INTERNAL MEDICINE

□ CASE REPORT □

Unique Mutations of the Cystic Fibrosis Transmembrane Conductance Regulator Gene of Three Cases of Cystic Fibrosis in Nagasaki, Japan

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Abstract

Cystic fibrosis (CF), the most common lethal hereditary disorder in Caucasians, is quite rare in Southeast Asia including Japan. Here, we report three CF cases encountered in Nagasaki, Japan. Case 1; a 24-year-old man with dyspnea and cough was diagnosed as CF with a missense mutation Q98R in exon 4 and a polymorphic 125C in exon 1 in the cystic fibrosis transmembrane conductance regulator (CFTR) gene. Case 2; a 13-year-old woman born of consanguineous parents was diagnosed as CF with homozygous Q98R mutations in exon 4. Case 3; a 29-year-old woman complaining of cough and sputum was diagnosed as CF with a heterozygous R347H mutation in exon 7 and a polymorphic 125C in exon 1. These mutations have been previously reported in Caucasian patients, but are considered very rare. Although the numbers of individuals with CF are very limited, the profiles of CFTR mutations in those patients are likely diverse in Japan.

Key words: cystic fibrosis, mutation, CFTR

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Introduction

Cystic fibrosis (CF) is an autosomal recessive fatal hereditary disease in Caucasian populations with an incidence ranging around 1 to 2,500-3,500 in U.S (1, 2). In contrast, the incidence of CF in Japan is extremely rare and is estimated as 1 to 350,000 (3). To date, a total of approximately 150 cases have been reported.

The gene responsible for CF is named cystic fibrosis transmembrane conductance regulator (CFTR) and localized to chromosome 7q31.2 (1). The CFTR gene consists of a total of 27 exons and encodes for cAMP-dependent chloride channel. The mutations of CFTR gene cause clinical manifestations of CF including pulmonary disease, meconium ileum, pancreatic insufficiency and elevated concentrations of chloride in sweat (4). More than 1,500 different mutations have been reported in the genes of patients (5, 6). The most common mutation of CFTR gene in Caucasian populations is F508del and approximately 70% of CF patients possess the mutation (4), whereas the incidences of other mutations remain about 2-3% (5).

Interestingly, F508del is not a common mutation in the Japanese population in the national surveillance by Japan Intractable Diseases Research Foundation (JIRDF), although the number of Japanese CF cases is quite few (data not published). The other novel or rare mutations such as R347H, D 979A, 1724delAG, H1085R, M152R and 1540del10 have

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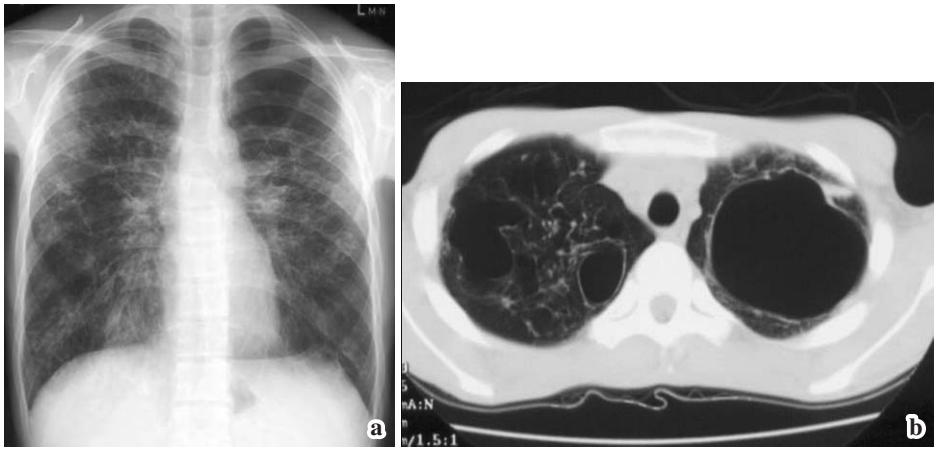

Figure 1. Radiological characteristics of Case 1. a) Chest X-ray film taken 10 years after the diagnosis of CF indicates diffuse bilateral cystic and bronchiectatic changes. b) Chest computed-tomography (CT) film shows advanced cystic and bronchiectatic changes in both lungs.

been identified in Japanese patients and reported previously (7-10). These data suggested that the profiles of mutations of CFTR gene in Japanese patients are different from those of Caucasian populations (1, 4). Here, we report three CF cases with rare CFTR mutations encountered in our institute in Nagasaki, Japan.

