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Research

The Correlation of Cystic Fibrosis Screening Test Results with Ultrasonographically Detected Fetal Anomalies in Prenatal Diagnosis

Prenatal Tanıda Kistik Fibroz Tarama Testi Sonuçlarının Ultrasonografik Olarak Saptanan Fetal Anomalilerle Korelasyonu

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ABSTRACT

Objective: In a multiethnic community, our goal was to assess the applicability of this method. Here we offer a collection of 112 diagnostic prenatal samples for which a comprehensive study of exons, exons/intron boundaries, and major rearrangements has been investigated in prenatal samples of fetuses with suspected cystic fibrosis over the past decade.

Methods: For the CFTR mutation study, 112 prenatal samples (amniotic fluid, chorionic villi, or cultured cells from amniotic fluid or chorionic villi) were brought into our lab. QIAseq Targeted NGS DNA Panel (Qiagen, Hilden, Germany) was performed to analyze the *CFTR* gene (27 exons).

Results: The pathogenic variation NM000492.4(CFTR):c.3454G>C was the most often found (p.Asp1152His), which accounted for 50% of the classic pathogenic CF variants in the study population. Compound heterozygous CFTR pathogenic variations were detected in one of our patients. NM000492.3(CFTR):c.2620-15C>G and NM000492.3(CFTR):c.2756A>G Two variants, one of which was reported as VUS and the other as pathogenic, were detected in a 17-week - old fetus (0.89%). Fetus inherited the NM000492.3(CFTR):c.2756A>G variant from mother and the NM000492.3(CFTR):c.2620-15C>G variant from father. There is an isolated hyperechoic bowel sign at 17 weeks of pregnancy.

Conclusion: In our case series, genetic analyzes suggest that an affected child may be heterozygous for CFTR mutations, compound heterozygous for two clinically significant recessive mutations inherited from healthy carrier parents. Early prenatal genetic testing pretesting and posttesting genetic counseling is crucial in the management of future pregnancies in heterozygous couples which are healthy carriers for CFTR mutations.

Keywords: Cystic fibrosis, genetic testing, CFTR mutations

ÖZ

Amaç: Çok ırklı bir popülasyonda kistik fibrozun prenatal dönemde genetik açıdan analizini ve sonuç olarak varyant sıklığını belirlemeyi amaçladık. Son on yılda kistik fibrozdan şüphelenilen fetüslerin doğum öncesi örneklerinden ekzonlar, ekzonlar/intron sınırları ve yeniden düzenlemeler hakkında kapsamlı bir çalışmanın araştırıldığı 112 doğum öncesi tanı örneğinden oluşan bir hasta popülasyon verisini sunuyoruz.

Gereç ve Yöntem: CFTR mutasyon analizi için 112 prenatal örneğin (amniyotik sıvı, koryonik villus veya amniyotik sıvı veya koryonik villustan kültüre edilmiş hücrelerin) laboratuvarımızda analizleri yapıldı. CFTR genini (27 ekzon) analiz etmek için QIAseq Hedefli NGS DNA Paneli (Qiagen, Hilden, Almanya) kullanılmıştır.

Bulgular: En yaygın olarak tanımlanan patojenik varyantımız, çalışma popülasyonundaki klasik patojenik varyantların %50'sini oluşturan NM000492.4(CFTR):c.3454G>C (p.Asp1152His) idi. Hastalarımızdan birinde bileşik heterozigot CFTR patojenik varyasyonu tespit edildi. NM000492.3(CFTR):c.2620-15C>G ve NM000492.3(CFTR):c.2756A>G olmak üzere, 17 haftalık fetüste sırayla ilki VUS diğeri patojenik olarak bildirilen iki varyant tespit edildi (%0,89). Fetus, NM000492.3(CFTR):c.2756A>G varyantını anneden ve NM000492.3(CFTR):c.2620-15C>G varyantını babadan kalıtım olarak almıştır. Gebeliğin 17. haftasında izole hiperekojen bağırsak bulgusu mevcuttur.

Sonuç: Bizim olgu serimizde, genetik analizler, etkilenen bir çocuğun CFTR mutasyonları için heterozigot olabileceğini, sağlıklı taşıyıcı ebeveynlerden kalıtılan klinik olarak anlamlı iki resesif mutasyon için bileşik heterozigot olabileceğini düşündürmektedir. Erken doğum öncesi genetik testler, ön test ve test sonrası genetik danışmanlık, CFTR mutasyonları için sağlıklı taşıyıcılar olan heterozigot bir çiftte gelecekteki gebeliklerin yönetiminde çok önemlidir.

