Research Article / Araştırma Makalesi

Epidemiological Evaluation of Next-Generation Sequencing and MLPA Results in Patients with a Presumptive Cystic Fibrosis Diagnosis

Kistik Fibrozis Ön Tanılı Hastaların Yeni Nesil Dizileme ve MLPA Sonuçlarının Epidemiyolojik Değerlendirilmesi

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Abstract: Cystic fibrosis is an autosomal recessive disease caused by pathogenic variants in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. The spectrum and frequencies of CFTR mutations vary among populations. As a result of continuous migration around the world, the frequency of CF variants may change and is still unclear in some geographies. We aimed to define the CFTR gene variants we observed as a result of our single-center experience. This research assessed the outcomes of 353 patients who underwent next-generation sequencing to identify variations in the CFTR gene. Variants classified as clinically uncertain significance, likely pathogenic or pathogenic detected in patients with pre-diagnosis of cystic fibrosis who underwent genetic testing were included in the evaluation. The variants detected in the vast majority of cases were comparable to those found in other populations. However, some variants showed significant differences in allele frequencies when compared to European and Asian populations. Mutations were detected in 25.2% of cases. This dataset revealed that the most common mutations in patients presenting to our center were c.2991G>C, c.2856G>C, c.1545_1546delTA, c.1521_1523 del and c.202A>G. This research presents data on CFTR variations to determine the frequency of CF in the Istanbul province of our nation and to identify additional frequently occurring pathogenic variants that are currently unknown. This kind of research has the potential to facilitate the creation of a localized strategy for maximizing healthcare provision for individuals with CF.
Keywords: Allele Frequency; Preliminary Diagnosis Of CF; CFTR Gene; MLPA; Cystic Fibrosis; Likely Pathogenic Variants, Variants Of Uncertain Significance.

Özet: Kistik fibröz, kistik fibroz transmembran iletkenlik düzenleyici (CFTR) genindeki patojenik varyantların neden olduğu otozomal resesif bir hastalıktır. CFTR mutasyonlarının spektrumu ve frekansları popülasyonlar arasında farklılık gösterir. Dünyada sürekli gerçekleşen göçler neticesinde KF varyantlarının görülme sıklığı değişebilmekte ve bazı coğrafyalarda hala netlik göstermemektedir. Tek merkez deneyimimiz neticesinde gözlemlediğimiz CFTR geni varyantlarını tanımlamayı amaçladık. Bu çalışmada, CFTR genindeki varyantların yeni nesil dizileme yöntemi ile araştırıldığı 353 hastanın sonuçları değerlendirilmiştir. Kistik fibroz ön tanısı almış ve genetik test yaptırmış hastalarda klinik olarak belirsiz öneme sahip, muhtemelen patojenik veya patojenik olarak sınıflandırılan varyantlar değerlendirmeye dahil edildi. Vakaların büyük çoğunluğunda tespit edilen varyantlar diğer popülasyonlarda bulunan varyantlarla karşılaştırılabilirdi. Ancak bazı varyantlar Avrupa ve Asya popülasyonlarıyla karşılaştırıldığında alel frekanslarında önemli farklılıklar gösterdi. Mutasyonlar vakaların %25,2'sinde tespit edildi. Bu veri seti, merkezimize başvuran hastalarda en sık görülen mutasyonların c.2991G>C, c.2856G>C, c.1545_1546delTA, c.1521_1523 del ve c.202A>G olduğunu ortaya koydu. Bu çalışma, ülkemiz İstanbul ili KF prevalansını tahmin etmek ve bilinmeyen ancak sık görülen diğer patojenik varyantları ortaya çıkarmak için CFTR varyantları hakkında bilgi sağlamaktadır. Bunun gibi çalışmalar, KF hastalarının tıbbi bakımını optimize etmek için bölgesel bir yaklaşımın geliştirilmesine yardımcı olabilecektir.
Anahtar Kelimeler: Alel frekansı; CF öntanısı; CFTR geni; MLPA; Kistik fibrozis; Patojenik ve Muhtemel patojenik varyantlar, Klinik Önemi Belirsiz varyant

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1. Introduction

Cystic fibrosis (CF) is a prevalent and serious genetic disorder that mostly affects children of Caucasian descent. It is inherited in an autosomal recessive manner, meaning that both parents must have the defective gene for their kid to develop the illness. CF has a rather high occurrence rate, with around 1 in every 2500-3500 live births being affected [1-3]. The rate of consanguineous marriages in Turkey is around 18.5 percent. Out of all the marriages, 57.8% of them are between first degree cousins. Due to this factor, the prevalence of cystic fibrosis in our nation, along with other autosomal recessive disorders, is greater compared to industrialised countries [4].

