a positive newborn screening test result for CF; borderline ST levels (30–60 mmol/L) were observed in 4 patients and positive ST levels (>60 mmol/L) were observed in 6 patients. ST was performed in 45 of the 327 patients who were referred with a preliminary clinical diagnosis of CF; borderline ST levels were detected in 14 patients and positive ST levels were observed in 8 patients.

In the group of 116 patients with a positive newborn screening test result for CF, 21 (18%), 9 (8%), and 5 (4%) patients exhibited heterozygous, compound heterozygous, and homozygous variants, respectively (Table 1). No variation was detected in 81 patients (70%). In the group of 327 patients with a preliminary clinical diagnosis of CF, 52 (16%), 26 (8%), and 11 (3%) patients exhibited heterozygous, compound heterozygous, and homozygous variations, respectively. ST results and genotypes of patients with compound heterozygous and homozygous variations are provided in Table 2. When the two groups were cumulatively evaluated, the most common mutant alleles were Pro1013Leu (10.4%), Tyr515* (9.6%), and Phe508del (4.8%). In the total study sample of 443 patients, 51 different variants were detected. Most variations were missense variations. No pathogenic results were observed in patients in whom CFTR MLPA analysis was performed to evaluate gross deletions and duplications.

Table 1. CF Newborn Screening Positive Patients with at least one CFTR variant

Patient	Sweat Test (mmol/L	Allele 1	Allele 2	Diagnosis
1	81	c.1545_1546delTA (p.Tyr515*)	c.1545_1546delTA (p.Tyr515*)	CF
2	56	c.1545_1546delTA (p.Tyr515*)	c.1545_1546delTA (p.Tyr515*)	CF
3	-	c.1210-11T>G	c.2856G>C (p.Met952Ile)	CF-SPID
4	-	c.443T>C (p.Ile148Thr)	-	CF carrier
5	-	c.1545_1546delTA (p.Tyr515*)	-	CF carrier
6	-	c.2421A>G (p.Ile807Met)	-	CF carrier
7	-	c.349C>T (p.Arg117Cys)	c.3038C>T (p.Pro1013Leu)	CF-SPID
8	-	c.581G>T (p.Gly194Val)	-	CF carrier
9	-	c.2991G>C (p.Leu997Phe)	-	CF carrier
10	-	c.902A>G (p.Tyr301Cys)	-	CF carrier
11	-	c.443T>C (p.Ile148Thr)	-	CF carrier
12	-	c.443T>C (p.Ile148Thr)	-	CF carrier
13	-	c.3454G>C (p.Asp1152His)	c.4426C>T (p.Gln1476*)	CF-SPID
14	-	c.117A>C (p.Gln39His)	-	CF carrier
15	-	c.3454G>C (p.Asp1152His)	c.3454G>C (p.Asp1152His)	CF-SPID
16	-	c.744-11_744-6delTTGATT	-	CF carrier
17	-	c.1521_1523delCTT (p.Phe508del)	-	CF carrier
18	-	c.443T>C (p.Ile148Thr)	-	CF carrier
19	-	c.1545_1546delTA (p.Tyr515*)	-	CF carrier
20	-	c.3162T>A (p.His1054Gln)	-	CF carrier
21	-	c.3140-5C>G	-	CF carrier
22	-	c.473G>C (p.Ser158Thr)	c.3038C>T (p.Pro1013Leu)	CF-SPID
23	-	c.2991G>C (p.Leu997Phe)	c.3154T>G (p.Phe1052Val)	CF-SPID
24	-	c.1897C>A (p.Leu633Ile)	c.3454G>C (p.Asp1152His)	CF-SPID
25	-	c.2758G>T (p.Val927Leu)	-	CF carrier
26	35	c.2991G>C (p.Leu997Phe)	c.2991G>C (p.Leu997Phe)	CF-SPID
27	25	c.2620-15C>G	-	CF carrier
28	-	c.1399C>T (p.Leu467Phe); c.3454G>C (p.Asp1152His)	c.1521_1523delCTT (p.Phe508del)	CF
29	-	c.2998delA (p.Ile1000Leufs*2)	c.2998delA (p.Ile1000Leufs*2)	CF
30	-	c.443T>C (p.Ile148Thr)	-	CF carrier
31	40	c.3154T>G (p.Phe1052Val)	-	CF carrier
32	21	c.1897C>A (p.Leu633Ile)	-	CF carrier
33	17	c.3038C>T (p.Pro1013Leu)	-	CF carrier
34	66	c.3935A>G (p.Asp1312Gly)	c.3454G>C (p.Asp1152His)	CF-SPID
35	35	c.2620-15C>G	-	CF carrier