Anahtar Kelimeler: Kistik fibrozis, genetik test, CFTR mutasyonları

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INTRODUCTION

One of the most prevalent autosomal recessive disorders in Caucasians is cystic fibrosis (CF). The condition is marked by progressive lung damage brought on by chronic infection, pancreatic exocrine insufficiency, male infertility, and excessive sweat chloride levels, although individuals with CF have an average life expectancy of approximately 33 years. Data from registries show that more than 72,000 people worldwide are living with CF (1). The CF gene was located on chromosome 7 in 1989. Phosphorylation of a transport protein activates the gene product's chloride channel activity, cAMP-dependent protein kinase A, referred to as the transmembrane conductance regulator in cystic fibrosis (CFTR). Different aspects of the *CFTR* protein synthesis (mRNA and protein), maturation, and channel function are impacted by pathogenic variations of the *CFTR* gene. Ion flow in epithelial cells of different organs is disturbed by the absence or malfunctioning of the CFTR protein. *CFTR* gene, which is inherited from one parent, has these mutations in both alleles, which causes CF (2).

The phenotype of each patient is the consequence of the combination two (or more) than 2,000 CFTR variations that have been discovered, which are dispersed across the gene structure and have various clinical ramifications. When CF-causing mutations are coupled, severe CF clinical characteristics result. CFTR-related abnormalities, which are single organ disorders, are associated with moderate or mild variations (CFTR-RD: diffuse bronchiectasis, congenital bilateral vas deferens absence in male infertility, pancreatitis). While some people with this condition experience severe pulmonary and/or gastrointestinal symptoms, others only experience moderate symptoms during adolescence and early adulthood. Regarding pancreatic function, genotype and phenotype are closely correlated; however, it has not been established that the discovery of certain CFTR mutations may more accurately predict the severity of pulmonary illness. There is no doubt that modifier genes and environmental variables contribute to this variety of expressions. Recently, CFTR2 research has produced incredibly helpful descriptions of the specific clinical indications and symptoms that might be anticipated with mutations (3).

Women with CF are at risk of fertility. They might signify a delayed beginning of menarche; anovulatory cycles with secondary amenorrhea are common in those with severe lung illness and malnutrition. Sperm entry of the cervical os may also be physically impeded by dehydrated and thicker cervical secretions. However, as more CF patients reach reproductive age, they need to be properly informed about contraception, pregnancy, and any potential elevated hazards related to CF (4).

Although the *CFTR* gene has more than 2,000 known mutations, only around 10% of them results in the illness, as previously indicated. Although the relative frequency of the F508del mutation varies depending on the region, it is found in all populations. Particularly among the white population of Northern European heritage, which makes up between 70 and 75 percent of the CF alleles, F508del is found at the highest frequency (3,5). The significance of the varying rates of mutation detection among different populations is the number of couples whose definitive prenatal diagnosis (PND) becomes possible when both partners are indeed identified as CF carriers.

There are a few CFTR polymorphisms that are linked to various phenotypes, from CF to CFTR-RD or CFTR-RD to the asymptomatic group. Finally, due to their rarity and lack of functional research, there are many clinical spectrum variations that are unknown (6).

Because of the outstanding development achieved regarding molecular biology, PND of genetic abnormalities is rapidly advancing. For couples who are both known to be CF mutation carriers, PND of CF is now advised. Additionally, hyperechogenic bowel and dilatation are ultrasound-detected digestive anomalies in babies that may lead to CF. This occurs primarily in the second trimester of pregnancy. CF affects approximately 1 newborn in 3500, and approximately one in 30 individuals are carriers of CF with marked regional variations (7,8).

When the fetal echogenic bowel is thought to be present, testing the fetus for CF mutations is also beneficial. Data on the incidence of additional CF prenatal diagnostic indicators and the implications of carrier screening recommendations are few (9-11). The American College of Obstetricians and Gynecologists the American College of Medical Genetics (ACMG), and the National Institutes of Health released guidelines for CF screening in the general population in 2001 (12,13). When both parents have a CF mutation, guidelines recommend prenatal testing for that pregnancy as screening is most successful when done before conception. In terms of regional demography, they also advise that laboratories providing CF screening include a minimum of 25 particular mutation sites in their panel in addition to other variants (14-16).