The CFTR gene mutations that result in the lack of chloride channel activity are responsible for the genetic disorder known as cystic fibrosis (CF) [5]. Malfunctions in CFTR function are associated with conditions such as severe diarrhoea and the hereditary ailment cystic fibrosis (CF), which is one of the most prevalent genetic illnesses with a limited lifespan in Caucasian populations. Common symptoms include repeated or persistent lung infections, chronic cough, frequent bronchitis episodes, and malnutrition. The presence of CFTR malfunction in the development of hypersensitivity in acute pancreatitis, chronic obstructive pulmonary disease (COPD), and asthma has a significant impact on the morbidity and mortality of patients [2, 5].

Traditionally, the diagnosis of CF has relied on a mix of clinical symptoms and elevated sweat chloride levels. However, with the discovery of the CFTR gene in 1989, genetic analysis has gained significance in the diagnostic process. While sweat testing and genetic analysis have made it easier to diagnose CF in most instances, they are especially crucial for individuals who do not fulfil all the diagnostic criteria. Challenges persist in the identification of instances. These disorders are often seen in people who have a specific illness affecting just one organ, such as the lack of the vas deferens, pancreatitis, or bronchiectasis. This leads to a diagnosis of CFTR-related disorder. Another situation where CF cannot be confirmed or ruled out is when newborn screening is inconclusive [6].

Cystic fibrosis is the result of disease-causing genetic mutations in the CFTR (CFTR/ABCC7: MIM*602421) gene, which may be either homozygous or compound heterozygous. Over 2000 CFTR variations have been documented in scientific literature since the identification of the CFTR gene three decades ago (Cystic Fibrosis Mutation Database http://www.genet.sickkids.on.ca). The most prevalent variant is the F508del variant [7, 8].

The advancement of next generation sequencing technology has greatly contributed to the elucidation of the molecular causes of several illnesses, particularly during the last 20 years [9]. This technique investigates pathogenic mutations in the CF gene that change the structure of the CF protein and impair the functionality of chloride channels. Consequently, the movement of chloride ions, which carry a negative charge, in and out of the cell is inhibited. Around 45% of the pathogenic variants seen in the CF gene are point mutations, whereas 18% are nonsense mutations, 22% are frameshift mutations, and 8% are exonic deletions and insertions. The first stage in the current CFTR variant identification technique is screening for prevalent SNV/INDEL variants. For patients who do not have these genetic variations, we analyse changes in the number of copies of DNA segments (known as copy number changes or CNV) in the regions where exons and introns meet, as well as in the exons themselves. Cystic fibrosis patients who carry the G551D variation in at least one allele of the CFTR gene undergo treatment with the medication Ivacaftor. The Pulmonary Clinical Guidelines Practice Committee highly endorses the use of this medication to enhance pulmonary function and enhance quality of life [7, 10].

Analysing the range of CF variations that are unique to a particular community aids in identifying high-risk populations for cystic fibrosis. This enables earlier interventions to avoid secondary complications, optimise treatment strategies, and eventually improve

the prognosis of the illness. Hence, the objective of this research is to examine CF variations that are unique to our group. In this research, we compared the data from our centre with the frequency of variance in CF patients in the Turkish community.

2. Materials and Method:

The Genetic Diseases Evaluation Center of Ümraniye Education and Research Hospital, University of Health Sciences, conducted a study in which specialist physicians collected family histories of individuals with a preliminary diagnosis of cystic fibrosis and conducted comprehensive examinations.

From 2017 to 2023, 353 patients were referred to the Medical Genetics Department of Ümraniye Education and Research Hospital, Istanbul with a preliminary diagnosis of cystic fibrosis; detailed information, including clinical and family history, was collected. Consent forms were obtained from the patients. The diagnosis of cystic fibrosis was routinely made with clinical features consistent with the cystic fibrosis phenotype and high sweat chloride concentration.

The diagnostic criteria for patients were based on repeated positive sweat chloride tests and typical lung/gastrointestinal disease findings. Patients with a wide range of respiratory diseases or unspecified pancreatic and borderline sweat chloride values were also analyzed. The group studied consisted of 190 male and 163 female patients. The researchers initially screened for several variants identified using next-generation sequencing of the CFTR gene, followed by MLPA analysis.This retrospective investigation was authorised by the Ethics Committee of the University of Health Sciences Ümraniye Training and Research Hospital. Essentially, the laboratory procedure entails amplifying the gene region(s) associated with the illness using polymerase chain reaction (PCR) and then analysing this area using next-generation sequencing technology. We used the CFTR MASTR Assay (Multiplicom) kit for this objective. The sequencing reaction was conducted using the Illumina MiSeq® equipment and appropriate reagent kits.

