

Relapsing Pancreatitis due to a Novel Compound Heterozygosity in the CFTR Gene Involving the Second Most Common Mutation in Central and Eastern Europe [CFTRdele2,3(21 kb)]

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Key Words

Idiopathic pancreatitis · CFTR compound heterozygosity · CFTRdele2,3(21 kb) · R117H · Cationic pancreatic trypsinogen · PRSS1 · Pancreatic secretory trypsinogen inhibitor · SPINK1

Abstract

A 43-year-old otherwise healthy female patient presented with mild pancreatitis. Her family history revealed that her only son had cystic fibrosis. Genotyping of the patient demonstrated CFTR compound heterozygosity CFTRdele2,3(21 kb) and R117H and wild type alleles of the poly-T-tract in intron 8 (7T/7T). No mutations were detected in the cationic pancreatic trypsinogen (PRSS1) and the pancreatic secretory trypsinogen inhibitor (SPINK1) genes. CFTRdele2,3(21 kb) has only been described in 2000 and is the second most frequent severe CFTR mutation after $\Delta F508$ in central and eastern Europe. This haplotype should be included in the genetic panel when evaluating patients of central or eastern European genetic background for possible CFTR related pancreatitis.

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Introduction

Acute relapsing pancreatitis may result from alcohol ingestion, cholelithiasis, autoimmune disease or congenital or acquired abnormalities of the ductal system. A minor fraction of patients may harbor a genetic defect predisposing them to acute relapsing pancreatitis. The classical genetic defects are mutations in the cationic pancreatic trypsinogen that generally result in a gain of function of the enzyme [1]. These mutations are often inherited in an autosomal-dominant fashion, have a high penetrance and manifest early in life. Mutations in the pancreatic secretory trypsinogen inhibitor (serine protease inhibitor Kazal type 1, SPINK1) [2] have a lower penetrance and SPINK1 may act as a disease modifier in some cases of acute relapsing or chronic pancreatitis [3] as well as in alcoholic chronic pancreatitis [4]. Classical and atypical cystic fibrosis (CF) is due to mutations in the cystic fibrosis transmembrane regulator (CFTR) and principally follows an autosomal-recessive trait. The type of disease is mainly determined by the degree of reduced function of the protein translated from the two alleles [5]; it includes classic CF with varying degrees of pancreatic involvement, congenital bilateral absence of the vas deferens, chronic obstructive pulmonary disease and pancreatitis [6]. Here we describe a case of relapsing pancreatitis in a female patient due to compound heterozygosity

for CFTR mutations (CFTRdele2,3(21 kb) and R117H), which has not been implicated with pancreatic disease previously.

Case Report

A 43-year-old female patient was admitted to the hospital because of severe upper abdominal pain of 3 days duration. Four months earlier, the patient had suffered from a similar episode, which was treated with a proton pump inhibitor without further diagnostic work up. There were no previous episodes of pancreatitis.

Physical examination revealed a soft abdomen with marked upper abdominal and periumbilical pain on palpation, but no guarding. The bowel sounds were positive, the rectal examination was unremarkable.

Laboratory data on admission revealed pancreatitis: white blood cells (WBC) 6,450/ μ l, hemoglobin 13.4 g/dl, platelets 235,000/ μ l, lipase 1406 U/l (<60 U/l), amylase 175 U/l (<100 U/l), gamma-GT 32 U/l (<28 U/l), AP 126 U/l (<180 U/l), GOT 13 U/l (<22 U/l), GPT 8 U/l (<18 U/l), bilirubin <1 mg/dl (<1 mg/dl), CRP 2.5 mg/dl (<0.5 mg/dl). The calcium concentration was slightly elevated (2.9 mmol/l, normal <2.6 mmol/l) as was the level of parathormone (4.9 mmol/l). Both parameters as well as the urine excretion of calcium and phosphate were normal on several controls. The stool weight and stool fat excretion were normal on repeated testing but the stool pancreas elastase was subnormal (135 and 156 μ g/g, normal >200 μ g/g) in two of three tests. There was no hypertriglyceridemia.

The patient convincingly reported one glass of wine per day (13 g alcohol). She had not suffered major abdominal trauma.

A spiral CT scan disclosed edematous pancreatitis, a renal cyst and a focal hepatic lesion consistent with a hemangioma. On two occasions during the acute phase of the disease abdominal ultrasound permitted visualization of the entire common bile duct and did not show cholelithiasis, choledocholithiasis or cholestasis.

The patient had a 7-year-old son suffering from classical cystic fibrosis (CF). Genotyping six years earlier had revealed a Δ F508 mutation in one of the CFTR alleles. Thus at that time the child was believed to carry another unknown severe mutation resulting in the classic CF phenotype.