When individuals have a severe mutation in trans, clinical categorization based on how severe the most prevalent accompanying manifestation is: Classical CF with pancreatic insufficiency is predominantly caused by CF mutations, whereas CFTR-RD variations have residual function and

are linked to monosymptomatic illness. Variations of unclear clinical significance (VUS), which have not yet been characterized, and benign variants, which have no clinical repercussions, are present in the milder phenotype (6). In recent times, intermediate variations have been classed as variations with variable clinical outcomes, and they can occasionally cause the more typical but typically pancreatic adequate form of CF, which can have a significant impact on some people's health status and CFTR-related disorders in others (17). It remains a dangerous condition in which a PND is possible when both parents carry the disease for the genes that cause CF, despite significant advancements in treatment techniques in recent years, notably with focused therapy for specific mutations (18). It remains a dangerous condition in which a PND is possible when both parents carry the disease for the genes that cause CF, despite significant advancements in treatment techniques in recent years, notably with focused therapy for specific mutations (9,10,18-26).

The rate of asymptomatic heterozygous carriers of CF is reported to be approximately 1/30 and has long been considered predominate among Caucasians with substantial regional differences (http://www.genet.sickkids.on.ca/cftr) (7,27). Molecular biology techniques have made great strides in genetic analysis and recent years are routinely much quicker now than it was a decade ago. We have the chance in our lab to detect variations according to the geographic history of our patient base.

The objective of this systematic review was to assess the efficacy of prenatal genetic testing for identifying pathogenic CFTR mutations in pregnancies with a high risk of CF. The choice of acceptable endpoints to assess the clinical value of a prenatal diagnostic is a significant assessment difficulty. To help the interpretation of clinical findings, we also address the ethical issues surrounding genetic testing.

Therefore, we chose to perform a full CFTR analysis if CF was suspected on prenatal ultrasound. Our goal was to evaluate the applicability of this method in a multiethnic community. Here we provide a collection of 112 prenatal diagnostic samples for which a thorough analysis of exons, exon/intron boundaries, and significant rearrangements in prenatal samples of babies with suspected CF during the last ten years has been explored. By focusing on the three digestive symptoms of CF, we explain the genotype/phenotype link in a CF fetus and offer fresh information on the regional distribution of CFTR variations. Finally, we recommend that a comprehensive CFTR study be performed in all parents, regardless of their ethnic origin, in the case of a ultrasonography (USG) abnormality such as fetal bowel abnormalities.

METHODS

Patients who underwent chromosomal microarray analysis (CMA) in addition to karyotype analysis were included in the study. Invasive test indications, fetal ultrasonographic screening reports, karyotype analysis, CMA, and next-generation sequencing (NGS) results of the patients were retrospectively obtained from the electronic data system of the genetic unit.

Depending on the results of the fetal USG, only karyotype analysis or both karyotype analysis and CMA are carried out in the initial step. All invasive testing begins with speedy diagnostic procedures like fluorescence *in situ* hybridization or quantitative fluorescence polymerase chain reaction (QF-PCR), and once aneuploidy is found during these quick procedures, CMA is not recommended. Additionally, in cases where structural abnormalities are seen by USG, CMA and karyotype analysis are frequently performed.

In the second step, NGS is recommended based on ultrasonographic findings in patients whose karyotype and CMA results are reported as normal.

Between January 2012 and February 2022, for the CFTR mutation study, 112 prenatal samples (amniotic fluid, chorionic villi, or cultured cells from amniotic fluid or chorionic villi) were supplied to our lab. Dilated intestinal loops, unable to see the fetal gallbladder Non-visualization of the fetal gallbladder, and sonographic intensity more than or equal to that of the surrounding bone during ultrasound were all considered to be Digestive Ultrasound Signs; each mark was either solitary or related with other traits (Table 1).

During a genetic counseling session, the parents provided their informed permission for genetic analysis in line with Turkish law. After a thorough discussion of the research methodology, all individuals provided written authorization with notice. The Ethical Committee of the Trakya University Faculty of Medicine (decision no: 03/11, date: 27.02.2023) and the Declaration of Helsinki were followed for all procedures in the study that included people.

