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The Genetic Analysis of Cystic Fibrosis Patients with Seven Novel Mutations in the *CFTR* Gene in the Central Anatolian Region of Turkey

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Background: Cystic fibrosis, a pulmonary disease which is an autosomal recessive, inherited, multisystemic genetic disease commonly seen in the Caucasian race, is the most frequent cause of mortality and morbidity. So far, more than 2000 disease-causing gene variants have been found and this number has been increasing with the studies conducted. Although there is not yet enough data that include the Turkish population, the recent increase of studies is noteworthy.

Aims: To discover the genetic variation in patients diagnosed with cystic fibrosis in the Central Anatolian region.

Study Design: Cross-sectional study.

Methods: The study was carried out in the Central Anatolian region in 3 pediatric pulmonology departments (Kayseri, Konya, and Ankara) in Turkey between July 2014 and December 2017. The Sanger and Next Generation Sequence analyses were used for exon and exon–intron boundaries in the cystic fibrosis transmembrane conductance regulatory (CFTR) gene, and in selected patients, mutation analysis was performed using the Multiplex Ligation-dependent Probe Amplification technique for large deletions and duplications.

Results: *CFTR* gene analysis was performed for 316 patients and 215 of them were genetically diagnosed with cystic fibrosis. Sixty-three different variants were defined in these patients and 7 of these were large deletions/duplications detected with the MLPA method. The most frequent variants were F508del (29.6%), G85E (8.2%), N1303K (8.2%), Y515* (7.5%), and G542* (3.4%).

Conclusion: Using sequencing and Multiplex Ligation-dependent Probe Amplification methods, the identification of seven new mutations that were not previously reported in the literature contributes to a better understanding of the heterogeneous nature of CFTR mutations in the Turkish population. When no mutations are detected (pathogenic/probably pathogenic) in clinically compatible cases, Multiplex Ligation-dependent Probe Amplification analysis contributes significantly to the diagnosis.

INTRODUCTION

Cystic fibrosis (CF; OMIM #219700) is the most frequent autosomal recessive inherited genetic disease of the Caucasian race. Its incidence is 1/2500-3500 newborns.[1,2] It is more frequent in European and European-origin communities when compared with other communities.[3] Recently, we showed the incidence of CF to be 1/3400 live births in our region, similar to other European communities.[4] While the percentage of carriers differs according to ethnic origins, it is 1/25 in Northern Europe, 1/29 among Ashkenazi Jews,[5,6] 1/46 in Hispanic Americans, and 1/65 in African Americans.[7]

The cystic fibrosis transmembrane conductance regulatory (*CFTR*) gene contains 27 exons and is localized on 7q31.

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The protein is composed of 1480 amino acids. *CFTR* is an integral membrane protein that operates as a regulated chloride channel in the epithelia. Cystic fibrosis occurs due to homozygous or compound heterozygous pathogenic variants in the *CFTR* (*CFTR/ABCC7*: MIM*602421) gene. Today, more than 2000 disease-causing variants of the *CFTR* gene have been found and their ethnic and geographical distributions show differences.[8] The most common variant seen in almost all the studies is F508del. In addition, the frequency of more than 20 variants is more significant than 0.1% (www.genet.sickkid.on.ca/Home.html.).

A variety of *CFTR* mutations have a different effect on the CFTR protein. Therefore, the traditional classification of *CFTR* mutations consists of 6 groups. Class I: Nonsense, frameshift, or splice junction variants such as c.1717-1G>A, c.1624G>T, and c.3846G>A can cause reduced or absent synthesis. Class II: Some missense variants and amino acid deletions such as c.1521_1523delCTT, c.3909C>G, and c.1682C>A disrupt the traffic. Class III: Block the regulation of *CFTR* chloride channel (such as c.1652G>A, c.1645A>C , c.4046G>A). Class IV: Alter conductance of the *CFTR* chloride channel (c.350G>A, c.1000C>T, c.1364C>A). Class V: Some missense or splice site variants such as c.3272-26 A>G can reduce the synthesis and cause less protein production. Class VI: Some mutations, such as c.120del123 may result in a less stable CFTR protein.[9,10]

The present study was conducted to describe the allele distribution and frequency of *CFTR* gene mutations, including rare variants, in patients diagnosed with CF in the Central Anatolian region of Turkey.

