Impact of cystic fibrosis transmembrane conductance regulator gene mutation on the occurrence of chronic pancreatitis in Japanese patients

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Impact of Cystic Fibrosis Transmembrane Conductance Regulator Gene Mutation on the Occurrence of Chronic Pancreatitis in Japanese Patients

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DNA analyses of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene in Japanese patients with idiopathic chronic pancreatitis (ICP) were performed to determine the relationship between the *CFTR* mutation and ICP. The study included patients with alcoholic pancreatitis (*n* = 20), patients with ICP (*n* = 20) and healthy volunteers (controls; *n* = 110). The poly-T region in intron 8 of the *CFTR* gene was analysed by direct sequencing. The *CFTR* coding region was screened using single-strand conformational polymorphism and direct sequencing. In the controls, frequencies of the 5T genotype and 5T allele were 4.5% and 3.6%, respectively. The frequency of the 5T genotype was significantly higher in the ICP group (20%) versus controls, but was not significantly different in alcoholic chronic pancreatitis patients (5%). Thus, the *CFTR* gene mutation, especially the 5T genotype, appears to have some relationship to ICP prevalence in Japanese patients independent of cystic fibrosis.

KEY WORDS: Idiopathic chronic pancreatitis; Alcoholic pancreatitis; Japanese patients; Cystic fibrosis transmembrane conductance regulator (CFTR)

Introduction

Chronic pancreatitis is clinically characterized by acinar cell degeneration and fibrosis that may lead to the destruction of exocrine and endocrine organ functions,[1,2] and results in maldigestion and diabetes mellitus. Several underlying conditions appear to play a role in the pathogenesis of chronic pancreatitis, however the most common types of chronic pancreatitis are alcohol-related and idiopathic.[3]

A strong association between mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene and idiopathic chronic pancreatitis (ICP) has been reported.[4] Cystic fibrosis is the most common autosomal recessive disease in Caucasians, affecting 1 in 1600 to 1 in 2500 live births and corresponding to a carrier frequency of about 1 in 25.[5] In contrast, cystic fibrosis is rare among Asian populations.[6] In Japan, there have been

approximately 130 cases of cystic fibrosis reported in the literature during the last five decades, with an estimated incidence of 1 in 350 000 live births.[7] It is thought that the frequency difference between the Japanese and Caucasian populations might be due to a lack of Japanese population exposure to the founder gene effect of *CFTR*.[6,7]

Since it was anticipated that some of the common mutations found in Western countries may not have not been detected in Japanese cases, the present study was not designed to examine disease-causing mutations but, rather, to investigate predisposing genetic factors. Variations in the poly-T sequence in intron 8 of the *CFTR* gene can affect the function of this gene and the 5T allelic variant has been shown to decrease *CFTR* gene function even when heterozygous.[8,9] The aims of the present study were, therefore, to confirm the frequency of the 5T genotype in the Japanese population, to evaluate its frequency in Japanese patients with ICP and alcoholic pancreatitis, and to examine mutations of the *CFTR* gene and the trypsinogen-encoding gene (protease serine 1 [*PRSS1*]) in Japanese ICP patients.

Patients and methods

PATIENTS

This study was approved by the Medical Ethics Committee of Kanazawa University and written informed consent to carry out genetic analysis was obtained from each patient and the family.

Consecutive Japanese patients who visited Kanazawa University Hospital (Kanazawa, Japan) with either ICP or alcoholic pancreatitis were enrolled in the study. The control group comprised healthy volunteers.

The diagnosis of chronic pancreatitis was based on visible calculi on X-ray film or unequivocally abnormal findings from endoscopic retrograde cholangiopancreatography. The criterion for diagnosis of alcoholic chronic pancreatitis was ethanol intake of ≥ 60 g/day for ≥ 2 years before manifestation of pancreatitis. The diagnosis of ICP was performed by exclusion of diseases of the biliary system, alcoholism, autoimmune disease, abnormalities of the pancreatic ducts, drug-induced pancreatitis, trauma, or autosomal-dominant pancreatitis.

