



Cholelithiasis in Cystic Fibrosis Patients in a Tertiary Care Center in Saudi Arabia

Banjar H^{1*}, Al-Ghuneim L¹, Al-Shammari A¹, Al-Mulhim FA², Al-Eid M³, Ghomraoui R⁴, Ghomraoui F⁵

¹Department of Pediatrics, [King Faisal Specialist Hospital & Research Centre \(KFSHRC\)](#), Riyadh, Saudi Arabia

²[King Fahd University Hospital](#), Al Khobar, KSA

³Biostatistics, Epidemiology, and Scientific Computing Department, [King Faisal Specialist Hospital & Research Centre \(KFSHRC\)](#), Riyadh, Saudi Arabia

⁴[Elm University of Dentistry and Pharmacy](#), Riyadh, Saudi Arabia

⁵Hospital: [University at Buffalo-Catholic Health](#), Buffalo, New York, USA

Corresponding Author: **Hanaa Banjar, MD, FRCPC** [ORCID ID](#)

Address: Professor of Pediatrics, Al-Faisal University, Consultant Pediatric Pulmonology, Department of Pediatrics, (KFSHRC). P.O.Box. 3354, MBC-58, Riyadh 11211, Saudi Arabia; Tel: +9661-442-7761; Fax No: +966-1-442-7784, +966-503493022; E-mail: hanaa@kfshrc.edu.sa

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Abstract

Introduction: Cholelithiasis has been reported in 12%-24% of Cystic Fibrosis (CF) patients, and is usually made up of cholesterol gallstones. These abnormalities are frequently asymptomatic and can include intra and extrahepatic ducts, gallbladder thickening and contraction, micro gallbladders, and cholelithiasis. Abdominal sonography is routinely used in order to detect these abnormalities.

Objectives: To obtain the prevalence of gall stones (Cholelithiasis) in CF patients and its relation to other clinical, laboratory, radiological, and genetic data.

Methodology: A retrospective chart review as part of the CF registry data from the period 1st January 1984 - 1st June 2018. All confirmed CF the patients of all age groups that have US studies done were included in the study. Patients with positive gallstones or sludge were evaluated and discussed.

Results: A total of 391 confirmed CF patients were involved. Out of them, 252 patients had an abdominal ultrasound, 7 patients (3%) had gallstones on the abdominal US, 8 patients (3%) were revealed to have sludge and 237 patients (94%) had normal gallbladders. Pancreatitis was found in 4 patients (2%). 191 patients (76%) had pancreatic insufficiency. 77 patients had follow up abdominal ultrasounds and 5 patients (7%) were found to have persistent gallstones, 4 patients (5%) had persistent sludge and 68 patients (88%) remained negative for gallstones. 2 patients required cholecystectomy.

Conclusion: Cholelithiasis is a common complication of CF disease; its incidence is more than the general population. Thus, we recommend that every CF patient get an ultrasonography study as part of liver disease screening to rule out any Gallbladder pathology.

Keywords

Cystic Fibrosis, Gallstone, Ultrasound, CFTR, Arab, Saudi Arabia, Cholelithiasis

Introduction

Cystic fibrosis (CF) is a progressive disease that affects many systems including respiratory, gastrointestinal, urogenital, and sweat glands [1-3]. It is the most common life-shortening autosomal recessive disease among Caucasian populations, with a frequency of 1 in 2000 to 3000 live births (3). CF is inherited as an autosomal recessive disease due to the mutation of the CF transmembrane conductance regulator (CFTR) gene [1]. This causes an abnormal chloride ion transport on the apical surface of the exocrine glands epithelial cells [2]. This results in abnormally thickened, viscous secretions, which affect multiple organ systems [2]. Deletion of phenylalanine in amino acid position 508 ($\Delta F508$) on chromosome 7 is considered the most common mutation in North America and western Europe [4], in contrast to Saudi Arabia, the most common mutation was 1548delG and I1234V [5,6].

One-third of Cystic Fibrosis patients have abnormal gallbladders and cystic ducts, regardless of their age, the severity of the disease, and hepatic pathology [7,8]. These abnormalities can include the intra and extrahepatic ducts, gallbladder thickening and contraction, micro gallbladders, and cholelithiasis [9]. Cholelithiasis occurs in 12%-24% of Cystic fibrosis patients and is usually made up of cholesterol gallstones. These abnormalities are frequently asymptomatic [10]. Abdominal sonography is routinely used to detect these abnormalities. However, Magnetic resonance (MR) cholangiography is considered to be more accurate [10].