Following the manufacturer's instructions, BioRobot EZ1 equipment (Qiagen Hilden, Germany) was used to extract DNA from samples of amniotic fluid, chorionic villi, or cultivated cells from these tissues. Before building libraries, isolated DNA samples were checked for quantity and quality using a Qubit 2.0 fluorometer (Invitrogen, Life Technologies). Based on GenBank accession NM 000492.3, the variant nomenclature (CFTR). For exonic variations, we

Table 1. Different genotypes finding in the 112 pregnancies analyzed

Fetus genotype	Inh.	Considerations for variant classification	Fetus phenotype
NM_000492.3(CFTR):c.2620-15C>G /NM_000492.3(CFTR): c.2756A>G(p.Tyr919Cys),	Com. Het	VUS Likely pathogenic (LP)	Hyperechogenic bowel
NM_000492.4(CFTR):c.2991G>C (p.Leu997Phe)	Het	VUS	Hyperechogenic bowel
NM_000492.3(CFTR):c.2991G>C (p.Leu997Phe)	Het	VUS	Double bubble, input VSD, PEV
NM_000492.3(CFTR):c.125C>T (p.Ser42Phe)	Het	VUS	Hyperechogenic cardiac focus, PEV
NM_000492.3(CFTR):c.3485G>T (p.R1162L)	Het	VUS	Age risk (double screening test), Grade 1 Hyperechogenic bowel
NM_000492.3(CFTR):c.3659C>T (p.Thr1220Ile)	Het	VUS	Hyperechogenic focus in left ventricle, Grade 1 hyperechoic bowel
NM_000492.3(CFTR):c.2354G>A (p.Arg785Gln), NM_000492.3(CFTR):c.224G>A (p.Arg75Gln)	Com. Het	VUS	Drug use during pregnancy, Grade 1 hyperechoic bowel
NM_000492.4(CFTR):c.3038C>T (p.Pro1013Leu)	Het	VUS	Hyperechoic bowel, triple TT: T21 risk 1/97
NM_000492.3(CFTR):c.202A>G (p.Lys68Glu)	Het	VUS	Hyperechogenic bowel
NM_000492.4(CFTR):c.1519A>G (p.Ile507Val)	Het	VUS	Grade 1 Hyperechogenic bowel
NM_000492.4(CFTR):c.3454G>C (p.Asp1152His)	Het	LP	Hyperechogenic bowel
NM_000492.4(CFTR):c.4333G>A(p.Asp1445Asn)	Het	VUS	Choroid plexus cyst (Bilateral), hyperechogenic bowel
NM_000492.4(CFTR):c.3038C>T, (p.Pro1013Leu)	Het	VUS	Age risk in double TT: 1/308 (high risk), Hyperechogenic bowel (Grade 1)
NM_000492.4(CFTR):c.650A>G, (p.Glu217Gly)	Het	VUS	Age risk in double TT: 1/308 (high risk), hyperechogenic bowel (Grade 1)
NM_000492.3(CFTR): c.1521_1523delCTT (p.F508del)	Het	LP	Hyperechogenic bowel + Bilateral Pelviectasis
NM_000492.4(CFTR):c.2991G>C (p.Leu997Phe)	Het	VUS	Dextrocardia, muscular VSD, Right ectopic kidney, Hyperechogenic bowel
NM_000492.4(CFTR):c.3454G>C (p.Asp1152His)	Het	LP	Age risk:1/168, Grade 1 Hyperechogenic bowel
NM_000492.4(CFTR):c.2991G>C (p.Leu997Phe)	Het	VUS	Hyperechogenic bowel

Inh.: Inhertitance, Het.: Heterozygous, Com.Het.: Compound heterozygous

employed the HGVS nomenclature at the protein level. To examine the *CFTR* gene, the QIAseq Targeted DNA Panel (Qiagen, Hilden, Germany) was used (27 exons). According to the manufacturer's instructions, libraries were set up. With the use of the Qubit dsDNA BR Assay system, the quality of the created libraries was checked (Invitrogen, Carlsbad, CA). Illumina NextSeq550 performed Fastq files (Illumina Inc., San Diego, CA, ABD). According to the QIAseq Targeted DNA Panel procedure, libraries encompassing the target genes were created (Qiagen, Hilden, Germany). Libraries were sequenced on the Illumina NextSeq 550 after target enrichment (Illumina Inc., San Diego, CA, ABD). Variant Call Format file ordering and quality control were performed using QCI analysis (Qiagen, Hilden, Germany). Using Ingenuity software, variation analysis was carried out (Qiagen, Hilden, Germany).

