Clonal chromosomal abnormalities in congenital bile duct dilatation (Caroli's disease)

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Abstract

Background—Caroli's disease is a rare congenital disorder characterised by cystic dilatation of the intrahepatic bile ducts and an increased risk of cholangiocellular carcinoma. The cause is unknown, but occasional familial clustering suggests that some cases are inherited, in particular when occurring in association with polycystic kidney disease and germline *PKD1* gene mutations. To date, no gene responsible for familial isolated Caroli's disease has been identified, and no genetic investigations of liver tissue from patients with Caroli's disease have been reported.

Patient/Method—A liver biopsy specimen from a patient with isolated Caroli's disease, without any signs of cholangiocellular carcinoma, was short term cultured and cytogenetically investigated after G banding with Wright's stain.

Result—Cytogenetic analysis disclosed the karyotype 45-47,XX,der(3)t(3;8)(p23;q13),+2mar[cp6]/46,XX[18].

Conclusions—The finding of an unbalanced translocation between chromosomes 3 and 8 suggests that loss of distal 3p and/or gain of 8q is of pathogenetic importance in Caroli's disease. Alternatively, structural rearrangements of genes located in 3p23 and 8q13 may be of the essence. These chromosomal breakpoints may also pinpoint the location of genes involved in inherited forms of Caroli's disease not associated with polycystic kidney disease.

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patients with Caroli's disease, strongly suggesting a premalignant nature of this disorder.

The cause of Caroli's disease is unknown, but occasional familial clustering suggests that some cases are inherited, in particular when occurring together with polycystic kidney disease,⁵ a disorder that in its autosomally dominant form is associated with germline mutations of at least two different genes—that is, *PKD1* and *PKD2* at 16p13.3 and 4q13-23 respectively—and in its autosomally recessive form with mutations of a gene mapping to 6p21.⁶⁻¹⁰ *PKD1* mutations have been shown in some patients with autosomal dominant polycystic kidney disease and Caroli's disease.⁷ However, no gene responsible for familial isolated Caroli's disease has been identified, and no genetic investigations of liver tissue from patients with Caroli's disease have been reported to date.

Cytogenetic abnormalities, constitutional as well as acquired, have proved important in identifying the genomic localisation of genes involved in inherited disorders and in neoplasia. For example, the finding of a constitutional deletion of chromosome arm 5q in a patient with colon polyposis contributed significantly to the physical mapping of the *APC* gene, which is now known to be mutated in most patients with adenomatous polyposis of the colon.^{11,12} Furthermore, detecting acquired abnormalities of chromosomes 8, 11, and 13 in exostoses, Wilms' tumour, and retinoblastoma respectively was instrumental in identifying the *EXT1*, *WT1*, and *RB1* genes involved in these inherited tumour types.¹³ As part of a cytogenetic study of liver tumours, we have investigated a liver biopsy specimen from a patient with Caroli's disease, and we here describe and briefly discuss the chromosomal abnormalities detected.

Case report

A 75 year old woman with a history of duodenal ulcer, Barrett's oesophagus, ischaemic heart disease, hypertension, and venous thrombosis was admitted to hospital because of abdominal pain. She had previously had an appendicectomy, cholecystectomy, total hip replacement, subtotal thyroidectomy for thyrotoxicosis, and mastectomy for breast cancer. On admission, elevated serum alkaline phosphatase levels were found (7.7 μ kat/l; normal <4.3 μ kat/l). Ultrasonography showed gallstones in a dilated common hepatic bile duct, and endoscopic retrograde cholangiopancreatography disclosed a stricture close to the junction between the choledochal and the common hepatic ducts with gallstones in the dilated left

