Structural Analysis of CFTR Gene in Congenital Bilateral Absence of Vas Deferens

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Congenital bilateral absence of the vas deferens (CBAVD) is found in most males with cystic fibrosis (CF), but this malformation can be observed without any pulmonary or digestive features. We have analyzed 13 exons of the CF gene in a cohort of 25 CBAVD patients. Among the 50 chromosomes studied, 24 mutations were identified: ΔF508 (14 cases), R117H (7 cases), R1070W (2 cases), 621+1 G→T (1 case), and A1067V (1 case). Except for ΔF508, the most frequent mutations (R117H, R1070W) were not observed in the CF group (109 patients) studied in our laboratory. We discuss the significance of these results.

Indexing Terms: cystic fibrosis/gene mutations/gene sequencing/denaturing gradient gel electrophoresis/genetic disorders

Congenital bilateral absence of the vas deferens (CBAVD) accounts for 1–2% of male sterility. Moreover, CBAVD is found in most males with cystic fibrosis (CF). For this reason, since 1990, the hypothesis of a nosologic identity between these two disorders has been proposed. The discovery of the cystic fibrosis transmembrane conductance regulator (CFTR) gene (1, 2) was quickly followed by the development of a technology permitting identification of numerous mutations. In cystic fibrosis, diagnosed on the basis of clinical, pulmonary, and pancreatic features and increased sweat chloride concentration, the first anomaly found was a 3-bp deletion called ΔF508. Today, 501 mutations have been identified. The prevalence of these mutations varies in populations of different ethnic origins. Nevertheless, ΔF508 is the most frequent one worldwide (68%), with a clear northwest (88%) to southeast (30%) gradient of relative frequency (3). Analysis for the CFTR gene in CBAVD patients showed that 45–50% of them were heterozygous for ΔF508 vs only 2–4% in the normal population (4, 5). These results led us to look for additional mutations.

In this study, we report the analysis of 13 exons of the CFTR gene in CBAVD with reference to the CF group studied in our laboratory. We also analyzed the intron-8 polypyrimidine tract length variants that can affect the phenotype produced by some mutations (6). Together with these results, we present the identification of a rare mutation in exon 17b.

Materials and Methods

Patients

Twenty-five patients (ages 26 to 39 years) originating from the Brittany region and followed for sterility by CECOS de l'Ouest (Centre d'Etude et de Conservation du Sperme Humain) were studied. Diagnosis of CBAVD was based on the following clinical and biological characteristics: azospermia with low semen volume (<1.5 mL), decrease of fructose (vesicular marker) and carnitine (epididymal marker) concentrations, normal testicular volumes, caput epididymis palpably enlarged, and normal follitropin concentrations. None of the patients showed pulmonary manifestation typical of CF. Chest radiographic studies were normal. No sputum microbiology was done because no recurrent respiratory infection was observed. Other respiratory problems included asthma in three cases and rhinitis in one case. All patients were pancreatic sufficient. One had pancreatitis. In all cases, results of sweat tests were normal except for one patient (chloride = 72 mmol/L), who did not show any pulmonary or gastrointestinal symptoms of CF.

Some 109 unrelated CF patients originating from the Brittany region were also studied in our laboratory. The diagnosis of CF was based on the clinical symptoms, pulmonary and pancreatic features, and confirmed by three sweat chloride concentrations >70 mmol/L.

Detection of Mutations

Total genomic DNA was isolated from the patients’ peripheral blood cells and analyzed for mutations in the CFTR gene. Chromosomes were first tested for the presence of the ΔF508 mutation by heteroduplex formation (7) and by polymerase chain reaction (PCR)-mediated site-directed mutagenesis (8). The non-ΔF508 chromosomes were then screened for mutations in 13 exons (2, 3, 4, 7, 10, 11, 12, 14a, 17b, 19, 20, 21, and 23) by denaturing gradient gel electrophoresis (9, 10). Computer analysis was performed by using the Melt 87 and SQHTX programs, generously provided by L. Lerman (11). The primers used, the range of denaturant concentration, and the run time are given in Table 1. Samples displaying a modified migration pat-
tern were sequenced with Sequenase version 2.0 (Amersham USB, Bucks, UK).

