Original Article

Identification of cystic fibrosis transmembrane conductance regulator gene (CFTR) variants

A retrospective study on the western and southern regions of Saudi Arabia

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ABSTRACT

الأهداف: دراسة التوزيع الجغرافي للمتغيرات الجينية لمرضى التليف الكيسي (CF) في المناطق الغربية والجنوبية من المملكة العربية السعودية.

المنهجية: أجريت دراسة بأثر رجعي على 69 من المرضى الذين تم تشخيص إصابتهم بالتليف الكيسي في مستشفى الملك فيصل التخصصي ومركز الأبحاث بجدة. تم جمع بيانات المرضى بين يونيو 2000 ونوفمبر 2021.

النتائج: أظهرت الدراسة 26 متغيرًا جينياً في 69 من المرضى الذين يعانون من التليف الكيسي، بما في ذلك متغير جديد لم يتم نشره من قبل (1549del G) في 2 من المرضى. المتغيرات الجينية الست الأكثر شيوعًا كما يلي: c.1521_1523delCTT (19%)، c.1418delG (10.2%)، c.579+1G>T (8.8%)، c.2988+1G>A (8.8%)، c.3419 T>A (7.2%)، و c.4124A>C (5.8%). كما أظهرت الدراسة أن إصابة الجهاز التنفسي بعدوى *Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae*، و *Streptococcus pneumoniae* شائعة جدًا بين المرضى الذين يعانون من التليف الكيسي.

الخلاصة: سلطت هذه الدراسة الضوء على سمات مرضى التليف الكيسي المقيمين في المناطق الغربية والجنوبية من المملكة العربية السعودية. أوضحت الدراسة المتغيرات الجينية الست الأكثر شيوعاً في هؤلاء المرضى، كما قدمت الدراسة متغير جيني جديد لم يتم نشره من قبل. نتائج هذه الدراسة قد تساهم في فحص وتشخيص التليف الكيسي في المملكة العربية السعودية.

Objectives: To investigate the geographic distribution of common cystic fibrosis (CF) variants in the western and southern regions of Saudi Arabia.

Methods: A retrospective study was conducted on 69 patients diagnosed with CF at King Faisal Specialist Hospital & Research Center, Jeddah. Patient data were collected retrospectively between June 2000 and November 2021. Various parameters were considered, including patient demographic information, CFTR variants, and respiratory cultures.

Results: We identified 26 CFTR variants in 69 patients with CF, including one novel variant that had not been reported or published before (1549del G) in 2 patients with CF. The 6 most prevalentvariants were as follows: c.1521_1523delCTT (19%), c.1418delG (10.2%), c.579+1G>T (8.8%), c.2988+1G>A (8.8%), c.3419 T>A (7.2%), and c.4124A>C (5.8%). In addition, respiratory cultures revealed that *Pseudomonas aeruginosa, Staphylococcus aureus, Haemophilus influenzae*, and *Streptococcus pneumoniae* were highly common among patients with CF.

Conclusion: This study highlighted features of patients with CF residing in the Western and Southern regions of Saudi Arabia. Six of the 26 CFTR variants were common in these patients. We also report, for the first time, a novel variant and other CFTR variants that are yet to be reported in Saudi Arabia. These findings could help establish a foundation for cystic fibrosis screening in Saudi Arabia and may assist in clinical diagnosis and prognosis.

Keywords: cystic fibrosis, CFTR, gene variants

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Cystic fibrosis (CF) is an autosomal recessive disease due to a mutation of the gene on human chromosome 7 that encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein.[1] The primary function of CFTR is to create chloride channels for mucus transport via chloride secretion.[2] The CFTR gene mutations can affect chloride release, sodium reabsorption, and water transport.[1,3] This disruption leads to dehydrated mucus secretion and an inflammatory response, contributing to severe bronchiectasis and, ultimately, respiratory failure.[4] Imaging techniques including chest radiography, magnetic resonance imaging, and computed tomography, are commonly used to diagnose CF.[5,6]

More than 2000 variants of CFTR gene have been described in the Cystic Fibrosis Mutation Database.[7] In addition, there are 6 different classes of CFTR gene mutations depending on the defects. Class I is characterized by a deficiency in CTFR protein production, whereas class II arises from abnormalities in post-translational processing.[8,9] Classes III, IV, V, and VI arise from defects in channel activity, reductions in ion flow rate and duration, functional CFTR activity, and CFTR stability.[8-10]

The incidence of CF in the Middle East is 1 in 2500-5000, and one in 4243 in Saudi Arabia.[11,12] Deletion of phenylalanine at position 508 (F508del) is the most common variant detected in CF, specifically in the Caucasian population.[8,13] Other variants, including G542X, W1282X, and N1303K, have been found in Europe, North Africa, and Latin America.[10,14] Between 1998 and 2017, 312 families were positive for CFTR variants, and consanguinity was observed in 85% of the parents.[15]

In this study, we provide an overview of the characteristics of patients with CF and the spreading of CFTR mutations among Saudi citizens in the western and southern regions of Saudi Arabia. This investigation will help identify the most common CFTR variants and understand the colonialization of bacteria in patients with CF, which could support clinical screening and prognosis.