The underlying pathophysiology of the increased cholelithiasis prevalence in Cystic Fibrosis patients is not yet discovered; nonetheless, multiple theories have been developed. One of these theories suggests that disturbed bile acid metabolism in CF patients causes increased bile acid fecal losses; leading to cholesterol supersaturation in the gallbladder and subsequent stone formation [11]. Nucleating factors, such as mucin glycoproteins can aid in the process of stone formation [12].

Wasmuth et al. [13], proposed that the coinheritance of the common UGT1A1 promoter mutation associated

with Gilbert syndrome is an additional lithogenic risk factor for gallstone formation in CF. The study included 52 patients with CF. Twenty-six patients were heterozygous for the A(TA)₇TAA allele, while five patients were homozygous for the A(TA)₇TAA allele. Compared with stone free patients with CF, significantly more CF patients with gallstones either carried the heterozygous A(TA)₇TAA allele or the heterozygous and/or homozygous A(TA)₇TAA allele. Only one patient with gallstones did not carry the mutated UGT1A1 allele. The study concluded that CF patients with homozygous Gilbert mutation appear to have an increased risk for gallstones, followed by CF patients with the heterozygous mutation and homozygous wild type alleles. Hence, genetic factors that increase the biliary secretion of bilirubin monoglucuronide raise the risk of forming gallstones [14].

Philippe et al. [8], reported the incidence of gallbladder diseases in 84 cystic fibrosis patients who underwent a cholecystographic evaluation, which evaluated the opacification of the gallbladder, gallbladder size and the presence of calculi. Thirty-nine patients (46.4%) had abnormal cholecystogram, mostly due to non-visualizations of the gallbladder. Ten of the 84 patients (11.9%) had gallstones; most of which were found in patients aged 10-20 years. It was suggested that this incidence could be attributed to some factors promoting stone development in CF patients; such as biliary stasis and as a result of cholestatic drugs like sulfonamides, anabolic agents, and oral contraceptives [11-14,15].

It has been proposed that the proper use of pancreatic enzymes in these patients can help in fat absorption, hence, decrease the lithogenic bile [16]. In the case of symptomatic gallstones in CF patients with suitable pulmonary function, cholecystectomy has been favored [17].

One case report has described the dissolution of gallstones in two CF patients post the administration of ursodeoxycholic acid. The authors explained that the use of ursodeoxycholic acid in selected cases, where cholesterol gallstones are established and the gallbladder shows opacification on oral

cholecystography, could result in dissolution or reduction in the size of the stones [18].

Objective

To obtain the incidence and the prevalence of gallstones (Cholelithiasis) in CF patients and its relation to genetic data from the period 1st January 1984- 1st June 2018.

Methodology

A retrospective chart review of 391 confirmed CF patients from the period 1st January 1984- 1st June 2018.

Definition:

The diagnosis of CF was established in all patients according to one of the following:

1. Typical pulmonary manifestations and/or typical gastrointestinal manifestation and/or a history of CF in the immediate family in addition to sweat chloride concentration >60mmol/L,
2. Pathologic CFTR mutation in both chromosomes.

Inclusion Criteria:

All confirmed CF patients of all the age groups that have positive gallstones in their radiological investigations namely ultrasound of abdomen (US) during their follow up period in CF clinic from the period 1st January 1984- 1st June 2018. The US was considered to be abnormal if it confirmed the presence of either gallstones or biliary sludge.

CFTR Identification:

CFTR Gene Screen Methodology: DNA Isolation, PCR amplification of genomic DNA, mutational analysis, and sequencing methods have been described before in a previous study from the same center [6]. Genomic DNA from the patient's lymphocytes was used to amplify the 27 exons and flanking sequences of the CFTR (NM_000492.3; NP_000483.3). The PCR products were analyzed by sequencing in both the forward and reverse directions [19] Polymerase Chain Reaction (PCR) [19] PCR evaluation, purification of PCR products for direct, and Direct Sequencing method were applied according to the recommended methods [19].