Primer sets were created for all required areas to execute Sanger sequencing on an ABI 3130 (Applied Biosystems, USA) capillary electrophoresis machine and validate the variations and segregation analyses.

The classification of all the variations was done in accordance with ACMG-2015 (28) rules, and the descriptions of the variants were done in accordance with the Human Genome Variation Society (29) recommendations (30). The following bioinformatics tools were used to evaluate the variants once they had been annotated by wANNOVAR: SIFT (http://sift.jcvi.org/), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), ClinVar Miner (https://clinvarminer.genetics.utah.edu/), CADD score (https://cadd.gs.washington.edu/snv), CFTR2 (https://cftr2.org/), CYSMA (https://cftr.iurc.montp.inserm.

fr/cysma/), Human Splicing Finder3.1 (HSF3.0) (http://www.umd.be/HSF/).

Statistical Analysis

The SPSS software version 21 (SPSS Inc., Chicago, IL, USA) package program was used to analyze the data. The format for descriptive data was number (%), mean standard deviation. To examine the distribution of continuous variables, the Kolmogorov-Smirnov test was performed. Continuous variables were compared using Student's t-test because they were regularly distributed. To ascertain the statistical significance of the categorical variables, the chi-square test was used. Fetal structural deformities related to pathogenic copy number variants (pCNVs) were examined by univariate analysis, and CMA results of fetuses with normal karyotypes were categorized as pCNVs and benign copy number variations/variants of unknown clinical significance. Possible fetal structural abnormalities linked to pCNVs discovered in univariate analysis were considered in logistic regression analysis. The threshold for statistical significance was set at $p<0.05$.

RESULTS

All 112 fetuses were screened for CF throughout the research period, three of which were twin pregnancies that had an echogenic bowel on a thorough ultrasound inspection. The mean gestational week of our patients was determined to be 19+6. Hyperechoic bowel is a common USG finding in all pregnancies. Table 1 provides an overview of the distribution of detected CF variations. The most frequently found pathogenic variation was NM000492.4(CFTR):c.3454G>C (p. Asp1152His), which was responsible for 50% of the research population's classic pathogenic CF variants. Compound heterozygous CFTR pathogenic variations were detected in one of our patients. NM000492.3(CFTR):c.2620-15C>G ve NM000492.3(CFTR):c.2756A>G two variants, one of which was reported as VUS and the other as pathogenic, were detected in a 17-week-old fetus (0.89%). Fetus inherited the NM000492.3(CFTR):c.2756A>G variant from mother and the NM000492.3(CFTR):c.2620-15C>G variant from father. There is an isolated hyperechoic bowel sign at 17 weeks of pregnancy.

Among the variants evaluated as VUS, the most detected variation was NM000492.3(CFTR):c.2991G>C (p. Leu997Phe). This VUS variant, which was detected most frequently, was encountered in the amniocentesis material of five patients.

Variations evaluated as VUS were detected in 12 patients. The total number of detected VUS was determined as 17. All of these variants, which were evaluated as VUS, had data toward likely pathogenicity according to the information obtained from different databases (ClinVar, VarSome, Franklin, HGMD-DM). All of these VUS variants detected in patients were heterozygous, except for one patient. A compound heterozygous VUS pattern was observed in one of our patients, including NM000492.3(CFTR):c.2354G>A (p. Arg785Gln) and NM000492.3(CFTR):c.224G>A (p. Arg75Gln). In the USG scan of this patient, there was a history of Grade I hyperechoic bowel and drug use during pregnancy. Except for all detected variations, only one NM000492.3(CFTR):c.1666A>g(p. Ile566Val) variation, which was reported as benign, was detected in only one of our patients (Table 1).