ANALYSIS OF THE POLY-T REGION IN INTRON 8 OF *CFTR*

Genomic DNA was isolated from peripheral blood. The poly-T region in intron 8 of the *CFTR* gene was amplified by polymerase chain reaction (PCR) and direct sequencing was performed using a non-radioactive sequencing method (Sequencing High Plus™ kit; Toyobo, Osaka, Japan).

ANALYSIS OF CODING REGIONS AND SPLICE SITES OF *CFTR* AND *PRSS1*

Each exon was amplified by PCR using specific primers (Tables 1 and 2), and screened using the single-strand conformational polymorphism (SSCP) method.[10] When abnormal bands were detected by SSCP, the PCR products were sequenced using a BigDye® Terminator Version 3.1 Cycle Sequencing Kit and ABI PRISM® 3100/Avant Genetic Analyzer (Applied Biosystems, Foster City, CA, USA).

RESTRICTION FRAGMENT LENGTH POLYMORPHISM

Restriction fragment length polymorphism (RFLP) was used to confirm the detection of mis-sense mutations in the *CFTR* gene according to the method of Botstein *et al*.[11]

STATISTICAL ANALYSIS

The χ^2 test was performed to assess the

TABLE 1:
Polymerase chain reaction primers used for amplification of cystic fibrosis transmembrane conductance regulator (*CFTR*) gene

Exon	Forward primer	Reverse primer
Exon 1F	5′-TTGGCATTAGGAGCTTGAGC-3′	5′-ACGTGTCTTTCCGAAGCTCG-3′
Exon 2F	5′-CATAATTTTCCATATGCCAG-3′	5′-CACCATACTTGGCTCCTATT-3′
Exon 3F	5′-CTTGGGTTAATCTCCTTGGA-3′	5′-ATTCACCAGATTTCGTAGTC-3′
Exon 4F	5′-CTTGTGTTGAAATTCTCAGGG-3′	5′-CAGCTCACTACCTAATTTATGA-3′
Exon 5F	5′-GAGAAGATAGTAAGCTAGAT-3′	5′-TATTAACAACAGGCTAAGGT-3′
Exon 6aF	5′-ACACCTGTTTTTGCTGTGCT-3′	5′-CTATGCATAGAGCAGTCCTG-3′
Exon 6bF	5′-GGAGGCATTTACCAAACAGT-3′	5′-ATATGAGGTGGAAGTCTACC-3′
Exon 7AF	5′-TTCCATTCCAAGATCCCT-3′	5′-AACACCACAAAGAACCCTGA-3′
Exon 7BF	5′-AAGGCAGCCTATGTGAGATA-3′	5′-TGTCCAGAGAAATGCTAGGA-3′
Exon 8F	5′-TCCTAGTGCTTGGCAAATTA-3′	5′-ACAGTTAGGTGTTTAGAGCA-3′
Exon 9F	5′-AAAATATCTGACAAACTCATC-3′	5′-AAAATACCTTCCAGCACTAC-3′
Exon 10F	5′-AGTGAATCCTGAGCGTGATT-3′	5′-GTGTGAAGGGTTCATATGCA-3′
Exon 11F	5′-CAACTGTGGTTAAAGCAATAGTGT-3′	5′-GCACAGATTCTGAGTAACCATAAT-3′
Exon 12F	5′-TGACCAGGAAATAGAGAGGA-3′	5′-TATGATGGGACAGTCTGTCT-3′
Exon 13AF	5′-GAATTCACAAGGTACCAATT-3′	5′-CTGTAGATTTTGGAGTTCTG-3′
Exon 13BF	5′-TTTGCATGAAGGTAGCAGCT-3′	5′-GGGAGTCTTTTGCACAATGG-3′
Exon 13CF	5′-ACTGGAGAGTTTGGGGAAAA-3′	5′-GCCAGTGACACTTTTCGTGT-3′
Exon 13DF	5′-GTCCTGAACCTGATGACACA-3′	5′-TGAAATACCCCCAAGCGATG-3′
Exon 14aF	5′-AAAAGGTATGCCACTGTTAA-3′	5′-GTATACATCCCCAAACATTCT-3′
Exon 14bF	5′-GGGAGGAATAGGTGAAGATG-3′	5′-CCACTACCATAATGCTTGGG-3′
Exon 15AF	5′-GTAAGTAACTTTGGCTGCCA-3′	5′-ATTAGAGTATGCACCAGTGG-3′
Exon 15BF	5′-AGTAGCCGACACTTTGCTTG-3′	5′-CCTATTGATGGTGGATCAGC-3′
Exon 16F	5′-GGGTTCTGAATGCGTCTACT-3′	5′-GACAGGACTTCAACCCTCAA-3′
Exon 17aF	5′-TTGTCCACTTTGCAATGTGA-3′	5′-TACAAGATGAGTATCGCACA-3′
Exon 17bAF	5′-AAGAATGGCACCAGTGTGAA-3′	5′-CTCATTTGGAACCAGCGCAG-3′
Exon 17bBF	5′-AGCCTTACTTTGAAACTCTG -3′	5′-GATAACCTATAGAATGCAGC-3′
Exon 18F	5′-GCCCTAGGAGAAGTGTGAAT-3′	5′-ACAGATACACAGTGACCCTC-3′
Exon 19F	5′-CCGACAATAACCAAGTGAC-3′	5′-GCTAACACATTGCTTCAGGC-3′
Exon 20F	5′-TCACAGAAGTGATCCCATCA-3′	5′-TTCTGGCTAAGTCCTTTTGC-3′
Exon 21F	5′-GATGGTAAGTACATGGGTGT-3′	5′-TGGTATGAGTTACCCCTTTC-3′
Exon 22F	5′-GCTTTCAGAACTCCTGTGTT-3′	5′-CTGTTCCTGTGCTATTA-3′
Exon 23F	5′-CTGATTGTGCGTAACGCTAT-3′	5′-AGGGCAATGAGATCTTAAGT-3′
Exon 24F	5′-CATAGAAGAGAACAAATGGC-3′	5′-GTGACTGTCCCACGAGCTCC-3′