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Caroli's disease, first described by Caroli *et al* in 1958, is a rare congenital disorder characterised by cystic dilatation of the intrahepatic bile ducts.¹ Two forms have been distinguished: a simple type and a periportal fibrosis type. The latter is associated with cirrhosis, portal hypertension, and periportal fibrosis, whereas such features are not seen in the simple form.² Recurrent cholangitis is the most common clinical manifestation, and biliary colic, jaundice, and acute pancreatitis occur as a consequence of intrahepatic calculi formation.³ A further complication of this disease is its association with biliary epithelial dysplasia and cholangiocellular carcinoma. Dayton *et al*⁴ have reported that the incidence of cholangiocellular carcinoma is more than 100 times greater in

hepatic ducts and in the common hepatic bile duct. The stricture and the gallstones remained despite endoscopic sphincterotomy and attempts at stone extraction, percutaneous transhepatic cholangiography with stricture dilatation, laser lithotripsy, and extracorporeal shock wave lithotripsy. Because the patient continued to have episodes of biliary colic and cholangitis, she was referred for surgery. Intraoperative ultrasonography confirmed the presence of gallstones in the lateral and medial segmental ducts of the left liver, and the left liver lobe was resected. The hepatic duct stricture was found to be due to scar tissue, which was divided. The postoperative course was complicated by congestive heart failure but was otherwise uneventful. The patient was discharged on the 15th day after the operation. She has had no biliary tract symptoms during two years of follow up.

Histological and cytogenetic studies

The resected liver tissue ($12 \times 11 \times 4$ cm) was divided into two parts: one for histopathological examination, which was stained with haematoxylin/eosin, and one for cytogenetic analysis, which was brought to the laboratory immediately after surgery. The specimen used for cytogenetic investigation was washed in Ham's F12 medium, minced with scalpels, and enzymatically disaggregated in collagenase type II (Sigma, St Louis, Missouri, USA). The resulting suspension was then washed twice by centrifugation in Ham's F12 medium. Short term cultures were set up in collagen coated plastic flasks in medium consisting of Dulbecco's modified Eagle's medium/Ham's F12 (1:1), supplemented with 10% fetal bovine serum, penicillin (100 IU/ml), streptomycin (0.2 mg/ml), hydrocortisone (0.5 µg/ml), dibutyryl cAMP (10 nM), and 1% ITS (insulin/

transferrin/selenious acid; Collaborative Biomedical Products, Bedford, Massachusetts, USA). After six days, the cultures were exposed to colcemid (0.02 µg/ml) for four hours. The cells were detached from the surface by trypsin/EDTA, treated in a 0.06 M KCl hypotonic solution for 30 minutes, subjected to repeated fixation in methanol/acetic acid (3:1), and dropped on to wet slides. The chromosomes were G banded with Wright's stain. In the cytogenetic analysis, the clonality criteria and the description of the karyotype followed the recommendations of ISCN (1995).¹⁴

Results

Macroscopically, the liver tissue was pale, but without signs of cirrhosis. Dilated cystic bile ducts with thick walls were observed in the hilar region. These ducts were mainly situated outside the hepatic tissue and contained concretions and bile stained fluid. Microscopic examination showed that the liver architecture was conserved without cholestasis. However, moderate steatosis in zone 3 around the hepatic veins was observed. Slightly enlarged bile ducts and ductules, as well as branches of arteries, were seen in some portal tracts, similar to preserved fetal structures of the ductal plate. The dilated segmental bile ducts were lined with columnar biliary epithelium with foci of papillary and basophilic epithelium, but without histological signs of malignancy. Bile and polymorphonuclear cells were seen in the ductal lumen. Arteries, veins, and nerves accompanied the abnormal bile ducts. Based on the clinical and histological features, the diagnosis was Caroli's disease.

A total of 24 metaphases were analysed from the short term cultured cells. Eighteen metaphases had a normal female chromosomal complement, whereas six mitoses displayed

Figure 1 Representative karyogram, showing the karyotype 47,XX,der(3)t(3;8)(p23;q13),+2mar. The arrow indicates the breakpoint on the derivative chromosome 3. The loss of chromosome 5 is non-clonal.

two marker chromosomes and an unbalanced translocation involving chromosomes 3 and 8. Thus the karyotype was: 45-47,XX,der(3)t(3;8)(p23;q13),+2mar[cp6]/46,XX[18] (fig 1). No more material was available for fluorescence in situ hybridisation analyses of the two marker chromosomes.

