EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

ABCB4/MDR3 gene mutations and cholangiocarcinomas

To the Editor:

Multidrug resistance protein 3 (MDR3), also known as ATP-binding cassette subfamily B, member 4 (ABCB4), is predominantly expressed in the liver and is the critical translocator for phospholipids across canalicular membranes of hepatocytes [1,2]. MDR3 mutations are responsible for progressive familial intrahepatic cholestasis type 3 (PFIC3), low phospholipid-associated cholelithiasis (LPAC syndrome) and intrahepatic cholestasis of pregnancy (ICP) [3]. Cholangiocarcinoma (CCa) is a rare malignant tumour of the biliary tract, characterized by a dramatically poor prognosis usually due to a delayed diagnosis and inefficient non-surgical treatments [4]. To our knowledge, up to now no association between CCa and MDR3 mutations has been reported. In this letter to the editor, we report two cases of association between *MDR3* gene mutations and CCa in two non-related families.

In the first family, patient 1, a 53-year-old man, was investigated for a perihilar tumoral obstruction and treated by right hepatic lobectomy. The pathological examination showed a CCa with one lymph node metastasis. Non-tumoral peripheral tissues showed signs of cholangitis and ductopenia (Fig. 1). After surgery, itching persisted, alkaline phosphatase level was 894 UI/L and MRI showed multiple partial stenosis of the remaining biliary tract. The patient was given ursodeoxycholic acid (UDCA) with a marked decrease in alkaline phosphatase to 258 UI/L. Twenty-six months after surgery, the CCa recurred with peritoneal carcinosis and the patient died 13 months after the diagnosis of CCa recurrence. Patient 2, patient 1's daughter, had a cholecystectomy at 14 years old for symptomatic gallstones. At 23 years old, she presented with jaundice and itching, which resolved spontaneously. A MDR3 familial biliary disease was suspected. The missense variation NM_000443.3: c.1469T>C in exon 13 was found with heterozygous status in the liver tissue of patient 1 and in the blood leucocytes of patient 2. This heterozygous variation leads to NP_000434: p.Ile490Thr at MDR3 protein level. It is a new variant, but according to SIFT software specialized in mutation interpretation, it predicts a non-functional protein.

In the second family, a 55-year-old woman (patient 3), was treated by right hepatectomy for a large perihilar CCa. The pathological examination revealed major ductopenia and inflammatory biliary ducts in the non-tumoral peripheral liver tissue. Ten months after the surgery, a recurrence was detected by CT scan and the patient died 31 months after the initial surgery. Patient 4, patient 3's sister, a 38-year-old woman, had a cholecystectomy at 20 years old for gallstones and surgical ablation of residual gallstones in the common bile duct at 24 years old. She also had an ICP during her first pregnancy. A resection of the right liver was decided due to post-operative common bile duct stenosis and intrahepatic gallstones in segments V and VIII. Histological analysis showed biliary duct inflammation and liver fibrosis probably subsequent to chronic cholestasis. After surgery, she still presented with pruritus, elevated liver enzymes (alkaline phosphatase at 300 IU/L) and developed signs of portal hypertension due to liver fibrosis. Due to intrahepatic lithiasis suggesting LPAC syndrome, we carried out research in order to find the *MDR3* mutation. A heterozygous missense sequence variation in exon 24, NM_000443.3:c.2932T>C leading to NP_ 000434: pSer978Pro at the MDR3 protein level was found. Using her paraffin-embedded hepatic tissue, we found the same MDR3 variant in her sister (patient 3), at heterozygous status. The mutation has already been encountered in five other families in France.

Cholangiocarcinomas represent less than 5% of digestive tumours and commonly identified risk factors are primary sclerosing cholangitis, congenital biliary abnormalities and hepatolithiasis [5]. Nevertheless, they represent only a minority of CCa cases. Neither genomic mutation nor hereditary predisposition has been found to be associated with the development of CCa.

Cases of intrahepatic CCa from pediatric patients affected by the homozygous mutations of BSEP (Bile Salt Export Pomp), the main bile acid transporter in hepatocytes, have been reported [6]. The CCa mechanisms associated during the process with *BSEP* and *MDR3* mutations are probably the same, but have yet to be satisfactorily explained. It is well-known that inactive MDR3 is responsible for the accumulation of altered bile in hepatocytes and cholangiocytes, thereby causing microscopic forms of liver damage such as inflammation and fibrosis [7]. Chronic biliary inflammation can increase cholangiocyte turnover, thereby

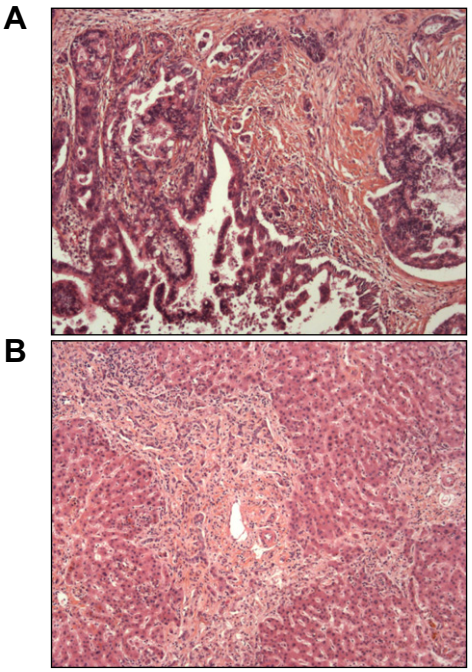

Fig. 1. Cholangiocarcinoma with (A) vascular invasion and (B) non-tumoral peripheral tissues with cholangitis.

enhancing the growth of altered cholangiocytes and leading to increased CCa susceptibility [8,9].

