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Cystic Fibrosis Presenting as Acute Pancreatitis and Obstructive Azoospermia in a Young Adult Male With a Novel Mutation in the CFTR Gene

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Summary. Cystic fibrosis is rare in the Asian population, and is often associated with consanguinity and rare genotypes. We report on a 23-year-old Asian man from a consanguineous pedigree referred to the regional cystic fibrosis unit after a diagnosis of congenital bilateral absence of the vas deferens during investigations for infertility. A detailed history revealed several previous episodes of acute pancreatitis. Full diagnostic appraisal showed homozygosity for a novel cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation, but normal sweat test and nasal potential difference studies. An endoscopic retrograde cholangiopancreatogram (ERCP) showed chronic pancreatitis with bulky side branches. The vas deferens and the pancreas appeared exquisitely sensitive to mild CFTR dysfunction. Patients with cystic fibrosis and unexplained upper abdominal pain should be screened for pancreatitis, and consideration should be given to screening patients with idiopathic pancreatitis for mutations in the CFTR gene. **Pediatr Pulmonol. 2002; 34:491–495.** © 2002 Wiley-Liss, Inc.

Key words: cystic fibrosis; pancreatitis; infertility; ABCC7.

INTRODUCTION

Cystic fibrosis (CF), the commonest genetic disease in the Caucasian population, is inherited in an autosomal-recessive fashion. The carrier frequency in the UK white population is approximately 1 in 25. Over 900 different mutations have been isolated since the identification of the gene on the long arm of chromosome 7 in 1989.[1] A 3 base-pair deletion termed ΔF508 accounts for about 70% of the known mutations worldwide. Its absence results in the loss of phenylalanine at amino-acid position 508 of the protein product, the cystic fibrosis transmembrane regulator protein (CFTR).[1] Other mutations are rare, their relative frequency varying in different populations, and many are unique.

CF is much less common in the Asian population, with an estimated prevalence of 1 in 10,000 reported in the UK.[2] A survey in the USA found an approximate prevalence of 1 in 40,000.[3] This figure, however, was probably an underestimate, as the authors believed that a number of older patients were not identified. Affected Asian patients very often have consanguineous parents,[3,4] an important factor in the expression of CF in this population, especially in those homozygous for unusual mutations. They also show a low frequency of ΔF508 and other common Caucasian mutations, and a high frequency of rare or unidentified mutations.[3,5]

CFTR is found in the apical membrane of epithelial cells in many body tissues, where it exerts a modifying influence on various aspects of cellular metabolism. Its prime function is to act as a cyclic adenosine monophosphate (cAMP)-regulated chloride channel. Reduced CFTR function results in a dehydrated intraluminal fluid with an altered ionic content and consequent inspissation of secretions. The most damaging effects are found in the lungs (recurrent infection, persistent inflammation), pancreas (fibrosis), hepatobiliary system (primary biliary fibrosis potentially progressing to multilobular cirrhosis), and intestinal tract (reduced motility and hydration).

Approximately 97–98% of men with CF are infertile. In 1968, Kaplan et al.,[6] attributed this to a congenital bilateral absence of the vas deferens (CBAVD). We now

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know that CFTR function is necessary for proper embryonic development of the Wolffian ducts. Otherwise healthy men presenting with infertility because of obstructive azoospermia show a significantly higher frequency of CFTR gene mutations than the general population. Although the severe ΔF508 mutation is significantly less common in men with CBAVD than in classic CF,[7,8] approximately 40–70% have at least one CFTR gene mutation, and 15–30% a CF mutation on both copies of the gene. A tract of thymidine (T) residues can run for 5, 7, or 9 bases at the end of intron 8 of the CFTR gene. The 7T variant is most commonly seen in the general population. The 5T variant causes skipping of exon 9 and consequent reduction of functional CFTR. If inherited on the same chromosome as a mild CFTR mutation, the 5T variant can enhance the severity of the latter.[9] About 60% of men with CBAVD and one CFTR gene mutation carry the 5T allele on the other CFTR gene.[7,10–12] The frequency of the 5T allele in the general population is 5%. In men with CBAVD, this may rise to 20% ($P < 0.0001$).[8,12] Sweat chloride concentration is usually normal or only marginally abnormal in men with CBAVD related to mild CF genotypes.[7,8,10] These men usually also have normal respiratory and pancreatic function.