MATERIAL AND METHODS

Study Populations

The study was carried out in the Central Anatolian region in 3 pediatric pulmonology departments (Kayseri, Konya, and Ankara) in Turkey between July 2014 and December 2017. The patients were assessed in the Erciyes University School of Medicine, Kayseri Training and Research Hospital, Gazi University School of Medicine and Necmettin Erbakan University Meram Medicine Faculty. The Erciyes University Clinical Research Ethical Board was referred to for the study.

The diagnosis of CF was based on sweat chloride levels (>60 mEq/L) and/or 2 identified CF mutations, as well as on characteristic symptoms of CF or a positive newborn screening test.[11] Of the total of 316 patients who were screened for genetic analysis, 215 patients who had homozygous or compound heterozygous mutations participated in the study. One hundred one patients (the variant was found only in one *CFTR* gene in 49 patients, no disease-causing mutation was found in 52 patients) were excluded from the study.

Genomic DNA isolation of the patients based on peripheral blood was made by using a DNA isolation kit according to the instructions of the manufacturer (Zinexts Life Science Corporation, Taiwan).

Sanger Sequencing Analysis

In 169 cases, genetic mutation analysis was conducted by Sanger sequencing. The GML SeqFinder Sequencing System CFTR FULL Gene Kit (GML AG Altendorf, Switzerland) was used for this process. Products of the polymerase chain reaction (PCR) were observed after gel electrophoresis, and these products were purified using the ExoSAP kit (Exo SAP PCR purification kit, UAB Corporation, Cleveland, OH, USA). The same products were sequenced with the BigDye Terminator version 3.1, according to the manufacturer's instructions. Later, they were purified with Sephadex. In the last stage, the products were analyzed on the Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzers device, and bioinformatics analysis was conducted using the SeqScape software v2.6.

Next Generation Sequencing Analysis

Mutation analysis was conducted on 147 patients using Next Generation Sequencing (NGS). The NEXTflex Cystic Fibrosis Amplicon panel (BIO SCIENTIFIC, a PerkinElmer Company, Austin, TX, USA) kit was used for mutation analysis. This panel includes 10.4 kb, 28 coding exons, 1 promoter region, and 3 deep intronic regions. Sixty-one pairs of primers provide the amplification and sequencing of all exons coding the *CFTR* locus. The total length of the related amplicons differ between 83 and 226 bp. The related reading areas of libraries and primary pad areas have an average size of 137-280 bp. The products were uploaded to the MiSeq Illumina device (Illumina, San Diego, California). The data obtained were analyzed with the Integrative Genomics Viewer software (version 2.3.98). Sequence annotation and variant calling were performed by the Genomize SEQ platform (https://seq.genomize.com).

MLPA (Multiplex Ligation-dependent Probe Amplification) Analysis

When no pathogenic/likely pathogenic allele was detected with Sanger Sequencing and NGS, Multiplex Ligation-dependent Probe Amplification (MLPA) analysis was performed for deletion/duplication in the *CFTR* gene according to the manufacturer's recommendations. DNA samples were denatured at 95°C, MRC-HOLLAND MLPA Probemix-P091 *CFTR* Probe was added and left for hybridization at 60°C for 17.5 hours, and was continued the next day with the ligation step. At this step, hybridized probe oligos were ligated with a ligation enzyme. After the PCR cycle was completed, bound probes were amplified with a PCR primer. After these steps were completed, the amplified DNA products were read by capillary electrophoresis in an Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzer device. These data obtained from the device were analyzed in the Coffalyser software version v.140701.0000 (MRC-Holland, Amsterdam, the Netherlands), and *CFTR* (NM_000492.3) was accepted as a reference. This performs a quality check and calculates probe ratios.

Interpretation of Results

CFTR region-specific MLPA probes are allele copy numbers of 2 (normal), 0 (homozygous deletion), 1 (heterozygous deletion),

3 (heterozygous duplication), and occasionally 4 (homozygous duplication or heterozygous triplication).

