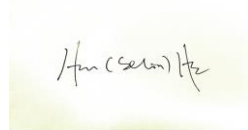


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Yours sincerely,



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c.753_754delAG, a novel CFTR mutation found in a Chinese patient with cystic fibrosis

Running title: Novel CFTR mutation in a Chinese CF patient

Abstract

Background: Cystic fibrosis (CF) is rare in Asian populations relative to the Caucasian population. In this study, we aimed to analyze the CFTR variation in a family of Chinese CF patients.

Methods: Genomic DNA was extracted from the CF patient and her parents. CFTR and all 27 coding exons were amplified by polymerase chain reactions (PCR), and then the PCR-amplified gDNA were analyzed by Sanger sequencing.

Results: A 30-month-old Chinese girl was diagnosed with CF based on her history and symptoms such as recurrent productive cough, wheezing with repeated infection of *Pseudomonas aeruginosa*, and paranasitis. The CFTR gene was sequenced from the blood samples of her and her parents and showed a heterozygous novel missense mutation of c.753_754delAG in exon 7. In addition, a heterozygous c.1240 C>T mutation was found in exon 10 of the *CFTR*. The mutation c.753_754delAG was verified to have been inherited from her mother, and the c.1240 C>T mutation was from her father who was diagnosed with congenital absence of vas deferens.

Conclusion: A novel mutation of CFTR, c.753_754delAG, was found. All CFTR mutation types in 61 Chinese CF patients indicated that c.2909G>A (11%) is the most common mutation among Chinese CF patients.

Key words: cystic fibrosis (CF), cystic fibrosis transmembrane conductance regulator (CFTR), mutation, Chinese children

Abbreviations:

CF: cystic fibrosis; CFTR: cystic fibrosis transmembrane conductance regulator; MLPA: multiplex ligation-dependent probe amplification; PCR: polymerase chain reaction

Introduction

Cystic fibrosis (CF) is an autosomal recessive inherited disease caused by mutations in the CF transmembrane conduction regulator (CFTR) gene. CF is most common in the Caucasian population, with a prevalence of 1/2500-3500 among those with Northern European ancestry [1,2]. CF was once considered extremely rare among the Chinese population, and to date, only about 60 cases of CF have been diagnosed in China [3]. CFTR is responsible for regulating the flow of chloride ions across the epithelial membrane. Since CFTR was first identified as the pathogenic gene of CF in 1989, more than 2000 mutations have been found in CF patients, according to the Cystic Fibrosis Mutation Database (<http://www.genet.sickkids.on.ca>). $\Delta F508$ is the most common mutation, accounting for greater than two-thirds of CF alleles worldwide, though it is not a predominant mutation in Chinese CF patients [4]. The most common gene mutation in Chinese children with CF is c.2909G-A [5]. With increased awareness of this disease and improvements in diagnostic techniques, we have found that CF is not as rare as once believed in the Chinese population. The novel variants c.699 C-A, c.579+1_579+2insACAT, c.1117-1G>C c.3140-454_c.3367+249del931ins13, and p.R1048_G1123del have been reported in CF patients from China in recent years [6-8]. Interestingly, the gene mutation spectrum of CFTR in Chinese patients with CF is significantly different from that in Caucasian patients. Therefore, it is necessary to establish the Chinese CFTR gene mutation database, which will facilitate the genetic

diagnosis of CF patients in China. In the present study, we identified a novel homozygous complex rearrangement involving CFTR exon 7 deletion (c.753_754delAG chr7-117176607-117176608) using multiplex ligation-dependent probe amplification (MLPA) analysis in a Chinese child with CF. We further reviewed the literature regarding Chinese CF patients from the 1970s to 2017s. The clinical data of all identified CF patients are summarized.

Methods

CFTR gene sequence analysis

This study was approved by the Ethics Committee of Soochow University, and written informed consent was obtained from the parents of the patient. Genomic DNA of the patient and her parents were extracted from peripheral blood using a Genomic DNA Purification kit (Life Invitrogen Qubit, USA) according to manufacturer's instructions. The DNA Library was constructed using KAPA LTP Library Preparation Kit reagent(Illumina Corporation, USA). All 24 coding exons of CFTR were amplified by polymerase chain reaction (PCR) in an automatic PCR cyclor (ETC 811, Beijing, China). Sanger sequencing for the 27 coding exons of CFTR and the flanking sequences was performed using the Illumina HiSeq2500 system (Illumina Corporation). Sequencing traces and generated results were analyzed using Illumina sequence control software (Illumina Corporation). All of the processes above were performed by Beijing Precision Gene Technology Company (Beijing, China).