Detection of CFTR Intron-8 Polypyrimidine Tract Length Variants

Samples bearing the R117H mutation were analyzed for the intron-8 splice variants 5T or 7T (6). The oligonucleotides used for amplification were C: 5’TGGGCCATGTGCTTTTCACAC 3’; D: 5’CTTCTA-ATGTTGATGACAGC 3’. The PCR product was purified on a Chromaspin-100 column (Clontech, Palo Alto, CA) and sequenced with the PCR primers. 

Results

Different genotypes are listed in Table 2, in which two patients’ cohorts are compared: one composed of 25 CBAVD and the other of 109 CF cases studied in our laboratory. Among the 50 chromosomes studied, 24 mutations (48%) were identified as follows: 13 patients were heterozygous for the AF508 deletion (62%), 6 of whom were compound heterozygous for the ΔF508 and missense mutations such as R117H (3 cases), R107W (2 cases), and a rare mutation designated as A1067V (1 case) recently identified in a CBAVD patient (12); 5 patients had only one identified mutation: R117H (4 cases) and 621+1G→T (1 case); and 7 patients had no mutation in the 13 studied exons.

In the intron-8 splice acceptor site the 7T variant was found in all seven R117H carriers.

Discussion

Is CBAVD a mild form of CF? Because CBAVD is a constant feature of CF, some authors agree with this definition (4); according to others, CBAVD rather represents a genital form of CF (13–16). The present work might shed light on this, inasmuch as clinical and biological analysis of the 25 patients showed that none of them had pulmonary or digestive pathology, and only one had a mildly increased sweat chloride concentration but without pancreatic involvement. Apparently, therefore, this group of patients belongs to a particular nosologic entity as the CBAVD as the clinical presentation. This entity is different from CF, in which the numerous clinical signs, the precocity of symptoms, and the severe complications result in medical and therapeutic-specific problems.

From all this, it follows that CBAVD and CF can be considered as resulting from structural abnormalities in the same gene. Several pathologies in which a unique gene is mutated can offer a variable phenotypic expression, either with varying intensity or even quite different aspects. A case in point is the β-globin gene, where a unique mutation produces sickle cell disease but with a gradually decreasing gravity from West to East: Occidental Africa, Central Africa, Middle East, and India (17). In contrast, anomalies in the androgen receptor gene are responsible for two quite different diseases, testicular feminization (18) and spinal and bulbar muscular atrophy (Kennedy disease) (19).
for the CFTR gene, varying phenotypic expression might result from two nonmutually exclusive causes, either the nature or the genetic background of the mutations. In addition to ΔF508, we have characterized four mutations: R117H, R1070W, 621+1G→T, and A1067V. Three of these seem to be more frequent for CBAVD, since they were not identified in the 218 CF chromosomes originating from the same population and previously analyzed in our laboratory. Similar results were reported in other CBAVD studies (4, 13, 20, 21). However, none of the most frequent mutations described in our CF population (G551D, N1303K, 3272-26G→A, G542X) was identified in our CBAVD cohort. CBAVD might well be a consequence of particular mutations in the CFTR gene, associated either with one another or with ΔF508. Some authors have clearly shown that the R117H mutation found in typical CF was associated with a 5T variant in intron 8 of the CF gene. In CBAVD, this same mutation was associated with a 7T variant (6). The present results corroborate this study, and it is quite possible that additional genetic markers may assist in specifying this genetic context and its functional role.

In this work, structural analysis of 13 exons of the CFTR gene allowed identification of mutations in 48% of CBAVD chromosomes. These results strengthen the hypothesis that the absence of vas deferens corresponds to a particular expression of CFTR abnormalities during development. Other factors may be involved in phenotype modulation of this disease, such as chromosomal environment or other genes. Microsurgical techniques aimed at obtaining sperm from patients' epididymis have recently permitted successful in vitro fertilization. Consequently, CBAVD patients and their partners should be advised to submit to DNA analysis and to seek genetic counseling.

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References