Methods. This retrospective study utilized the medical records of patients with CF between June 2000 and November 2021 at King Faisal Specialist Hospital & Research Centre in Jeddah, Saudi Arabia. Data from patients residing in the western (Makkah, Jeddah, and Tabuk provinces) and southern regions (Baha, Jizan, Asir, and Najran provinces) who visited the hospital were incorporated in our study. To investigate the CFTR variants in the 69 patients, we used 3 CF mutation databases: Cystic Fibrosis Mutation Database, The Human Gene Mutation Database, and Clinical and Functional Translation of CFTR (CFTR2).

Data were recruited for analysis after the incidence of CF was confirmed by: i) Assessing the standard clinical image of sputum, cough production, and family history of CF. ii) High sweat chloride levels (>60mmol/L) in 2 consequent samples using the Wescor quantitative test. iii) Genetic detection of CFTR variants by next generation sequencing. Demographic information, such as gender, geographic location, and age of patients with CF, was initially included in the analysis.

This study was approved by the ethics committee International Review Board in King Faisal Specialist Hospital & Research Center, Jeddah, (IRB#: 2023-41) following the Declaration of Helsinki and good clinical practice guidelines. All collected data were reviewed and supervised by the principal investigator.

Statistical analysis. Descriptive analyses were completed to calculate the distribution patterns of parameters in the sample, such as gender, age, the geographic distribution of CF patients, common CFTR variants, and bacterial detection in percentages.

Results. Of the 69 diagnosed patients with CF, 40 (58%) were male and 29 (24%) were female. Patients with CF were categorized into 6 groups based on age: <1, 1–5, 6–10, 11–15, 16–20, and >20 years old (Table 1). In addition, 89.9% of the patients with CF in the King Faisal Specialist Hospital & Research Centre were residents of the western region, whereas 10.1% were residents of the southern region of Saudi Arabia.

The identification of CFTR variants. This study identified 26 CFTR variants in 69 patients with CF (Table 2). The variants have been described in the CFTR

Table 1 - The age distribution of patients with CF featured in this study.

Age group (years)	n	%
< 1	4	5.9
1–5	14	20.3
6–10	21	30.4
11–15	13	18.8
16–20	15	21.7
> 20	2	2.9

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databases and documented in the literature. However, we identified a novel variant that has not been reported or published in the literature. In addition, the prevalence of the variants was variable, as some appeared in more than 5 patients, while others appeared only once. For example, the c.220C>T and c.530T>C variants were observed in more than one patients.

Among the 26 CFTR variants observed in this study, 6 common variants were detected in 69 patients with CF. The most common variants identified were as follows: i) c.1521_1523delCTT (19%), ii) c.1418delG (10.2%), iii) c.579+1G>T (8.8%), iv) c.2988+1G>A (8.8%), v) c.3419 T>A (5.8%), and vi) c.4124A>C (5.8%), as shown in **Table 3**.

We also found 19 variants in 34 patients with CF residing in Jeddah, where the most prevalent variant was c.1521_1523delCTT (26.3%). In contrast, 12 variants were identified in 14 patients with CF from Makkah; the most common variant was c.3419 T>A (33%).

Interestingly, 3 patients harbored more than one variant. The first patient was homozygous for 3 variants: c.1408G>A, c.2562T>G, and c.4389G>A. The second patient was heterozygous for 3 variants (c.869+11C>T, c.1408G>A, and c.91C>T), and the third was homozygous for 2 variants (c.1399C>T and c.1521_1523delCTT).

Notably, a novel CFTR variant was identified in 2 patients (a female and a male). Both patients were from the Western region. However, their ages varied,

Table 2 - Cystic fibrosis transmembrane conductance regulator (CFTR) variants identified in patients with cystic fibrosis from Saudi Arabia. The identity of the complementary deoxyribonucleic acid (cDNA), protein, legacy, and exon region of each identified CFTR variant is listed.

