

DETERMINATION OF CYSTIC FIBROSIS MUTATION FREQUENCY IN PRETERM AND TERM NEONATES WITH RESPIRATORY TRACT PROBLEMS

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ABSTRACT

Cystic fibrosis (CF) is an autosomal recessive disease. The genetic transition occurs with CF transmembrane conductance regulator (*CFTR*) gene mutation. We aimed to determine the frequency of CF mutations and also new mutations in the *CFTR* gene in neonates with respiratory distress. Newborn babies hospitalized due to respiratory distress were included in the patient group. The control group consisted of infants who had no respiratory distress. The *CFTR* genes of both groups were analyzed using polymerase chain reaction (PCR) and restriction fragment length polymorphism (RFLP) methods. A total of 40 patients (20 in the patient group and 20 in the control group) were evaluated. The *CFTR* gene analysis was normal in 16 neonates in the patient group, whereas in others: A46D (c.137C>A) ($n = 1$), D1312G (c.3935A>G) ($n = 1$), R117H (c.350G>A) ($n = 1$), S1426P (c.4276T>C) ($n = 1$) heterozygotes were detected; *CFTR* gene analysis was normal at 14 neonates in the control group, whereas in others: E1228G (c.3683A>G) ($n = 1$), E217G (c.650A>G) ($n = 1$), E632TfsX9 (c1894_1895delAG) ($n = 1$), I807M (c.2421A>G) ($n = 2$), S573F (c.1718C>T) ($n = 1$) heterozygotes were detected. There was no significant difference in the patient and control groups' *CFTR* gene analysis ($p = 0.340$). This study demonstrates the importance of *CFTR* gene analysis in asymptomatic newborn infants for follow-up and early diagnosis of *CFTR*-related disorders. In this

study, a c.1894_1895delAG (E632TfsX9) heterozygous mutation detected in the *CFTR* gene in an asymptomatic newborn infant, was first encountered in the literature.

Keywords: Cystic fibrosis (CF); Cystic fibrosis trans-membrane conductance regulator (*CFTR*) gene; Mutation; Newborn.

INTRODUCTION

Cystic fibrosis (CF) is an autosomal recessive genetic disease that affects all systems with epithelial surfaces, including the lungs, pancreas, glands that secrete mucus in the intestines, liver and sweat glands. It has a high mortality and morbidity, and develops as a result of the mutation of CF transmembrane conductance regulator (*CFTR*) gene [1,2]. Cystic fibrosis is characterized by recurrent lung infections, exocrine pancreatic insufficiency, and elevated levels of sweat chloride. It may present with mild or atypical symptoms. Therefore, clinicians should be aware of the possibility of CF, even if there are only a few of these features. Diagnosis of CF is based on the finding of genetic and/or functional abnormalities in the *CFTR* gene. Functional criteria and genetic analysis are required in cases with mild clinical symptoms or normal or borderline sweat chloride levels. A *CFTR*-related disorder is a clinical disease restricted to a single organ system with *CFTR* dys-functions and without complete genetic or functional criteria. Isolated obstructive azoospermia, chronic sinusitis, chronic pancreatitis can be seen in *CFTR*-related disorder. Patients should be followed-up to prevent the emergence of new disease symptoms and to provide genetic counseling [3,4]. Metabolic syndrome associated with *CFTR* is a temporary diagnosis and requires follow-up. The patient is usually an asymptomatic infant with positive neonatal screening results. In some cases, although the disease is usually mild, the clinical characteristics of CF

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may continue to develop along with a positive sweat test. Symptoms of *CFTR*-related disease, including isolated male infertility, may develop or the disease may remain completely asymptomatic. The onset of clinical symptoms varies widely due to differences in the *CFTR* genotype and other individual factors. Even in the absence of symptoms, respiratory function abnormalities can often be detected [5]. In this study, we aimed to determine the frequency of CF mutations, and also new mutations on the *CFTR* gene in neonates with respiratory distress.