TABLE 2:
Polymerase chain reaction primers used for amplification of the protease serine 1 (*PRSS1*) gene

Exon	Forward primer	Reverse primer
Exon 1F	5′-GAGTGGCCAAACATAGCCAG-3′	5′-GCATTTGTCGGCCAGGAACG-3′
Exon 2F	5′-CGCCACCCCTAACATGCTAT-3′	5′-CTCTCCCAGGCAGACTGGCC-3′
Exon 3F	5′-AAGGTGGGATAGGTGCCCTG-3′	5′-GGATGGAGGGAAGTAGAAGGACT-3′
Exon 4F	5′-GACCCACATTTCTACTTCCTTTGATC-3′	5′-CTCAGCATGGGAAGGGTTGG-3′
Exon 5F	5′-TATTCCTCCTCCATCTCTCCATAC-3′	5′-CAGTGTGAAGGAGTGAGAGG-3′

frequency of the 5T genotype between patients and controls. A *P*-value < 0.05 was considered to be statistically significant. Stat View® version 5.0 (SAS Institute, Cary, USA) was used for data analysis.

Results

CLINICAL CHARACTERISTICS

A total of 40 patients with chronic pancreatitis were enrolled in the study: 20 were diagnosed with ICP (four males, 16 females, mean ± SD age 42.6 ± 10.2 years) and 20 with alcoholic chronic pancreatitis (17 males, three females, mean ± SD age 53.2 ± 12.2 years). The control group comprised 110 healthy volunteers (56 males, 44 females).

DNA STUDIES

Following PCR amplification and direct sequencing of the poly-T region in intron 8 of the *CFTR* gene, genotypes with the 5T and 7T alleles were identified (Fig. 1). Genotypes that had at least one 5T allele were defined as a 5T genotype. The frequencies of the 5T genotype and 5T allele in the control group were 4.5% and 3.6%, respectively. The frequencies for the 5T allele in the ICP and alcoholic chronic pancreatitis patients were 12.5% and 5.0%, respectively. The frequency of the 5T genotype in ICP patients was 20.0%, which was significantly higher than in the control group ($P = 0.012$). In contrast, the frequency of the 5T genotype in alcoholic chronic pancreatitis patients was 5.0% and was not significantly different from the control group (Table 3). In this study, there were three patients with early onset chronic pancreatitis, i.e. diagnosed at < 30 years of age. All were idiopathic and two had the 5T genotype. This study was unable to show the frequency of the 5T genotype in alcoholic chronic pancreatitis patients to be statistically significantly different from that in the IPC group.