Variant number	cDNA name	Protein name	Legacy name	Region	Reference
1	c.1521_1523delCTT	p.Phe508del	[delta]F508	Exon 11	(16, 17)
2	c.1418delG	p.Gly473GlufsX54	1548delG	Exon 11	(18)
3	c.579+1G>T	-	711+ 1G- >T	Intron 5	(19)
4	c.2988+1G>A	-	3120+ 1G- >A	Intron 18	(20, 21)
5	c.3419T>A	p.Met1140Lys	M1140K	Exon 21	(22)
6	c.4124A>C	p.His1375Pro	H1375P	Exon 25	(23)
7	c.3700A>G	p.Ile1234Val	I1234V	Exon 22	(24)
8	c.2739T>A	p.Tyr913X	Y913X	Exon 17	(25)
9	c.443T>C	p.Ile148Thr	I148T	Exon 4	(26)
10	c.2657+5G>A	-	2789+ 5G>A	Intron 16	(27)
11	*1549 deIG	-	-	-	In this study
12	delExon19-21	-	-	-	(17)
13	c.1697C>A	p.Ala566Asp	-	Exon 12	(28)
14	c.2620-26A>G	-	2752- 26A- >G	Intron 15	(29)
15	c.2051_2052delAAinsG	p.Lys684SerfsX38	2183AA- >G	Exon 14	(30)
16	c.1660_1661insA	p.Ala554AspfsX14	-	Exon 12	(31)
17	c.4251delA	p.Glu1418ArgfsX14	4382delA	Exon 27	(32)
18	c.3717+12191C>T	-	3849+ 10kbC- >T	Intron 22	(23)
19	c.220C>T	p.Arg74Trp	R74W	Exon 3	(33)
20	c.530T>C	p.Ile177Thr	I177T	Exon 5	(34)
21	c.1408A>G	p.Met470Val	M470V	Exon 11	(35)
22	c.2562T>G	-	2694T/G	Exon 15	(36)
23	c.4389G>A	-	4521G/A	Exon 27	(37)
24	c.869+11C>T	-	1001+ 11C/T	Intron 7	(38)
25	c.91C>T	p.Arg31Cys	R31C	Exon 2	(39)
26	c.1399C>T	p.Leu467Phe	1531C/T (L467F)	Exon 11	(40)

Table 3 - The number of patients retaining common cystic fibrosis transmembrane conductance regulator variants and their genotype.

Variant number	cDNA Name	n (%)	Heterozygous	Homozygous
1	c.1521_1523delCTT	13 (19.0)	4	9
2	c.1418delG	7 (10.0)	1	6
3	C.579+G>T	6 (8.7)	-	6
4	c.2988+1G>A	6 (8.7)	1	5
5	c.3419T>A	5 (7.2)	-	5
6	c.4124A>C	4 (5.8)	-	4

cDNA: complementary deoxyribonucleic acid

where the females were 5 and males were 11 years old. In addition, the CFTR trait in both patients was homozygous. The respiratory cultures for the female patient revealed *Staphylococcus aureus* (*S. aureus*) while the culture for the male patient showed *Streptococcus pneumoniae* (*S. pneumonia*).

Respiratory cell culture. Respiratory culture data from 54 patients with CF revealed the presence of several bacterial species (**Appendix 1**). The predominant species observed in most of the samples were *Pseudomonas aeruginosa (P. aeruginosa)* (44.4%), *Haemophilus influenzae (H. influenzae)* (18.5%), *S. aureus* (18.5%), *S,pneumoniae* (13%), and *Branhamella catarrhalis (B. catarrhalis)* (9%). Although *P. aeruginosa* was the most common bacterial type detected in all patients, its prevalence was lower in females (12.9%) than in males (31.5%). We also found that these species were all associated with the 4 most common CFTR variants: c.1521_1523delCTT, c.1418delG, c.579+1G>T, and c.2988+1G>A. Other bacterial species, such as *Klebsiella sp.* (3%) and *Stenotrophomonas maltophilia (S. maltophilia)* (1.8%), were found only rarely (**Table 4**).

Table 4 - Types of bacterial colonization from the first respiratory culture in patients with CF with common CFTR variants.

Variant number	cDNA Name	First respiratory culture
1	c.1521_1523delCTT	*Streptococcus pneumoniae* *Pseudomonas aeruginosa* *Haemophilus influenzae* *Escherichia coli* *Klebsiella* sp. *Branhamella catarrhalis* *Aspergillus* sp. *Pseudomonas* sp.
2	c.1418delG	*Pseudomonas aeruginosa* *Haemophilus influenzae* *Streptococcus pneumoniae* *Staphylococcus aureus*
3	c.579+G>T	*Streptococcus pneumoniae* *Haemophilus influenzae* *Pseudomonas aeruginosa* *Branhamella catarrhalis* *Pseudomonas aeruginosa* *Klebsiella pneumoniae*
4	c.2988+1G>A	*Pseudomonas aeruginosa* *Streptococcus pneumoniae* *Pseudomonas aeruginosa* *Branhamella catarrhalis* *Staphylococcus aureus*
5	c.3419T>A	*Staphylococcus aureus*
6	c.4124A>C	*Stenotrophomonas maltophilia* *Enterobacter cloacae*

CFTR: cystic fibrosis transmembrane conductance regulator, cDNA: complementary deoxyribonucleic acid

