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Identification of Novel Mutations of the CFTR Gene in a Japanese Patient with Cystic Fibrosis

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SEKI, K., ABO, W., YAMAMOTO, Y. and MATSUURA, A. *Identification of Nobel Mutations of the CFTR Gene in a Japnese Patient with Cystic Fibrosis.* Tohoku J. Exp. Med., 1999, **187**(4), 323-328 —— Cystic fibrosis (CF) is an inheritable disorder characterized by defective epithelial chloride transport and progressive lung disease, caused by mutations in the CF transmembrane conductance regulator (CFTR) gene. The subject of this study was an 8-year old Japanese boy, who developed typical CF symptoms including meconium ileus, pancreatic insufficiency, an elevated sweat chloride concentration and pulmonary disease. Analysis of the CFTR gene of this patient revealed compound heterozygous mutations in exon 11 (1742 delAC) and intron 9 (1525-18 GtoA) of the CFTR gene. ———— cystic fibrosis; CFTR gene; hereditary disease; molecular diagnosis

Cystic fibrosis (CF) is an autosomal recessive hereditary disease. The gene responsible for CF (CFTR, cystic fibrosis transmembrane conductance regulator) was identified in 1989, and localized to chromosome 7q31.2 (Rommens et al. 1989). CFTR gene consists of 27 exons and encodes a cAMP-dependent chloride channel. A deficiency in CFTR causes the typical symptoms of CF, such as meconium ileus, pancreatic insufficiency, an elevated sweat chloride concentration and pulmonary disease (Welsh et al. 1995).

CF is one of the most common hereditary diseases in Caucasian populations, with an incidence ranging from 1 in 2000 to 1 in 4000 (Farrell and Mischler 1992). Following its identification, more than 700 different mutations have been characterized in the gene of the patients. According to the Cystic Fibrosis Registrys

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1995 Annual Report, 48.7% of patients in the US were homozygous for a common mutation (⊿F508), while 86.6% were carriers of the ⊿F508 mutation (Kerem et al. 1990; Gregg et al. 1997). Two other mutations (G542X, G551D) also occur at relatively high frequencies (2.2% and 1.9% in all mutant alleles, respectively). In the Japanese population, CF is a rare disease. Approximately 50 cases have been reported to date in Japan, but these have not been analyzed at the molecular level.

In this study, we analyzed the CFTR gene of a Japanese CF patient and his family, and identified two heterozygous mutations which have not been reported previously.

Patient Report

Our patient was an 8-year old boy with severe CF. He was the second child of healthy non-consanguineous Japanese parents. His parents, elder sister and younger brother had no symptoms of CF. The patient was born after 39 weeks of gestation without any delivery complications, and his birth weight was 3192 g. On the third day after delivery, he developed meconium ileus, for which he underwent surgery. He required two further operations for a perforated intestine and paralytic ileus at 4 and 9 months of age, respectively. He experienced further episodes of paralytic ileus at 3 and 7 years of age, and was treated conservatively. He was also diagnosed as having bronchitis, pneumonia and bronchial asthma, and frequently needed antibiotics.

When he was 8 years old, he was admitted to our hospital because of a high fever, cough and general malaise. His chest x-ray and computer tomograph showed diffuse obstructive lung disease with pneumonia. His serum analysis showed pancreatic insufficiency (lipase, 3 IU/liter; esterase I, 43 ng/100 ml; trypsin, 30 ng/ml; phospholipase, undetectable), elevated IgG and IgA levels (2210 and 424 mg/100 ml, respectively) and a normal IgM level (212 mg/100 ml). Methicillin-resistant Staphylococcus aureus (MRSA) was detected from his sputum. A pulmonary function test showed a restrictive pattern (% VC, 55.8 and $FVC_{1\%}$, 95.1). His sweat sodium and chloride levels were markedly elevated (163 and 153 mEq/liter, respectively).

Materials and Methods

Analysis of the nucleotide sequences of exons 10 and 11 of the CFTR gene

Initially, 5 ml of heparinized blood was obtained from the patient, his parents, his elder sister and his younger brother by venipuncture. Genomic DNA was isolated from the blood samples using Wizard Genomic DNA Purification Kit (Promega, Madison, WI, USA). PCR amplification was carried out using CFTR10F as the sense primer and CFTR10R as the antisense primer for exon 10, and CFTR11F as the sense primer and CFTR11R as the antisense primer for exon 11 as listed in Table 1. The PCR mixture contained 2 pmol of each primer, 4

