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CFTR gene mutations in Japanese individuals with congenital bilateral absence of the vas deferens

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Abstract

Congenital bilateral absence of the vas deferens (CBAVD) is a monosymptomatic disease confined to the male reproductive system with similarity to the phenotype of cystic fibrosis (CF), and mutations in the CFTR gene are highly prevalent in Caucasian CBAVD patients. While CF is very rare in Japan, CBAVD is not. Our previous study demonstrated high prevalence of the 5T allele in the CFTR gene in Japanese CBAVD patients. We analyzed whole exons of the CFTR gene in 19 CBAVD patients and 53 normal individuals using polymerase chain reaction amplification-single strand conformation polymorphism analysis and direct sequencing. Three missense mutations (W216X, G1349S, Q1352H) were found in seven CFTR alleles, and the 5T allele was positive in 11 of 38 CFTR patient alleles. Consequently, 47% of CFTR chromosomes in the patients were affected, and 11 individuals (58%) had at least one mutated CFTR allele. In contrast, three of 53 normal individuals (5.7%) had a missense mutation in one of the CFTR genes, but no 5T allele was detected (both $P < 0.0001$). Mutations of the CFTR gene are closely associated with Japanese patients with CBAVD.

Keywords: Cystic fibrosis; 5T allele; Male reproductive system; Single-strand conformation polymorphism analysis; Direct sequencing

1. Introduction

Congenital bilateral absence of the vas deferens (CBAVD) accounts for approximately 6% of cases of obstructive azoospermia [1] and is responsible for 1% of male infertility [2]. It is well known that CBAVD is also present in approximately 95% of male patients with cystic fibrosis (CF), which is a disease of exocrine organs characterized by chronic sinusitis, severe obstructive respiratory impairment, pancreatic exocrine insufficiency and elevated concentrations of sweat electrolytes [3].

CF is caused by mutations of the gene coding for cystic fibrosis transmembrane conductance regulator (CFTR) [3–5]. To date, more than 1000 pathogenic CFTR (ABCC7) gene mutations have been reported worldwide [6,7]. Mutations of the CFTR gene have also been frequently identified in patients with CBAVD [8–13]. In particular, the 5T allele in intron 8 of the gene, which causes reduced levels of normal CFTR mRNA [14], has significant clinical effects related to male infertility [15]. These observations strongly suggest that CBAVD is one of the monosymptomatic diseases with CFTR etiology [16], which has only genital presentations of the classical form of CF.

In Caucasians, a carrier frequency of mutations of the CFTR gene is estimated as approximately 1 in 25, and an incidence of the disease is 1 in 2500 live births [3,6]. In contrast, CF had been believed to be rare in the Asian or Oriental populations [3,6,17]. Thus far, approximately 130 typical cases of CF have been reported in Japan [18–22], and the incidence was estimated as approximately 1 in 100 000–350 000 live births [18].

Interestingly enough, however, CBAVD itself is not uncommon in Japan, with an estimated prevalence of 1% in male infertile patients, which is comparative with that previously reported in the United States [1,2]. Our

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Table 1
CFTR gene mutations in 19 male patients with CBAVD

Patient number	Age at study (years)	CFTR genotype	(TG)mTn genotype
1	41	G1349S/Q1352H	(TG)11T7/(TG)11T7
2	31	W216X/Q1352H	(TG)11T7/(TG)11T7
3	29	Q1352H/WT	(TG)11T7/(TG)13T5
4	29	Q1352H/WT	(TG)11T7/(TG)13T5
5	42	Q1352H/WT	(TG)11T7/(TG)13T5
6	29	WT/WT	(TG)13T5/(TG)13T5
7	29	WT/WT	(TG)13T5/(TG)13T5
8	36	WT/WT	(TG)13T5/(TG)12T7
9	36	WT/WT	(TG)13T5/(TG)12T7
10	32	WT/WT	(TG)13T5/(TG)11T7
11	29	WT/WT	(TG)12T5/(TG)12T7
12	35	WT/WT	(TG)12T7/(TG)12T7
13	34	WT/WT	(TG)12T7/(TG)12T7
14	26	WT/WT	(TG)12T7/(TG)12T7
15	29	WT/WT	(TG)12T7/(TG)12T7
16	34	WT/WT	(TG)12T7/(TG)12T7
17	32	WT/WT	(TG)12T7/(TG)11T7
18	31	WT/WT	(TG)11T7/(TG)11T7
19	48	WT/WT	(TG)11T7/(TG)11T7

WT denotes wild type in which mutations with a codon or alternative splicing were all negative.

previous study has shown that any of 32 or 70 frequent mutations observed in CF patients in North America was not present, but the allelic frequency of the 5T variant was 30%, as observed in Caucasians CBAVD patients [23]. Importantly, analyses for CF in Japan have identified several quite novel or extremely rare mutations in the CFTR gene [19–22].