The Sophia DDM® data analysis platform processed raw data as part of the bioinformatics analysis. Pepper®, an exclusive underlying algorithm developed by Sophia Genetics, conducted alignment and variant identification using the hg19 human genome reference. We used the Ensembl VEP software to conduct variant annotation. This included estimating the impact of each variation on the protein sequence, such as missense or stop gain mutations. Additionally, we determined the frequency of these variants in several populations, including 1000G, ESP, ExAC, and gnomAD. Furthermore, we assessed the potential destructive effect of the variants using prediction techniques such as SIFT and PolyPhen. We have integrated this information. The CNV identification was carried out using Sophia Genetics' MUSKAT® programme. We used the "pick-order" functionality of the VEP programme to arrange transcripts in the following prioritised sequence: "rank, ccds, refseq, mane, tsl, biotype, appris, ensemble, canonical, length". Predicted transcripts were eliminated from consideration. The transcripts chosen using Ensembl VEP may vary from those shown on the Sophia DDM® platform. The variant categorization in question was based on references from the Clinvar database's expert working groups and databases established by Maxwell et al. We evaluated the criteria set out by Maxwell et al. for further variations that were not included in the databases. The parameters used in this assessment were informed by the sequencing and sequence variant categorization recommendations of the American College of Medical Genetics and Genomics (ACMG).
We conducted Multiplex Ligation-Dependent Probe Amplification (MLPA) study on the CFTR gene according to the instructions provided by the manufacturer. This analysis was undertaken since Next-Generation Sequencing (NGS) did not identify any pathogenic or potentially pathogenic allele.

The DNA samples were subjected to denaturation at a temperature of 95°C. Subsequently, the MRCHOLLAND MLPA Probemix-P091 CFTR Probe was applied, and hybridization was allowed to occur at a temperature of 60°C for a duration of 17.5

hours. The ligation phase was then carried out on the following day. During this phase, a ligation enzyme joined together the hybridised probe oligonucleotides. After the completion of the PCR cycle, a PCR primer was used to amplify the probes that were bound. To analyse the amplified DNA products, we utilise capillary electrophoresis on the Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzer. The Coffalyser software version v.140701.0000, developed by MRC-Holland in Amsterdam, Netherlands, was used to examine the data acquired from the device. The programme used CFTR (NM_000492.3) as a reference.

3. Results

The mean age of the patients evaluated was 10.7 years. Women constituted 46% of the total number at the time of application, while men constituted 54% (Figure 1).

Of the 353 individuals who underwent genetic testing, 89 had variants classified as clinically uncertain, likely pathogenic, or pathogenic. These variants were found in heterozygous, homozygous, and compound heterozygous forms. The proportion of patients with homozygous variants was 2.5% (9 patients), compound heterozygous was 3.1% (11 patients), and heterozygous was 19.5% (69 patients). The mean age of patients with any mutation was 9.2 years; 43 of these patients were female and 46 were male.

The first table shows the age, gender, and specific mutations discovered for the 69 individuals who were heterozygous. Of these variants, 28.9% (20 variants) were pathogenic, 24.6% (17 variants) were likely pathogenic, and 46.3% (32 variants) were of uncertain clinical significance. According to the typical autosomal recessive transmission pattern of the disease, 27 of these 69 patients who could not be diagnosed with CF were patients who were brought to our clinic with high sweat test results during infancy. These patients and patients who were not detected to have any mutations were followed up for re-evaluation and further investigation. Patients who did not have mutations as a result of the test were not included in the tables.

The demographic data of the second group, which included 20 individuals with homozygous or compound heterozygous mutations, and the cDNA and protein codes of the variants are summarized in Table 2. In these patients, the homozygous patients had clinical severity and consanguinity between the parents. The variants of the compound heterozygous patients were in trans position.

Of the variations identified in a heterozygous form, 75.3% were missense mutations, 7.2% were splice site mutations, 7.2% were intronic mutations, 5.7% were frameshift mutations, 2.8% were nonsense mutations, and 1.4% were inframe mutations. Out of the 22 alleles found in the 11 individuals with compound heterozygosity, 77.2% were missense mutations, 13.6% were nonsense mutations, 4.5% were frameshift mutations, and 4.5% were inframe mutations.

The frequencies of nonsense, frameshift, and inframe homozygous variations were all equal, with a frequency of 22.2%. Missense variations accounted for the remaining 33.3% of homozygous variants.

The overall percentage of missense variations identified was 68.8% when considering each allele individually.

As shown in graph-1 and figure-2, the most frequently found variants in patients were c.2991G>C, c.2856G>C, c.1545_1546delTA, c.1521_1523 del and c.202A>G, respectively. No particular area of the exon was identified as a hot spot for the observed variations.

During the MLPA study conducted on patients, the use of NGS technology allowed for copy number variation analysis. However, no significant variation was observed in terms of possible large deletions and duplications that may have been overlooked.