Materials and Methods

Sweat chloride concentration and exocrine pancreatic function were measured for clinical diagnosis in all cases. Sweat chloride concentration was measured by SWEAT CHECK 3,120 and MACRODUCT 3,700 (Wescor, Inc., Logan, Utah). The normal range of sweat chloride concentration was 0-40 mmol/L. Exocrine pancreatic function was evaluated by pancreatic functional diagnostant (PFD) test, and the normal range of PFD test was 73.4-90.7%.

The peripheral blood from all patients was collected in Nagasaki University School of Medicine and genomic DNA was extracted from each peripheral blood mononuclear cells by standard methods. For Case 1, single strand conformation polymorphism (SSCP) analysis was performed for all of 27 exons of the CFTR gene, as described elsewhere (11). A temporal temperature gradient electrophoresis (TTGE) with modification was used to scan for sequence variations for Cases 2 and 3 (Ambry Genetics, Aliso Viejo, CA) (12). Once any mutation or sequence variation was detected by SSCP or modified TTGE, further sequencing using genomic DNA from sense and anti-sense directions was performed to confirm their eligibilities.

Analysis of CFTR mutations of all cases was approved by each patient and their family member if the patient was a minor and informed consent from all patients and relatives were obtained. The patient confidentiality has been maintained.

Case 1

A 24-year-old man with dyspnea on effort and productive cough was diagnosed as CF with chronic sinusitis at the age of 15 years old. He was the second child of non-consanguineous parents and his mother had systemic lupus eryhtematosus and Graves' disease. He had pseudo-Bartter syndrome and cholangiolitis as a past medical history. On physical examination, he was emaciated and coarse crackles were heard in whole lung. He had congenital bilateral absence of the vas deferens (CBAVD) and pancreatic insufficiency (17.0 % in PFD test). Sweat chloride concentration was elevated (94.0 mmol/L). Missense mutation Q98R was detected in exon 4 and polymorphic 125C was present in exon 1 by CFTR mutation screening. Further analysis revealed that Q98R was derived from his father and 125C from his mother. Heterozygous Q98R was also recognized in his otherwise healthy elder sister. He had been treated with clarithromycin at the daily dose of 200 mg. However, he developed repeated exacerbations of chronic respiratory infection due to *Staphylococcus aureus* followed by *Pseudomonas aeruginosa*, and his lung function deteriorated to the status of respiratory failure (Fig. 1). He is on a waiting list for lung transplantation.

Case 2

A 13-year-old woman with productive cough and abnormal chest X-ray findings was diagnosed as CF with chronic sinusitis at the age of 12 years old. She was the third child of consanguineous parents with healthy elder brother and sister, and younger brother. She had no particular past medical history. Coarse crackles were audible in the lower chest and clubbed fingers were noted on physical examination. Chest X-ray revealed diffuse infiltration, bronchiectatic and cystic changes (Fig. 2). The presence of missense mutation Q98R was detected in a homozygous fashion in exon 4 of