Because of this family history and the lack of an alternative etiology of her pancreatitis the patient was genotyped for mutations in the cationic pancreatic trypsinogen (PRSS1), the pancreatic secretory trypsinogen inhibitor (SPINK1) and the CFTR gene. Analysis of the CFTR gene (addressing 18 CFTR mutations which comprise 84% of all CFTR mutations in the German population) showed compound heterozygosity for the mutations CFTRdele2,3(21 kb) and R117H. The poly-T-tract in intron 8 revealed wild-type alleles. No mutations were detected in the PRSS1 and the SPINK1 genes. Because the genotyping could not rule out that the two CFTR mutations were located on the same chromosome, genotyping of the son was repeated and disclosed CFTR compound heterozygosity for the mutations Δ F508 (inherited from the father) and CFTRdele2,3(21 kb) (inherited from the patient), indicating that the patient's compound heterozygosity originated from the two CFTR alleles and not from the same chromosome.

Given this heterozygosity the patient was examined for nasal polyps and her sweat chloride concentration was determined to test

whether she may have a mild manifestation of classic CF. There were no nasal polyps and the sweat chloride concentration was in the normal range (34 mmol/l).

The patient recovered from her pancreatitis uneventfully. But she was readmitted 6 months later because of another episode of pancreatitis. At this time, a MRI study of the pancreas and a MRCP study ruled out pancreatic or biliary duct abnormalities but showed atrophy of the head of the pancreas. Notably, at this time the serum levels of calcium and phosphorous were normal. The patient again recovered uneventfully from this episode.

Discussion

The patient described presented with two episodes of mild pancreatitis of unknown origin. Typical etiologies of acute relapsing pancreatitis were ruled out by the history, by laboratory test, by abdominal ultrasound and by the MRI/MRCP study. ERCP or endoscopic ultrasound to rule out biliary pancreatitis were not performed because in this tall patient the common bile duct was visualized in its entire length by transabdominal ultrasound on two occasions and no cholelithiasis or cholestasis was detected. Also, the laboratory values did not suggest cholestasis (only the gamma-GT was minimally elevated). Therefore, biliary pancreatitis was thought very unlikely. It may be possible though that the patient's mild alcohol consumption (13 g per day) had an additional effect on top of her severely altered CFTR function (see below) in the pathogenesis of her relapsing pancreatitis. Hypercalcemia may also cause acute pancreatitis [7] and it is tempting to speculate that mild hypercalcemia may have triggered the first episode; on the other hand, no hypercalcemia was recorded during the second attack. Hyperparathyroidism was ruled out and familial hypocalcemic hypercalcemia, which has recently reported as a contributing factor in chronic pancreatitis [8], is unlikely given the normal calcium and phosphorous excretion in the urine.

The patient's family history was significant in that her only son had cystic fibrosis including pancreatic insufficiency. Therefore, the patient was supposed to be a carrier of a CF-causing mutation.

The CFTR protein is a 1480 amino acid plasma membrane protein. At the DNA level, more than 1,000 mutations and polymorphisms have been identified in the CFTR gene. They are grouped into six categories based on the mechanism by which the mutation affects CFTR function. They are also stratified according to their severity as 'severe', 'moderate', 'mild' and 'normal' (i.e. a polymorphism but not a mutation) [5]. CFTR acts as a chloride and bicarbonate channel and as an inhibitor of the epithelial sodium channel (ENaC) [9]. Loss of function of

the CFTR channel results in diminished secretion of chloride and bicarbonate and/or hyperabsorption of sodium in the affected epithelia leading to an increased viscosity of the pancreatic juice. Because of the high expression of CFTR in the pancreatic ductal system mutations in the CFTR gene typically result in pancreatic disease, the type and the severity of which are determined by the degree of residual CFTR function [5, 10]. The most common manifestation is pancreatic insufficiency in the setting of classical cystic fibrosis as a result of complete or near complete loss of function of the CFTR protein. Such pronounced loss of function requires both alleles of the CFTR gene to be affected by severe mutations resulting in 1% or less residual CFTR activity. Less severely impaired CFTR function (~5% residual activity) as a result of one severe and one moderate mutation still results in CF lung disease but lacks pancreatic insufficiency; instead acute relapsing pancreatitis is common. Pancreatitis is also common in the presence of one severe and one mild CFTR mutation, which typically results in about 10% residual CFTR activity. It is not well understood why the most severe loss of CFTR function results in pancreatic insufficiency while less severely impaired function predisposes to acute pancreatitis. But, in general, pancreatic disease is thought to be due to precipitation of secreted proteins in the ductal system and subsequent clogging [10].

Because the patient was supposed to be a carrier of a severe CFTR mutation, genotyping for CFTR mutations was performed and revealed the deletion of a 21-kb fragment spanning exons 2 and 3 and part of the adjacent introns [CFTRdele2,3(21 kb)] as well as the point mutation R117H. Of note the analysis showed wild-type alleles (IVS8-T7) of the poly-T-tract in intron 8.

CFTRdele2,3(21 kb) is a common (severe) mutation in central and eastern Europe [11]. It has only been described in 2000 and is usually not included in the widely used mutation kits for CFTR genotyping. Besides, sequencing is not able to detect this mutation. Therefore, analysis for the CFTR2,3dele(21 kb) mutation, which can be done by a simple duplex PCR [11], needs to be set up separately.