MATERIALS AND METHODS

Patients. Term and preterm newborn babies hospitalized due to respiratory distress in the Neonatal Intensive Care Unit at Manisa Celal Bayar University Hospital, Manisa, Turkey, between May 2017 and January 2018, were included in this study. The control group consisted of term and preterm infants who had no respiratory distress. The study was approved by the Manisa Celal Bayar University Medical School Ethics Review Board. Infants with congenital anatomic respiratory disease, congenital heart disease, perinatal asphyxia, meconium aspiration syndrome, and respiratory distress due to metabolic and hematological causes, were not included in the study. Infants with respiratory distress due to cesarean section (C-section) were also excluded from the study.

Genetic Analysis. DNA isolation from blood samples obtained from each infant was performed at the Medical Genetics Laboratory, Ege University Medical Faculty, Izmir, Turkey. DNA amplification was performed using thermal cycler (GeneAmp PCR System 9600; Applied Biosystems, Foster City, CA, USA). A Sanger sequence [capillary electrophoresis (CE)] platform was used for sequence analysis. All exons of the *CFTR* gene were reproduced and purification procedures were carried out. Related products were run on a CE device. Each sample was analyzed using the sequencing analysis software program (<http://technelysium.com.au/wp/chromas/>). Using the Chromas program, a comparison was made with the normal sequence at the <http://www.ncbi.nlm.gov> website, and the detected mutations were identified.

Statistical Analyses. Statistical analysis was performed using the Statistical Package for the Social Sciences (SPSS) version 18.0 for Windows program (<https://www.ibm.com/spss/statistics>). The *t*-test, χ^2 test, logistic regression, nominal regression and Cohen's κ coefficient methods were used to evaluate the data. Odds ratio (OR) and 95% confidence interval (95% CI) values were calculated and genotype and allele differences were compared using the χ^2 test. As there was no significant difference between the patient group with respiratory distress and

the control group without respiratory distress in terms of the genotypes, allele frequency was not analyzed by the Hardy-Weinberg equivalence test. The relationship of genotype and allele groups with clinical characteristics was tested with the Pearson correlation test. A *p* value of <0.05 was considered to be statistically significant.

RESULTS

Forty babies, 20 with respiratory distress and 20 babies without respiratory distress, were included in the study. Demographic characteristics of the infants revealed that the mean gestational age was 36.22 ± 3.89 weeks. Twenty-one of the infants were born preterm and 19 were born at term. Twenty-two were girls and 18 were boys. The mean birth weight was 2775.5 ± 952.9 g, the mean birth height was 47.07 ± 5.45 cm and the mean head circumference was 33.02 ± 3.2 cm. Demographic characteristics of the babies in the control and patients groups are shown in Table 1. In the patient group, the mean gestational age was 34.25 ± 4.65 weeks; 14 infants were born preterm and six were born at term. In the control group, the mean gestational age was 38.2 ± 1.15 weeks; seven of them were born preterm and 13 were born at term. The prenatal histories taken from the patient and control groups revealed that the maternal age was similar in both groups. No significant difference was found between the patient and the control groups with regard to incidence of placental abruption, placenta previa, chorioamnionitis, gestational diabetes, diabetes mellitus, preeclampsia and eclampsia, and prenatal steroid administration.

The pathologies and treatments of infants admitted to the neonatal intensive care unit in the patient group are listed in Table 2. The mean duration of stay on mechanical ventilation in the patient group was 2.25 ± 4.49 days, and the mean duration of oxygen therapy was 13.35 ± 20.71 days. The mean length of hospital stay in the patient group was 24.65 ± 21.32 days. Of the 20 infants in the control group, 14 were admitted to the neonatal intensive care unit due to hyperbilirubinemia, one for urinary tract infection, four for hypernatremic dehydration, and one for intrauterine growth restriction. The mean length of hospital stay in the control group was 4.75 ± 1.97 days.