Discussion

The paradigmatic view of acquired chromosomal abnormalities is that they are a *sine qua non* for neoplastic transformation and tumour growth. Apart from providing information about genetic mechanisms underlying neoplasia, chromosomal changes are also clinically useful for diagnosis and prognostication, to date mainly in haematological malignancies.¹⁵ For example, the translocations t(9;22)(q34;q11) and t(15;17)(q22;q12-21) are diagnostic for chronic myeloid leukaemia and acute promyelocytic leukaemia respectively. During the last decade, it has been shown that such characteristic translocations also occur in some solid tumour types, primarily mesenchymal tumours, such as t(12;16)(q13;p11) in myxoid liposarcoma and t(11;22)(q24;q12) in Ewing's sarcoma. In general, less is known about disease associated translocations in epithelial tumours, which rather seem to be characterised by chromosomal imbalances, such as loss of 1p, 3p, and 6q and gain of 1q in breast cancer and loss of 1p, 8p, 17p, and 18 and gain of 7, 8q, and 20q in colon cancer. Only a few cytogenetic studies of liver tumours have been reported, but some characteristic patterns have nevertheless emerged: trisomy 20 and total or partial trisomy 2 are common in hepatoblastoma, and rearrangements of chromosomes 1, 7, and 8, resulting in gains of 1q, 7p and 8q and losses of 1p and 8p, are common in primary liver cancer.^{16 17}

The strong association between acquired chromosomal changes and neoplasia notwithstanding, clonal chromosomal alterations are occasionally also found in non-neoplastic lesions, such as Dupuytren's contracture, Peyronie's disease, and osteoarthritis.¹⁷ Also some tumour-like lesions of the liver have been reported to harbour cytogenetic aberrations (table 1). Thus acquired chromosomal abnormalities are not necessarily associated with neoplasia. The present finding of clonal changes in a liver biopsy specimen from a patient with Caroli's disease should hence be interpreted with caution. Thus, although a gain of 8q is common in primary liver cancer, including cholangiocellular carcinoma,¹⁶ and the identified unbalanced 3;8 translocation resulted in a gain of 8q13-qter, one cannot conclude that neoplastic transformation had

occurred. However, even though there were no clinical or histopathological signs of cholangiocellular carcinoma, we cannot exclude the possibility that the cytogenetic investigation disclosed a very early change in hepatocarcinogenesis. If so, loss of 3p and gain of 8q would be candidate initiating changes for liver cancer. Alternatively, these genomic imbalances may be associated with development of Caroli's disease rather than with an ensuing malignancy.

A similar translocation—that is, t(3;8)(p21;q12) involving the *PLAG1* gene at 8q12 and the β -catenin (*CTNNB1*) gene at 3p21—has been reported in pleomorphic adenomas of the salivary glands and has been suggested to play a pivotal role in salivary gland tumorigenesis.¹⁸ Unfortunately, lack of material precluded molecular analyses of the present t(3;8), and we hence do not know whether these genes were also rearranged in Caroli's disease. However, the chromosomal breakpoints identified—3p23 and 8q13—are different from those in pleomorphic adenomas, and it seems reasonable to assume that other genes may have been involved, although rearrangement of these genes cannot be excluded by G banding alone. Finally, considering that acquired genetic changes have previously been instrumental in mapping chromosomal regions harbouring genes involved in inherited disorders,¹³ the cytogenetic abnormalities presented here may pinpoint the location of genes involved in inherited forms of Caroli's disease not associated with polycystic kidney disease.

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Table 1 Cytogenetic data on tumour-like lesions of the liver.¹⁷

Morphology	Karyotype
Focal liver steatosis	47,XX+7/46,XX
Focal nodular hyperplasia	45-46,XY,add(4)(q21-25),add(11)(p14),add(19)(p13), der(20)t(1;20)(q25;p12),add(21)(q22),-22,+mar
Mesenchymal hamartoma	46,XY,t(15;19)(q15;q13)
Mesenchymal hamartoma	46,XY,t(11;19)(q13;q13)
Focal liver steatosis (unpublished)	70-75,XXX,+X,+2,+7,+8,-16,-18,+19,+21,+22[cp4]/ 46,XX[96]
Caroli's disease (present report)	45-47,XX,der(3)t(3;8)(p23;q13),+2mar[cp6]/46,XX[18]