Mutation Detection [19]:

Mutation detection was scored using a publicly available mutation database for Cystic Fibrosis such as "Cystic Fibrosis Mutation Database" (<http://www.genet.sickkids.on.ca/CFTR/Home.html>) and "The Human Gene Mutation Database - Professional Edition" (<http://www.hgmd.cf.ac.uk/ac/index.php>). Both mutation databases provided an extensive repertoire of up-to-date sequence variants, deletions, and insertions for the CFTR gene.

Ethical Considerations:

This is a retrospective study. All data were stored in the pediatrics research unit, accessed only by the principal investigator and the assigned Clinical Research Coordinator. The entire patient's information was kept strictly confidential. Each patient was given a study number, and all patient data were entered into the designated data sheet (EXCEL) without any patient's identification. The Declaration of Helsinki and GCP guidelines were followed.

Statistical Statement:

The data collected from this study were electronically entered into a database. Multiple variables were collected and analyzed such as Abdominal Ultrasound findings, presence of pancreatitis, presence of pancreatic insufficiency based on chart review, and the administration of Ursodeoxycholic acid.

Results

A total of 391 CF patients were included in our study. However, some patients were excluded due to the lack of abdominal sonography, 252 patients had an abdominal ultrasound. Out of the 391 patients, there were 196 females (50%) and 195 males (50%) patients.

The Eastern region had the highest percentage of CF patients (38%), followed by the central region, which involved (25%) of the patients. The Southern region had the least amount of patients (11%) (**Fig-1**).

The initial abdominal US of these patients revealed

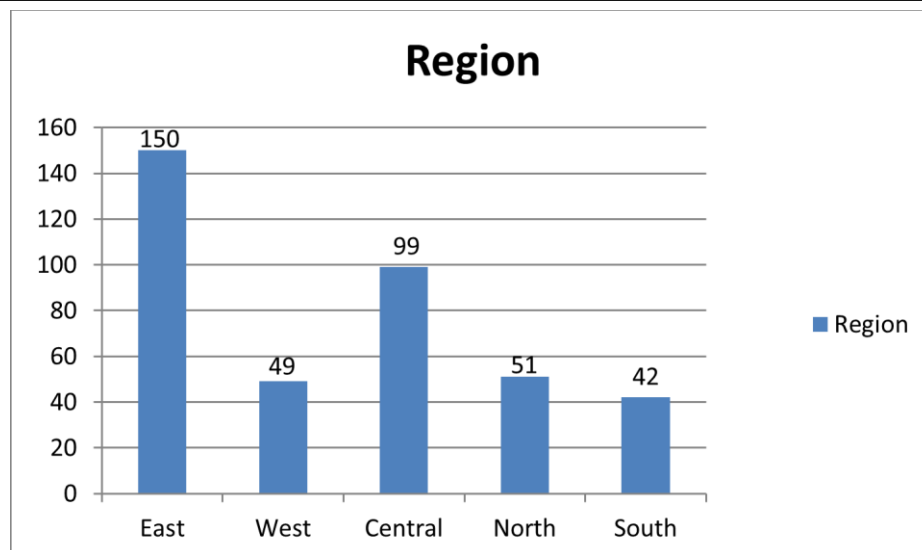


Fig-1: Geographic distribution of CF population Total 391 patients

that 7 patients (3%) had gallstones (2 females and 5 males), 8 patients (3%) had findings consisting with biliary sludge (2 females and 6 males) and 237 (94%) patients had normal gallbladders on US (**Table-1**).

Gallstones	1 st US	2 nd US
Total	252	77
Yes	7 (2.77%)	5 (6.49%)
No	245 (97.22%)	72 (93.50%)

Review of Ultrasound studies showed that: 86 (34%) had normal US liver, 28 (11%) mild hepatomegaly, 40 (16%) echogenic liver, 33 (13%) fatty infiltration, 1 (0.5%) peri-portal fibrosis, and 5 (2%) developed liver cirrhosis.

Pancreatitis was found in 4 patients (2%). A total of 191 patients (76%) had evidence of pancreatic insufficiency. Furthermore, nineteen patients were on Ursodeoxycholic acid as a result of concurrent hepatic enzyme abnormalities. Moreover, 77 patients had follow-up abdominal ultrasounds and 5 patients (7%) either developed or remained positive for gallstones, 4 patients (5%) had sludge and 68 patients (88%) remained negative for gallstones. Only two patients with cholelithiasis underwent cholecystectomy.