Variant Classification

The guidelines of the American College of Medical Genetics and Genomics and Association for Pathology,[12] with the classification—benign, likely benign, uncertain significance, likely pathogenic, and pathogenic—were used to classify a variant. The pathogenicity of the novel variants was determined with the recommendation of these guidelines. There are 2 groups of criteria: (1) pathogenic/likely pathogenic and (2) benign/likely benign. Every pathogenic criterion is classified as: very strong, PVS1; strong, PS1-4; moderate, PM1-6; and supporting, PP1-5; and each benign criterion is classified as: stand-alone, BA1; strong, BS1-4; or supporting, BP1-7. Then, a class was chosen from the 5-tier system, as follows: Class 1, pathogenic; Class 2, likely pathogenic; Class 3, variant of uncertain significance (used if it did not meet one of the criteria/showed conflicting benign or pathogenic evidence); Class 4, benign; and Class 5, likely benign.

Use of Databases

Allele frequency is an important and striking indicator for benign/pathogenic characterization of a variant in Mendelian disorders. The gnomad database (https://gnomad. broadinstitute.org/) was used for the annotation of variant frequency. The CFTR mutation database (http://www.genet.sickkids.on.ca/) was also used for known variants. ClinVar (https://www.ncbi.nlm.nih.gov/clinvar) gave us an important clinical phenotype, Additionally, these databases were also used: http://exac.broadinstitute.org; http://browser.1000genomes.org; http://www.ncbi.nlm.nih.gov/snp (dbSNP); http://www.omim.org (OMIM); http://www.hgmd.org; http://www.hgvs.org/dblist/dblist.html (HGVS); http://www.ncbi.nlm.nih.gov/genome; and http://www.ncbi.nlm.nih.gov/refseq/rsg.

Several in silico computational methods were used to identify the possible degree of pathogenicity of *CFTR* variants: (a) Mutation taster (http://www.mutationtaster.org/); (b) Polyhen-2 (http://genetics.bwh.harvard.edu/pph2/), used for missense alterations; (c) Human Splicing finder (http://umd.be/Redirect.html), used for slice site variants; (d) CADD, used for analyzing evolutionary conservation and allelic diversity; and (e) SIFT (http://sift.jcvi. org), which predicts whether an amino acid substitution affects protein function.

RESULTS

A molecular genetic analysis of 215 patients diagnosed with CF from the provinces of Ankara, Konya, and Kayseri in the Central Anatolian region of Turkey was performed. Of the patients, 105 were male, while 110 were female. The median age at diagnosis was 5 months (10 days-20 years). *CFTR* gene analysis was performed for 316 patients and 215 of them were genetically diagnosed with CF. Sixty-three different mutations were found. One hundred twenty-six patients (39.8%) were homozygous for a variant and 89 (27.8%) were compound heterozygous. In 49 patients, only 1 variant was found; no disease-causing mutation was found in 52 patients, and these were excluded from the study.

Variant Spectrum

A total of 63 different variants were found and the frequency of 20 of these was >1%. These variants are shown in Table 1. In our study, 29 missense, 12 nonsense, 13 splice sites, 7 frameshifts, and 2 large del/dup mutations were found.

In 5 of the patients, 3 different variants were found at the same time. Three of these were found in 3 siblings from the same family (N1303K/c.1210-11T>G /E217G). While the N1303K variant was in transposition with the c.1210-11 T>G and E217G variants, the F508del variant was in transposition with the F1052V and Q2P (novel variant) in one patient, and the R170C variant was in transposition with the F1052V and Q2P in another patient. MLPA analysis was conducted in patients with a mutation in one *CFTR* gene or who had no mutation. Exon 2 deletion was found in 5 alleles, while exon 22 duplication was found in 2 alleles. Exon 2 deletion was homozygous in one patient, with Y515X in 2 patients and with F508del in 1 patient. In 2 patients, exon 22 duplication was with F508del.

Seven of the 63 variants were novel, and they were defined in this study for the first time. The clinical and laboratory findings of the new variants are listed below and shown in Table 2.

The **c.1393-1G>T** splice site variant was found in intron 10. This variant was found in 1 case and it was in a patient who was referred with a complaint of growth retardation and chronic diarrhea at the age of 4 months, due to steatorrhea. The result of the sweat chloride test was 102 mmol/L. The patient was from the Kayseri region in Central Anatolia.