TABLE 1. *Primers for analysis of CFTR gene*

Primer	Type	Sequence	Position
CFTR10F	Sense	5′-GAATATACACTTCTGCTTAGGATG-3′	Intron 9
CFTR10R	Antisense	5′-CTAACCGATTGAATATGGAGCC-3′	Intron 10
CFTR11F	Sense	5′-ACTGTGGTTAAAGCAATAGTGTG-3′	Intron 10
CFTR11R	Antisense	5′-GTGATTCTTAACCCACTAGCC-3′	Intron 11

nmol of each of the four deoxynucleotide triphosphates, 100 ng purified genomic DNA and 0.5 U EX-Taq DNA polymerase (TAKARA, Kyoto) mixed with an equal volume of TaqStartTM antibody (Clonetech, Palo Alto, CA, USA) to a total volume of 20 μl. The mixture was cycled 35 times using Gene Amp PCR System 9600 (Perkin Elmer, Norwalk, CA, USA). Each cycle consisted of a denaturation step (94°C for 1 minutes), an annealing step (58°C for 1 minutes) and an elongation step (72°C for 1 minutes). The PCR products were directly sequenced using a Dye Terminator Cycle Sequencing Kit (PE Applied Biosystems, Foster, CA, USA).

RESULTS

The analysis of the CFTR gene was focused on exons 10 and 11, in which the most frequently identified mutations leading to CF have been found (⊿F508 in

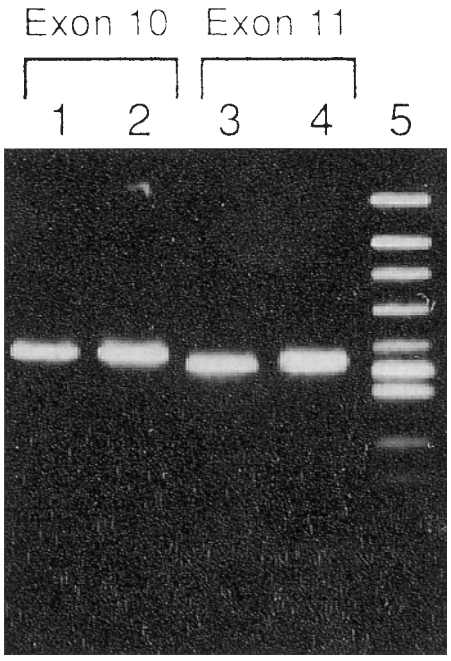

Fig. 1. PCR amplification of exons 10 and 11 of the human CFTR gene.
The PCR reaction was carried out as described in materials and methods, then the PCR products were separated on 3% agarose gel. The PCR products from the patient were run in lanes 1 and 3 and identical products from a normal control were run in lanes 2 and 4. In lane 5, fX174 DNA digested with HincII was run as a standard size marker.