Screening for CFTR mutations for both gallstones and sludge groups showed that only one patient from each group showed similar CFTR mutation (c.2988+1G>A), otherwise the remaining patients from both groups showed different mutations as shown in **Table-2**. Furthermore, there were 4 patients with splice donor-site mutational effect (2 in intron 18 and 2 in intron 5) (**Table-2**). Its significance has not been reported before.

Discussion

In this retrospective study, we tried to determine the involvement of gallbladder abnormalities in CF patients via the use of abdominal sonography. Furthermore, we tried to report some findings related to CF in our patients such as; pancreatitis and pancreatic insufficiency.

Nazer H, showed that Cystic fibrosis has an estimated incidence of 1:4243 live births in Saudi Arabia [20]. Agrone showed that Cholelithiasis occurs in 12%-24% of Cystic fibrosis patients [10]. In comparison to our study, which revealed a 3% prevalence of cholelithiasis in CF. Nazer H, has reported a prevalence of 0.25% in a large population of hospitalized children [20]. Many of our patients were asymptomatic at the time of diagnosis.

Table-2: Genetic Mutation associated with gallstones in CF patients							
Ref	Mutation	Number of Patients	Ref SNP	Nucleotide Change	Location	Legacy Name	Mutation effect
24	DF508	1	rs113993960	c.1521_1523delCTT	Exon 11	[delta]F508; Exon 10	In-frame Deletion
25	711+1G>T	1	rs77188391	c.579+1G>T	exon6	711+1G>A; Intron 5	Splice Donor-Site
26	IVS18+1G>A	2	Rs75096551	c.2988+1G>A	Intron 18	3120+1G>A; Intron 16	Splice Donor-Site
Gall Bladder Sludge							
27	R709X	1	rs121908760	c.2125C>T	Exon 14	p.R709X	Nonsense Mutation
27	1507del9	2	_____	_____	Exon 9	_____	_____
26	IVS18+1G>A	1	Rs75096551	c.2988+1G>A	Intron 18	3120+1G>A; Intron 16	Splice Donor-Site
25	IVS5+1G>A	1	rs77188391	c.579+1G>T	Intron 5	711+1G>A; Intron 5	Splice Donor-Site
28	p.G473EfsX54	1	rs397508205	c.1418delG	Exon 11	1548delG; Exon 10	Frameshift Deletion
29	p.Q637HfsX26	1	rs1554389296	c.1911delG	Exon 14	2043delG; Exon 13	Single Nucleotide Deletion
30	p.S549R	1	rs121909005	c.1647T>G	Exon 12	S549R 'T>G'; Exon 12	Missense

Two out of our 7 patients with gallstones were females, and five were males; which is contrary to the common 4:1 female to the male ratio which was shown by Nazer H and his group [20]. Likewise, 2 out of the 8 patients who had sludge were females and 6 patients were males. Zimmerman HJ, has recommended that adolescent females with cystic fibrosis should avoid oral contraceptive pills as it can increase their chances of having cholelithiasis through cholesterol supersaturating in the bile [14].

The pancreas is involved in 85-90% of CF patients, commonly in the form of exocrine insufficiency while 30-50% only is in the form of endocrine insufficiency [8,21]. Our study showed a similar prevalence of pancreatic insufficiency 191 out of 391. Pancreatitis is usually an infrequent finding in CF patients with an estimated occurrence rate of 1.2% [22,23]. Compared

to 3% in our study.

One case report demonstrated the complete dissolution of gallstones after the administration of ursodeoxycholic acid in two CF patients [17]. Whereas 19 patients of our cystic fibrosis population were on ursodeoxycholic acid for cholestasis, which may have contributed to the low prevalence of cholelithiasis in our study.

We have reported for the first time the CFTR results of our CF gall stones/ sludge group. We have shown that there were 4 patients with splice donor site CFTR mutations (2 in gall stones group and 2 in sludge group). Its effect is unknown if it has any relation to cause liver cirrhosis (Table-2).

Conclusion

We recommend that abdominal sonography should be done in every CF patient in order to rule out the gallbladder and pancreatic diseases. As most of the cholelithiasis patients are asymptomatic, medical treatment is probably sufficient to improve the symptoms. However, surgical intervention should be considered in some cases.

Limitations

Our CF patients reflected approximately 80% of the CF population in KSA.

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Conflict of Interest

All authors have read and approved the final version of the manuscript. The authors have no conflicts of interest to declare.