DISCUSSION

The risk of CF diagnosis associated with gastrointestinal abnormalities on ultrasonographic scans varies based on research from 0.5% to 9% (24,25). Since the *CFTR* gene was identified in 1989, technological developments have allowed for a deeper understanding of this gene. Current applications for CFTR analysis in the context of heterozygote co-analysis or ultrasonographic the possibility of CF serves to identify the most common mutations by geographic origin. However, these procedures have not been modified for people descended from broad areas where mutations are yet unknown. A thorough CFTR investigation, however, also entails finding uncommon variations that could be challenging to interpret. Most of the rare variants we discovered were categorized in our research as having uncertain therapeutic significance (77%). VUS should not be used in clinical decision-making, according to ACMG recommendations. However, regular variant updates are recommended if information has changed in any VUS. It is likely that under these situations, measuring the amniotic fluid containing fetal digestive enzymes may be beneficial. However, for this invasive surgery to be effective, it must be done before 22 weeks of gestation because digestive disorders are typically noticed beyond this point. Interpretation of VUS is particularly complex in the case of ultrasound intestinal abnormalities as the specificity of fetal findings is not always significant: the CFTR variants identified are not necessarily associated with CF.

We believe that the present study is important because it includes patients with positive prenatal 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 Northwest Anatolian Region of Türkiye.

In summary, we present the first comprehensive study showing the distribution of *CFTR* gene variation in the Northwest Anatolian Region of Türkiye. In this study, the mutation distribution was highly heterogeneous, and we believe that analysis of the entire *CFTR* gene is necessary and will increase the diagnosis rates for the Turkish population.

The analytical step for thorough CFTR analysis is available to many laboratories in the context of mass sequencing that is now available. However, although bioinformatics databases and tools have been created to support correct clinical interpretation, proper molecular diagnosis requires thorough understanding of variations, their penetrations, and known complex alleles (31,32). Experience and expertize in this area are especially crucial when dealing with extremely uncommon or unidentified varieties where clinical observations are insufficient and in ultrasound scans where the phenotype is not specific. In this study, the incidence of CF was close to that reported from previous studies as 0.9% to 2.3% (0.89%) (23,33,34). Inheritance patterns for the CFTR variation of parents in prenatally diagnosed pregnancies were unknown. Maternal and paternal genotypes were determined by retrospective analysis, especially for our patient in whom we detected only compound heterozygous variants.

The NM000492.3(CFTR):c.2991G>C (p. Leu997Phe) variation, which we detected the most among the variations we evaluated as VUS, has been reported to increase the susceptibility to pancreatic ductular obstruction in patients with CF (Table 2) (35).

The transmembrane domain of the encoded protein sequence, ABC transporter type 1, experiences a non-conservative amino acid mutation because of CFTR c.2991G>C (p. Leu997Phe). A negative impact of variation on protein function was identified in four out of five distinct *in silico* vehicles. According to these findings, it is unlikely that the mutation would cause CF or any of the illness phenotypes that are related to it and have a variable expression. Most databases such as CFTR2 state that this variant does not cause disease (3). In addition, functional studies have also reported that the variant may have a role in organ bicarbonate permeability, in which CFTR is used for bicarbonate secretion and significantly reduces chloride conductance; however, the effect of these functional defects in vivo is unknown (36,37). However, evidence from the literature combined with allele frequency data from public databases when available was insufficient to determine whether this variant causes disease. This mutation is therefore categorized as a variant of uncertain significance.

The NM000492.4(CFTR):c.3454G>C (p. Asp1152His) variant is the variant evaluated as pathogenic. In our cases, this mutation was discovered to be heterozygous. This sequence change converts aspartic acid to the amino acid histidine at codon 1152 of the CFTR protein (p. Asp1152His). The residue