Discussion. Cystic fibrosis has attracted the attention of researchers attempting to understand and explore the incidence and genetic mutations in the CFTR gene. Likewise, this study aimed to investigate the genetic variation in CFTR genes in Saudi patients with CF. However, we focused mainly on the western and southern regions of Saudi Arabia. Various parameters were considered, such as demographic information, CFTR gene variant identity, and bacterial colonization associated with the gene variants. Since the primary goal of this study was to understand the characteristics of Saudi patients diagnosed with CF in the western and southern regions only, 69 CF cases were identified between 2000 and 2021. This explains why the sample size of this study was comparatively small compared to another study (396 patients with CF) that targeted the general population.[15] Several studies have suggested that gender can predict the clinical outcomes and severity of CF. It has been demonstrated that females risk a worse prognosis than males because of increased respiratory infections, and *P. aeruginosa* has been implicated in poor survival.[41,42] This suggests that female patients with CF from the western and southern regions presumably have a better survival rate since the number of female patients and the incidence of *P. aeruginosa* were low.

Banjar et al[43] provided the first description of CFTR gene variants in the Saudi population in 1999. Several studies by the Banjar group have explored CF variants in Saudi Arabia over various time frames.[12,15,17] The high incidence of homozygous CF cases observed in this study (81%) reflected the occurrence of consanguineous marriages. This has also been reported in other studies, where consanguinity accounted for 89% of the families with CF.[44]

The c.1521_1523delCTT variant, responsible for the deletion of phenylalanine at position 508 in the CFTR gene (p.Phe508del), is considered the most common variant in CF.[45] This is consistent with the findings of this study, as 19% of CF cases had p.Phe508del. It has also recently been reported that 70% of patients with CF in the western region of Saudi Arabia exhibit this mutation.[17,43,46] According to the findings of our study, the c.1418delG variant was found to be most common in the northern region. The third most common variant, c.579+1G>T, was discovered to be more common in the southern region. Lastly, c.2988+1G>A, the fourth common variant identified in our study, was most frequently found in the eastern region.[17] Interestingly, c.3419T>A (p.Met1140Lys), which accounted for 7.2% of the cases in this study, has been reported in only one patient in Saudi Arabia,

and c.4124A>C (p.His1375Pro) (in 5.8% of our cases) has not been previously reported in Saudi patients with CF.[47]

In addition, our study reports variants of the CFTR gene that have not been previously reported in the Saudi population. However, these variants have been reported or published in the literature in other Arab and American populations.[46,48] The variants are c.443T>C, c.2657+5G>A, c.1697C>A, c.2620-26A>G, c.2051_2052delAAinsG, c.1660_1661insA, c.4251delA, c.3717+12191C>T, c.220C>T, c.530T>C, c.1408A>G, c.2562T>G, c.4389G>A, c.869+11C>T, c.91C>T and c.1399C>T. The clinical significance of these variants differs; they are considered to be either pathogenic, likely pathogenic, or benign, or there are conflicting interpretations of pathogenicity (**Appendix 1**).[23-30,32-38,40] Further studies are essential to clarify the clinical significance of these variants. We also found a CFTR variant (delExone 19-21) present in 2 patients with CF, which has been reported previously in the Saudi population.[15]

Interestingly, we identified a novel variant of the CFTR gene. The variant is (1549delG), located on exon 10. It is predicted to cause deletion of a "G" at nucleotide position 1549 in the CFTR gene and subsequently causes a frameshift mutation which alters the sequence of the amino acid.

Pathogenic bacteria are among the primary factors contributing to morbidity and mortality in CF.[49] *Pseudomonas aeruginosa*, *S. aureus*, and *H. influenzae* are among the most prevalent pulmonary bacterial infections associated with CF.[50,51] In addition, infections caused by multiple species have been identified. These include co-infections with bacteria and fungi.[52] Although culture-based studies are the most common method used to detect and assess infection in patients with CF, further studies are recommended to explore the role of these microorganisms in pathogenesis.[53,54] In this study, most patients who underwent respiratory culture examination displayed colonization by *P. aeruginosa*, *S. aureus, H. influenzae, S. pneumoniae,* and *B. catarrhalis*. This is consistent with the most common CF lung bacterial species.[54,55] These species were also associated with the 4 most common CFTR variants (c.1521_1523delCTT, c.1418delG, c.579+1G>T, and c.2988+1G>A) and with other less common variants. Notably, no correlation was found between the type of mutation and bacterial species, corresponding to Banjar et al's[56] findings.

Some bacterial species, such as *S. maltophilia* and *Klebsiella pneumoniae* (*Klebsiella sp.)*, were detected infrequently in this study. Several studies have suggested that *S. maltophilia* is less pathogenic than other species and is not related with the lung diseases.[57-59] Another study suggested that *Klebsiella sp.* is rarely found in patients with CF, which reflects what we found in Saudi patients with CF. [60]

Study limitations. Since the diagnosis within a medical record is a prevalent theme, health care institutions should be provided with adequate training and facilities to enable them to make accurate early diagnoses and, in turn, appropriate treatments with high cure rates. Data from medical reports and registries that have been analyzed have shown considerable limitations in the reporting of standardized information on cystic fibrosis from health care settings. The implementation of medical staff training and infrastructure is crucial to sustaining this concern and bringing forth clear conclusions. One of the limitations of our study was the presence of incomplete medical records or patients who did not follow up after receiving care.