Case report

General findings

A girl aged 2 years and 10 months was admitted to Children's Hospital Soochow University in May 2018 due to recurrent productive cough and wheezing lasting for 1 month. She had experienced recurrent pneumonia (2–3 times every year) beginning 4 months after birth, with repeated infection by *Pseudomonas aeruginosa* and paranasitis, but without a history of chronic diarrhea or pancreatic involvement. The child was conceived through in vitro fertilization. Her father had been diagnosed with congenital absence of vas deferens, and her mother was healthy. On physical examination, she weighed 11 kg and presented with shortness of breath and dyspnea. Crackles and wheezing rales were present in bilateral lungs. No clubbed digits were found. Laboratory examination showed a white blood cell count of $15.59 \times 10^9/L$, a C reactive protein concentration of 55.4 mg/L, and positivity for *Pseudomonas aeruginosa* on sputum culture. Findings on other tests, including serum electrolyte measurement, fungus culture, Glactomannan test, T-SPOT tuberculosis test, allergic bronchopulmonary aspergillosis and aspergillus fumigatus specific IgE detection, and echocardiography of pancreas, were all negative.

CT scanning

Chest computed tomography (CT) scanning revealed obvious exudative lesions and bilateral bronchiectasis (Figs. 1, 2). Sinus CT scanning revealed bilateral paranasitis. Liver CT scanning

revealed a low-density lesion in the left lobe of the liver.

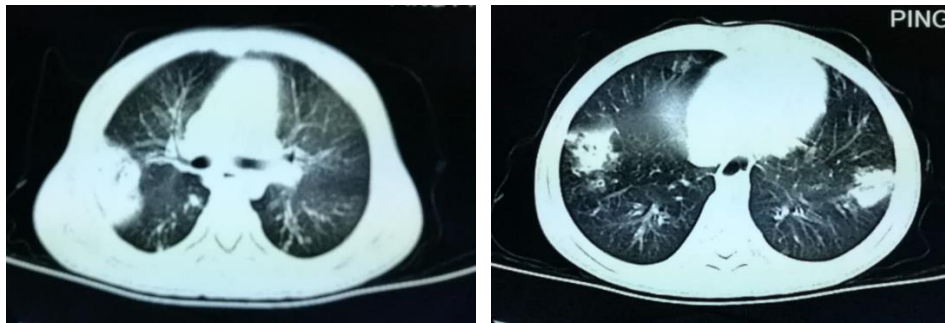


Fig 1. Chest CT scan of the CF patient. Chest CT scans showed obvious exudative lesions and bilateral bronchiectasis in lung of the CF patient.

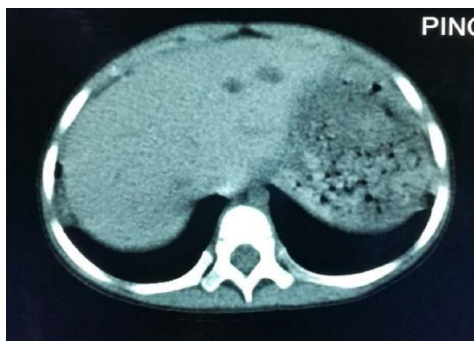


Fig 2. Liver CT scan of the CF patient. Liver CT scan revealed a low-density lesion in the left lobe of the liver.

CFTR gene sequence analysis

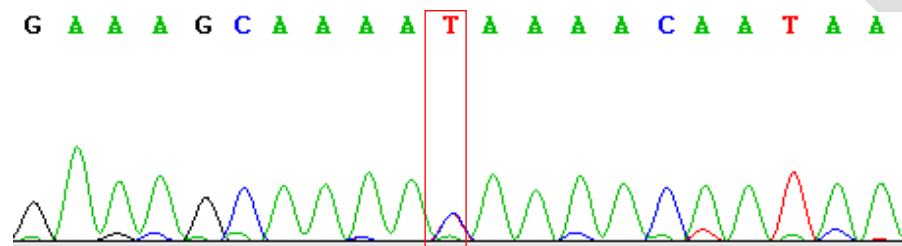
Two heterozygous mutations were found in the CF patient by Sanger sequencing analysis. A heterozygous novel missense mutation of c.753_754delAG chr7-117176607-117176608 was identified in exon 7 (Fig. 3), which was inherited from her mother based on its identification in the mother's sample as well (Fig. 3). This novel mutation has not yet been recorded in the CFTR

mutation database (<http://www.genet.sickkids.on.ca>). In addition, a heterozygous c.1240 C>T mutation in exon 10 was observed in CFTR of the CF patient (Fig. 4), which was inherited from her father and had already been included in the CFTR mutation database.