When the *CFTR* coding regions of patients with ICP were screened, a mis-sense mutation was detected in one allele in one patient: a C-to-T substitution at position 4353 of the cDNA, which led to one amino acid substitution of arginine to tryptophan at codon 1453 (Arg1453Trp) (Fig. 2). This result was confirmed by performing RFLP. This mutation created a *Bsr 1* digestion site (*Bsr 1* restriction fragments are shown in Fig. 3) in ICP patients but it was not detected in the 50 control subjects.

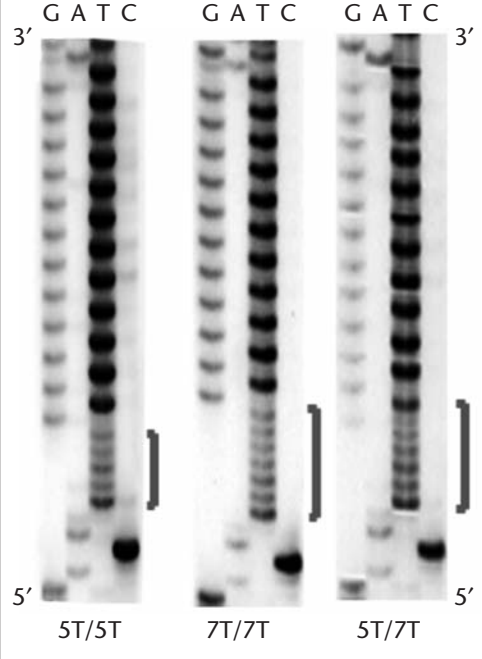

FIGURE 1: The poly-T region in intron 8 of the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene amplified by polymerase chain reaction and analysed by a direct sequencing method showed genotypes with at least one 5T allele, which were defined as the 5T genotype; the 9T allele was not detected in this study. Bars signify the location of the poly-T region.

TABLE 3:
Frequency of the 5T genotype in patients with chronic pancreatitis compared with healthy control subjects

	Control subjects (*n* = 110)	Alcoholic pancreatitis (*n* = 20)	Idiopathic pancreatitis (*n* = 20)
5T genotype	4.5% (*n* = 5)	5.0% (*n* = 1)	20.0% (*n* = 4)*

*$P = 0.012$ (χ^2-test) versus control subjects; other between-group comparisons were not statistically significant ($P > 0.05$).

The *PRSS1* coding regions of the 20 patients with ICP were also screened, but no mutation was detected.

Discussion

Alcohol is one of the major causes of pancreatitis. Since only a minority of drinkers develops pancreatitis, studies have sought to identify factors that may increase susceptibility to the disease, however, so far no susceptibility factor has been identified.[12] The present study examined the poly-T genotype frequencies in intron 8 of the *CFTR* gene; however, no difference between the 5T genotype frequencies of patients with alcoholic chronic pancreatitis and healthy control subjects was observed. Studies on the acinar cell itself as the principal site for ethanol-related damage have been carried out[13,14] and have also investigated the molecular mechanism of alcoholic chronic pancreatitis.[12] Further studies in these areas may help to resolve the factors that affect susceptibility to pancreatitis.

In the present study, the frequency of the 5T genotype in ICP was significantly higher

