Acute recurrent biliary pancreatitis associated with the ABCB4 gene mutation

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]. Acute recurrent pancreatitis who relapsed after cholecystectomy but responded to oral ursodeoxycholic acid. Both had the same, as yet undescribed, heterozygous mutation of the ABCB4 gene that led to 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 (Adepol® 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. She underwent a cholecystectomy (figure 1). She had been treated with oral contraceptives (Adepol® 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; Meladia® from 2001 to 2003).

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Acute recurrent biliary pancreatitis associated with the ABCB4 gene mutation

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 septicaemia from *Pseudomonas Aeruginosa*. Jaundice and cholestasis appeared. Fever and cholestasis resolved with vancomycin, amikacin and imipenem intravenously. In October 1993, abdominal pain and fever recurred 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 hyperchoeic 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 occurred 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 hyperchoeic 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 cyclosporine were started in February 2001. In May 2001 an increase in serum alkaline phosphatase (4 times above the upper limit of normal), μGT (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 pericentrolobular cholestasis and eosinophils within the portal space (figure 3). Biliary and pancreatic endoscopic ultrasonography examination was normal. Cyclosporine 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 hyperchoeic 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 started.

Fig. 1 – Pedigree of family of the 2 patients studied. Four members of this family had a history of cholelithiasis. Patient II-7 had a cholecystectomy at 45 for biliary colic, search for ABCB4 was not performed. Patient 1 had her first biliary symptoms at 24 and was found to be mutated for ABCB4. Patient 2 had his first biliary symptoms at 34 and was found to be mutated for ABCB4. Patient III-14 had his first biliary symptoms at 30; search for ABCB4 mutation has not been performed.

Arbre généalogique des 2 malades présentés. Quatre membres de cette famille ont présenté un ou plusieurs épisodes en rapport avec une lithiase biliaire. Le malade II-7 a eu une cholécystectomie à l’âge de 45 ans pour colique hépatique, la recherche du gène ABCB4 n’a pas été effectué. Le malade 1 a eu ses premiers symptômes à l’âge de 24 ans et présentait une mutation du gène ABCB4. Le malade 2 a eu ses premiers symptômes biliaires à l’âge de 34 ans et présentait une mutation du gène ABCB4. Le malade III-14 a eu les premières manifestations biliaires à l’âge de 30 ans; la recherche de la mutation n’a pas été réalisée.

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 septicaemia from *Pseudomonas Aeruginosa*. Jaundice and cholestasis appeared. Fever and cholestasis resolved with vancomycin, amikacin and imipenem intravenously. In October 1993, abdominal pain and fever recurred 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 hyperchoeic 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 occurred 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 hyperchoeic foci in segment VI. The patient was seen in July 2004, she was in good health, and symptom-free.

Fig. 2 – Liver ultrasound in patient 1 showing hyperchoeic foci within the liver parenchyma (arrow, panel A) and the intrahepatic bile ducts (arrow, panel B).

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exon 15 and del 3683>3688 within exon 28.

and localization of ABCB4 gene mutations and SNPs were assessed
Biosystems, Applera France SA, Courtaboeuf, France). Identification
juncions. PCR reaction products were purified on a Sephadex (Pharma-
genomic DNA was obtained using standard procedures. Polymerase
chain reaction was performed using specific primers of exons and splice

continued. After twenty four months of follow up, the patient is in good
health and symptom-free.

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 splicing
juncions. PCR reaction products were purified on a Sephadex (Pharma-
cia) 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).

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.

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) recurred 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, sphincter of Oddi dysfunction was not
consistent with other features of the case such as intrahepatic
cholestasis and hyperchoic foci; moreover, endoscopic sphinc-
terotomy did not improve the patient’s condition. In case 2, the
presence of posterior uveitis suggested associated primary scler-
osing 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 pancrea-
titis. 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
contribute to cholelithiasis and in patient 2 cyclosporine con-
tributed to cholelithiasis. ABCB4 mutations and drugs may have
both contributed to cholelithiasis and cholestatic liver disease.
However, the role of ABCB4 mutations in drug-induced cholesta-
sis 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 pancrea-
titis of a biliary origin. The search for mutations should be per-
formed in patients with acute biliary pancreatitis who exhibit
other features of the LPAC syndrome.

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