From MDR3 to LPAC: Cross talk between molecular biology and clinical medicine

Du MDR3 au LPAC : intéractions entre la biologie moléculaire et la médecine clinique

In this issue of this journal, Poupon et al. [1] describe a new form of the low phospholipid-associated cholelithiasis (LPAC) syndrome, characterized by large intrahepatic bile ducts dilatations without stenosis. The chain of events that led to this clinical advance is fascinating.

The MDR (multidrug resistance) protein family was discovered in the mid-1980s. MDR, or cross resistance to various lipohilic anticancer drugs, had intrigued clinicians and biologists for several decades, until it was discovered that this phenomenon was related to over-expression of a 170 kDa membrane glycoprotein, designated P-glycoprotein or MDR1 [2]. This over-expression leads to an energy-dependent increase in drug efflux out of the cell, and thus to a decrease in the intracellular concentration of the drug. MDR1 (now known as ABCB1) is a member of a superfamily of proteins, the ATP-binding cassette (ABC) proteins. When MDR1 was discovered, only two members had been recognized: MDR1, responsible for the MDR phenotype, and MDR3 (now known as ABCB4) (mdr2 in rodents), whose function remained unknown until a landmark observation in 1993. That year, Smit et al., using the new technique of knockout mice, showed that mdr2−/− mice, whose two copies of the mdr2 gene were inactivated, suffered from a characteristic liver disease and had no phospholipids in bile [3]. Liver histology in these mice showed marked portal and periportal fibrosis and extensive bile ductular proliferation. The lesions were explained by the toxicity of physiological bile acids, which are hydrophobic, on the membranes of hepatocytes and cholangiocytes. In these mdr2−/− mice, bile acids are transported normally and, in the absence of phospholipids, they are highly toxic for surrounding membranes.

After this initial observation, MDR3/ABCB4 was shown to act as a flippase, moving phospholipids (mostly phosphatidylcholine) from the inner leaflet of the canalicular membrane of the hepatocyte to the outer leaflet, which faces the canalicular lumen [4,5]. From there, phosphatidylcholine is washed out into the lumen by bile acids. The gene, now designated as ABCB4, was mapped to chromosome 7q21.1. Another important observation was made shortly thereafter: in mdr2−/− mice, increasing the hydrophobicity of the bile salt pool by cholate feeding leads to more severe liver lesions, whereas decreasing the hydrophobicity by ursodeoxycholate feeding improves liver histology [6], a finding entirely consistent with the role of bile salts in the development of hepatic lesions in this animal model.

These pioneering experimental observations led to three major clinical advances, when progressive familial intrahepatic cholestasis (PFIC), intrahepatic cholestasis of pregnancy (ICP) and intrahepatic cholesterol cholelithiasis (here designated as LPAC syndrome), three entities thought to be unrelated, were shown to be due to mutations of ABCB4.

PFIC is an heterogeneous group of autosomal recessive hepatic diseases of childhood in which cholestasis of hepatocellular origin often presents in the neonatal period or the first years of life. It leads to death from hepatic failure at ages ranging from infancy to young adulthood. There are three known phenotypes of PFIC. Type 3 is characterised by cholestasis starting between 1 month and 20 years of age, elevated aminotransferases, alkaline phosphatase and, in contrast to types 1 and 2, high serum γ-glutamyl transferase activity [7]. Liver histology shows portal fibrosis and marked ductular proliferation, with a mixed inflammatory infiltrate. At a later stage, typical biliary cirrhosis develops. The analogy with the lesions of mdr2−/− mice led Deleuze et al. to postulate that a genetic defect of ABCB4 could be the cause of the disease. They found an abnormal expression of ABCB4 (low amount of mRNA in the liver) and low phospholipid concentration in bile of these patients [8]. The genetic defect was soon confirmed, with two types of mutations: stop codons leading to a truncated protein and missense mutations [7,9,10]. Children with missense mutations have a less severe disease than those with a truncated protein, with...
an onset later in life, a slower progression and a favourable effect of ursodeoxycholic acid (which is never observed in children with a truncated protein) [9].

ICP is defined by the occurrence of cholestasis in pregnant women with an otherwise normal medical history. Familial cases have been reported and a genetic predisposition had been suspected for a long time. This was proven when Jacquemin et al. found that, within the families of several children with PFIC, type 3, women had experienced episodes of ICP and were heterozygotes for ABCB4 mutations [7,9,11]. Since then, other cases have been observed, and, in a large cohort of 50 unrelated women with ICP, the prevalence of ABCB4 mutations was estimated to 16% [12]. This discovery opened the way to the identification, in ICP, of mutations of the bile salt export pump, the canalicular transporter of bile salts [13,14].

The third disease related to ABCB4 mutations is the LPAC syndrome. It was first described by Rosmorduc et al. [15,16]. It is characterized by gallbladder and intrahepatic cholesterol gallstones, and clinically by biliary symptoms, often recurring after cholecystectomy. The diagnosis can be suspected when at least one of the following is present: age less than 40 years at onset of symptoms, recurrence after cholecystectomy, intrahepatic hypercholeic foci with a topography compatible with lipid deposits along the luminal surface of the intrahepatic biliary tree, intrahepatic sludge, microthiasis, familial history of cholelithiasis in first-degree relatives, or clinical history of ICP [17]. The syndrome can, occasionally, be associated with PFIC, type 3 [18], with ICP or with biliary cirrhosis in adults [19]. In this new paper, Poupon et al. [1] describe the cases of eight patients with a peculiar form of LPAC characterised by large dilatations of intrahepatic bile ducts containing gallstones. Five of these patients had severe cholangitis. Other causes of bile ducts dilatation, including biliary stenosis, Caroli’s disease, sclerosing cholangitis or cholangiocarcinoma, were carefully excluded, and analysis of ABCB4 showed heterozygous or compound heterozygous mutations, confirming the diagnosis of LPAC. Interestingly, these mutations were not different from those observed in patients with the LPAC syndrome without large bile duct dilatations. Ursodeoxycholic acid had a favourable effect and five of the eight patients remained free of symptoms under treatment. An improvement of the biliary dilatations, together with a decrease in the number of stones, was observed after 3 years or more of treatment. In one patient, the stones disappeared at ultrasound and magnetic resonance imaging. The authors estimate that the prevalence of this peculiar phenotype is in the range of 5 to 10% of patients with the LPAC syndrome.

In clinical practice, the diagnosis of LPAC should now be suspected in a patient with biliary symptoms (biliary colic, cholangitis or cholestatic jaundice) and intrahepatic bile duct dilatations. Genetic analysis of ABCB4 should be performed to confirm the diagnosis. This is another remarkable example of the interrelationship between molecular biology and clinical medicine.

Conflict of interest statement

None.

References


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