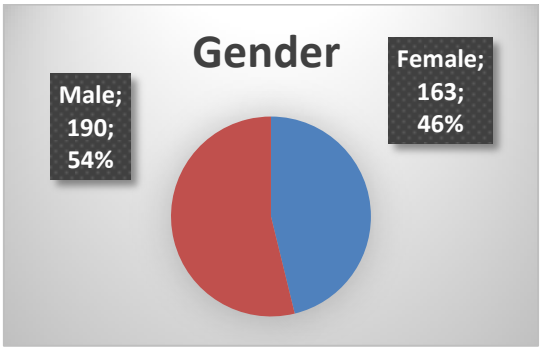

Figure 1. Gender of individuals evaluated with a Preliminary Diagnosis of Cystic Fibrosis

Table 1. Patients with heterozygous variants in the CFTR gene

Patient	Age/Gender	c.DNA	Protein	Zygosity	Change	dbSNP	Classification
1	1/F	c.3909C>G	p.Asn1303Lys	HET.	missense	rs80034486	P
2	1/F	c.2991G>C	p.Leu997phe	HET.	missense	rs1800111	VOUS
3	0/F	c.202A>G	p.Lys68Glu	HET.	missense	rs397508332	VOUS
4	28/M	c.3053 C>T	p.Ala1018Val	HET.	missense	NA	VOUS
5	9/F	c.3389_3402del GTATTATCCTGACT	p.Gly1130Valfs*21	HET.	frameshift	NA	P
6	1/F	c.164+9A>T	NA	HET.	İntronic	rs397508245	VOUS
7	1/M	c.3389G>C	p.G1y1130Ala	HET.	missense	rs397508550	VOUS
8	31/F	c.2973A>G	p.Ile991Met	HET.	missense	rs370181570	VOUS
9	8/F	c.890G>A	p.Arg297Gln	HET.	missense	rs143486492	VOUS
10	1/M	c.1766+1G>A	NA	HET.	splice-site	rs121908748	LP
11	40/M	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
12	7/F	c.2856G>C	p.Met952IIe	HET.	missense	rs151048781	P
13	5/F	c.3025G>A	p.Ala1009Thr	HET.	missense	rs184724618	VOUS
14	7/M	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
15	1/M	c.1897C>A	p.Leu633Ile	HET.	missense	rs397508317	LP
16	3/M	c.2476G>A	p.Glu826Lys	HET.	missense	rs397508381	VOUS
17	1/F	c.2491G>T	p.Glu831*	HET.	nonsense	rs397508387	P
18	5/M	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
19	11/M	c.328G>C	p.Asp110His	HET.	missense	rs113993958	P
20	30/F	c.3154T>G	p.Phe1052Val	HET.	missense	rs150212784	LP
21	2/M	c.2909-15T>G	NA	HET.	intronic	rs397508455	LP
22	2/M	c.3454G>C	p.Asp1152His	HET.	missense	rs75541969	P
23	14/F	c.1516A>G	p.Ile506Val	HET.	missense	rs1800091	VOUS
24	0/F	c.3154T>G	p.Phe1052Val	HET.	missense	rs150212784	LP
25	11/F	c.1545_1546delTA	p.Tyr515*fs*1	HET.	frameshift	rs121908776	P
26	1/M	c.890G>A	p.Arg297Gln	HET.	missense	rs143486492	VOUS
27	7/F	c.2476G>A	p.G1u826Lys	HET.	missense	rs397508381	VOUS
28	15/F	c.902A>G	p.Tyr301Cys	HET.	missense	rs150691494	LP
29	1/M	c.220C>T	p.Arg74Trp	HET.	missense	rs115545701	VOUS