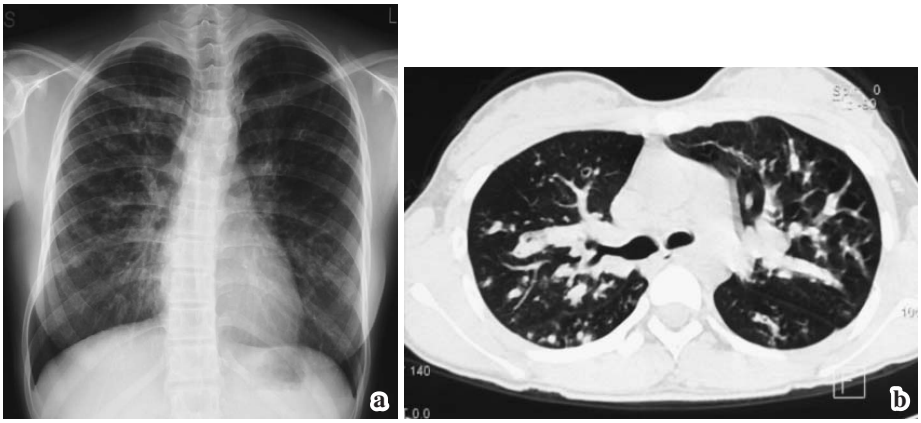

Figure 2. **Radiological characteristics of Case 2. a) Chest X-ray film indicates diffuse bilateral cystic and bronchiectatic changes. b) Chest CT film shows mucoid impaction, bronchiectatic and cystic changes in both lungs.**

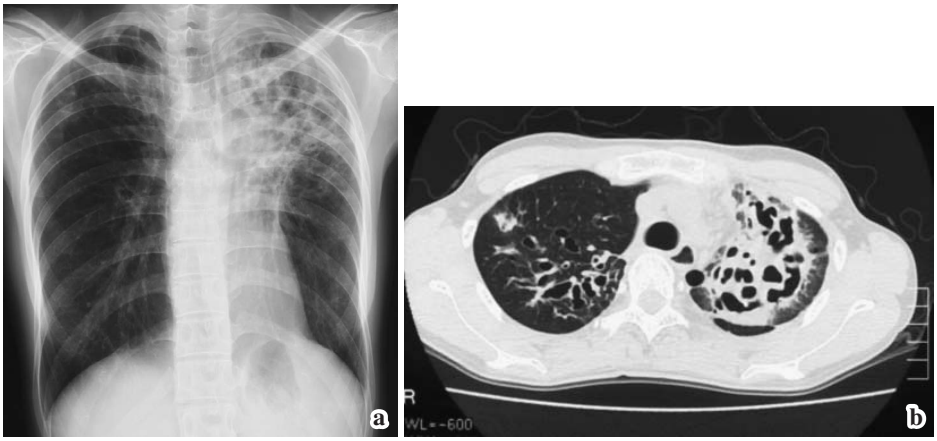

Figure 3. **Radiological characteristics of Case 3. a) Chest X-ray film demonstrating cystic and bronchiectatic changes in both upper fields, predominantly in the left lung. Both lower lung fields remained nearly intact. b) Chest CT film shows bronchiectasis and cystic changes in both lungs with minor parenchymal infiltrations.**

both CFTR alleles in mutation analysis. Her father possessed heterozygous Q98R mutation (test was not performed to mother). Sweat chloride level was intermediate (54.8 mmol/L) and pancreatic secretion test revealed low function (67.0% in PFD test). She was under continuous administration of azithromycin (250 mg/every other day) and inhalation of hypertonic saline for prophylaxis of acute exacerbation. Although methicillin-resistant *S. aureus* (MRSA) was isolated from sputum since the first admission to our hospital, *P. aeruginosa* has not yet been isolated.

Case 3

A 29-year-old woman with productive cough and purulent sputum was diagnosed as CF with chronic sinusitis at the age of 28 years old. She was the first child of non-consanguineous parents and her younger sister suffered from epilepsy. On physical examination, coarse crackles were audible in her left upper back. She had been diagnosed as bronchiectasis for 15 years and clarithromycin was administrated occasionally at outpatient clinics. Chest X-ray films indicated bronchiectatic and cystic changes predominantly in the left upper lung field (Fig. 3). Heterozygous R347H mutation in exon 7 and a polymorphic 125C in exon 1 were present by CFTR mutation screening. Sweat chloride level was abnormal (60 mmol/L) and pancreatic secretion test revealed insufficiency (69.8 % in PFD test). *S. aureus* was isolated from sputum and 200 mg/day of clarithromycin was restarted daily. Clinical characteristics of all three cases are summarized in Table 1.

Discussion

CF is considered to be extremely rare in Southeast Asia, and it is a nearly unknown disease in Japan. Interestingly however, we encountered three cases of CF all of whom were residents of Nagasaki prefecture, a southwest part of Japan.