The **c.865_869delAGACA** variant was found in exon 7. The sweat chloride test result was high (74mmol/L) in the patient who referred with Pseudo Bartter syndrome (PBS) at 4 months. Bronchiectasis and diabetes developed in the follow-up. Pseudomonas and MRSA grew in the cultures of sputum. This novel variant was also in homozygous form and the patient came from the Konya region.

The **c.2909-1G>C** variant found in intron 18 in a patient who was from the Kayseri region. The patient had diarrhea, vomiting, and failure to thrive. PBS was developed in the follow-up. He had steatorrhea and a high sweat chloride test result (93mmol/L). This variant was a homozygous mutant.

The **c.5A>C** variant was found in 2 patients. In the first of the 2 different cases, which were found to have c.5A>C with F1052V, this novel variant was in transposition with the R170C variant. In this patient, steatorrhea and bronchiectasis were found in the neonatal period. In the second case, the same variant was with F1052V and in transposition with F508del. This patient had a diagnosis of PBS, and the sweat chloride test result was high (74.1 mmol/L).

TABLE 1. CFTR Mutation Spectrum and Allele Frequency of Central Anatolian CF Patients in Turkey. Mutations Are Named as Recommended by the HGVS (Human Genome Variation Society)

	Mutation/HGVS Nomenclature	Protein Name	Exon/Intron	No. of Alleles	%	dbSNP	HGMD ID	ClinVar Accession
1	c.1521_1523delCTT	p.F508del	E10	129	29.6	rs113993960	CD890142	VCV000007105
2	c.254G>A	p.G85E	E3	36	8.2	rs75961395	CM910068	VCV000007143
3	c.3909C>G	p.N1303K	E24	36	8.2	rs80034486	CM910076	VCV000007136
4	c.1545_1546delTA	p.Y515X	E10	28	7.5	rs1301983423	CD910490	VCV000638949
5	c.1624G>T	p.G542X	E12	15	3.4	rs113993959	CM900049	VCV000007115
6	c.3454G>C	p.D1152H	E21	14	3.2	rs75541969	CM950256	VCV000035867
7	c.2051_2052delAAinsG	p.K684SfsX38	E14	12	2.7	rs121908746	CD972972	VCV000053432
8	c.1000C>T	p.R334W	E8	12	2.7	rs121909011	CM910070	VCV000007139
9	c.2657+5G>A		I16	9	2.1	rs80224560	CS900235	VCV000038497
10	c.3151A>T	p.I1051V	E20	8	1.8	rs374403559	CM043473	VCV000495927
11	c.3154T>G	p.F1052V	E20	7	1.6	rs150212784	CM930125	VCV000035865
12	c.2491G>T	p.E831X	E15	7	1.6	rs397508387	CM960283	VCV000053501
13	c.1210-11T>G		I9	7	1.6	rs73715573	CS1718581	VCV000178713
14	c.3717+5G>A		I22	7	1.6	rs193922520	CS972968	VCV000035875
15	c.3846G>A	p.W1282X	E23	6	1.3	rs77010898	CM900061	VCV000983867
16	c.1127_1128insA	p.Q378AfsX4	E9	6	1.3	rs397508163	CI972570	VCV000487392
17	c.328G>C	p.D110H	E4	5	1.1	rs113993958	CM900042	VCV000007108
18	c.2353C>T	p.R785X	E14	5	1.1	rs374946172	CM941979	VCV000053480
19	c.1393-1G>A		I10	5	1.1	rs397508200	CS930767	VCV000053242
20	Exon2 del		E2	5	1.1	NA	NA	NA
21	c.2991G>C	p.L997F	E19	4	0.9	rs1800111	CM920171	VCV000007229
22	c.2195T>G	p.L732X	E14	4	0.9	rs397508350	CM970286	VCV000053451
23	c.4364C>G	p.S1455X	E27	4	0.9	rs121909043	CM980357	VCV000007223
24	c.650A>C	p.E217G	E6	3	0.7	rs121909046	CM972939	VCV000007238
25	c.3472C>T	p.R1158X	E22	3	0.7	rs79850223	CM920180	VCV000007144
26	c.2909-15C>T		I17	3	0.7	rs397508455	CS015368	VCV000053592
27	c.443T>C	p.I148T	E4	3	0.7	rs35516286	CM920145	VCV000038850
28	c.14C>T	p.P5L	E1	2	0.4	rs193922501	CM970256	VCV000035824
29	c.224G>A	p.R75Q	E3	2	0.4	rs1800076	CM980331	VCV000035839
30	c.1519_1521delATC	p.I507del	E10	2	0.4	rs121908745	CD900275	VCV000007106
31	c.3929G>A	p.W1310X	E24	2	0.4	rs397508645	CM920192	VCV000053856
32	c.3484C>T	p.R1162X	E22	2	0.4	rs74767530	CM910074	VCV000007137
33	c.2998delA	p.I1000LfsX2	E19	2	0.4	rs397508475	CD043671	VCV000053620
34	c.3700	p.I1234V	E22	2	0.4	rs75389940	CM920184	VCV000007215
35	c.1704G>T	p.L568F	E13	2	0.4	rs397508275	CM970281	VCV000053350
36	c.350G>A	p.R117H	E4	2	0.4	rs78655421	CM900043	VCV000007109
37	c.1766+2T>C		I13	2	0.4	rs1554389062	CS102313	VCV000558608