Table 2. **CFTR variants identified in the 112 patients**

Location	cDNA change	Protein change	dbSNP138	HGMD	Variant type	gnomAD Popmax Filtering AF (95% confidence interval)
Intron 15	c.2620-15C>G	p.(?)	rs139379077	CS004690	Definitely pathogenic	0.002618
Exon 17	c.2756A>G	p.(Y919C)	rs397508430	-	Uncertain significance	0.0002506
Exon 19	c.2991G>C	p.(L997F)	rs1800111	CM920171	Uncertain significance	0.003646
Exon 2	c.125C>T	p.(S42F)	rs143456784	-	Uncertain significance	0.0001750
Exon 22	c.3485G>T	p.(R1162L)	rs1800120	-	Uncertain significance	0.001111
Exon 22	c.3659C>T	p.(T1220I)	rs1800123		Uncertain significance	0.0004605
Exon 14	c.2354G>A	p.(R785Q)	rs141880790		Uncertain significance	0.00001439
Exon 03	c.224G>A	p.(R75Q)	rs1800076	CM980331	Uncertain significance	0.02769
Exon 19	c.3038C>T	p.(P1013L)	rs193922516		Uncertain significance	0.0001298
Exon 03	c.202A>G	p.(K68E)	rs397508332	CM972935	Uncertain significance	0.0005404
Exon 11	c.1519A>G	p.(I507V)	rs1801178		Uncertain significance	0.00008816
Exon 21	c.3454G>C	p.(D1152H)	rs75541969		Likely pathogenic	0.0006254
Exon 27	c.4333G>A	p.(D1445N)	rs148783445	CM962488	Uncertain significance	0.001486
Exon 06	c.650A>G	p.(E217G)	rs121909046	CM972939	Uncertain significance	0.008363
Exon 11	c.1521_1523del	p.(F508del)	rs113993960	CD890142	Likely pathogenic	-

of aspartic acid remains moderately unchanged, and there is a moderate physicochemical difference between aspartic acid and histidine. This variant is available in population databases (rs75541969, ExAC 0.05%). Although this variant can be reported in most individuals affected by congenital absence of the vas deferens, chronic pancreatitis (CP), atypical CF, and bronchiectasis, it has rarely been reported in individuals with classical CF. Experimental studies have shown that this missense change does not affect protein stability or maturation but has a negative effect on the function of CFTR in cell culture. Because of these results, the variant has been classified as pathogenic (38).

A 17-week-old fetus with isolated hyperechogenic bowel carries 2 variants detected as compounds, NM000492.3(CFTR):c.2620-15C>G, NM000492.3(CFTR):c.2756A>G. The NM000492.3(CFTR):c.2620-15C>G variant has entries in different databases as benign and VUS conflict criteria [Conflicting interpretations of pathogenicity, Uncertain significance (2); Benign (2); Likely benign (2) (Last evaluated: Sep 5, 2022)] (39,40).

The encoded protein sequence has a nonconservative amino acid change in the ABC transporter type 1 transmembrane domain (IPR011527) caused by the CFTR mutation c.2756A>G (p. Tyr919Cys). All five in-silicon vehicles showed that the mutation had a negative impact on protein function. The variation was discovered in the control chromosome 251398 with a frequency of 7.6e-05 (gnomAD). This ratio is not significantly higher than would be expected for a pathogenic variant causing CF in CFTR (7.6e-05 vs 0.013) and does not allow conclusions about the significance of the variant. c.2756A>G has been reported in the literature in individuals affected by CF, however, no second mutation was identified in these patients, and at least one of these individuals had a truncated mutation on the same allele. These investigations do not offer any firm conclusions about the link between variation and CF. These and other transmembrane 8 variations have an impact on channel transition according to at least one study, but the data is insufficient to draw firm conclusions about how the variants affect CFTR protein function (41).

In another compound heterozygous patient, 2 VUS (NM000492.3(CFTR):c.2354G>A (p. Arg785Gln) and NM000492.3(CFTR):c.224G>A (p. Arg75Gln)) was inherited as heterozygous. NM000492.3(CFTR):c.2354G>A (p. Arg785Gln), in this sequence change, it replaces arginine with glutamine at codon 785 of the CFTR protein (p. Arg785Gln). The arginine residue is poorly conserved and there is a small physicochemical difference between arginine and glutamine. This variation is available in population databases (rs141880790, ExAC 0.01%). This variation has been reported in an individual suffering from pancreatitis (PMID:17003641). This variation has a record in ClinVar (Variation ID: 573871). The following outcomes were obtained from algorithms designed to forecast how missense mutations may affect protein structure and function: Align-GVGD is "Class C0," PolyPhen-2 is "Benign," and SIFT is "Tolerated."