Table 1. Summary of Three Cystic Fibrosis Cases

Case	Age	Sex	CP	sinusitis	CBAVD	PFD test (%)	sweat chloride concentration (mmol/L)	Locus of CFTR mutation			
								Mutation (variant)	Exon	Mutation (variant)	Exon
1	24	M	-	+	+	17.0	94.0	125C	1	Q98R	4
2	13	F	+	-	-	67.0	54.8	Q98R	4	Q98R	4
3	29	F	-	+	-	69.8	60.0	125C	1	R347H	7

CP, consanguineous parents; CBAVD, congenital bilateral absence of the vas deferens; PFD, pancreatic functional diagnostant. Normal reference ranges 73.4-90.7% for PFD test, 0-40 mmol/L for sweat chloride concentration.

The CFTR Q98R mutation was first reported by Romey et al (13), and the patient possessed F508del mutation on the other allele. In the present Case 1, the patient carried Q 98R mutation on the one allele and 125C polymorphism in exon 1 on the other allele of CFTR gene. Although father and elder sister of the patient carried the same Q98R mutation heterozygously, they were phenotypically healthy. This indicated 125C polymorphism delivered from his mother might play an important role for causing CF. Although there is no apparent or strong evidence that this specific polymorphism is solely responsible for clinical phenotype of CF even in the CF Mutation Database, Yoshimura reported the profile of CFTR gene mutations among 23 Japanese CF patients in the national surveillance by JIDRF, and the polymorphic 125C was detected in 13 alleles out of 46 (28%) (unpublished data). The data suggests that this 125C polymorphism is likely disease causing, although further functional elucidation may be necessary.

In Case 2, the Q98R mutation was detected homozygously and it was likely due to the consanguineous marriage of her parents. The patient was a resident of a small community district and consanguineous marriage was not rare in such districts. Thus, the mutation was considered to have accumulated through generations. No cases with Q98R homozygous mutation in CFTR gene was reported among Japanese CF patients in the JIDRF surveillance (unpublished data). Since Cases 1 and 2 lived in close proximity in Nagasaki prefecture, the possible connection of these two families was carefully researched by interview. However, no apparent relationship was discovered. The common findings observed in these two cases with Q98R were the distribution of lung lesions and the pattern of onset. Chest X-ray findings of Case 2 quite resembled those of the early phase of Case 1 (data not shown). Case 1 has developed severe respiratory failure with chronic respiratory tract infection with *P. aeruginosa* and is currently on the waiting list for lung transplantation.

S. aureus is always the first isolated pathogen of lung infection prior to *P. aeruginosa* in CF patients. The treatment and prophylaxis with anti-staphylococcal agents would result in a greater occurrence of *Pseudomonas* in the sputum cultures of CF patients (14).

In Case 2, representing the early phase of CF compared to Case 1 as mentioned above, MRSA was isolated from sputum from the very beginning of first admission. Recently, infection of community-acquired MRSA (CA-MRSA) is a major clinical concern in U.S. (15). In this context, the isolate detected in Case 2 possessed no *Mec A* (16) nor Panton-Valentine leukocidin gene (17), indicating that the strain was not likely a typical CA-MRSA strain.

The R347H mutation which was detected in Case 3 was originally reported in 1992 in a CF patient with mild phenotype (18). The clinical features of CF patients with R347H mutation were characterized as mild pulmonary symptoms and all men were infertile accompanied by CBAVD (7). Although a few cases with R347H mutations with F508del on the other allele were previously reported in Japan and Italy (7, 18), no cases with R347H/125C mutation have been reported before. The chest X-ray findings in Case 3 are completely different from those of Cases 1 and 2. The cystic changes observed in the lungs of Case 3 are extremely minor and localized only in the left upper lobe, which are comparable with the data from previous studies (18). Such differences in terms of lung destruction led to the delayed diagnosis in Case 3 compared to Cases 1 and 2. In addition, *S. aureus* was already colonized in the airway and occasionally caused the chronic respiratory tract infection.

In summary, we have described three Japanese cases of CF in the Nagasaki region. The mutations detected were Q 98R in Cases 1 and 2, and R347H in Case 3, although they were heterozygous in Cases 1 and 3. Since CF is considered extremely rare in Southeast Asia populations including Japan, the present report would suggest that the profiles of CFTR mutations in Japanese individuals with CF are likely different geographically and ethnically.

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