To verify our findings, a larger multicenter cohort investigation is required. Further, the majority of errors in sweat chloride tests are caused by insufficient sweat collection, technological problems, and misinterpretation of data. The apparent low incidence of diagnosed CF patients in Saudi Arabia could be owing to a lack of clinical suspicion, as well as technical errors in sweat chloride level detection and a lack of autopsy data. A higher level of suspicion is required for an early diagnosis. A comprehensive national program would be advantageous in raising awareness among general physicians and pediatricians in Saudi Arabia about the occurrence and potential devastating consequences of CF.

In conclusion, this study highlighted the characteristics of patients with CF in the western and (to a lesser extent) southern regions of Saudi Arabia. Multiple CFTR variants were found in patients with CF, but only 6 were common. Here, we report for the first time a novel CFTR variant (1549del G). In addition, the majority of patients representing these regions were infected with *P. aeruginosa, S. aureus*, *H. influenzae*, and *S. pneumoniae*. These findings could help assess clinical prognosis and predict life expectancy, outcomes, and possible treatment strategies. However, a patient's follow-up with these clinical features will assist in measuring the pathological consequences of CF on health.

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References

1. Turcios NL. Cystic fibrosis lung disease: An overview. *Respir Care* 2020;65: 233-251.
2. Stoltz DA, Meyerholz DK, Welsh MJ. Origins of cystic fibrosis lung disease. *N Engl J Med* 2015; 372 : 1574-1575.
3. Mall MA, Hartl D. CFTR: cystic fibrosis and beyond. *Eur Respir J* 2014; 44: 1042-1054.
4. Rubin BK. Cystic fibrosis: myths, mistakes, and dogma. *Paediatr Respir Rev* 2014; 15: 113-116.
5. Wielputz MO, Eichinger M, Biederer J, Wege S, Stahl M, Sommerburg O, et al. Imaging of cystic fibrosis lung disease and clinical interpretation. *Rofo* 2016; 188: 834-845.
6. Woods JC, Wild JM, Wielputz MO, Clancy JP, Hatabu H, Kauczor HU, et al. Current state of the art MRI for the longitudinal assessment of cystic fibrosis. *J Magn Reson Imaging* 2020; 52: 1306-1320.
7. Lopes-Pacheco M. CFTR modulators: The changing face of cystic fibrosis in the era of precision medicine. *Front Pharmacol* 2019;10:1662.
8. Chen Q, Shen Y, Zheng J. A review of cystic fibrosis: Basic and clinical aspects. *Animal Model Exp Med* 2021; 4: 220-232.
9. Moskowitz SM, Chmiel JF, Sternen 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: 851-868.
10. Antunovic SS, Lukac M, Vujovic D. Longitudinal cystic fibrosis care. *Clin Pharmacol Ther* 2013; 93: 86-97.
11. Nazer H, Riff E, Sakati N, Mathew R, Majeed-Saidan MA, Harfi H. Cystic fibrosis in Saudi Arabia. *Eur J Pediatr* 1989; 148: 330-332.
12. Banjar H, Angyalosi G. The road for survival improvement of cystic fibrosis patients in Arab countries. *Int J Pediatr Adolesc Med* 2015; 2: 47-58.
13. Habib AR, Kajbafzadeh M, Desai S, Yang CL, Skolnik K, Quon BS. A systematic review of the clinical efficacy and safety of CFTR modulators in cystic fibrosis. *Sci Rep* 2019; 9: 7234.
14. Estivill X, Bancells C, Ramos C. Geographic distribution and regional origin of 272 cystic fibrosis mutations in European populations. The biomed CF mutation analysis consortium. *Hum Mutat* 1997; 10: 135-154.
15. Banjar HH, Tuleimat L, El Seoudi AAA, Mogarri I, Alhaider S, Nizami IY, et al. Genotype patterns for mutations of the cystic fibrosis transmembrane conductance regulator gene: a retrospective descriptive study from Saudi Arabia. *Ann Saudi Med* 2020; 40: 15-24.
16. Kerem B, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. *Science* 1989; 245 :1073-1080.