In the presence of an SPINK1 mutation, the CFTR p. R75Q variation has been linked to an increased risk of pancreatitis and has also been observed in more CP patients in several other studies. Researchers have discovered that CFTR p. R75Q is processed and developed in cells like CFTR WT, and physiological experiments demonstrate no gate or Cl- channel malfunction, although this variation has previously been examined for producing CF. Such data strengthen the argument that the sequence variation pR75Q does not affect the autosomal recessive condition CF. However, repeated reporting of CFTR p. R75Q as a CFTR variant in some patients with atypical CF and CF-related disorders, such as sarcoidosis, chronic obstructive pulmonary disease, and CP, suggests that normal function is somewhat impaired. Schneider et al. (42) showed for the first time that p. R75Q changes bicarbonate but not chloride conductivity. This prompts the development of the CP model, which identifies CFTR as the bicarbonate channel in the cells of the pancreatic duct and predicts that CFTR mutations that impair bicarbonate conductance will significantly raise the risk of developing pancreatic disease by completely disrupting protein synthesis or altering duct characteristics. This discovery also raises the possibility that there are other CFTR mutations that particularly affect bicarbonate conductivity and are risk factors for CP but not CF. According to data from Schneider et al. (42), heterozygous CFTR p. R75Q or CFTR p. F508del variants are inconsequential in the presence of an SPINK1 WT, while multiplying CP denotes an elevated risk. The relatively frequent CFTR variation p. R75Q corresponds to an SPINK1 mutant. This suggests that SPINK1 variations should also be investigated in patients with suspected CP (42).

The F508del variant was detected in one patient in our patient group. This is less than the rate reported in the literature for the variant. When the studies were investigated; Heltshe et al. (43). According to the US Cystic Fibrosis Foundation Patient Registry, the pregnancy rate in women with the F508del heterozygous variant has been reported as 31-34%. The second important point; in individuals carrying the F508del heterozygous variant, the rate of achieving a live birth is between 72-74%. These two conditions can be seen as the reason for the low F508del variant rate in our study (43,44).

Because our patient group was evaluated over a ten-year period. At the time of writing this article, current variant statuses have been checked and final classifications have been reported.

CONCLUSION

In our case series, genetic analyzes suggest that an affected child may be heterozygous for CFTR mutations, compound heterozygous for two clinically significant recessive mutations inherited from healthy carrier parents. Early prenatal genetic testing pretesting and posttesting genetic counseling is crucial in the management of future pregnancies in heterozygous couples which are healthy carriers for CFTR mutations. Less CF-affected babies are born because of CF testing. It is difficult to determine if this indicates that the test is worthwhile because patients may not value maternal or fetal health outcomes in their primary motivations, whether psychologically or otherwise. It might be argued that rather than health, the value of testing should be evaluated in terms of increasing patient autonomy.

In view of these findings, CF carrier screening should be made available to all couples who have a confirmed CF family history. This applies to all spouses of people with CF and to all Caucasian couples who are trying to get pregnant or seeking prenatal treatment and who are of European or Ashkenazi Jewish origin. Screening should ideally occur either before conception or during the first or early second trimester. Patients from other ethnic and racial groups should be informed about screening for CF. In addition, those in lower risk categories should be able to obtain counseling and screening upon need. Based on family history and the identification of the spouses' racial and ethnic backgrounds at the time of the initial research, the doctor should select couples who should be examined. When necessary, the obstetrician should perform CF screening and may decide to offer or request pretest counseling. It takes specialized understanding of elements and calculation of genetic risk to provide post-test counseling for couples with positive/negative, positive/untested, or positive/positive screening findings, CF severity, prognosis, treatment choices, etc. In this situation, a geneticist or physician with specialized knowledge in CF testing must be consulted. If there is a family history of CF, if carriers have been found with CF mutations that may be linked to congenital absence of the vas deferens in male offspring, if an affected adult or affected fetus has been identified, or if any of these situations apply, referral to a geneticist or person with specific expertize in CF testing should also be considered.

ETHICS

Ethics Committee Approval: The institutional review committee (Trakya University Faculty of Medicine, TUMF Scientific Research Ethics Committee Directive TUTF-BAEK, decision no: 03/11, date: 27.02.2023) approved the study.

Informed Consent: During a genetic counseling session, the parents provided their informed permission for genetic analysis in line with Turkish law.

Authorship Contributions

Surgical and Medical Practices: S.Y., H.G., Concept: E.A., H.G., Design: E.A., Data Collection or Processing: E.İ.A., H.G., Analysis or Interpretation: E.İ.A., S.D., Literature Search: E.İ.A., S.D., S.Y., Writing: E.İ.A.

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