CF: Cystic fibrosis, CF-SPID: CF screen positive, inconclusive diagnosis

Table 2. Compound Heterozygous and Homozygous Patients with Clinical Preliminary Diagnosis of CF

Patient	Sweat Test (mmol/L)	Allele 1	Allele 2
1	-	c.443T>C (p.Ile148Thr)	c.2002C>T (p.Arg668Cys)
2	-	c.202A>G (p.Lys68Glu)	c.2597G>A (p.Cys866Tyr)
3	-	c.3503A>G (p.Asp1168Gly)	c.1516A>G (p.Ile506Val)
4	-	c.2991G>C (p.Leu997Phe)	c.1040G>A (p.Arg347His)
5	-	c.3700A>G (p.Ile1234Val)	c.3700A>G (p.Ile1234Val)
6	-	c.2856G>C (p.Met952Ile)	c.3454G>C (p.Asp1152His)
7	-	c.349C>T (p.Arg117Cys)	c.1521_1523delCTT (p.Phe508del)
8	89	c.1545_1546delTA (p.Tyr515*)	c.1545_1546delTA (p.Tyr515*)
9	68	c.1043T>A (p.Met348Lys)	c.1043T>A (p.Met348Lys)
10	62	c.3700A>G (p.Ile1234Val)	c.443T>C (p.Ile148Thr)
11	77	c.2991G>C (p.Leu997Phe)	c.443T>C (p.Ile148Thr)
12	-	c.2991G>C (p.Leu997Phe)	c.2421A>G (p.Ile807Met)
13	-	c.2991G>C (p.Leu997Phe)	c.2421A>G (p.Ile807Met)
14	106	c.2491G>T (p.Glu831*)	c.2988+1G>A
15	80	c.2491G>T (p.Glu831*)	c.2988+1G>A
16	-	c.328G>C (p.Asp110His)	c.1521_1523delCTT (p.Phe508del)
17	-	c.1727G>C (p.Gly576Ala)	c.2002C>T (p.Arg668Cys)
18	-	c.91C>T (p.Arg31Cys)	c.650A>G (p.Glu217Gly)
19	-	c.3718-2477C>T	c.3718-2477C>T
20	-	c.1521_1523delCTT (p.Phe508del)	c.1521_1523delCTT (p.Phe508del)
21	-	c.328G>C (p.Asp110His)	c.1545_1546delTA (p.Tyr515*)
22	-	c.91C>T (p.Arg31Cys)	c.1043T>A (p.Met348Lys)
23	-	c.650A>G (p.Glu217Gly)	c.1043T>A (p.Met348Lys)
24	-	c.3454G>C (p.Asp1152His)	c.3038C>T (p.Pro1013Leu)
25	-	c.117A>C (p.Gln39His)	c.1408G>A (p.Val470Met)
26	84	c.1393-1G>A	c.1393-1G>A
27	88	c.3909C>G (p.Asn1303Lys)	c.3909C>G (p.Asn1303Lys)
28	-	c.544A>C (p.Ser182Arg)	c.1545_1546delTA (p.Tyr515*)
29	-	c.274G>A (p.Glu92Lys)	c.1210-11T>G
30	-	c.2998delA (p.Ile1000Leufs*2)	c.2998delA (p.Ile1000Leufs*2)
31	-	c.2051_2052delAAinsG (p.Lys684Serfs*38)	c.2051_2052delAAinsG (p.Lys684Serfs*38)
32	-	c.328G>C (p.Asp110His)	c.328G>C (p.Asp110His)
33	-	c.224G>A (p.Arg75Gln)	c.3038C>T (p.Pro1013Leu)
34	-	c.1897C>A (p.Leu633Ile)	c.3038C>T (p.Pro1013Leu)
35	-	c.2991G>C (p.Leu997Phe)	c.2991G>C (p.Leu997Phe)
36	-	c.224G>A (p.Arg75Gln)	c.3038C>T (p.Pro1013Leu)
37	-	c.1040G>A (p.Arg347His)	c.2195T>G (p.Leu732*)

Discussion

It should be "CF is characterized by recurrent respiratory infections and pancreatic insufficiency. It presents with delayed weight gain and recurrent lung infections in the early neonatal period. Respiratory disease is the most severe clinical manifestation and is the most common cause of death. With the standardization and improvement of the treatment and follow-up protocols in recent years, most patients can survive up to adulthood. A delay of a few weeks in CF diagnosis leads to the occurrence of bronchiectasis at an early stage. Most patients are diagnosed at a later stage despite complaints of chronic cough and poor weight gain, which are typical symptoms [19]. Because early diagnosis is vital in CF, this disease has been included in the newborn screening program in several countries. Newborn screening for CF using the IRT/IRT algorithm is being conducted in Turkey since January 1, 2015.