When the clinical symptoms and signs of respiratory distress in 20 infants in the patient group were taken into account, four had a cough, five were wheezing, 10 were grunting, three had apnea, 19 had tachypnea, 15 had retraction, and 12 had findings in lung auscultation (rales/ rhonchi) (Table 3). When the laboratory findings of 20 infants in the patient group were examined, five of them had a positive C-reactive protein (CRP). The mean CRP value was 0.59 ± 1.14 mg/dl. Ten of the infants had

Table 1. Demographic characteristics of the control group and the patient group.

Parameters	Control Group (n = 20)	Patient Group (n = 20)	p Value
Gender:			
females	11	11	1.000
males	9	9	
Mean birth weight (g) ± SD (min-max)	3278.00 ± 585.62 (1970.00-4180.00)	2273.00 ± 994.63 (610.00-3850.00)	0.297
Mean birth height (cm) ± SD (min-max)	49.95 ± 1.80 (45.00-53.00)	44.20 ± 6.37 (33.00-51.00)	0.186
Mean head circumference (cm) ± SD (min-max)	34.55 ± 1.15 (31.50-37.00)	31.50 ± 3.84 (23.00-37.00)	0.134
Delivery type:			
NspD	5	0	0.017
C-section	15	20	
Gestational age (weeks)	38.20 ± 1.16 (37.00-41.00)	35.25 ± 4.65 (26.00-40.00)	0.062
Intrauterine growth retardation (n)	2	4	0.091
Median Apgar score (1st min.) (min-max)	8 (7-9)	7 (5-8)	0.009
Median Apgar score (5th min.) (min-max)	9 (9-10)	8 (7-9)	0.001

n: number; NspD: normal spontaneous delivery; C-section: cesarean section.

Table 2. Pathologies and treatments of infants in the patient group.

Parameters	Patient Group (n = 20)
Respiratory distress syndrome (RDS) (n)	9
Congenital pneumonia (n)	2
Pneumonia (n)	4
Transient tachypnea of newborn (n)	5
Surfactant treatment (n)	5
Mechanical ventilation treatment (n)	11
Mean duration of mechanical ventilation (days)	2.25 ± 4.49 (1.00-20.00)
nCPAP treatment (n)	13
Mean duration of nCPAP (days)	9.10 ± 15.43 (1.00-51.00)
Oxygen treatment (n)	17
Mean duration of oxygen therapy (days)	13.35 ± 20.71 (1.00-66.00)
Bronchopulmonary dysplasia (BPD) (n)	3
Steroid treatment (n):	
IV	0
inhaled	5
Patent ductus arteriosus (n)	4
Pulmonary hypertension (n)	1
Sepsis (n):	
clinical	18
proven	2
Intraventricular hemorrhage (n)	1
Mean length of stay in hospital (days)	24.65 ± 21.32 (9.00-71.00)

n: number; nCPAP: nasal continuous positive airway pressure; IV: intravenous.

leuko-cytosis with a mean leukocyte value of $11651 \pm 5109.40 \text{ mm}^3$. Two of the infants had thrombocytopenia, the average platelet count was $258.45 \pm 11.6402.42 \text{ mm}^3$. Five infants had metabolic acidosis. The mean values of

the parameters were as follows: pH 7.31 ± 0.11 , bicarbonate (HCO_3) $21.44 \pm 3.34 \text{ mmol/L}$, base deficit -3.36 ± 4.42 , partial pressure of carbon dioxide (PCO_2) $47.61 \pm 13.16 \text{ mmHg}$ and partial pressure of oxygen (pO_2) 57.39

± 40.56 mmHg. With regard to the radiological findings of 20 infants in the patient group, 14 had lung infiltration and six had a ground-glass appearance compatible with respiratory distress syndrome (RDS).