These case reports suggest a need for prospective follow-up of patients with *MDR3* mutations, in order to identify the CCa risk. It would probably also be interesting to look for an *MDR3* mutation in young patients with CCa, especially if there exists a familial history of CCa or biliary disease.

Conflict of interest

The authors declared that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Acknowledgements

The authors thank Jeffrey Arsham for editing the manuscript. The authors thank Valerie Paradis, Dominique Valla, Jacques Belghiti and Christine Silvain for their contributions to this work.

References

[1] van Helvoort A, Smith AJ, Sprong H, Fritzsche I, Schinkel AH, Borst P, et al. MDR1 P-glycoprotein is a lipid translocase of broad specificity, while MDR3 P-glycoprotein specifically translocates phosphatidylcholine. Cell 1996;87:507–517.
[2] Davit-Spraul A, Gonzales E, Baussan C, Jacquemin E. The spectrum of liver diseases related to ABCB4 gene mutations: pathophysiology and clinical aspects. Semin Liver Dis 2010;30:134–146.
[3] Jacquemin E. Role of multidrug resistance 3 deficiency in pediatric and adult liver disease: one gene for three diseases. Semin Liver Dis 2001;21:551–562.
[4] Tyson GL, El-Serag HB. Risk factors of cholangiocarcinoma. Hepatology 2011;54:173–184.
[5] Cardinale V, Semeraro R, Torrice A, Gatto M, Napoli C, Bragazzi MC, et al. Intra-hepatic and extra-hepatic cholangiocarcinoma: new insight into epidemiology and risk factors. World J Gastrointest Oncol 2010;2:407–416.
[6] Scheimann AO, Strautnieks SS, Knisely AS, Byrne JA, Thompson RJ, Finegold MJ. Mutations in bile salt export pump (ABCB11) in two children with progressive familial intrahepatic cholestasis and cholangiocarcinoma. J Pediatr 2007;150:556–559.
[7] Trauner M, Fickert P, Halilbasic E, Moustafa T. Lessons from the toxic bile concept for the pathogenesis and treatment of cholestatic liver diseases. Wien Med Wochenschr 2008;158:542–548.
[8] Wise C, Pilanthananond M, Perry BF, Alpini G, McNeal M, Glaser SS. Mechanisms of biliary carcinogenesis and growth. World J Gastroenterol 2008;14:2986–2989.
[9] Jaiswal M, LaRusso NF, Burgart LJ, Gores GJ. Inflammatory cytokines induce DNA damage and inhibit DNA repair in cholangiocarcinoma cells by a nitric oxide-dependent mechanism. Cancer Res 2000;60:184–190.

David Tougeron*
Department of Hepatology and Gastroenterology, Poitiers University Hospital, Poitiers, France
Laboratoire Inflammation Tissus Epithéliaux et Cytokines, EA 4331, University of Poitiers, Poitiers, France
*Corresponding author. Address: *Department of Hepatology and Gastroenterology, Poitiers University Hospital, 2 rue de la Milétrie, 86000 Poitiers Cedex, France*
Tel.: +33 5 49 44 37 51; fax: +33 5 49 44 38 35
E-mail address: davidtougeron@hotmail.fr

Ginette Fotsing
Department of Hepatology and Gastroenterology, Poitiers University Hospital, Poitiers, France

Veronique Barbu
AP-HP, LCBGM, Saint-Antoine Hospital, UPMC Univ Paris 06 & INSERM, UMR_S 938, CdR Saint-Antoine, Paris, France

Michel Beauchant
Department of Hepatology and Gastroenterology, Poitiers University Hospital, Poitiers, France

The optimal dose of omega-3 supplementation for non-alcoholic fatty liver disease

To the Editor:

Non-alcoholic fatty liver disease (NAFLD) is a burgeoning health problem that affects one-third of adults and an increasing number of children in developed countries [1]. It was previously reported that various therapeutic regimens can be adopted for NAFLD [2], including weight loss agents, bariatric surgery, insulin-sensitizing agents, lipid-lowering agents, antioxidants, and other novel compounds. However, there is no consensus on its treatment. Both lifestyle therapy and pharmacotherapy have limitations due to poor compliance and side effects. Therefore, new therapeutic approaches to managing NAFLD are urgently needed.

We read with interest the study by Parker *et al.* [3]. This is the first meta-analysis to investigate the effect of omega-3 polyunsaturated fatty acid (PUFA) on liver fat in humans. The authors found that omega-3 PUFA could decrease liver fat and that benefits were seen with a consumption of >0.83 g/day. The results are instructive for the therapeutic regimen of NAFLD; however, we have some concerns over the optimal dose mentioned in the article.

Given the complications of NAFLD, especially cardiovascular disease (CVD), the optimal dose of omega-3 PUFA should be much higher than 0.83 g/day. Targher *et al.* revealed a strong association between NAFLD and CVD risk by reviewing accumulating clinical evidence [4]. Previous evidence suggests that CVD dictates the outcome in patients with NAFLD more frequently and to a greater extent than does the progression of liver disease, and CVD is the most important cause of death in NAFLD patients [5]. On the other hand, many investigators have demonstrated that omega-3 PUFA could prevent CVD [6]. In a large-scale intervention trial of secondary prevention after myocardial infarction, GISSI-Prevenzione investigators identified a substantial reduction in all-cause and cardiovascular mortality with 1 g per day of n-3 PUFA supplementation [7]. In addition, McKenney *et al.* recommend n-3 PUFA supplementation at a dose of 2–4 g per day to patients with high triglyceride concentrations [8]. At present, Saravanan *et al.* found that omega-3 PUFA could act as beneficial pleiotropic agents to prevent CVD, by conducting a review of numerous clinical trials [9]. As fish is rich in omega-3 PUFA, the