17. Banjar H, Al-Mogarri I, Nizami I, Al-Haider S, AlMaghamsi T, Alkaf S, et al. Geographic distribution of cystic fibrosis transmembrane conductance regulator (CFTR) gene mutations in Saudi Arabia. *Int J Pediatr Adolesc Med* 2021; 8: 25-28.
18. el-Harith EA, Dörk T, Stuhrmann M, Abu-Srair H, al-Shahri A, Keller KM, et al. Novel and characteristic CFTR mutations in Saudi Arab children with severe cystic fibrosis. *J Med Genet* 1997; 34: 996-999.
19. Zielenski J, Bozon D, Kerem B, Markiewicz D, Durie P, Rommens JM, et al. Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics* 1991; 10: 229-235.
20. Dörk T, El-Harith EH, Stuhrmann M, Macek M, Jr., Egan M, Cutting GR, et al. Evidence for a common ethnic origin of cystic fibrosis mutation 3120+1G-->A in diverse populations. *Am J Hum Genet* 1998; 63: 656-62.
21. Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, et al. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. *J Pediatr* 1995; 127: 705-710.
22. Clavel C, Pennaforte F, Pigeon F, Verlingue C, Birembaut P, Férec C. Identification of four novel mutations in the cystic fibrosis transmembrane conductance regulator gene: E664X, 2113delA, 306delTAGA, and delta M1140. *Hum Mutat* 1997; 9: 368-369.
23. Lucarelli M, Bruno SM, Pierandrei S, Ferraguti G, Stamato A, Narzi F, et al. A Genotypic-oriented view of CFTR genetics highlights specific mutational patterns underlying clinical macrocategories of cystic fibrosis. *Mol Med* 2015; 21: 257-275.
24. Molinski SV, Gonska T, Huan LJ, Baskin B, Janahi IA, Ray PN, et al. Genetic, cell biological, and clinical interrogation of the CFTR mutation c.3700 A>G (p.Ile1234Val) informs strategies for future medical intervention. *Genet Med* 2014; 16: 625-632.
25. Schrijver I, Ramalingam S, Sankaran R, Swanson S, Dunlop CL, Keiles S, et al. Diagnostic testing by CFTR gene mutation analysis in a large group of Hispanics: novel mutations and assessment of a population-specific mutation spectrum. *J Mol Diagn* 2005; 7: 289-299.
26. Rohlfs EM, Zhou Z, Sugarman EA, Heim RA, Pace RG, Knowles MR, et al. The I148T CFTR allele occurs on multiple haplotypes: a complex allele is associated with cystic fibrosis. *Genet Med* 2002; 4: 319-323.
27. Schwartz KM, Pike-Buchanan LL, Muralidharan K, Redman JB, Wilson JA, Jarvis M, et al. Identification of cystic fibrosis variants by polymerase chain reaction/oligonucleotide ligation assay. *J Mol Diagn* 2009; 11: 211-215.
28. Alibakhshi R, Kianishirazi R, Cassiman JJ, Zamani M, Cuppens H. Analysis of the CFTR gene in Iranian cystic fibrosis patients: identification of eight novel mutations. *J Cyst Fibros* 2008; 7: 102-109.
29. Bergougnoux A, Viart V, Miro J, Bommart S, Molinari N, des Georges M, et al. Should diffuse bronchiectasis still be considered a CFTR-related disorder? *J Cyst Fibros* 2015; 14: 646-653.
30. Nemat Farahzadi H, Akbari MT. Analysis variants of the CFTR gene in iranian cystic fibrosis patients. *J Human Gen Genom* 2020; 4: e123474.
31. Sharma N, Singh M, Kaur G, Thapa BR, Prasad R. Identification and characterization of CFTR gene mutations in Indian CF patients. *Ann Hum Genet* 2009; 73: 26-33.
32. Castaldo G, Polizzi A, Tomaiuolo R, Cazeneuve C, Girodon E, Santostasi T, et al. Comprehensive cystic fibrosis mutation epidemiology and haplotype characterization in a southern Italian population. *Ann Hum Genet* 2005; 69: 15-24.
33. Steiner B, Rosendahl J, Witt H, Teich N, Keim V, Schulz HU, et al. Common CFTR haplotypes and susceptibility to chronic pancreatitis and congenital bilateral absence of the vas deferens. *Hum Mutat* 2011;32: 912-920.
34. Sobush KT, Thornburg CD, Voynow JA, Davis SD, Peterson-Carmichael SL. A 2-year-old girl with co-inherited cystic fibrosis and sickle cell-β+ thalassemia presenting with recurrent vaso-occlusive events during cystic fibrosis pulmonary exacerbations: a case report. *J Med Case Rep* 2013; 7: 203.