The pancreatic ducts may show an exquisite sensitivity to a lack of CFTR, similar to that of the vas deferens. In the pancreas, CFTR is found on the apical membrane of the duct cell. Recent studies identified CFTR gene mutations as risk factors for idiopathic acute and chronic pancreatitis.[13,14] Cohn et al. described 3 patients with idiopathic chronic pancreatitis and CF genotypes.[13] One of the 3 also had CBAVD. Their mutations (ΔF508/wild-type, 9T/5T in two, and ΔF508/R117H, 9T/7T in one) were those described most commonly in men whose only clinical manifestation of CF was obstructive azoospermia.[7,8,10,11] Chillon et al., suggested that these mutations may produce CFTR protein with a normal structure but low levels of expression.[10] CFTR function in these patients is about 10% of normal, compared to only 1% of normal in patients with classic CF. This results in pathological changes only in those organs most sensitive to CFTR dysfunction.[15,16] We report on a young Asian man with a novel CFTR gene mutation. His first clinical manifestation of CF was recurrent acute pancreatitis. His second presentation was infertility, subsequently diagnosed as due to CBAVD.

ABBREVIATIONS

ATP	Adenosine triphosphate
cAMP	Cyclic adenosine monophosphate
CF	Cystic fibrosis
CFTR	Cystic fibrosis transmembrane conductance regulator
CBAVD	Congenital bilateral absence of the vas deferens
ERCP	Endoscopic retrograde cholangiopancreatogram
FEV_1	Forced expiratory volume in 1 sec
FVC	Forced vital capacity
SaO_2	Arterial blood oxygen saturation
T	Thymidine

CASE REPORT

In 1998, after 2 years of marriage, patient Mr. A, a 23-year-old Asian male born in the UK of Pakistani ancestry, was referred to the urology clinic for investigation of infertility. Bilateral absence of the vas deferens was diagnosed on clinical examination. Histological examination of a testicular biopsy showed seminiferous tubules with complete spermatogenesis, but many tubules with low numbers of mature spermatozoa (Fig. 1), consistent with obstruction of the vas deferens. Mr. A. was referred to the regional CF center for further investigation.

His past medical history was of recurrent abdominal pain from age 3 years. Diagnoses of nonspecific abdominal pain were punctuated by acute appendicitis at 7 years and acute hepatitis A at 9 years. In 1992, when he was 17 years old, acute pancreatitis was diagnosed (serum amylase 4,750 IU). Abdominal ultrasound showed a hyperechoic pancreas, a characteristic feature in CF but reported as unusual for acute pancreatitis, and a normal gallbladder (Fig. 2). An endoscopic retrograde cholangiopancreatogram (ERCP) demonstrated a normal pancreatogram and ampulla. In 1993, gastroscopy showed a normal stomach and duodenum.

Mr. A. continued with intermittent but not severe abdominal pain until presenting again with acute intense epigastric pain at age 20 years (serum amylase >4,500 IU). Abdominal ultrasound was reported as normal. No cause for the acute pancreatitis was found. There was no history of alcohol or drug abuse, gallstones, or relevant family history. A further attack had occurred in 1998 at age 22 years (serum amylase 889 IU).

A comprehensive assessment was performed at the CF center in the summer of 1999. Clinical examination was

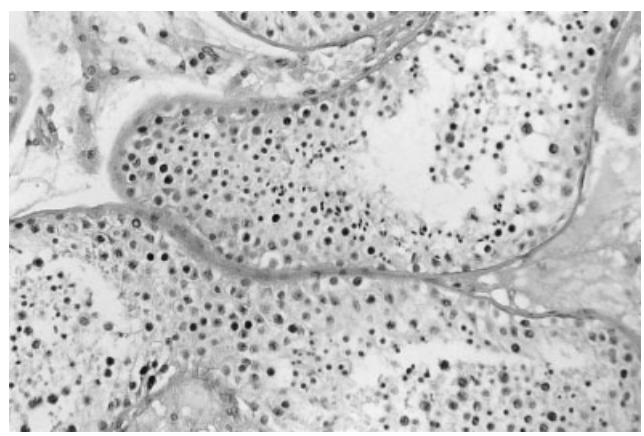

Fig. 1. Testicular biopsy, showing seminiferous tubules with complete spermatogenesis.

Fig. 2. Abdominal ultrasound, showing hyperechoic pancreas.

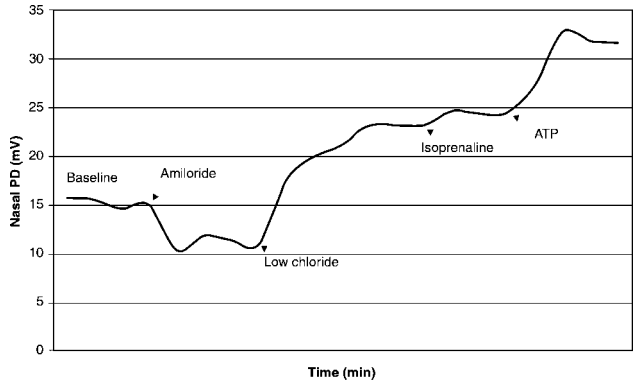

Fig. 3. Nasal potential difference study, showing normal baseline measurement, apical sodium channel inhibition by amiloride, sustained hyperpolarization with perfusion (with low chloride solution showing normal functioning CFTR channels), and normal response to ATP.

normal, and body mass index was 20.3 kg/m^2 (normal range, 18.5–25). Respiratory function tests showed forced vital capacity (FVC) and forced expired volume at 1 sec (FEV_1) at 84% predicted normal. Arterial oxygen saturation (SaO_2) in air was 98%.

