Acute recurrent biliary pancreatitis associated with the ABCB4 gene mutation

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Introduction

Acute pancreatitis is a severe complication of biliary stones or sludge. Cholelithiasis is thought to account for 35-43% of all cases of acute pancreatitis [1-3]. After routine investigations, no obvious aetiology is identified in 16-30% of the cases [1, 3]. After intensive biliary investigations (such as ERCP or endoscopic ultrasonography) or follow up 21-73% of patients with unexplained acute pancreatitis are shown to have cholelithiasis [3, 4]. Previous studies have shown that patients with acute recurrent pancreatitis and biliary sludge have a low phospholipid concentrations in bile [5].

The ABCB4 gene codes for a protein involved in the transport of phosphatidylcholine across the canalicular membrane of the hepatocyte. ABCB4 gene defects have been associated with progressive familial intrahepatic cholestasis type 3, intrahepatic cholestasis of pregnancy, adult biliary cirrhosis and the more recently described low phospholipid associated cholelithiasis syndrome. The present paper describes 2 probands with a long history of recurrent pancreatitis and cholelithiasis and the same heterozygous, as yet undescribed del 3683>3688 within exon 28 of the ABCB4 gene resulting in a loss of function. This report shows that ABCB4 mutations may cause acute recurrent biliary pancreatitis.

Case reports

Patient 1

This patient was born in 1961. She has a brother (case 2) and 2 sisters. Her aunt (her mother’s dizygotic twin sister) had cholelithiasis for which she underwent a cholecystectomy (figure 1). She had been treated with oral contraceptives (Adepal® from 1979 to 1982 and Diane 35® from 1982 to 1989). In 1981 she had severe epigastric pain for 15 days that resolved spontaneously. From 1981 to 1985, she was symptom-free. In 1983, she had her first pregnancy which was uneventful. In February 1985, a diagnosis of acute pancreatitis was made on the basis of prolonged, severe epigastric pain and increased serum amylase levels, 30 times above the upper limit of normal values. Serum ALAT and ASAT activity was increased 6 times above the upper limit of normal values. Abdominal CT scan was normal and an ultrasound examination showed biliary sludge within the gallbladder. A cholecystectomy was performed in March 1985; the intraoperative cholangiography was normal. Between 1985 and 1993 she had several bouts of acute epigastric pain with or without increased serum amylase and transaminase activity. Her second pregnancy in 1989 was uneventful. Oral contraceptives (Diane 35®) were prescribed for 3 months after delivery; and were replaced by an intrauterine device for 3 years during which she was symptom-free. Then, oral contraceptives were prescribed from 1992 to 2003 (Diane 35® from 1992 to 2001; Melodia® from 2001 to 2003).

RÉSUMÉ

Le gène ABCB4 code pour une protéine impliquée dans le transport de la phosphatidylycholine à travers la membrane canaliculaire de l’hépatocyte. Des anomalies du gène ABCB4 ont été associées à la cholestase intraépithéliale progressive familiale de type 3, la cholestase intraépithéliale de la grossesse, les cirsoses biliaires de l’adulte et le syndrome récemment décrit sous le nom de « low phospholipid associated cholelithiasis ». Le présent article décrit deux malades, frère et sœur, atteints de pancréatites aiguës récidivantes et de lithiase biliaire, porteurs de la même délétion (jusqu’à présent non décrite) 3 683 > 3 688 de l’exon 28 du gène ABCB4, résultant en une perte de fonction. Ces observations montrent que les mutations du gène ABCB4 peuvent être à l’origine de pancréatites biliaires récidivantes.

SUMMARY

Pancréatites aiguës biliaires récidivantes associées à une mutation du gène ABCB4

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In May 1993 she was admitted for acute pancreatitis. An ERCP was performed and was found to be normal; an endoscopic sphincterotomy was performed. She developed sepsis from *Pseudomonas Aeruginosa*. Jaundice and cholestasis appeared. Fever and cholestasis resolved with vancomycin, amikacin and imipenem intravenously. In October 1993, abdominal pain and fever reappeared and resolved with the same antibiotics. Liver ultrasound showed that the intrahepatic bile ducts of the left lobe were enlarged. Biliary and pancreatic endoscopic ultrasonography were normal, a liver biopsy showed intrahepatic cholestasis. Several abdominal ultrasound examinations were performed and showed intrahepatic hyperchoic foci within the liver parenchyma and the intrahepatic bile ducts (figure 2). Treatment with ursodesoxycholic acid at a dose of 10 mg/kg/d was started in 1995. Acute epigastric pain recurred in November 1996 while the patient was being treated with ursodesoxycholic acid at a dose of 15 mg/kg/d. In February 1998 while she was still receiving ursodesoxycholic acid at a dose of 15 mg/kg/d, she had acute epigastric pain. A stone was found in the common bile duct and removed during an ERCP. Liver function tests returned to normal values. Ursodesoxycholic acid was maintained at a dose of 10 mg/kg. In 2001, a liver ultrasound examination showed an intrahepatic hyperchoic foci in segment VI. The patient was seen in July 2004; she was in good health, and symptom-free.