The CFTRdele2,3(21 kb) is a severe mutation because it deletes introns 1–3 of the CFTR gene resulting in the loss of exons 2 and 3 of the CFTR mRNA and thereby creating a premature termination signal within exon 4 [11]. Depending on the splicing efficiency of exon 9, which is determined by the length of the poly-T-tract in intron 8, the R117H mutation can result in mildly or severely reduced function of the transcribed CFTR protein [12]. Because the splicing efficiency of exon 9 appeared normal (wild-type IVS8–7T alleles), the R117H mutation in this

patient has to be judged as ‘mild’. Compound heterozygosity of the CFTR gene with one severely and one moderately or mildly affected allele has been established as a cause of acute pancreatitis [13–16]. In line with these genetic findings there were no clinical signs of classic CF, there were no nasal polyps and the sweat chloride concentration was within the normal range.

The particular genotype found in this patient has not been implicated with recurrent pancreatitis before. At first, this appears not to be surprising given the vast number of theoretically possible allelic combinations and the limited number of patients published in three studies in 1998 and 1999, which looked at an Anglo-Saxon [16], a US-American [15] and an Italian population [13]. On the other hand, the CFTRdele2,3(21 kb) deletion was only described in 2000 [11]. Like other CFTR mutations, it has a specific geographic distribution and is the second most common mutation after $\Delta F508$ in central and eastern Europe, with an allele frequency of about 1.5% in German and up to 6.4% in Czech CF patients [11]. Up to this time, allele frequencies for the CFTRdele2,3(21 kb) mutation have not been determined among patients with idiopathic pancreatitis. The R117H mutation also has a fairly high allele frequency of 0.3% in northern Europe. Therefore, the described genotype may be comparably frequent in Europe [11]. One of the two compound heterozygotes included in the originally description of the CFTRdele2,3(21kb) deletion had exactly this genotype [11]. This patient had congenital bilateral absence of the vas deference (CBAVD), which – based on residual CFTR activity – belongs to the same group of CFTR-associated diseases such as idiopathic pancreatitis [17].

This is the first description of recurrent acute pancreatitis with compound heterozygosity in the CFTR gene involving the quite recently discovered severe CFTR mutation CFTRdele2,3(21kb), which is frequent in eastern and central Europe. We suggest that in eastern and central Europe and in patients with that particular genetic background the CFTRdele2,3(21kb) mutation should be included in the panel of mutations studied when addressing possible CFTR associated pancreatitis.

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Invited Commentary

A Further CFTR Mutation in Pancreatitis – Another Small Piece of a Large Puzzle?

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Already in 1994, CFTR mutations were hypothesized to play a role in the pathogenesis of pancreatitis [1] but only Sharer et al. [2] and Cohn et al. [3] in 1998 were able to prove a significantly increased rate of CFTR variants in idiopathic pancreatitis. These mutations were found in 20–40% of the patients and were later also identified in 10% of patients with alcoholic chronic pancreatitis [4]. Within recent years, an increasing number of CFTR mutations were described which extended the knowledge on the genetic base of pancreatitis considerably. The paper by Lamprecht et al. [5] published in the present issue of *Pancreatology* is a very recent example of this progress. In one female patient with acute recurrent pancreatitis, they found a compound heterozygosity consisting of

R117H and the CFTR variant dele2,3(21kB) that has been described in CF patients belonging to the slavic population of eastern and central Europe [6].

We must now face the difficulty of proving the association between genotype and phenotype in a single patient. This is a particular problem in rare or isolated mutations. Should, for example, a mutation be found in less than 2% of subjects with either cystic fibrosis (CF) or chronic pancreatitis (CP) as described for dele2,3 (21 kb) in central Europe, one will have to analyze at least 100 patients and more than 500 controls to confirm an association to pancreatitis. This number of patients can easily be found in the CF literature [6], but the controls are generally lacking. This holds also true for the original de-

scription of *dele2,3(21 kb)* which may cast doubts on the relation of this particular mutation to either CF or CP.

The situation is even more complex as genetic risk factors are only one part of the story. The patient described by Lamprecht et al. [5] drank small amounts of alcohol which could play a role in the manifestation of disease. In addition, it is very difficult to rule out a recurrent biliary pancreatitis in this subject. It is my opinion that a single factor will not be sufficient to explain the manifestation of the disease. Therefore, the mere separation into alcoholic and non-alcoholic pancreatitis will have to be given up in the future. It seems to be more accurate to understand pancreatitis as a multifactorial disease with a varying mixture of both genetic and non-genetic risk factors.

In order to analyze the relative role of each of the risk factors, a very large group of patients with chronic pancreatitis will have to be investigated thoroughly. As this is not done yet we do not exactly know whether the piece described by Lamprecht's group is really part of our large puzzle.

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