To elucidate further the molecular basis of CBAVD in Japan, we have analyzed the whole 27 exons and the 5′- and 3′- intron–exon boundaries of the CFTR gene, as well as the genotype of TG-repeat and polythymidine tract [(TG)mTn] in intron 8 in patients with this disease in the present study. Our data clearly demonstrate the close association of CFTR gene mutations and CBAVD in the Japanese population.

2. Materials and methods

2.1. Subjects for study

We have analyzed the CFTR genotype in 19 male Japanese individuals with CBAVD after obtaining informed consent. Their age ranged from 26 to 48 years (average 33.3 ± 5.5) at the time of the study (Table 1). A total of 10 individuals were also subjects in the previous study [23]. No patient was reported to have pulmonary or gastrointestinal manifestations of typical CF, and four individuals tested for sweat chloride levels showed normal results. The diagnosis of CBAVD was made on the basis of scrotal exploration, analysis of semen, such as volume, number and concentration of sperm, and transrectal and abdominal ultrasonography [23]. As control, 53 normal individuals were also evaluated for CFTR gene mutations.

2.2. Analyses of CFTR gene mutations

Genomic DNA was isolated from nucleated blood cells using a commercially available DNA extraction kit (Qiagen, Tokyo, Japan). First, all 27 exons of the CFTR gene including both the 5′- and 3′- intron–exon junctions were subjected to single-strand conformation polymorphism (SSCP) analysis [24]. DNA from CBAVD patients or normal individuals was amplified by polymerase chain reaction (PCR) with specific sets of primers as described elsewhere [25], and the PCR products of each exon were electrophoresed on GeneGel Excel 12.5/24 (Pharmacia Biotech, Tokyo, Japan) and evaluated by silver staining. Those individuals whose PCR products showed different mobility from those of the reference control were forwarded to further evaluation by direct sequencing using the dideoxy chain-terminating method (Pharmacia Biotech, Tokyo, Japan) [25]. Once any mutation was detected, its eligibility was further confirmed by additional PCR amplification with the mutant allele-specific primer sets in patients, as well as in the normal control subjects.

Second, the (TG)mTn polymorphism at the splice acceptor site in intron 8 was evaluated [14,26]. The region containing the intron 8–exon 9 junction and exon 9 was PCR-amplified with primers CF9iS2 (5′-CATAAAACAAGCATCTATTG-3′) and CF9iAS2 (5′-AGAGACATGGACACCAAATT-3′), and was subjected to direct sequencing as described above [25].

All data were expressed as mean $\pm$ standard deviation. The chi-square statistics with Yates' correction were used appropriately. P values of less than 0.05 were considered to be of statistical significance.

3. Results

Using PCR-SSCP analysis followed by direct sequencing, we could detect three missense mutations in a total of seven CFTR alleles: W216X ($779G \rightarrow A$) in exon 6a; G1349S ($4177G \rightarrow A$); and Q1352H ($4188G \rightarrow C$) in exon 22 (Table 1). Q1352H mutation was the most frequent one detected in as many as five individuals with the disease. W216X and G1349S were novel mutations and have been deposited in the CF database [7]. The presence of these missense mutations in patients was further verified by separate PCR amplification with sets of allele-specific oligonucleotide primers at the more stringent hybridization (data not shown). In contrast, in 53 normal individuals similarly tested, E217G was found in one chromosome and Q1352H was in two, but no other mutations were detected (data not shown). These individuals were totally free from clinical symptoms of CF. The frequency of codon mutations was

Table 2
Association of abnormal CFTR genotypes with Japanese individuals with CBAVD

	Alleles		P value
	CBAVD	Normal subjects	
Mutation causing codon change	7/38 (18.4%)	3/106 (2.8%)	0.0041
5T variant	11/38 (28.9%)	0/106 (0%)	<0.0001
Abnormal CFTR allele including 5T variant	18/38 (47.4%)	3/106 (2.8%)	<0.0001
Individuals with at least one affected CFTR allele	11/19 (57.9%)	3/53 (5.7%)	<0.0001

significantly higher in the patients with CBAVD than in the normal control subjects ($P = 0.0041$) (Table 2).

Evaluation of the (TG)mTn polymorphism of intron 8 in Japanese patients with CBAVD demonstrated that (TG)13T5 was present in 10 alleles, (TG)12T5 in one, (TG)12T7 in 14 and (TG)11T7 in 13 (Table 1). Therefore, the allelic frequency of 5T was 28.9% in individuals with CBAVD (Tables 1 and 2). In contrast, the 5T allele was not detected in any of the 106 CFTR alleles of the normal subjects ($P < 0.0001$).

Importantly, including the 5T allele, 18 CFTR alleles (47.4%) were affected in CBAVD individuals, and a total of 11 patients with the disease (57.9%) had at least one mutated allele (Tables 1 and 2). In particular, five patients were compound heterozygotes: patient 1 was associated with G1349S and Q1352H, patient 2 with W216X and Q1352H, and patients 3, 4 and 5 with Q1352H and 5T. In addition, two were homozygotes of the 5T allele (patients 6 and 7) (Table 1). In marked contrast, only three out of 106 alleles (2.8%) or 53 individuals (5.7%) in the normal population were affected as described above. Both the prevalence of the mutated alleles and the number of individuals positive for at least one abnormal CFTR gene were significantly higher in the patients with CBAVD than in the normal control subjects (both $P < 0.0001$) (Table 2).