In the control group, *CFTR* gene analysis was normal in 14 infants, E1228G (c.3683A>G) heterozygosity was detected in one, E217G (c.650A>G) heterozygosity in one, E632TfsX9 (c.1894_1895delAG) heterozygosity in one, I807M (c.2421A>G) heterozygosity in two, and S573F (c.1718C>T) heterozygosity in one (Table 4). Of the six patients in the control group with heterozygous mutations, five were hospitalized for hyperbilirubinemia and one for hypernatremic dehydration. The *CFTR* gene analysis was normal in 16 infants with respiratory distress in the patient group, A46D (c.137C>A) heterozygosity was detected in one, D1312G (c.3935A>G) heterozygosity in one, R117H (c.350G>A) heterozygosity one and S1426p (c.4276T>C)

heterozygosity in one (Table 5). Heterozygous mutations on the *CFTR* gene were detected in 10 of 40 infants in the patient and control groups included in the study. The symptoms and diagnoses of these patients are presented in Table 6. There was no significant difference in *CFTR* gene analysis in the control and the patient groups ($p = 0.340$). In the patient group, the mean hospitalization time of 20 symptomatic babies was 24.65 ± 21.32 days, while the mean hospitalization time of four babies with symptomatic and heterozygous mutations was 27.50 ± 26.33 days, no statistically significant difference was found ($p = 0.128$).

Table 3. Symptoms and signs of respiratory distress in the patient group.

Symptoms	Patient Group (n=20)
Cough	4
Wheezing	5
Grunting	10
Apnea	3
Tachpnea	19
Retraction	15
Rales/rhonchi	12
Prolonged expiration	1
Tachycardia	2
Oxygen requirement	17
Hypotonia	6

Table 4. *CFTR* gene analysis of infants in the control group.

<i>CFTR</i> Gene Analysis	Control Group (n=20)
Normal (n)	14
E1228G (C.3683A>G) het. (n)	1
E217G (c.3683A>G) het. (n)	1
E632TfsX9 (c.1894_1895delAG) het. (n)	1
I807M (c.2421A>G) het. (n)	2
S573F (c.1718C>T) het. (n)	1

CFTR: cystic fibrosis transmembrane conductance regulator gene; het.: heterozygous; n: number.

Table 5. *CFTR* gene analysis of infants in the patient group.

<i>CFTR</i> Gene Analysis	Patient Group (n=20)
Normal (n)	16
A46D (c.137C>A) het. (n)	1
D1312G (c.3935A>G) het. (n)	1
R117H (c.350G>A) het. (n)	1
S1426P (c.4276T>C) het. (n)	1

CFTR: cystic fibrosis transmembrane conductance regulator gene; het.: heterozygous; n: number.

Table 6. The symptoms and diagnoses of infants that heterozygous *CFTR* gene mutation was detected in the control and patient groups.

Heterozygous <i>CFTR</i> Gene Mutation (n=10)	Symptoms of the Infant	Diagnosis of the Infant
E1228G (c.3683A>G) heterozygous	asymptomatic	hyperbilirubinemia
E217G (c.650A>G) heterozygous	asymptomatic	hyperbilirubinemia
E632TfsX9 (c.1894_1895delAG) het.	asymptomatic	hyperbilirubinemia
I807M (c.2421A>G) heterozygous	asymptomatic	hyperbilirubinemia
I807M (c.2421A>G) heterozygous	asymptomatic	hyperbilirubinemia
S573F (c.1718C>T) heterozygous	symptomatic	hypernatremic dyhydration
A46D (c.137C>A) heterozygous	tachypnea; oxygen requirement	respiratory distress syndrome (RDS)
D1312G (c.3935A>G) heterozygous	tachypnea; retraction	respiratory distress syndrome (RDS)
R117H (c.350G>A) heterozygous	tachypnea; retraction	transient tachypnea of newborn
S1426P (c.4276T>C) heterozygous	cough; wheezing	pneumonia

CFTR: cystic fibrosis transmembrane conductance regulator gene; n: number.