References

- [1] Zielenski J, Rozmahel R, Bozon D, Kerem B, Grzelczak Z, Riordan JR, Rommens J, Tsui LC. Genomic DNA sequence of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics*. 1991 May;10(1):214-28. [PMID: 1710598]
- [2] Robertson MB, Choe KA, Joseph PM. Review of the abdominal manifestations of cystic fibrosis in the adult patient. *Radiographics*. 2006 May-Jun;26(3):679-90. [PMID: 16702447]
- [3] Cystic Fibrosis Foundation Patient Registry: Annual Data Report to the Center Directors, 2014. Available from: <https://www.cff.org/Research/Researcher-Resources/Patient-Registry/>
- [4] Rana M, Munns CF, Selvadurai H, Donaghue KC, Craig ME. Cystic fibrosis-related diabetes in children--gaps in the evidence? *Nat Rev Endocrinol*. 2010 Jul;6(7):371-78. [PMID: 20498678]
- [5] Banjar H. Geographic distribution of cystic fibrosis transmembrane regulator gene mutations in Saudi

- Arabia. *East Mediterr Health J*. 1999 Nov;5(6):1230-35. [PMID: 11924117]
- [6] Kambouris M, Banjar H, Moggari I, Nazer H, Al-Hamed M, Meyer BF. Identification of novel mutations in Arabs with cystic fibrosis and their impact on the cystic fibrosis transmembrane regulator mutation detection rate in Arab populations. *Eur J Pediatr*. 2000 May;159(5):303-9. [PMID: 10834512]
 - [7] Feigelson J, Pecau Y, Sauvegrain J. Liver function studies and biliary tract investigations in mucoviscidosis. r58 C/*539-44* A/Feigelson J, Pecau Y, Sauvegrain J: Liver function studies and biliary tract investigations in mucoviscidosis. *Acta Paediatr Scand*. 1970 Sep;59(5):539-44. [PMID: 5455520]
 - [8] Jebbink MC, Heijerman HG, Masclee AA, Lamers CB. Gallbladder disease in cystic fibrosis. *Neth J Med*. 1992 Oct;41(3-4):123-26. [PMID: 1470281]
 - [9] Soyer P, Spelle L, Pelage JP, Dufresne AC, Rondeau Y, Gouhiri M, Scherrer A, Rymer R. Cystic fibrosis in adolescents and adults: fatty replacement of the pancreas--CT evaluation and functional correlation. *Radiology*. 1999 Mar;210(3):611-15. [PMID: 10207457]
 - [10] Agrons GA, Corse WR, Markowitz RI, Suarez ES, Perry DR. Gastrointestinal manifestations of cystic fibrosis: radiologic-pathologic correlation. *Radiographics*. 1996 Jul;16(4):871-93. [PMID: 8835977]
 - [11] Weber AM, Roy CC, Morin CL, Lasalle R. Malabsorption of bile acids in children with cystic fibrosis. *N Engl J Med*. 1973 Nov 8;289(19):1001-5. [PMID: 4742200]
 - [12] Forstner J, Wesley A, Mantle M, Kopelman H, Man D, Forstner G. Abnormal mucus: nominated but not yet elected. *J Pediatr Gastroenterol Nutr*. 1984;3 Suppl 1:S67-73. [PMID: 6389816]
 - [13] Wasmuth HE, Keppeler H, Herrmann U, Schirin-Sokhan R, Barker M, Lammert F. Coinheritance of Gilbert syndrome-associated UGT1A1 mutation increases gallstone risk in cystic fibrosis. *Hepatology*. 2006 Apr;43(4):738-41. [PMID: 16557566]
 - [14] Zimmerman HJ: Hepatic injury caused by therapeutic agents, in *The Liver, Normal and Abnormal Function: Part A, The Biochemistry of Disease*, vol 5, edited by Becker FF, New York, Marcel Dekker; 1974. pp 225-302.
 - [15] Bennion LJ, Ginsberg RL, Gernick MB, Bennett PH. Effects of oral contraceptives on the gallbladder bile of