4. Discussion

The prevalence of CBAVD in Japan is estimated as approximately 1% in male infertile patients, which is compatible with the level observed in the United States [1,2]. Besides a high prevalence of absent or rudimentary vas deference in individuals with CF, CBAVD by itself is now considered to be one of the monosymptomatic diseases with CFTR etiology confined to the male reproductive system [16]. CF, the most common lethal autosomal recessive disease in the Caucasian population, is caused by mutations of the CFTR gene, and to date more than 1000 mutations have been reported worldwide to be responsible for developing clinical manifestations of CF [6,7]. Likewise, a large number of reports have described strong association between CBAVD and CFTR mutations in Caucasians [8–13]

The most frequent CFTR gene alteration identified in Caucasian individuals with CBAVD is the 5T variant in the intron 8 polythymidine tract [15]. This splicing variant, along with the preceding higher repetitive dinucleotide TG sequences such as (TG)13 or (TG)12, produces more mRNA transcripts with inframed deletion of exon 9 [14,26,27]. The exon 9^- mRNA results in synthesis of non-functional CFTR protein. The particular combination of the two CFTR alleles in a given individual (i.e. the genotype) results in specific levels of normal CFTR mRNA and in a specific clinical phenotype. When both CFTR genes have the 5T allele, this non-functional CFTR mRNA accounts for up to 92% of the total mRNA [14]. In general, a small amount of normal CFTR protein could prevent clinical manifestations of CF in the lungs or pancreas. However, a high level of CFTR gene expression in epithelial cells is required in the fetus for normal development of male genital organs, such as the epididymal gland and the vas deferens [28,29]. This is the presumed pathogenesis by which individuals with subtle abnormalities in the CFTR gene such as the 5T variant would have errors in development of the reproductive system, resulting in CBAVD without any typical clinical manifestation of CF [23].

In the present study, we have found that a significant proportion, as high as 30%, of Japanese males with CBAVD have the 5T allele, as was observed in Caucasian CBAVD patients [15]. In contrast, no 5T variant was detected among the normal control population. In this context, the 5T allele is also likely a disease-causing mutation in CBAVD in Japan. Furthermore, although our previous evaluation failed to detect any of 32 or 70 frequent mutations responsible for more than 90% of Caucasian CF patients in North America [30], by thorough analysis over all the 27 coding exons along with the 5′- and 3′- adjunct sequences, this study demonstrated that five patients had at least one codon mutation in their CFTR genes. Among the mutations found in CBAVD patients, G1349S and W216X are quite novel and which we deposited in the CF database in 1999 [7]. Another mutation, Q1352H, which was previously described by other Japanese investigators [7], is likely the most prevalent alteration in Japan. The 1352nd amino acid residue is coded by trinucleotide in exon 22

of the CFTR gene, and is located in the second ATP-binding domain. Thus, it is speculated that alteration of the codon for glutamine to histidine would cause decreased efficiency in activation of the CFTR Cl^- channel. Furthermore, it should be determined in the future whether or not this common Q1352H CF allele might be associated with other types of 'CFTRpathies' including the classical form of CF in the Japanese population [16].

Most importantly, we have demonstrated that 47% of CFTR chromosomes of the Japanese patients with CBAVD were affected, and 11 patients (58%) had at least one mutated CFTR allele. As observed in Caucasian CBAVD patients, mutations of the CFTR gene including the 5T allele were closely associated with CBAVD in the Japanese population. The prevalence of CFTR gene mutations seems to be much higher in the Japanese population than previously estimated [18]. This may be true, since three normal individuals had one mutated CFTR allele, two of which were Q1352H, as stated above. Interestingly enough, the profile of gene mutations in the Japanese population is likely quite distinct from that of the Caucasian counterpart [6]. In this regard, the screening system designed for CFTR mutations in Caucasians failed to detect any abnormal allele in the Japanese patients with CBAVD, as for CF [21,22].

However, we could not detect any CFTR mutation in the remaining eight patients (42%). This might be because of the SSCP method, since this approach is not foolproof for screening CFTR mutations and has its limitations in sensitivity, as well as in the capability of detecting mutations in general [24]. Moreover, it is suggested that Japanese may have uncharacterized mutations in CF alleles located in the intervening sequences, or that another gene could be associated with CBAVD. Further study is needed to demonstrate the more detailed profile of CFTR gene mutations in Japanese individuals with CBAVD. Moreover, sterility in patients with CBAVD can now be treated by modern assisted reproduction technology [23]. If the female partner is a heterozygous carrier of a CF mutation, their offspring might have the possibility of inheriting a currently undetected genetic defect and developing clinical manifestations of CF and/or CBAVD [16]. Therefore, all couples should be informed of this possibility and the importance of genetic testing for CFTR gene mutations in advance of intracytoplasmic sperm injection [16,23].

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