38	c.1766+3A>G		I13	2	0.4	rs397508298	CS791651	VCV000053379
39	c.714delt	p.L240X	E6	2	0.4	rs397508787	CD000925	VCV000054045
40	c.1040G>C	p.R347P	E8	1	0.2	rs77932196	CM900044	VCV000007110
41	c.3038C>T	p.P1013L	E19	1	0.2	rs193922516	CM980353	VCV000053634
42	c.3611G>A	p.W1204X	E22	1	0.2	rs121908764	CM920183	VCV000007212
43	c.579+3A>C		I5	1	0.2	rs397508761	CS941439	VCV000054010
44	c.274G>A	p.E92K	E4	1	0.2	rs121908751	CM920143	VCV000007181
45	c.1705T>G	p.Y569D	E24	1	0.2	rs397508276	CM980339	VCV000053352
46	c.3196C>T	p.R1066C	E20	1	0.2	rs78194216	CM920175	VCV000007162
47	c.1116+1G>A		I8	1	0.2	rs397508158	CS001827	VCV000053190
48	c.2506G>T	p.D836Y	E15	1	0.2	rs201386642	CM920986	VCV000053505
49	c.2290C>T	p.R764X	E14	1	0.2	rs121908810	CM960282	VCV000053471
50	c.509G>A	p.R170H	E5	1	0.2	rs1800079	CM15353	VCV000053983
51	c.1645A>C	p.S549R	E12	1	0.2	rs121908757	CM910071	VCV000038733
52	c.3718-24G>A		I22	1	0.2	rs374013084	CS094455	VCV000499621
53	c.1399C>T	p.L467F	E11	1	0.2	rs1800089	CM63898	VCV000053246
54	c.1040 G>A	p.R347H	E8	1	0.2	rs77932196	CM920152	VCV000007182
55	c.3872A>G	p.Q1291R	E23	1	0.2	rs397508621	CM940279	VCV000053827
56	c.508C>T	p.R170C	E5	1	0.2	rs578029902	CM941968	VCV000455782

The **c.3407C>A** variant was found in exon 21 and was in transposition with G85E. As in other patients, PBS was present, and the sweat chloride test result was high (79 mmol/L).

The **c.1210-2A>G** variant was found in intron 16. This variant and the R347P variant were in transposition with F508del and the patient had PBS, with a high sweat chloride test result (102mmol/L), and steatorrhea.

Novel Duplication Exon 22

A novel duplication was found in 2 patients who were siblings. The younger patient was diagnosed with PBS and steatorrhea; although the other sibling had PBS, the sweat chloride test was normal at first, while subsequent values were found to be high (84 and 86 mmol/L). No fat was detected in stool. In the genetic analyses of both siblings, there was heterozygous duplication in the whole exon 22 and at the same time, there was F508del in the other allele.

The N1303K/E217G and c.1210-11T>G variants were found in 3 family members. The first of these was found through neonatal screening. PBS, high sweat chloride test result, distal intestinal obstruction in advanced periods, and *S. aureus* colonization in the phlegm were found. These variants were found by *CFTR* molecular examinations carried out due to similar results in other siblings.