35. Gaikwad A, Khan S, Kadam S, Kadam K, Dighe V, Shah R, et al. The CFTR gene mild variants poly-T, TG repeats and M470V detection in Indian men with congenital bilateral absence of vas deferens. ***Andrologia*** 2018; 50.
36. Minarowski L, Minarowska A, Sands D, Kowalczuk O, Chyczewski L, Chyczewska E, et al. Single nucleotide polymorphims 1540G/A, 2694T/G and 4521G/A of CFTR gene and their association with cystic fibrosis phenotype. ***Eur Respir J*** 2014; 44: P1201.
37. Atag E, Bas Ikizoglu N, Ergenekon AP, Gokdemir Y, Eralp EE, Ata P, et al. Novel mutations and deletions in cystic fibrosis in a tertiary cystic fibrosis center in Istanbul. ***Pediatr Pulmonol*** 2019; 54: 743-750.
38. Farhat R, Puissesseau G, El-Seedy A, Pasquet MC, Adolphe C, Corbani S, et al. N1303K (c.3909C>G) mutation and splicing: implication of its c.[744-33GATT(6); 869+11C>T] complex allele in CFTR exon 7 aberrant splicing. ***Biomed Res Int*** 2015; 2015: 138103.
39. Grangeia A, Alves S, Gonçalves L, Gregório I, Santos AC, Barros H, et al. Spectrum of CFTR gene sequence variants in a northern Portugal population. ***Pulmonology*** 2018; 24: 3-9.
40. Ivanov M, Matsvay A, Glazova O, Krasovskiy S, Usacheva M, Amelina E, et al. Targeted sequencing reveals complex, phenotype-correlated genotypes in cystic fibrosis. ***BMC Medical Genomics*** 2018; 11: 13.
41. Harness-Brumley CL, Elliott AC, Rosenbluth DB, Rghavan D, Jain R. Gender differences in outcomes of patients with cystic fibrosis. ***J Womens Health (Larchmt)*** 2014; 23: 1012-1020.
42. Demko CA, Byard PJ, Davis PB. Gender differences in cystic fibrosis: Pseudomonas aeruginosa infection. ***J Clin Epidemiol*** 1995; 48: 1041-1049.
43. Banjar H, Kambouris M, Meyer BF, al-Mehaidib A, Mogarri I. Geographic distribution of cystic fibrosis transmembrane regulator gene mutations in Saudi Arabia. ***Ann Trop Paediatr*** 1999;19: 69-73.
44. El Mouzan MI, Al Salloum AA, Al Herbish AS, Qurachi MM, Al Omar AA. Consanguinity and major genetic disorders in Saudi children: a community-based cross-sectional study. ***Ann Saudi Med*** 2008; 28: 169-173.
45. Colak 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.
46. Al-Sadeq D, Abunada T, Dalloul R, Fahad S, Taleb S, Aljassim K, et al. Spectrum of mutations of cystic fibrosis in the 22 Arab countries: A systematic review. ***Respirology***. 2019; 24: 127-136.
47. Banjar H, Chaballout M, Karkour K, Al-Ghamdi H, Al-Mogarri I, Al-Haider S, et al. The prevalence of viral infections in children with cystic fibrosis in a tertiary care center in Saudi Arabia. ***Int J Pediatr Adolesc Med*** 2020; 7: 83-87.
48. Silva Filho LV, Castanos C, Ruiz HH. Cystic fibrosis in Latin America-Improving the awareness. ***J Cyst Fibros*** 2016; 15: 791-793.
49. Gibson RL, Burns JL, Ramsey BW. Pathophysiology and management of pulmonary infections in cystic fibrosis. ***Am J Respir Crit Care Med*** 2003; 168: 918-951.
50. Burns JL, Rolain J-M. Culture-based diagnostic microbiology in cystic fibrosis: Can we simplify the complexity? ***J Cyst Fibros*** 2014; 13: 1-9.
51. Steinkamp G, Wiedemann B, Rietschel E, Krahl A, Gielen J, Bärmeier H, et al. Prospective evaluation of emerging bacteria in cystic fibrosis. ***J Cyst Fibros*** 2005; 4: 41-48.
52. Camus L, Briaud P, Vandenesch F, Moreau K. How bacterial adaptation to cystic fibrosis environment shapes interactions between *Pseudomonas aeruginosa* and *Staphylococcus aureus*. ***Front Microbiol*** 2021; 12: 617784.
53. Burns JL, Gibson RL, McNamara S, Yim D, Emerson J, Rosenfeld M, et al. Longitudinal assessment of Pseudomonas aeruginosa in young children with cystic fibrosis. ***J Infect Dis*** 2001; 183: 444-452.
54. Hauser AR, Jain M, Bar-Meir M, McColley SA. Clinical significance of microbial infection and adaptation in cystic fibrosis. ***Clin Microbiol Rev*** 2011; 24: 29-70.
55. Godoy JM, Godoy AN, Ribalta G, Largo I. Bacterial pattern in chronic sinusitis and cystic fibrosis. ***Otolaryngol Head Neck Surg*** 2011; 145: 673-676.
56. Banjar H, Rt L, Alsheikh H, Abuzubida A, Almelhem J, Wagley Z, et al. Correlation of Cystic Fibrosis Mutations (CFTR) and Bacteria in a Tertiary Care Center in Saudi Arabia. ***Journal of MAR Pediatric***s 2021; 1:1-11 .
57. Waters V, Atenafu EG, Lu A, Yau Y, Tullis E, Ratjen F. Chronic Stenotrophomonas maltophilia infection and mortality or lung transplantation in cystic fibrosis patients. ***J Cyst Fibros*** 2013; 12: 482-486.
58. Goss CH, Otto K, Aitken ML, Rubenfeld GD. Detecting Stenotrophomonas maltophilia does not reduce survival of patients with cystic fibrosis. ***Am J Respir Crit Care Med*** 2002; 166: 356-361.
59. Granchelli AM, Adler FR, Keogh RH, Kartsonaki C, Cox DR, Liou TG. Microbial interactions in the cystic fibrosis airway. ***J Clin Microbiol*** 2018; 56.
60. Phang SH, Greysson-Wong J, Somayaji R, Storey DG, Rabin HR, Surette MG, et al. Incidence, impact and natural history of Klebsiella species infections in cystic fibrosis: A longitudinal single center study. ***Can J Respir Crit*** 2019; 3: 148-154.

