**DIAGNOSIS:** Oral contraceptive pill-induced cholestasis due to heterozygous \( \text{ABCB4} \) mutation (causing \( \text{ABCB4} / \text{MDR3} \) deficiency)

This case of drug-induced cholestasis is used to illustrate our evolving knowledge about the mechanisms involved in a spectrum of cholestatic disorders that are sometimes difficult to categorize. It is now well established that mutations in 3 of the canalicular membrane transporters responsible for bile formation can be responsible for severe cholestatic disease in children – progressive familial intrahepatic cholestasis (PFIC) types 1-3. Additionally some families or family members express a milder phenotype, benign recurrent intrahepatic cholestasis (BRIC) types 1-3, with the same transporter proteins responsible.

Subsequently, a range of biliary/cholestatic disorders have been linked to mutations of the same genes, and drug-induced cholestasis is one of these.\(^1\) In the current case, further history was obtained when the liver biopsy was discussed at the liver CPC. Her mother had a history not only of early cholecystectomy but also jaundice of pregnancy. There was no family history of unexplained cirrhosis. Because of the “cholestatic” family history and the picture of canalicular cholestasis with some injury to small bile ducts, it was suggested that this could represent a mild manifestation of \( \text{ABCB4} / \text{MDR3} \) deficiency. Gene testing (performed in Europe) showed heterozygous mutation of \( \text{ABCB4} \) (encoding \( \text{ABCB4} \) or multidrug resistance P-glycoprotein 3 [MDR3] - the term MDR3 is falling out of favour since, although there is close homology with the drug transporter MDR1, MDR3 transports only endogenous phospholipid, not drugs). It is highly likely that the same mutation was involved in her mother’s early cholelithiasis and intrahepatic cholestasis of pregnancy. The classical description of contraceptive steroid-induced cholestasis is of bland canalicular cholestasis, and vanishing bile duct syndrome has not been described.\(^2\) The liver biopsy in this case shows canalicular cholestasis, but there is also evidence of biliary epithelial injury with vacuolation, possible focal loss of occasional ducts and aberrant keratin-7 expression in periportal hepatocytes. The biliary injury may be related to toxicity either from free bile acids that fail to form micelles due to lack of phosphatidylcholine (see pathogenesis below), or from free cholesterol crystals. There is also mild portal fibrosis and a focal ductular reaction. This biopsy does not have any periductal fibrosis.

**Pathogenesis**

Bile salt translocation across the canalicular membrane is a critical function of the hepatocyte. Bile salts are needed for emulsification and digestion of fat and are also important for producing bile flow in the canaliculi. Several transporter proteins play a role in bile formation.\(^3\) Bile acids themselves (making up 50% of the bile) are transported by \( \text{ABCB11} \) (bile salt export protein, BSEP). Because bile acids are toxic to canalicular membranes once transported, a second component, phosphatidylcholine (PC) which comprises 25% of the bile, is secreted to form micelles with the bile acids and secreted cholesterol. This is done by “flopping” PC from the inner cell membrane leaflet to the outer leaflet on the canalicular luminal surface where it is released into the bile.\(^3\) The “floppase” performing this is \( \text{ABCB4}/\text{MDR3} \). This phospholipid transfer causes an unacceptable degree of membrane instability so a third protein ATP8B1 (FIC1, the protein associated with PFIC1 or Byler disease) transports phosphatidylserine in the other direction from the outer to inner leaflet, stabilising the canalicular membrane.\(^3\) All 3 transporters are needed for normal bile formation and flow.
There are many mutations of the genes expressing ABCB4/MDR3 and ABCB11/BSEP. Homozygous or compound heterozygous mutations with marked loss of activity result in early and severe cholestatic disease (PFIC3 and PFIC2 respectively). Heterozygosity and mutations causing a milder phenotype are probably more common than currently recognised and the functional outcome of different mutations is quite variable. It has been shown recently that some mutations cause premature stop codons (30% of cases). 70% are missense mutations that produce a mutated protein, which variously may be unable to exit the endoplasmic reticulum, is not transported normally to the canalicular membrane or has reduced floppase activity.

Expression of the bile acid/canalicular transporter proteins is regulated by orphan nuclear receptors, particularly FXR and PPARα. These nuclear receptors can be downregulated or blocked by estrogen and progesterone metabolites; when the transporters are already defective, any further reduced expression induced by these hormones (pregnancy, OCP) can then precipitate frank cholestatic disease. Loss of ABCB4/MDR3-transported phosphatidylcholine leads to defective micelle formation and bile acid-induced injury to the canalicular and bile duct luminal surface. Additionally, cholesterol (4% of the bile) is not incorporated into micelles and becomes lithogenic, leading to precipitation with stone formation and possibly direct cholesterol crystal injury to cells. As therapeutic targets, the nuclear receptors can be upregulated by agonists such as ursodeoxycholic acid (UDCA) and PPARα agonists, thereby upregulating the transporter proteins.