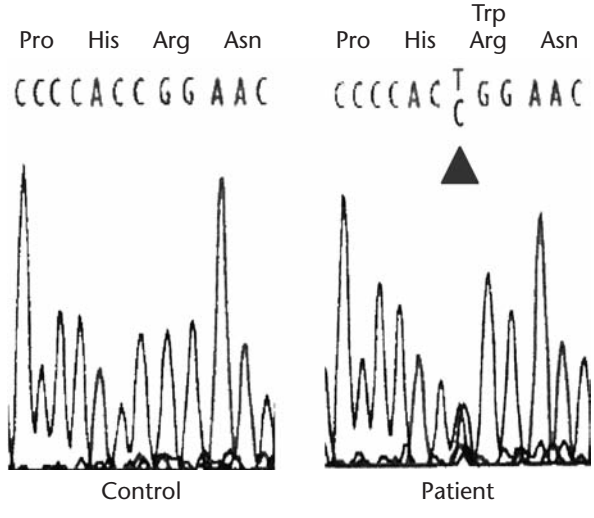

FIGURE 2: A mis-sense mutation in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene was detected in one allele in one patient: a C-to-T substitution at position 4353 of the cDNA, which led to one amino acid substitution of arginine to tryptophan at codon 1453 (Arg1453Trp)

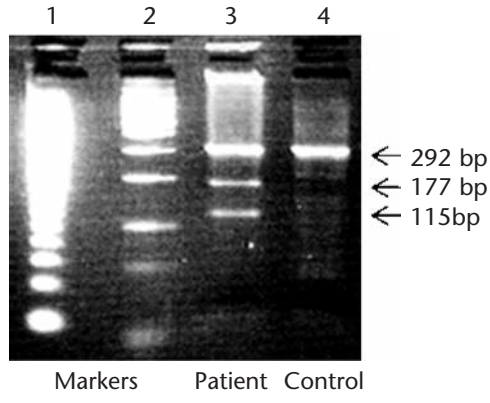

FIGURE 3: The mis-sense mutation, Arg1453Trp, in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene was confirmed using restriction fragment length polymorphism (RFLP). Column 1 is a 20-base pair (bp) marker and column 2 is a 100 bp size marker. The mutation created a *Bsr 1* digestion site: *Bsr 1* restriction fragments of 115 and 177 bp were detected using RFLP

than observed in healthy controls. Unlike the healthy controls with the 5T genotype, those patients with ICP who possessed the 5T genotype did not show cystic fibrosis symptoms. This suggests that the 5T genotype has some relation to ICP prevalence in Japanese patients, especially in young patients, independent of cystic fibrosis.

The poly-T sequence in intron 8 has been shown to affect the function of the *CFTR* gene.[8] Allelic variants of the poly-T sequence are 5T, 7T and 9T, and the 5T genotype of intron 8 in the *CFTR* gene can decrease the function of *CFTR* even when heterozygous.[8,9] The 5T variant occurs in 5% of chromosomes in the general population, and differs from the 7T and 9T variants because it hinders splicing of *CFTR* mRNA.[8,9] The 5T allele reduces the efficiency of exon 9 splicing,[8,9] and this abnormal splicing reduces the expression of functional CFTR protein.[8] For the purposes of the present study in terms of genetic structure and allelic frequency, it was assumed that the frequency of the 5T genotype was not different between Caucasian and Japanese populations in evaluating whether there was any relationship between the 5T genotype and chronic pancreatitis. The CFTR coding region of patients with ICP was also screened.

Autosomal-dominant pancreatitis was excluded in the present study when the *CFTR* mutations in patients with ICP were examined and we found no mutation in the *PRSS1* coding regions of the patients with ICP. Although it has been reported that 19% of patients with a presumed diagnosis of ICP have mutations in the cationic trypsinogen-encoding gene, *PRSS1*,[15] hereditary pancreatitis differs from ICP in that it leads to pancreatic adenocarcinoma with a cumulative risk approaching 40%.[16]

A mis-sense mutation, Arg1453Trp, in the *CFTR* gene was found in one patient with IPC in the present study. This mutation reportedly affects channel activity, but its overall effect on *CFTR* function appears to be mild.[17] As this mis-sense mutation appeared in only one patient, further studies are required to establish whether this manifestation is related to IPC or was merely coincidental.

In conclusion, mutation in the *CFTR* gene, especially the 5T genotype, appears to be

related to the prevalence of ICP in Japanese patients, independent of the presence of cystic fibrosis. No mutation of *PRSS1* was detected in Japanese patients with ICP. One limitation of this study was the small sample size of only 20 ICP patients and further studies are needed to establish the significance of the relationship between ICP and *CFTR* in Japanese patients.

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Conflicts of interest

The authors had no conflicts of interest to declare in relation to this article.

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