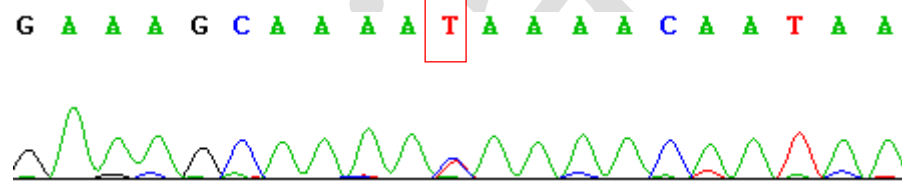


Fig. 3. Genomic sequence of exon 7 of CFTR. CFTR genomic sequencing results for exon 7 show a heterozygous mutation of c.753_754delAG chr7-117176607-117176608 p.R251Sfs*6 in the CF patient and her mother. Exon 7 of CFTR was normal in her father.

Patient



Father



Mother

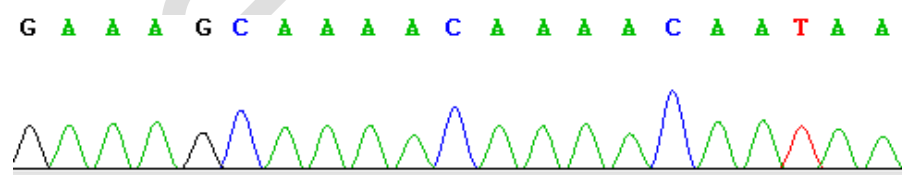


Fig. 4. Genomic sequence of exon 10 of CFTR. CFTR genomic sequencing results of exon 10 revealed a heterozygous mutation of c.1240C>T chr7-117188725 p.Q414* in the CF patient and her father. Exon 10 of her mother was normal.

Discussion

Table 1. Characteristics of CFTR gene mutations in 69 Chinese CF patients

Reference	Location	n	Gender	Age (years)	Mutation
Wang1993 ^[9]	Taiwan China	1	F	0.5	1898+5 G-->T, 2215insG+G2816A
Chen 1995 ^[10]	Mainland China	1	F	—	E2 del about 30 bp
Zielenski 1995 ^[11]	Taiwan China	1	F	8	1898+5 G-->T, 1898+5 G-->T
Crawford 1995 ^[12]	Chinese and Portuguese	1	F	3	1898 + 1G>T
Wagner 1999 ^[13]	Chinese	1	F	23	c.319-326delGCTTCCTA, c. 2909G>A
Wu 2000 ^[14]	Taiwan China	2	F	14	1898+5 G>T, 2215insG+G2816A
			M	17	1898+5 G>T, 2215insG+G2816A
Alper 2003 ^[15]	Chinese and Vietnamese	2	M	1.5	G151T, 989-992insA
	Taiwan China				1898+5G>T, 2215insG+G2816A

Chen 2005 ^[16]	Taiwan China	1	M	3	R553X, R553X
Li 2006 ^[17]	Mainland China	1	F	14	699C>A, 3821-3823delT
Wang 2012 ^[18]	Mainland China	1	F	14	W679X
Liu 2012 ^[19]	Mainland China	2	F	13	2909G>A, 362T>G
			F	10	3196C>T, 3196C>T
Cheng 2013 ^[20]	Mainland China	1	F	12	W679X, 1342-11TTT>G, 3120+2T>C
Liu 2015 ^[21]	Mainland China	7	M	12	c.95T>C, c.1657C>T
			M	10	c.293A>G, c.558C>G
			M	16	c.2052 dupA, Δ E18-E20(c.2909-?_3367 + ?del)
			F	16	c.2909G>A, Δ E7-E11†(c.744-?_1584 + ?del)
			F	10	c.1679 + 2T>C, c.2658-1G>C
			F	21	c.293A>G, c.293A>G
Shen 2016 ^[22]	Mainland China	19	F	28	c.1666A>G
			M	11.58	c.1699G>T, c.3909C>G
			F	10.58	c.263T>G, c.1766+5G>T, c.110C>G
			M	13.25	c.3700A>G, c.960_961insA
			F	13.67	c.263T>G, c.2909G>A
			M	7.17	c.326A>G, c.1000C>T, c.1666A>G