DISCUSSION

Cystic fibrosis is a multi-system disease affecting lungs, gastrointestinal system, sweat glands and reproductive system. It may lead to progressive respiratory failure months, even years after birth [6]. Cystic fibrosis is caused by mutations on a single large gene on chromosome 7, which encodes the CFTR protein. Clinical disease requires disease-causing mutations on both copies of the *CFTR* gene. The *CFTR* database lists more than 2000 different mutations on the *CFTR* gene that have the potential to cause disease. The most common mutation is F508del [7,8]. The *CFTR* gene sequencing should be done in cases with an uncertain diagnosis. It should be performed in patients with intermediate sweat chloride levels, and in patients with confirmed or suspected CF if the genotype is not previously known. In these patients, gene analysis confirms the diagnosis and knowledge of the specific *CFTR* mutation has important implications for treatment and prognosis [9-11]. Links between genetic and phenotypic information in CF are collected by an international consortium (Clinical and Functional Translation of *CFTR*) and the results are published on the consortium's website (www.cftr2.org). At the present time, information on specific phenotypic aspects of hundreds of *CFTR* mutations have been reported. The Cystic Fibrosis Mutation database lists more than 1500 different mutations in *CFTR* gene that have the potential to cause disease (<http://www.genet.sickkids.on.ca/cftr/>).

Mutations of the CFTR gene have been divided into five different classes. Class I mutations: defective protein production, class II mutations: defective protein processing, class III mutations: defective regulation, class IV mutations: defective conduction, class V mutations: reduced amounts of functional *CFTR* protein [12-14].

Clinical disease generally requires pathogenic mutations on both copies of the *CFTR* gene, but individuals with a single pathogenic variant (carrier status) occasionally develop disease limited to one organ system. Clinical manifestations of heterozygous carriers may include isolated obstructive azoospermia, chronic rhinosinusitis, chronic pancreatitis, pulmonary disease in adulthood or asymptomatic [15,16]. There was no significant difference in *CFTR* gene analysis in the control and patient groups ($p = 0.340$).

A c.1718C>T heterozygous mutation was detected in an infant without respiratory distress. The c.1718C>T heterozygous mutation is phenotypically reported to result in an elevated immunoreactive trypsinogen (IRT) in the neonatal period (<http://www.genet.sickkids.on.ca/cftr/>). In our case, the IRT screening test for the newborn was reported as normal.

In two infants who had no respiratory distress in the control group, a c.2421A>G heterozygous mutation was

detected. This has been evaluated as a polymorphism found in the French population [17]. In the Cystic Fibrosis Mutation Database, the c.2421A>G heterozygous mutation has been reported in eight patients (<http://www.genet.sickkids.on.ca/cftr/>). While one of them had asymptomatic compound heterozygous mutation, a *CFTR*-related disorder was detected in the remaining seven patients. Congenital bilateral absence of vas deferens was found in three of seven patients, chronic pancreatitis was found in three cases and various clinical findings were detected in one of the patients [17]. Patients with *CFTR*-related disorders should be followed-up periodically. The estimated prevalence and disease symptoms of individuals with *CFTR*-related disorders may show changes in the future [3,4]. Therefore, these two cases, who were not symptomatic in the neonatal period, were enrolled for follow-up.

A c.3683A>G heterozygous mutation was also found in an infant who had no respiratory distress. This mutation was reported in a 9-year-old Turkish girl by Kilinc *et al.* in the Cystic Fibrosis Mutation Database in 2000 (<http://www.genet.sickkids.on.ca/MutationDetailPage.external?sp=1084>) This patient was diagnosed at age one and her chloride level was <60 mEq/L in the sweat test and severe respiratory findings along with bronchiectasis were reported (<http://www.genet.sickkids.on.ca/cftr/>).

Another infant in the control group had a heterozygous mutation c.650A>G. In the Cystic Fibrosis Mutation Database, this mutation was detected in a 2-year-old Portuguese male patient who had a sweat chloride concentration of 60.0-80.0 mEq/L and had pancreatic insufficiency and moderate lung disease [18]. This type of mutation was also detected in one patient by Yoshimura *et al.* [19] in 1999 and it was reported that this patient had diffuse pan-bronchiolitis findings.