No abnormality was seen on the chest radiograph. A sweat test was normal; weight was 106 mg, chloride 25 mmol/L, and sodium 37 mmol/L (normal, <40). Apart from a hyperechoic pancreas, abdominal ultrasound was normal. Stool pancreatic elastase was 583 μg/g, (normal, >200). Complete blood count, clotting screen, plasma viscosity, C-reactive protein, blood liver function tests, urea, electrolytes, calcium, and plasma vitamins A and E were all normal. Plasma vitamin D was low at 2.2 ng/mL (normal, >3). Dietary assessment was insufficient for quantitative analysis, but a normal to high fat diet was eaten.

Nasal bioelectric potential differences were measured in both nostrils,[17] and showed a normal baseline measurement and apical sodium channel inhibition by amiloride. Perfusion with low chloride solution was associated with sustained hyperpolarization, although the response to isoprenaline was minimal. The response to adenosine triphosphate (ATP) was within the normal range (Fig. 3).

Gene screening for CF showed a 3 base-pair deletion in exon 18, Δ1123Glu (Fig. 4). This novel mutation was present in the homozygous state in Mr. A, an individual from a consanguineous pedigree. Both parents were heterozygous for Δ1123Glu. Intron 8 pyrimidine tract polymorphism was homozygous for the 7T allele.

In September and November 1999, Mr. A. suffered two further attacks of acute upper abdominal pain. Serum amylase values were 451 IU/L and >2,000 IU/L, respectively. Repeat ERCP demonstrated chronic pancreatitis with bulky side branches. The main pancreatic duct was normal. There were no strictures or stones (Fig. 5).

DISCUSSION

Approximately 4% of patients with CF present with monosymptomatic diseases such as pancreatitis, obstructive azoospermia, liver disease, or sinusitis with nasal polyposis.[18] In these cases, sweat chloride concentrations may be normal or only minimally raised. The recent Cystic Fibrosis Foundation consensus statement on the criteria for a diagnosis of CF requires at least one phenotypic feature, plus elevated sweat chloride concentrations or mutations in each CFTR gene known to cause CF, or in vivo characteristic abnormalities of ion transport across the nasal epithelium.[18] Our patient fulfills the phenotypic criteria for CF. We believe that he is homozygous for a

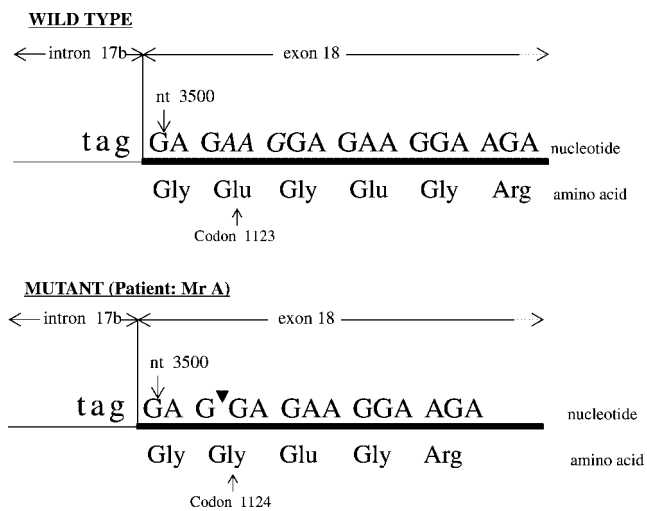

Fig. 4. Diagramatic representation of Δ1123Glu mutation. Bases AAG, which make up the last two bases of codon 1123 and the first base of codon 1124, are removed. The amino acid coded by the remaining base (G . . . GA) is a glycine. The net effect of this mutation is therefore a deletion of codon 1123, glutamic acid.

Fig. 5. ERCP, showing chronic pancreatitis with bulky side branches.

novel CF-causing mutation. This is defined in the consensus statement as ''a novel amino acid sequence that does not occur in the normal CFTR genes from at least 100 carriers of CF mutations from the patient's ethnic group.''