30	0/M	c.3659C>T	p.Thr1220Ile	HET.	missense	rs1800123	VOUS
31	0/F	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
32	10/F	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
33	0/F	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
34	16/F	c.3038C>T	p.Pro1013Leu	HET.	missense	rs193922516	VOUS
35	2/F	c.3472C>T	p.Arg1158*	HET.	missense	rs79850223	P
36	4/M	c.1516A>G	p.Ile506Val	HET.	missense	rs1800091	VOUS
37	5/M	c.1727G>C	p.G1y576Ala	HET.	missense	rs1800098	VOUS
38	22/F	c.2988+1G>A	NA	HET.	splice donor	rs75096551	P
39	26/M	c.2988+1G>A	NA	HET.	splice donor	rs75096551	P
40	1/M	c.2856G>C	p.Met952Ile	HET.	missense	rs151048781	P
41	0/F	c.3038C>T	p.Pro1013Leu	HET.	missense	rs193922516	VOUS
42	1/M	c.2856G>C	p.Met952Ile	HET.	missense	rs151048781	P
43	0/F	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
44	1/F	c.2856G>C	p.Met952Ile	HET.	missense	rs151048781	P
45	0/M	c.3935A>G	p.Asp1312Gly	HET.	missense	rs397508646	VOUS
46	27/F	c.3154T>G	p.Phe1052Val	HET.	missense	rs150212784	LP
47	0/M	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
48	6/M	c.2856G>C	p.Met952Ile	HET.	missense	rs151048781	P
49	2/M	c.2991G>C	p.Leu997Phe	HET.	missense	rs1800111	VOUS
50	1/F	c.4056G>T	p.Gln1352His	HET.	missense	rs113857788	LP
51	1/M	c.3038C>T	p.Pro1013Leu	HET.	missense	rs193922516	VOUS
52	1/M	c.2260G>A	p.VaI1754Met	HET.	missense	rs150157202	VOUS
53	2/F	c.4015C>G	p.Leu1339VaI	HET.	missense	NA	VOUS
54	4/M	c.1521_1523 del	p.Phe508del	HET.	inframe	rs113993960	LP
55	5/M	c.2909-15T>G	NA	HET.	intronic	rs397508455	LP
56	29/M	c.489+3A>G	NA	HET.	splice donor	rs377729736	LP
57	1/F	c.489+3A>G	NA	HET.	splice donor	rs377729737	LP
58	39/M	c.2657+5G>A	NA	HET.	splice site	rs80224560	P
59	33/F	c.3161A>G	p.His1054Arg	HET.	missense	rs1417435640	LP
60	1/M	c.332C>T	p.Pro111Leu	HET.	missense	rs140502196	LP
61	25/M	c.1545_1546delTA	p.Tyr515*	HET.	frameshift	rs121908776	P
62	23/M	c.1545_1546delTA	p.Tyr515*	HET.	frameshift	rs121908776	P
63	0/F	c.2926T>G	p.Phe976Val	HET.	missense	NA	LP
64	24/M	c.2057C>A	p.Ser686Tyr	HET.	missense	rs201444561	VOUS
65	21/F	c.489+3A>G	NA	HET.	intronic	rs377729736	LP
66	8/F	c.3170C>G	p.Thr1057Arg	HET.	missense	NA	LP
67	13/M	c.1545_1546del	p.Tyr515*	HET.	nonsense	rs121908776	P
68	16/M	c.274G>A	p.Glu92Lys	HET.	missense	rs121908751	P
69	27/F	c.2657+5G>A	NA	HET.	splice site	rs80224560	P

(HET.: Heterozygous; P: Pathogenic; LP: Likely Pathogenic; VOUS: Variant of Uncertain Significance)

Table 2. Patients with homozygous/compound heterozygous variants in the CFTR gene

Patient	Age/Gender	c.DNA	Protein	Zygosity	Change	dbSNP	Classification
1	8/F	c.2973A>G	p.Ile991Met	COMP. HET.	missense	rs370181570	VOUS
		c.4228T>C	p.Cys1410Arg		missense	NA	LP
2	1/F	c.3846G>A	p.Trp1282*	HOM.	nonsense	rs77010898	P
3	8/F	c.3503A>G	p.Asp1168Gly	COMP. HET.	missense	rs150326506	LP
		c.1516A>G	p.Ile506VaI		missense	rs1800091	VOUS
4	5/M	c.274G>A	p.Glu92Lys	COMP. HET.	missense	rs121908751	P
		c.266A>G	p.Tyr89Cys		missense	rs397508418	VOUS
5	0/M	c.1545_1546delTA	p.Tyr515*fs*1	HOM.	frameshift	rs121908776	P
6	0/M	c.2195T>G	p.Leu732*	COMP. HET.	nonsense	rs397508350	P
		c.2991G>C	p.Leu997Phe		missense	rs1800111	VOUS
7	0/M	c.1521_1523del	p.Phe508del	HOM.	inframe	rs113993960	LP
8	1/F	c.1397C>G	p.Ser466*	COMP. HET.	nonsense	rs121908805	LP
		c.3209G>A	p.Arg1070Gln		missense	rs78769542	LP
9	0/F	c.202A>G	p.Lys68GIu	HOM.	missense	rs397508332	VOUS
10	1/M	c.1521_1523 del	p.Phe508del	COMP. HET.	inframe	rs113993960	LP
		c.2991G>C	p.Leu997Phe		missense	rs1800111	VOUS
11	1/F	c.2991G>C	p.Leu997Phe	COMP. HET.	missense	rs1800111	VOUS
		c.202A>G	p.Lys68Glu		missense	rs397508332	VOUS
12	54/M	c.274G>A	p.Glu92Lys	COMP. HET.	missense	rs121908751	P
		c.2991G>C	p.Leu997Phe		missense	rs1800111	VOUS
13	0/M	c.2657+5G>A	NA	HOM.	splice site	rs80224560	P
14	39/M	c.1545_1546del	p.(Tyr515*)	COMP. HET.	nonsense	rs121908776	P
		c.2856G>C	p.(Met952Ile)		missense	rs151048781	P
15	1/F	c.1055G>A	p.Arg352Gln	HOM.	missense	rs121908753	LP
16	0/M	c.1043T>A	p.Met348Lys	COMP. HET.	missense	rs142920240	VOUS
		c.3935A>G	p.Asp1312Gly		missense	rs397508646	VOUS
17	42/F	c.3335_3336insGG	p.Ile1112Metfs*10	COMP. HET.	frameshift	NA	LP
		c.2018G>C	p.Gly673Ala		missens	NA	VOUS
18	2/F	c.2051_2052delinsG	p.Lys684Serfs*38	HOM.	frameshift	rs121908799	P
19	15/M	c.2491G>T	p.Glu831*	HOM.	nonsense	rs397508387	P
20	0/M	c.1521_1523del	p.Phe508del	HOM.	inframe	rs113993960	LP