Patient 2

The patient was born in 1965. In 1999, he was admitted for severe epigastric pain and increased transaminase levels. Biliary sludge and dilatation of the intra and extrahepatic bile ducts were found on abdominal ultrasound. A cholecystectomy was performed, intraoperative cholangiography was found to be normal. Liver biopsy was normal. One week after the cholecystectomy he had loss of vision in the left eye. A posterior uveitis and thrombosis of the central retinal vein were found. Corticosteroids and ciclosporine were started in February 2001. In May 2001 an increase in serum alkaline phosphatase (4 times above the upper limit of normal), γGT (13 times above the upper limit of normal), ASAT (4 times above the upper limit of normal) and ALAT (5 times above the upper limit of normal) were discovered. A liver biopsy was performed and showed pericentral cholestasis and eosinophils within the portal space (figure 3). Biliary and pancreatic endoscopic ultrasonography examination was normal. Ciclosporine was discontinued, liver function tests improved and returned to normal values in July 2001. Azathioprine was started in April 2002. In October 2002, the patient had severe and prolonged epigastric pain. Serum lipase levels were above 1500 IU/liter (Normal values 114-286) and serum amylase levels were 9.5 times above the upper limit of normal, ASAT and ALAT were also increased (4 and 2.5 times above the upper limit of normal). A new abdominal ultrasound examination showed intrahepatic hyperchoic foci. Abdominal CT scan and MR cholangiography were normal. Oral intake was resumed and pain did not recur. Ursodesoxycholic acid was begun at a dose of 10 mg/kg and azathioprine was...
exon 15 and del 3683>3688 within exon 28.

ABCB4 gene. Both were found to have a mutation 1769G>A within exon 15 by sequence comparisons with Seqscape Software (version 1; Applied Biosystems, Applera France SA, Courtaboeuf, France). Identification and localization of ABCB4 gene mutations and SNPs were assessed by polymerase chain reaction was performed using specific primers of exons and splice junctions. PCR reaction products were purified on a Sephadex (Pharmacia) column and sequenced with Big Dye Terminator chemistry (Applied Biosystems).

Patients gave informed consent to search for mutations within the ABCB4 gene. Both were found to have a mutation 1769G>A within exon 15 and del 3683>3688 within exon 28.

Mutation screening

Mutations were screened as previously described [10, 11]. Briefly, genomic DNA was obtained using standard procedures. Polymerase chain reaction was performed using specific primers of exons and splice junctions. Mutations were screened as previously described [10, 11]. Briefly, genomic DNA was obtained using standard procedures. Polymerase chain reaction was performed using specific primers of exons and splice junctions. PCR reaction products were purified on a Sephadex column and sequenced with Big Dye Terminator chemistry (Applied Biosystems, Applera France SA, Courtaboeuf, France). Identification and localization of ABCB4 gene mutations and SNPs were assessed by sequence comparisons with Seqscape Software (version 1; Applied Biosystems).

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Discussion

These two probands have a longstanding history of recurrent pancreatitis and cholelithiasis and the same mutation in the ABCB4 gene. The 1769G>A within exon 15 is a polymorphism and does not seem to have functional consequences. In contrast, the as yet undescribed, del 3683>3688 within exon 28 leads to a deletion in the ATP binding site of the protein resulting in a loss of function. These 2 case reports confirm the results reported by Rosmorduc et al. who found that ABCB4 gene mutation-associated cholelithiasis (including cholangitis and pancreatitis) occurred after cholecystectomy, was associated with intrahepatic hyperchoic foci and sludge with an onset of symptoms in patients less than 40 years old [10, 11]. In our patients, acute pancreatitis was associated with, and a prominent feature of, LPAC. Acute pancreatitis had all the characteristics of a biliary origin and was associated with the other typical features of LPAC.

Other causes of acute pancreatitis were eliminated in these 2 patients. In case 1, sphencter of Oddi dysfunction was not consistent with other features of the case such as intrahepatic cholestasis and hyperchoic foci; moreover, endoscopic sphincterotomy did not improve the patient’s condition. In case 2, the presence of posterior uveitis suggested associated primary sclerosing cholangitis. This diagnosis was eliminated by the normal MR cholangiography findings and liver biopsy results. Patient 2 was treated with azathioprine, a drug that can induce pancreatitis. However, azathioprine-induced pancreatitis generally occurs within 6 weeks after the drug is begun whereas 6 months elapsed between the start of azathioprine and pancreatitis and it did not recur even though azathioprine was continued.

It can be hypothesized that, in patient 1, oral contraceptives contributed to cholelithiasis and in patient 2 cyclosporine contributed to cholestasis. ABCB4 mutations and drugs may have both contributed to cholelithiasis and cholestatic liver disease. However, the role of ABCB4 mutations in drug-induced cholestasis deserves further study.

In conclusion, the present paper shows that acute biliary pancreatitis should be added to the clinical spectrum of diseases associated with the ABCB4 gene defect. The ABCB4 gene defect should be added to the list of causes of acute recurrent pancreatitis of a biliary origin. The search for mutations should be performed in patients with acute biliary pancreatitis who exhibit other features of the LPAC syndrome.

REFERENCES


Fig. 3 – Liver biopsy in patient 2 showing intrahepatic cholestasis within the cytoplasm of the hepatocytes. These abnormalities predominate in the centrolobular space (arrow panel a). Liver biopsy in patient 2 showing eosinophils within the portal space (PS) suggesting drug-induced injury (arrow panel b).

Biopsie du foie chez le malade 2 montrant un cholestase intrahépatique, intracytoplasmique à prédominance centrolobulaire (flèche image a). Chez le même malade, on note la présence d’éosinophiles dans l’espace porte (PS), suggérant l’origine médicamenteuse de la cholestase (flèche, image b).