			F	10.67	c.595C>T
			F	7.75	c.223C>T, c.326A>G
			F	7.33	c.1000C>T
			F	10.17	c.263T>G
			F	11.08	c.1666A>G
			M	8.25	c.293A>G, c.558C>G
			F	4.17	c.326A>G, c.2374C>T
			M	3.67	c.1666A>G
			F	12.67	c.293A>G
			M	11	c.648G>A, c.2491-126T>C
			F	10.33	c.3196C>T
			M	11.17	c.414_415insCTA
			F	3.42	c.1075C>T, c.3307delA
			F	14	c.2909G>A
Chu 2016 ^[23]	Mainland China	1	M	9	C.579+2insACAT, C.F481766+5G>T
Xu 2016 ^[24]	Mainland China	1	M	0.67	c.595C>T, C.2290C>T
Li 2016 ^[25]	Mainland China	1	M	0.42	c.214G>G/A, c.650A>A/G, c.3406G>G/A
Tian 2016 ^[26]	Mainland China	8	F	15	c.2909G>A, c.2374C>T

Leung 2017 ^[27]	HongKong China	4	F	1	c.2909G>A, c.2125C>T
			M	13	c.3700A>G, c.959-960insA
			M	15	c.3635delT
			F	4	c.2909G>A, c.263T>G
			F	13	c.2909G>A, c.2907A>C
			M	20	c.2909G>A, c.1521_1523delCTT
			F	22	c.2909G>A, c.1997T>G
			M	17	c.1766+5G>T, c.3068T>G
			M	0.5	c.1766+5G>T, c.3140-26A>G
			M	0.17	c.868C>T, c.3068T>G
Xie 2017 ^[28]	Mainland China	2	F	0.75	c.1657C>T, c.3068T>G
			M	12	c.865A>T, c.3651_3652insAAAT
			M	15	c.865A>T, c.3651_3653insAAAT
Zheng 2017 ^[29]	Mainland China	2	M	5	c.3196C>T, c.870-1G>C
			F	5	c.3G>A, c.1572C>A
Xu 2017 ^[30]	Mainland China	4	M	9	c.579+1_579+2insACAT, c.1766+5G>T
			M	5	c.595C>T
			F	6	c.1117-1G>C, c.2909G>A

			M	13	c.4056G>C
Liu 2017 ^[31]	Mainland China	1	M	11	c.3140-454_c.3367+249del931ins13
Yao 2017 ^[32]	Mainland China	1	F	0.5	c.532G>A
Sun 2017 ^[33]	Mainland China	1	F	2	C.1 666A>G
Guo 2017 ^[34]	Mainland China	1	F	0.75	c.1373G>A(p.G458E), c.271G>A(p.G91R)
Li 2017 ^[35]	Mainland China	1	F	1.33	R709X, G970D

CF is characterized by the abnormal transport of ions and fluid across epithelial cell membranes, resulting from mutations on both alleles in the gene encoding the CFTR [36,37]. CFTR mutations can cause secretions to obstruct the airway, pancreatic, and biliary tracts and lead to abnormal secretion by sweat glands. The most important organ to be invaded in CF is the lung, and lung disease is the most lethal factor (85%) [38]. The pancreas is also an important affected organ in CF. Disorders caused by CF include nutritional disorders (fat, protein malabsorption, fatty diarrhea) and growth retardation. Low body weight caused by pancreatic insufficiency is negatively correlated with lung function and survival rate, and thus, an important factor for poor prognosis [39]. Malnutrition and gastrointestinal symptoms are relatively mild and atypical in Chinese CF patients. Therefore, it is easy for CF diagnosis to be missed or delayed.