We have not analyzed exon 18 of CFTR in 100 Asian carriers, and therefore cannot confirm this. However, codon 1123 occurs in a region of CFTR highly conserved through evolution and showing 100% amino-acid sequence homology between human, sheep, mouse, bovine, and rabbit species. A change in the conserved sequence would therefore be expected to have a deleterious effect on the protein produced. This supports 1123delGlu as a pathogenic mutation.

Although the CF genotype does not absolutely predict phenotype, there is a correlation between the amount of normal CFTR function and disease in various organs. Thus patients with genotypes resulting in about 1% CFTR activity have classic CF with pulmonary disease, pancreatic insufficiency, CBAVD, and abnormal sweat chloride concentrations. Patients with approximately 5% CFTR function are pancreatic-sufficient. Those with about 10% CFTR function have CBAVD alone.[19,20] Recent papers suggest that such patients are also at risk for pancreatitis.[13,14]

In the pancreas, high levels of CFTR are found in the intralobular and proximal ductular epithelial cells.[21,22] In CF, impaired ductal chloride secretion[23] interferes with the secretion of a bicarbonate-rich alkaline fluid and with intraluminal hydration.[24] The consequent inspissation of pancreatic acinar secretions results in duct obstruction, cell destruction, and fibrosis.[25,26] Pancreatic insufficiency and steatorrhoea are usually present at birth, and are found in about 90% of the CF population.[27]

Patients with pancreatic sufficiency have less severe CFTR mutations,[28] less severe symptoms,[29] and more normal sweat chloride concentrations, and are usually diagnosed at a later age. They are, however, susceptible to attacks of acute pancreatitis, functional acinar tissue being a prerequisite for the inflammatory response.

1123delGlu occurs in exon 18 of CFTR, which encodes part of the second membrane-spanning domain of the protein. As three nucleotides are deleted, no frameshift occurs and the reading frame of CFTR is maintained. No new amino acids are created by the deletion, so there is no introduction of amino acids with charge or polarity unsuitable for a membrane-spanning domain. The effect on the protein is that caused by the loss of the glutamic acid residue alone. We speculate that this is the reason for the mild phenotype shown by our patient.

In 1975, Shwachman et al. described 10 young adult patients with CF and recurrent acute pancreatitis.[29] All were pancreatic-sufficient. Eight presented with recurrent pulmonary infection, and 2 with pancreatitis; all had developed bronchiectasis between 8–23 years of age. Subsequent authors documented further cases in young adults with CF confirmed by abnormal sweat chloride concentrations, but with minimal or no respiratory symptoms, pancreatic sufficiency, and normal nutritional status. These patients had no history of other risk factors for pancreatic problems.[30–32]

Identification of the CFTR gene has allowed further confirmation of the association of pancreatitis with CF. Just as searching for mutations in the CFTR gene in patients with CBAVD has shown that obstructive azoospermia may be the only phenotypic manifestation of a mild CF genotype, so a higher-than-expected frequency of abnormal CFTR alleles has been found in patients with pancreatitis. Both sets of patients may have normal sweat chloride values and basal nasal potential difference studies, but they carry two CFTR mutations.

Sharer et al. studied 134 patients with chronic pancreatitis.[14] Although none had mutations on both copies of the CFTR gene, the frequency of CF carrier status was $2.5 \times$ the normal population ($P < 0.001$), and that of the 5T allele $2 \times$ the expected frequency ($P < 0.008$). Four patients carried both a CFTR gene mutation and the 5T allele. No patient had any other laboratory or clinical feature of CF.

Cohn et al. studied 27 patients referred for idiopathic chronic pancreatitis, and found CFTR mutations in 8 patients ($11 \times$ the expected frequency).[13] The 5T allele was present in 5 patients. In 3 patients, both CFTR alleles were affected ($80 \times$ the expected rate), and resembled those CFTR gene mutations associated with CBAVD in otherwise healthy men.[7,8,10,11] None of the three had pulmonary disease typical of CF, raised sweat chloride values, or abnormal baseline nasal potential difference. Each, however, showed nasal cAMP-mediated chloride transport abnormalities resembling those shown by patients with CF. These studies suggest a strong link between chronic pancreatitis and CFTR gene mutations, and a similarity between the phenotype with pancreatitis and that with CBAVD.

In conclusion, we have described a new CFTR gene mutation in a young Asian man of consanguineous

pedigree. We have also emphasized the extreme sensitivity of the vas deferens and the pancreas to relatively mild CFTR dysfunction. CBAVD and pancreatitis appear to be minimal expressions of the molecular abnormality which results from mutations in the CFTR gene. Classic CF with pancreatic insufficiency, sino-pulmonary disease, and raised sweat chloride values is at the opposite end of the potential disease spectrum. We should consider screening patients with idiopathic pancreatitis for mutations in the CFTR gene. Patients with CF and unexplained upper abdominal pain should be screened for pancreatitis.

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