In the present study, a total of 443 patients with a positive newborn screening test result and a preliminary clinical diagnosis of CF in the Eastern Anatolia Region of Turkey were investigated in terms of CFTR gene variants using the new generation sequencing method. A homozygous or compound heterozygous CF-causing variant was detected in 6 (5.1%) of 116 patients with a positive newborn screening test result, and ST was found to be positive in all these patients. Further, 22 patients were found to be CF carriers and 7 (6%) patients were CF-SPID. According to the literature, 10%–50% of CF-SPID cases may progress into CF; therefore, these patients should be followed up [20]. In a study by Basaran et al., 5.7% of the patients were diagnosed with CF and 5.1% were diagnosed with CF-SPID [21]. In a study by Şaşihüseyinoğlu et al., these rates were 6.5% and 29.5%, respectively [22]. In terms of CF diagnostic rates, the ratio in the present study was similar to that in other studies conducted in the Turkish population. Owing to the popularization of newborn screening tests, the number of patients with CF-SPID has been increasing, leading to emotional stress in the families. Moreover, screening tests detect undesirable and unintended carrier status. Some researchers describe this as an "unwanted side effect", whereas others consider it an opportunity for genetic counseling [23]. Although newborn screening tests using the IRT/IRT algorithm are cheaper, their sensitivity is lower than tests using the IRT/DNA method. High levels of false positivity may be associated with prematurity, neonatal infections, and perinatal distress, which elevates the IRT level [22]. Also, a previous study conducted in the Turkish population reported that the revision of the cutoff values for the IRT levels would reduce false positivity rates [21].

The most common CFTR gene variation observed worldwide is Phe508del. The highest rates of this variant in terms of prevalence are observed in North European countries. The prevalence of this variation is 82%, 24.3%, 18%, and 19% in Denmark, Israel, Iran, and Palestine, respectively [24-26]. In studies conducted in different regions of Turkey, the prevalence of Phe508del was 4%–28.4% [27-29]. The lowest rates (4%) were observed in the studies conducted in Izmir, and the prevalence in the studies conducted in Adana and Şanlıurfa was 11.9% and 8.8%, respectively [29-31]. In the present study, the prevalence of this variation was found to be low (4.8%), similar to the studies conducted in Izmir. Pro1013Leu variation (10.4%), the most common variation in the present study, was heterozygous in 7 patients and compound heterozygous in 6 patients. A functional study demonstrated that this variation, which was first identified as compound heterozygous in a Turkish patient with CF in 1998 by Onay et al., affects channel activity and that it may be a Class III mutation [32,33]. The second most common variation, Tyr515* (9.6%), was observed to have a prevalence of 6.4% and 5.97% in two different studies conducted in the Turkish population [34,35]. This variation was homozygous in 3, compound heterozygous in 3, and heterozygous in 4 patients in the present study. In a study by Atag et al., this variation was the second most common variation, and it was homozygous in 10 patients. Pancreatic insufficiency was observed in all patients, and they were reported to have a clinical presentation of mild pulmonary disease [34].

The panel of 23 mutations screening recommended by ACOG and ACMG for CF does not include the most common mutations Pro1013Leu and Tyr515* [36,37]. This suggests that commercial kits, which are often used as screening tests for CF, are inadequate for the Turkish population. We believe it would be more effective to produce screening kits for mutations that are most commonly observed in each population.

We believe that the present study is important because it includes patients with positive newborn screening test results, evaluates the entire CFTR gene using new generation sequencing, and provides data, for the first time to the best of our knowledge, from the Eastern Anatolia Region of Turkey. However, because clinical data and ST results are not available for all patients, the inability to generate analysis in the context of genotype-phenotype correlation is a limitation of the study.

In summary, we present the first comprehensive study demonstrating CFTR gene variation distribution in the Eastern Anatolia Region of Turkey. In the present study, mutation distribution was highly heterogeneous, and we believe that analysis of the entire CFTR gene is necessary and would increase the diagnostic rates for the Turkish population. Further, mutation detection rates were examined in patients with positive newborn screening test results, as well as the diagnostic effectiveness of newborn screening tests for CF performed in Turkey for the last 4 years. False positivity rates of newborn screening tests for CF were found to be high, and it was considered that this could be associated with IRT cutoff values. Clinical findings, ST results, and genotypic data of patients in larger Turkish population cohorts would contribute to further elucidation of the etiopathogenesis of the disease.

Conflict of interests
The authors declare that they have no competing interests.

Financial Disclosure
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Ethical approval
This study was approved by the Ethics Committee of Erzurum Regional Training and Research Hospital (Approval number: 2020/19-187).

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