- normal women. *N Engl J Med*. 1976 Jan 22;294(4):189-92. [PMID: 1244533]
- [16] Watkins JB, Tercyak AM, Szczepanik P, Klein PD. Bile salt kinetics in cystic fibrosis: influence of pancreatic enzyme replacement. *Gastroenterology*. 1977 Nov;73(5):1023-28. [PMID: 332576]
- [17] Stern RC, Rothstein FC, Doershuk CF. Treatment and prognosis of symptomatic gallbladder disease in patients with cystic fibrosis. *J Pediatr Gastroenterol Nutr*. 1986 Jan;5(1):35-40. [PMID: 3003321]
- [18] Salh B, Howat J, Webb K. Ursodeoxycholic acid dissolution of gallstones in cystic fibrosis. *Thorax*. 1988 Jun;43(6):490-91. [PMID: 3420564]
- [19] Banjar HH, Tuleimat L, El Seoudi AAA, Mogarri I, Alhaider S, Nizami IY, AlMaghamisi T, Alkaf SA, Moghrabi N. Genotype patterns for mutations of the cystic fibrosis transmembrane conductance regulator gene: a retrospective descriptive study from Saudi Arabia. *Ann Saudi Med*. 2020 Jan-Feb;40(1):15-24. [PMID: 32026723]
- [20] Nazer H, Riff E, Sakati N, Mathew R, Majeed-Saidan MA, Harfi H. Cystic fibrosis in Saudi Arabia. *Eur J Pediatr*. 1989 Jan;148(4):330-32. [PMID: 2785036]
- [21] Newman DE. Gallstones in children. *Pediatric Radiology*. 1973 Jul 1;1(2):100-4.
- [22] Dietrich CF, Chichakli M, Hirche TO, Bargon J, Leitzmann P, Wagner TO, Lembcke B. Sonographic findings of the hepatobiliary-pancreatic system in adult patients with cystic fibrosis. *J Ultrasound Med*. 2002 Apr;21(4):409-16. [PMID: 11934098]
- [23] De Boeck K, Weren M, Proesmans M, Kerem E. Pancreatitis among patients with cystic fibrosis: correlation with pancreatic status and genotype. *Pediatrics*. 2005 Apr;115(4):e463-69. [PMID: 15772171]
- [24] el-Harith EA, Dörk T, Stuhmann M, Abu-Srair H, al-Shahri A, Keller KM, Lentze MJ, Schmidtke J. Novel and characteristic CFTR mutations in Saudi Arab children with severe cystic fibrosis. *J Med Genet*. 1997 Dec;34(12):996-99. [PMID: 9429141]
- [25] Zielenski J, Bozon D, Kerem B, Markiewicz D, Durie P, Rommens JM, Tsui LC. Identification of mutations in exons 1 through 8 of the cystic fibrosis transmembrane conductance regulator (CFTR) gene. *Genomics*. 1991 May;10(1):229-35. [PMID: 1710599]
- [26] Wilschanski M, Zielenski J, Markiewicz D, Tsui LC, Corey M, Levison H, Durie PR. Correlation of sweat chloride concentration with classes of the cystic fibrosis transmembrane conductance regulator gene mutations. *J Pediatr*. 1995 Nov;127(5):705-10. [PMID: 7472820]
- [27] Bonizzato A, Bisceglia L, Marigo C, Nicolis E, Bombieri C, Castellani C, Borgo G, Zelante L, Mastella G, Cabrini G, et al. Analysis of the complete coding region of the CFTR gene in a cohort of CF patients from north-eastern Italy: identification of 90% of the mutations. *Hum Genet*. 1995 Apr;95(4):397-402. [PMID: 7535742]
- [28] Sun Q, Xu X, Zhang Qq WH, Liu Y. Diagnostic Direct DNA Sequencing and Systemic Treatment with Voriconazole in *Scedosporium apiospermum* Keratitis? A Case Report. *J Clin Exp Ophthalmol*. 2013;4.
- [29] Fanen P, Ghanem N, Vidaud M, Besmond C, Martin J, Costes B, Plassa F, Goossens M. Molecular characterization of cystic fibrosis: 16 novel mutations identified by analysis of the whole cystic fibrosis conductance transmembrane regulator (CFTR) coding regions and splice site junctions. *Genomics*. 1992 Jul;13(3):770-76. [PMID: 1379210]
- [30] Kerem BS1, Zielenski J, Markiewicz D, Bozon D, Gazit E, Yahav J, Kennedy D, Riordan JR, Collins FS, Rommens JM. Identification of mutations in regions corresponding to the two putative nucleotide (ATP)-binding folds of the cystic fibrosis gene. *Proc Natl Acad Sci U S A*. 1990 Nov;87(21):8447-51. [PMID: 2236053]