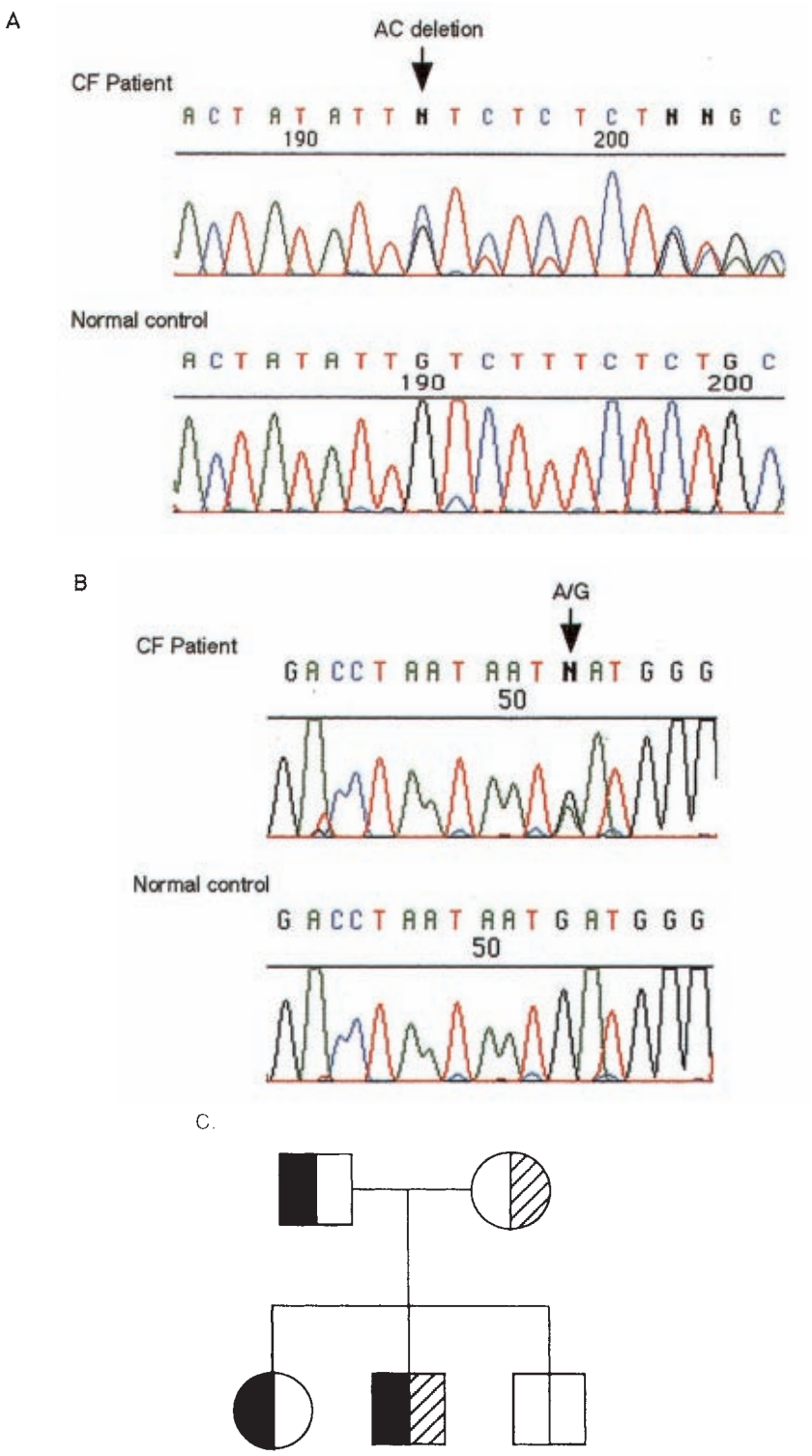

Fig. 2. Nucleotide sequences of PCR products around the observed mutations in the CFTR gene of the CF patient and the pedigree analysis. **A**, Nucleotide sequences of the antisense strand of the heterozygous 1742 delAC mutation in exon 11. **B**, Nucleotide sequences of the sense strand of the heterozygous 1525-18 GtoA mutation in intron 9. **C**, The pedigree of the CF patient. The alleles containing 1525-18 GtoA and 1742 delAC mutations were indicated by hatched lines and black boxs, respectively.

exon 10, and G542X and G551D in exon 11). DNA fragments containing exons 10 and 11 were efficiently amplified from the genomic DNA as 375-bp and 354-bp bands, respectively, on 3% agarose gel (Fig. 1). Sequencing analysis revealed a heterozygous mutation (1525-18 GtoA) in the intronic sequences flanking exon 10 (Fig. 2B) and a heterozygous mutation (1742 delAC) in exon 11 (Fig. 2A) of the patient gene. Analysis of the genome of his family indicated that one of the mutation in intron 9 (1525-18 GtoA) and in exon 10 (1742 delAC) were derived from his mother and his father, respectivery (Fig. 2C). His healthy younger brother was not a carrier of these mutations, however, his healthy elder sister was a carrier of the heterozygous 1742 delAC (Fig. 2C).

Discussion

Very few molecular studies of CFTR gene mutations in the Japanese population have been reported up to the present. Our patient was of Japanese parentage and had heterogeneous compound mutations in exon 11 and intron 9 (Figs. 2A and 2B, respectively). Analysis of the genome of his family showed that the mutation in intron 9 was derived from his mother, while the mutation in exon 11 was derived from his father (Fig. 2C). The mutation in exon 11 (1742 delAC) caused a reading frame shift. The transcript from this allele would encode a protein with 29 unrelated amino acids downstream of the mutation, thus losing the functional domain. The effect of the mutation in intron 9 (1525-18 GtoA) on CFTR gene function is unclear, however, analysis of the family pedigree showed that this mutation might correlate with functional loss of the CFTR gene.

Recently, twins with cystic fibrosis were reported in Japan (Hojo et al. 1998). The patients were of mixed parentage; they had a Japanese mother and a German father. Their CFTR genes exhibited a compound heterozygous ⊿F508 mutation from the father and missense mutations in exon 7 (R347H) and exon 16 (D979A) from the mother. ⊿F508, the most frequently identified CF mutation in Caucasian populations, might therefore not be the major mutation in Japanese CF patients.

Our patient is now receiving pancreatic enzyme, and vitamin supplements, together with low-dose erythromycin to control his chronic obstructive lung disease. This case shows that, although CF is very rare in Japan, its diagnosis is important so that risks to the patient's health can be reduced or controlled. Our PCR method might be useful in the diagnosis of CF in Japanese patients at the molecular level.

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