DISCUSSION

The present study provides an extensive review of the variants in the CF gene in a representative cohort in the Central Anatolian region of Turkey. As a result of the study, 63 different variations were found in 215 CF patients. In our study, variant variation shows high heterogeneity in the gene. As can be seen in studies conducted, the Mediterranean region, including Turkey, is the region with the highest mutation heterogeneity,[13-20] while 121 different variants were found in a study in the Spanish population,[21] 105 variants in the French population,[22] and 82 different variants in North-Eastern Italy.[23] In contrast, relatively homogeneous groups are also found in this region.[24] Genetically, the Anatolian population is very heterogeneous. Anatolia is a genetic bridge between the East and the West. In specific periods of history, these lands have hosted great migrations. This is thought to be the source of the high heterogeneity in the Turkish population.

The F508del variant was the most frequent in our study (29.6%). This variant was found as homozygous in 40 patients and as compound heterozygous in 40 patients. While this rate is low, especially when compared with European countries, it complies with the geographical distribution—decreasing from the north to the south.[17,18,21,25,26] The rates of F508del are similar to those in Turkey's southern and eastern neighbors, and higher than in Arab states.[20,27-31]

In our study, c.254G>A p.(G85E) and c.3909C>G p.(N1303K) were the second most frequent variants (8.2 %). G85E was found in a total of 36 CF chromosomes (homozygous in 13 cases and compound heterozygous in 10 cases). It was first defined by Zielenski et al.[32] This rate can be the highest among the studies conducted recently. The closest rate to this was found in a study conducted

TABLE 2. Novel Mutation Classification

Patient	Gender/Age	Mutation/HGVS Nomenclature. CFTR gene (NM_000492.4)	Protein Name	Exon/Intron	No. of Alleles	%	ACMG 2015 Criteria ID	Pathogenicity and Conservation Scores
1	F/3y	c.5A>G	p.Q2P	E1	2	0.4	PM2.PP2.PP3	-PolyPhen2:PD -SIFT:Damaging -Mutation Taster :DC -GERP:4.17 -DANN:0.9925
2	F/4m	c.865_869delAGACA	p.R289Nfs*17	E7	2	0.4	PVS1.PM2.PP3.PP5	-PolyPhen2:NA -SIFT:NA -Mutation Taster :DC -GERP:5.09 -DANN:NA
3	M/2y	c.3407C>A	p.A1136D	E21	1	0.2	PM1.PM2.PP2. PP3	-PolyPhen2:PD -SIFT:Damaging -Mutation Taster :DC -GERP:5.9 -DANN:0.9983
4	M/3 m	c.1210-2A>G	-	I9	1	0.4	PVS1.PM2.PP3	-PolyPhen2:NA -SIFT:NA -Mutation Taster: DC -GERP:4.84 -DANN:0.9949
5	F/4y	c.1393-1G>T	-	I10	2	0.4	PVS1.PM2.PP3.	-PolyPhen2:NA -SIFT:NA -Mutation Taster: DC -GERP:5.46 -DANN:0.9953
6	M/1y	c.2909-1G>C	-	I18	2	0.4	PVS1.PM2.PP3.PP5	-PolyPhen2:NA -SIFT:NA -Mutation Taster: DC -GERP:5.4 -DANN:0.9955
7	F/3y	Exon22 dup	-	E22	2	0.4	NA	NA

PVS1, Pathogenic; Very Strong PM1-6, Pathogenic; Moderate PP1-5, Pathogenic; SupportingBP1, Benign, Supporting; GERP, genomic evolutionary rate profiling; PolyPhen2, Polymorphism Phenotyping v2; SIFT, sorting intolerant from tolerant; DANN, it is based on deep neural networks; DC, disease-causing; NA, not available.

in Syria, a southern neighbor of Turkey; however, the patient population was quite limited.[30] The relative frequency was found to be around 0.2% in studies conducted (www.genet.sickkid.on.ca/Home.html). Although it is the second most frequent variant in our cohort, it was rare or nonexistent in previously conducted studies.[13-16] This data can recall that carriers are more intense in the region of Turkey. Data from a more extensive and higher number of participants should be analyzed to determine whether there is geographical specificity to the G85E variant.