Appendix 1 - A description of the consequence, clinical significance, genetic variation and condition of the 26 identified CFTR variants.

Variant #	cDNA Name	Consequence	Clinical significance	Variation Location	Condition(s)
1	c.1521_1523delCTT	deletion of Phe at 508	Pathogenic	NM_000492.3(CFTR):c.1521_1523delCTT (p.Phe508delPhe)	Cystic fibrosis, Hereditary pancreatitis,
2	c.1418delG	frameshift	Pathogenic	NM_000492.3(CFTR):c.1418delG (p.Gly473Glufs)	Cystic fibrosis, not provided
3	c.579+1G>T	mRNA splicing defect	Pathogenic	NM_000492.3(CFTR):c.579+1G>T	Cystic fibrosis, Hereditary pancreatitis, not specified,
4	c.2988+1G>A	mRNA splicing defect	Pathogenic	NM_000492.3(CFTR):c.2988+1G>A	Cystic fibrosis, Hereditary pancreatitis
5	c.3419T>A	Met to Lys at 1140	N/A	NM_000492.3(CFTR):c.3419T>A (p.Met1140Lys)	Cystic fbrosis
6	c.4124A>C	His to Pro at 1375	Likely pathogenic	NM_000492.3(CFTR):c.4124A>C (p.His1375Pro)	Cystic fibrosis
7	c.3700A>G	Ile to Val at 1234	Pathogenic	NM_000492.3(CFTR):c.3700A>G (p.Ile1234Val)	Cystic fbrosis
8	c.2739T>A	Tyr to Stop at 913	Pathogenic	NM_000492.3(CFTR):c.2739T>A (p.Tyr913Ter)	Cystic Fibrosis
9	c.443T>C	Ile to Thr at 148	Conflicting interpretations of pathogenicity	NM_000492.3(CFTR):c.443T>C (p.Ile148Thr)	
10	c.2657+5G>A	mRNA splicing defect	Pathogenic	NM_000492.3(CFTR):c.2657+5G>A	
11	1549 delG				
12	delExon19-21				
13	c.1697C>A		Uncertain significanc	NM_000492.3(CFTR):c.1697C>A (p.Ala566Asp)	Cystic fibrosis
14	c.2620-26A>G	mRNA splicing defect	Likely benign(2);Uncertain significance(1)	NM_000492.3(CFTR):c.2620-26A>G	
15	c.2051_2052delAA insG	frameshift	Pathogenic	NM_000492.3(CFTR):c.2051_2052delAAinsG (p.Lys684Serfs)	Cystic fibrosis
16	c.1660_1661insA		Not specifc	NM_000492.3(CFTR):c.1660_1661insA (p.Ala554Aspfs)	N/A
17	c.4251delA	frameshift	pathogenic	(p.Glu1418Argfs)	N/A
18	c.3717+12191C>T	creation of splice acceptor site	Pathogenic	NM_000492.3(CFTR):c.3718-2477C>T	Cystic fbrosis
19	c.220C>T	Arg to Trp at 74	Uncertain significance	NM_000492.3(CFTR):c.220C>T (p.Arg74Trp)	Cystic fibrosis
20	c.530T>C	Ile to Thr at 177	Uncertain significance	NM_000492.3(CFTR):c.530T>C (p.Ile177Thr)	Cystic Fibrosis
21	c.1408A>G	sequence variation	Benign/Likely benign	NM_000492.3(CFTR):c.1408G>A (p.Val470Met)	
22	c.2562T>G	sequence variation	Benign/Likely benign	NM_000492.3(CFTR):c.2562T>G (p.Thr854=)	
23	c.4389G>A	sequence variation	Benign/Likely benign	NM_000492.3(CFTR):c.4389G>A (p.Gln1463=)	
24	c.869+11C>T	sequence variation	Benign/Likely benign	NM_000492.3(CFTR):c.869+11C>T	
25	c.91C>T		Conflicting interpretations of pathogenicity	NM_000492.3(CFTR):c.91C>T (p.Arg31Cys)	
26	c.1399C>T	sequence variation	Conflicting interpretations of pathogenicity	NM_000492.3(CFTR):c.1399C>T (p.Leu467Phe	Cystic fibrosis, not provided