(COMP. HET.: Compound Heterozygous HET.: Heterozygous; P: Pathogenic; LP: Likely Pathogenic; VOUS: Variant of Uncertain Significance)

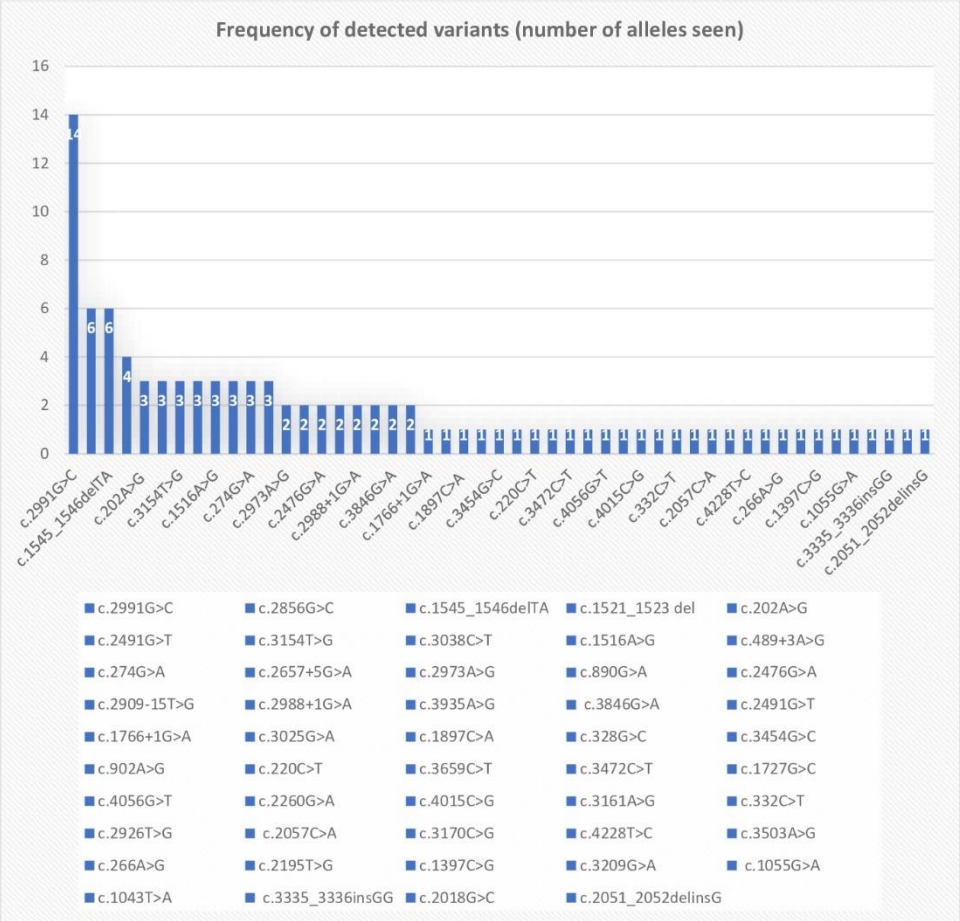

Figure 2: Frequency of detected variants

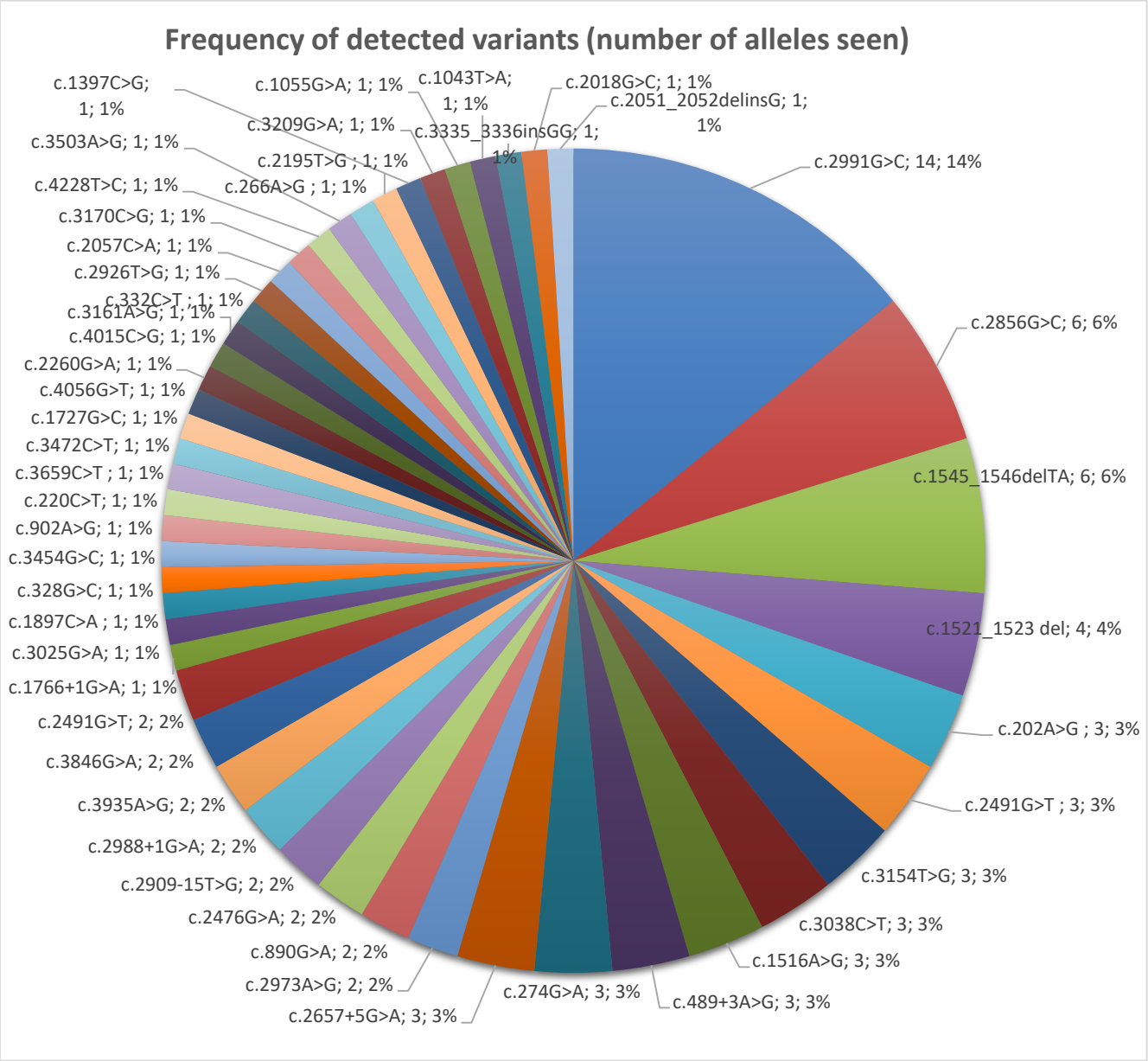

Figure 3. Frequency of detected variants

4. Discussion:

Cystic fibrosis is characterized by recurrent respiratory infections and inadequate pancreatic function. The main manifestations of this illness often include a delay in weight growth and frequent occurrences of lung infections throughout the early stages of infancy. Based on the data shown in this table, respiratory diseases constitute the primary cause of both sickness and mortality. Thanks to the establishment and improvement of standardized treatment and follow-up protocols in recent years, the majority of patients now achieve maturity.

Different age groups may experience different symptoms of this condition, such as bronchiectasis, exocrine pancreatic insufficiency, meconium ileus, hemoptysis, pneumothorax, progressive lung failure, diabetes, cirrhosis, portal hypertension, and osteoporosis [11]. Hence, the comprehensive management of CF patients necessitates a multidisciplinary approach. Several countries have included CF in their newborn screening programme due to its importance of early detection. Since January 1, 2015, Turkey has begun conducting newborn screening for

cystic fibrosis (CF) using the IRT/IRT algorithm [12].

However, some individuals may receive a diagnosis beyond the age of 40. Patients with cystic fibrosis (CF) might appear to healthcare providers at any age with a variety of symptoms caused by genetic variability.

The ΔF508 variation is the most prevalent mutation in the CFTR gene globally. The ΔF508 variation has a declining gradient from the northwest to the southeast in European and Asian nations. While the rate of this phenomenon is about 100% in Scandinavia, it stands at around 13–14% in the western part of our nation and 30% in the eastern part [13, 14].

When we look at the results of our own patients, nine out of 20 individuals who tested positive for any mutation had the homozygous form, which is consistent with the traditional inheritance pattern of cystic fibrosis disease. The remaining 11 patients exhibited variant positivity in both alleles, indicating compound heterozygosity. Among these individuals who exhibited familial segregation, the clinical progression was notably more severe, with the majority of them experiencing symptoms at a younger age.

Despite being classified as carriers for typical CF illness based on the OMIM database, the 69 individuals who had mutations discovered in just one allele (heterozygous) had CF-related characteristics of diverse severity but generally less severe.