A heterozygous mutation, c.1894_1895delAG, was detected in one infant in the control group. There was no reported case of this mutation in the literature review of the *CFTR* gene analyses. No reported case of this mutation was found in the Cystic Fibrosis Mutation Database.

A c.137C>A heterozygous mutation was detected in an infant who had respiratory distress. This mutation has so far been detected in patients with severe respiratory symptoms and high chloride levels in the sweat test. This mutation was found in two Greek patients with CF. One of these patients was 18 years old, his sweat chloride level was 80.0 mEq/L, respiratory function tests revealed FEV1 58.0% and he had pseudomonas infections; the other patient was 30 years old with a sweat chloride level of 92.5 mEq/L, respiratory function tests revealed FEV1 92.5%, and he had pseudomonas infections and pancreatic insufficiency [20].

A c.3935A>G heterozygous mutation was detected in an infant in the patient group. In the Cystic Fibrosis Mutation Database, this mutation was detected in a 14-year-old male patient in 2008. He had a negative sweat test and chronic sinusitis, pseudomonas-infected bronchitis, short stature and growth retardation were reported in this patient (<http://www.genet.sickkids.on.ca/cftr/>).

A c.4276T>C heterozygous mutation was found in an infant in the patient group. In the Cystic Fibrosis Mutation Database, this mutation was reported in a patient with congenital bilateral absence of vas deferens in 1999 (<http://www.genet.sickkids.on.ca/cftr/>).

A c.350G>C heterozygous mutation was found in an infant in the patient group. This mutation is one of the 10 common mutations seen in CF (F508del, I507del, V520F, G551D, G542X, R553X, R117H, 621+1G>T, N1303K, A455E). The disease phenotype of this mutation can range from asymptomatic to classic CF disease. Therefore, genetic counseling is also challenging because of the phenotypic changes associated with this mutation [21].

In this study, no significant difference was found in the *CFTR* gene analysis in the newborns, with or without respiratory distress in the neonatal period. A *CFTR* heterozygous mutation was detected in 20.0% of the infants with respiratory distress, while a *CFTR* gene heterozygous mutation was found in 30.0% of the infants without respiratory distress. This indicates that these infants who are not symptomatic in the neonatal period should be followed-up in terms of *CFTR*-related disorder and their families should be informed. In this study, in an asymptomatic infant, a heterozygous mutation c.1894_1895delAG (E632TfsX9) was also detected on the *CFTR* gene. The heterozygous mutation c.1894_1895delAG (E632TfsX9) is a novel mutation and is reported in the literature for the first time.

Conclusions. This study demonstrates the importance of *CFTR* gene analysis in asymptomatic newborn infants for follow-up and early diagnosis of *CFTR*-related disorders that may develop months or years after birth. In this study, the c.1894_1895delAG (E632TfsX9) heterozygous novel mutation was also detected in the *CFTR* gene, which was first encountered in the literature in an asymptomatic newborn infant. The limitations of this study was the low number of patients.

Declaration of Interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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REFERENCES