Clinical syndromes linked to ABCB4 (MDR3) mutation

ABCB4 mutation was initially described as an autosomal recessive cause of progressive familial intrahepatic cholestasis (PFIC3) in affected children, but it is now clear that milder cholestatic diseases can occur in an autosomal dominant pattern in some families. Recent studies have suggested that mutations in ABCB4 may also manifest as:

- intrahepatic cholestasis of pregnancy (~15% of cases, usually more severe ones)
- drug-induced cholestasis (some cases)
- unexplained episodic jaundice
- unexplained chronic cholestatic liver tests
- adult cryptogenic cirrhosis with biliary features
- adult ductopenia
- low phospholipid-associated cholelithiasis (LPAC) syndrome

LPAC is characterized by biliary symptoms before 40yrs, recurrent symptoms after cholecystectomy, recurrent hepatolithiasis / microlithiasis and a normal MRCP. The recurrent pain and intrahepatic microlithiasis are due to the precipitation of free cholesterol crystals due to the lack of phosphatidylcholine and failure to form micelles with bile acids.

As well as canalicular cholestasis seen in drug- and pregnancy-associated cholestasis, other relatively subtle histological manifestations are being described in mildly affected patients. A recent study of liver findings in 13 adults with proven heterozygous mutations found that most biopsies from patients with ABCB4 mutations had a ductular reaction, mild portal inflammation and fibrosis. In 3 patients aggregates of cholesterol crystals (or spaces where the crystals had dissolved) were identified, and the presence of frank hepatolithiasis was associated with biliary dysplasia and subsequent cholangiocarcinoma in one case. Two of 13 biopsies showed obliterative duct scars, commonly regarded as a feature that is suggestive of PSC. Progression to cirrhosis was rare.
Additionally, in another study of patients with unexplained cholestatic liver tests, ABCB4 mutations were found in 34% of cases, and liver biopsy usually showed portal fibrosis and a ductular reaction.\textsuperscript{14} In our hands and in others,\textsuperscript{13,15} MDR3 immunoperoxidase staining of the canicular membrane is generally normal in these mild cases of cholestatic disease, contrasting with the frequent diminution of canicular MDR3 staining that characterises full-blown PFIC3.

**Small duct PSC – is it all just ABCB4 (MDR3)?**

The study by Wendum and colleagues\textsuperscript{16} showed that some patients with ABCB4 mutation had bile duct injury and periductal onion-skinning fibrosis with duct scars, obviously a pattern resembling mild primary sclerosing cholangitis (PSC) and raising the question of whether some cases could be related to ABCB4 mutation. A study of conventional PBC and PSC cohorts found that there is no increased mutation rate in ABCB4 compared with the general population.\textsuperscript{16} This does not exclude a role in the minority of PSC patients who show only small duct lesions, without large duct strictures or MRCP abnormalities. These cases of small duct PSC (SD-PSC) require liver biopsy for diagnosis,\textsuperscript{17} showing either the characteristic lesions of duct scars and/or periductal onion-skinning fibrosis, or else a biliary ductular reaction consistent with some form of biliary disorder. Recently, it has been increasingly recognized that other disorders can have overlapping clinical features with PSC.\textsuperscript{18}

However, it is clear that SD-PSC must exist as a true variant of PSC, since around 25% of cases eventually progress to develop large duct changes, 50-88% have a history of IBD, and 10% of transplanted cases recur in the allograft, which would not be possible if an inherited intrahepatic transporter in the native liver were the cause of disease. The current EASL guidelines suggest that SD-PSC can be diagnosed in those who also have IBD as long as the biopsy shows features that are consistent with biliary disease, usually a ductular reaction. In short, most patients with apparent SD-PSC do in fact have PSC, but in patients without IBD, ABCB4 mutation causing ABCB4/MDR3 deficiency should be carefully considered since treatment with UDCA is beneficial.

In summary there is a range of mild cholestatic changes such as ductular reaction, mild portal fibrosis and sometimes canicular cholestasis that occur on a background of chronic cholestatic liver function tests, where the usual tests for PBC, PSC and large duct obstruction are negative. Some of these represent mild ABCB4/MDR3 disease. A careful family history is required and if this is suggestive then genetic testing is worth considering.

**References**