For patients with one or more clinical characteristics, such as chronic sinopulmonary disease, gastrointestinal and nutritional abnormalities, genital abnormalities in males resulting in

obstructive azoospermia, and/or a family history of CF, the measurement of sweat electrolyte concentrations has been the mainstay of CF diagnosis since the standardized procedure was introduced [40]. In the CF case reported here, the patient had chronic sinopulmonary disease, and her father had a CF mutation with obstructive azoospermia. These patients should undergo repeat sweat chloride testing and further evaluation, including detailed clinical assessment and more extensive CFTR gene mutation analysis. CF in Chinese patients is difficult to diagnose, due to insufficient understanding and because sweat examination as well as genetic testing cannot be carried out in most hospitals. It is necessary to educate Chinese pediatricians concerning the clinical manifestations and diagnostic criteria for CF and to promote the implementation of the sweat chloride test.

CFTR mutations are divided into five general classes: mutations affecting biosynthesis, mutations interfering with protein maturation, mutations influencing Cl^- channel regulation, mutations intervening Cl^- conductance or channel gating, and mutations that **reduce** CFTR synthesis [41]. Different types of CFTR mutations can cause different clinical phenotypes: I, II, and III mutations are prone to cause pancreatic insufficiency with more serious clinical manifestations. In contrast, because normal Cl^- channel function is partially retained, the clinical symptoms of IV and V mutations are relatively mild with pancreatic function remaining normal.

Several studies have demonstrated that p.F508del is the most common mutation in Caucasian CF patients, accounting for approximately 70% of cases [42,43]. The p. F508del mutation is a

type II mutation. We reviewed 82 different mutations among 69 Chinese CF patients (40 females and 29 males) reported from the 1970s to 2017. Among them, 53 were from mainland China, 9 were from Taiwan, and 4 were from HongKong, with the remaining patients being of Chinese and Vietnamese descent, Chinese and Portuguese descent [9-35] (Table 1). The age at diagnosis ranged from 0.17 months to 23 years.

Among the Chinese CF patients, the c.2909 G>A variant was the most common mutation type (11%), followed by 1898+5G>T (7.3%), c.293A>G (6.1%), and 2215insG+G2816A and c.263T>G (both 4.9%). Nevertheless, no p.F508del mutation was found in the Chinese patients (Table 1). In addition, with the exceptions of c.3909 C>G, R553X, and c.1000 C>T, none of the CFTR mutations in the Chinese patients were present in the common Caucasian CFTR mutation-screening panels, indicating that the mutations identified in Chinese CF patients are obviously different from the common gene mutations in Caucasian CF patients. Further, pulmonary lesions were more prominent in Chinese CF patients with or without pancreatic insufficiency [17,21,22,30,31]. Therefore, it is necessary to establish a Chinese gene mutation database to facilitate genetic diagnosis of CF in China to clarify the relationship between genotype and clinical phenotype.

In the case reported herein, the c.1240C>T mutation resulted in the alteration of amino acid p.Q414* (glutamine > termination). This mutation type has been reported already as a pathogenic mutation in the HGMD pro database [41]. c.753_754A del A.G is a novel mutation (deletion

mutation) that results in amino acid changes P.R251Sfs * 6 (frame-shifting mutation - 6 termination). According to the ACMG guidelines, the mutation site c.753_754delAG could be classified as a pathogenic mutation [44]. Both mutations could result in the early termination of CFTR protein translation, which might have a great impact on protein function. The double heterozygous mutation came from the patient's parents separately. As a compound heterozygous mutation, it is consistent with autosomal recessive inheritance and is a theoretically possible cause of disease. This case expands the mutation spectrum of CFTR in patients of Chinese origin. Several studies have shown that only pancreatic function correlates well with CFTR genotypes [45,46]. According to the pancreatic status of patients, CF mutations can be subdivided into two groups: mild and severe mutations [45]. Patients with pancreatic insufficiency are homozygous or compound heterozygous with two "severe" mutations, whereas patients with pancreatic sufficiency have at least one "mild" allele. Elevated serum lipase and malnutrition in the reported patient suggests that the two mutations were severe mutations. In conclusion, a novel compound heterozygous c.753_754delAG mutation was found in exon 7 of CFTR in the case reported herein. The common CFTR mutation spectrum in Chinese CF patients is quite different from that in Caucasian patients. Therefore, the Chinese common CFTR mutation spectrum provides valuable data for CF diagnosis in Chinese patients and the development of a commercial Chinese CFTR genetic screening kit. The relevant Chinese gene mutation database is urgently needed.