The N1303K variant is the second most common with the G85E, and its rate in the study population was 8.2%. The relative frequency found in the studies conducted was 1.3% (www.genet.sickkid.on.ca/Home. html). In terms of frequency, the related variant has been reported as over 1% in many studies conducted, and it is among the most frequent of 5 variants.[13-16] Although it differs in various ethnic groups and regions, it is the most prevalent in the Mediterranean region. The highest rates have been reported in studies conducted in Italian, Spanish, and Bulgarian populations.[19,21,33] Although it was among the most frequently seen in previously conducted studies in Turkey, its frequency was found to be relatively low in our study. Unlike F508del, the variant frequency was found to increase in the northern–southern gradient in the European population.

The Y515X variant, the fourth most frequent variant in our study, is important since it is rare or nonexistent in other European communities and in the Mediterranean basin. It is among the most frequent variants in studies conducted in Turkey[13,16] and the Turkish population in Bulgaria.[19] The related study also reported that the haplotype of this variant was related to the same haplotype in the Bulgarian population, and that these results supported the hypothesis that the variant can have only one origin. This information was not confirmed since our study did not include haplotype analysis.

The G542X variant was the fifth most frequent variant found in the study (3.4%). This variant is regular in the Mediterranean population. Its highest prevalence is in the Spanish population.[21] It can be said that this population is distribution-specific for the Mediterranean geography.[18,23,24,34] The frequency in our study was similar to the relative frequency (2.6-2.4%).

The most apparent difference between our study and the studies in the Mediterranean region and Turkey is that the 2 variants with a frequency of over 1% were not found.[18,21,23,24] These are c.2183AA>G and c.2789+5G>A. Although they were most frequently seen in 3 other studies in Turkey, they were not found in our study.[13,14,16] The reason for this should be the low carrier frequency of these variants in our study area.

Genetic heterogeneity is more frequent in European-origin communities.[17,18,21,23,25] Communities other than those with a European origin (Middle East, Arabia, Far East)[27-31] are weak in terms of *CFTR* alleles, with the Anatolian community as an exception. High heterogeneity should be expected in future Turkish population studies, as shown in the previous studies[13-16] and in the present study. Our study shows the highest heterogeneity reported in Turkey so far. At the same time, while it shows partial similarities with previously reported studies in terms of frequent variants, some variants are reported in literature and databases as rare variants.

Among the 215 patients with homozygous or compound heterozygous mutations, 38 were eligible for elexacaftor/tezacaftor/ivacaftor therapy, 1 of them was eligible for ivacaftor therapy, 17 were eligible for both tezacaftor/ivacaftor and ivacaftor therapy, 26 were eligible for elexacaftor/tezacaftor/ivacaftor, ivacaftor and lumacaftor/ivacaftor therapy, 16 were eligible for tezacaftor/ivacaftor, elexacafto r/tezacaftor/ivacaftor, and lumacaftor/ivacaftor therapy, 3 of them were eligible for elexacaftor/tezacaftor/ivacaftor, ivacaftor, and tezacaftor/ivacaftor therapy, 1 patient was eligible for both lumacaftor/ivacaftor and tezacaftor/ivacaftor therapy, and 1 patient was eligible for ivacaftor and elexacaftor/tezacaftor/ivacaftor therapy. Approximately 47.9 % of the study population was eligible for modulatory drug therapy. This rate was lower than that of the North American and European communities and might be due to the lower incidence of the F508del variant in the study population.

This study has some limitations. Although we analyzed ±50 bp to all coding regions of the *CFTR* gene and exon–intron boundaries, deep intronic *CFTR* mutations that may cause CF could not be analyzed for technical reasons. In addition, in some of the patients in whom we did not find any mutations or in whom only heterozygous pathogenic/likely pathogenic mutations were detected in the *CFTR* gene, we could not perform the MLPA test because they did not continue to patient follow-up. Another limitation of the study is the absence of consanguineous marriage rates.

In conclusion, the present study contributes to a better understanding of the heterogeneous structure of *CFTR* mutations in the Turkish population. Contrary to what is known, we report that the CF disease is observed in a high frequency and broad spectrum outside Europe and especially in Turkey.

Ethics Committee Approval: Ethical committee approval was received from the Ethics Committee of Erciyes University School of Medicine, (2016/355).

Patient Consent for Publication: Informed consent was obtained from all patients.

Data-sharing Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

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