The prevailing mutation seen in our patients in both groups was c.2991G>C p.Leu997Phe. This mutation resulted in a missense alteration and was categorized as having an uncertain clinical significance (VOUS). This variant was identified in 14 out of 89 cases. The allele was seen in compound heterozygous form on four occasions and in 10 out of 69 carriers. Homozygous patients did not exhibit any signs of it. This mutation was detected in many people who had compound heterozygosity and were diagnosed with cystic fibrosis, mild cystic fibrosis, or CFTR-related illnesses such as nasal polyposis, bronchiectasis, recurrent pancreatitis, and congenital bilateral absence of the vas deferens. Nevertheless, this particular variation was also detected in other people who are asymptomatic, both in compound heterozygous and homozygous states (15-19). Functional research conducted in vitro reveals that this variation leads to a significant decrease in chloride transport when compared to the wild type. The variation is located outside of the splicing consensus sequence and computational prediction software algorithms do not indicate any harmful impact on splicing. To summarize, given the information provided, it is now not possible to definitively identify the clinical relevance of this mutation. This variant is classified as a variant of uncertain significance (20).

The p.Phe508del variant, which is the prevailing mutation globally, was detected in our patients in two scenarios: homozygotes, compound heterozygotes on a single allele, and carriers. Presently, our group of patients diverges from the prevailing variation results documented in the literature. On an allelic basis, it was seen in 3.6% of our patients. This is much lower in comparison to the 20% prevalence rate discovered in prior research conducted in our nation (4, 9, 12).

The p.Phe508del variation was discovered at the greatest rate in two out of the nine individuals who had the identical alteration in both alleles and were homozygous, which is consistent with the existing literature. The remaining seven homozygous individuals exhibited the following variants: nonsense p.Trp1282*, frameshift p.Tyr515*fs*1, missense p.Lys68GIu, splice site c.2657+5G>A with unknown protein, missense p.Arg352Gln, frameshift p.Lys684Serfs*38, and nonsense p.Glu831*. Out of these homozygous variations, the p.Lys68GIu variant, which had a missense alteration, was categorised as having unknown clinical relevance, but the others were classed as pathogenic and likely pathogenic. The occurrence of this uncertain variation in the overall population is minimal, however, more data is required about its frequency in various ethnic groups and geographical regions. A research investigated the clinical implications of this variation and compound heterozygous states involving distinct CFTR mutations. The

findings indicated that the CF phenotype might differ based on the presence of the variation among other mutations (21).

The most common change seen in a single allele when it is heterozygous is the p.Leu997Phe alteration. This is followed by the p.Met952IIe variant, which is the second most frequent missense variant. The p.Tyr515* variant, characterized by a frameshift mutation, was classified as the third most common. Furthermore, the p.Phe1052Val variation, which is characterized by a missense mutation, was often seen. Seventy-five percent of the detected mutations in carriers were missense variations, which were categorized as having uncertain clinical significance.

The new study, in contrast to previous studies, discovered that there is no significant difference in the age at which symptoms of cystic fibrosis first appear. These aspects should be prioritised by physicians in order to ensure the identification of both known and novel variants detected by NGS technology in patients. Although it may not always be possible to comprehensively document the precise clinical observations and previous sweat tests when assessing patients with cystic fibrosis and similar phenotypes, as well as when conducting genetic tests in medical genetics outpatient clinics, physicians should consider these aspects. It is very important to be better classified and included into the body of literature. These nuances are something that medical professionals who deal with cystic fibrosis (CF) need to pay special attention to, as is customary for disorders that are investigated utilizing a multidisciplinary approach.

It is crucial to acknowledge this findings, particularly in nations like ours, where there is a high prevalence of consanguineous marriages. Cystic fibrosis is acquired by autosomal recessive inheritance. If both parents are proven to have one copy of a CFTR pathogenic variation, each afflicted person has a 25% probability of having a sibling affected, a 50% probability of being a carrier, and a 25% probability of not receiving any of the familial pathogenic variations during pregnancy. If CFTR pathogenic mutations are identified in a family member who is ill, relatives who are at risk may undertake targeted carrier testing. Occasionally, it may be necessary to do prenatal or preimplantation genetic testing for CF [1].
The study aims to identify high-risk groups for cystic fibrosis and implement prompt interventions to avoid secondary problems. Additionally, it seeks to refine treatment protocols and eventually enhance the prognosis and life expectancy of patients. In the future, our discoveries may be more efficiently used in molecular and therapeutic investigations. Optimal outcomes may be attained by conducting studies with bigger cohorts of patients and using a more comprehensive clinical categorization of persons who have received a preliminary diagnosis of cystic fibrosis.

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Ethics

Ethics Committee Approval: The study was approved by University of Health Sciences, İstanbul Ümraniye Training and Research Hospital, Clinical Research Ethical Committee (Decision no: 390, Date: 22.12.2022).
Informed Consent: The authors declared they get consent from the patients.
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Authorship Contributions: "Concept: SC, MHY, ME. Design: SC, MHY. Data Collection or Processing: SC, MHY, ME, HY. Analysis or Interpretation: SC, MHY, ME. Literature Search: SC. Writing: SC."
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