1. Anderson DH. Cystic fibrosis of the pancreas. *J Chronic Dis.* 1958; 7(1): 58-90.
2. Tuğ E, Tuğ T. Cystic fibrosis and molecular-genetic approaches. *Turk Thorac J.* 2003; 4(2): 198-204.
3. De Boeck K, Wilschanski M, Castellani C, Taylor C, Cuppens H, Dodge J, *et al.* Cystic fibrosis: Terminology and diagnostic algorithms. *Thorax.* 2006; 61(7): 627-635.
4. Farrell PM, White TB, Ren CL, Hempstead SE, Accurso F, Derichs N, *et al.* Diagnosis of Cystic fibrosis: Consensus guidelines from the Cystic Fibrosis Foundation. *J Pediatr.* 2017; 181S: S4-S15.e1
5. Kharrazi M, Yang J, Bishop T, Lessing S, Young S, Graham S, *et al.* Newborn screening for cystic fibrosis in California. *Pediatrics.* 2015; 136(6): 1062-1072.
6. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. *Am J Respir Crit Care Med.* 2003; 168(8): 918-951.
7. Collins FS. Cystic fibrosis: Molecular biology and therapeutic implications. *Science.* 1992; 256(5058): 774-779.
8. Drumm ML, Collins FS. Molecular biology of cystic fibrosis. *Mol Genet Med.* 1993; 3: 33-68.
9. Bear CE, Li CH, Kartner N, Bridges RJ, Jensen TJ, Ramjeesingh M, *et al.* Purification and functional reconstitution of the cystic fibrosis transmembrane conductance regulator (CFTR). *Cell.* 1992; 68(4): 809-818.
10. Guggino WB, Banks-Schlegel SP. Macromolecular interactions and ion transport in cystic fibrosis. *Am J Respir Crit Care Med.* 2004; 170(7): 815-820.
11. Johnson LG, Boyles SE, Wilson J, Boucher RC. Normalization of raised sodium absorption and raised calcium-mediated chloride secretion by adenovirus-mediated expression of cystic fibrosis transmembrane conductance regulator in primary human cystic fibrosis airway epithelial cells. *J Clin Invest.* 1995; 95(3): 1377-1382.
12. Ratjen F, Döring G. Cystic fibrosis. *Lancet.* 2003; 361(9358): 681-689.
13. Cutting GR. Cystic fibrosis genetics: From molecular understanding to clinical application. *Nat Rev Genet.* 2015; 16(1): 45-56.
14. Moskowitz SM, Chmiel JF, Stern DL, Cheng E, Gibson RL, Marshall SG, *et al.* Clinical practice and genetic counseling for cystic fibrosis and CFTR-related disorders. *Genet Med.* 2008; 10(12): 851-868.

15. Çolak Y, Nordestgaard BG, Afzal S. Morbidity and mortality in carriers of the cystic fibrosis mutation *CFTR* Phe508del in the general population. *Eur Respir J*. 2020; 56(3): 2000558.
16. Boyle MP. Nonclassic cystic fibrosis and *CFTR*-related diseases. *Curr Opin Pulm Med*. 2003; 9(6): 498-503.
17. Bombieri C, Claustres M, De Boeck K, Derichs N, Dodge J, Girodon E, *et al*. Recommendations for the classification of diseases as *CFTR*-related disorders. *J Cystic Fibrosis*. 2011; 10(Suppl 2): S86-S102.
18. Aznarez I, Bal J, Casals T, Estivill X, Moral N, Sands D, *et al*. Analysis of mutations in the *CFTR* gene in patients diagnosed with cystic fibrosis in Poland [Polish]. *Med Wieku Rozwoj*. 2000; 4(2): 149-159.
19. Yoshimura K, Wakazono Y, Iizuka S, Morokawa N, Tada H, Eto Y. Japanese patient homozygous for the H1085R mutation in the *CFTR* gene presents with a severe form of cystic fibrosis. *Clin Genet*. 1999; 56(2): 173-175.
20. Tzetis M, Kanavakis E, Antoniadis T, Traeger-Synodinos J, Doudounakis S, Adam G, *et al*. Identification of two novel mutations (296 + 1G-C and A46D) in exon 2 of the *CFTR* gene in Greek cystic fibrosis patients. *Mol Cell Probes*. 1995; 9(4): 283-285.
21. Thauvin-Robinet C, Munck A, Huet F, Génin E, Bellis G, Gautier E, *et al*.; Collaborating Working Group on R117H. The very low penetrance of cystic fibrosis for the R117H mutation: A reappraisal for genetic counselling and newborn screening. *J Med Genet*. 2009; 46(11): 752-758.

