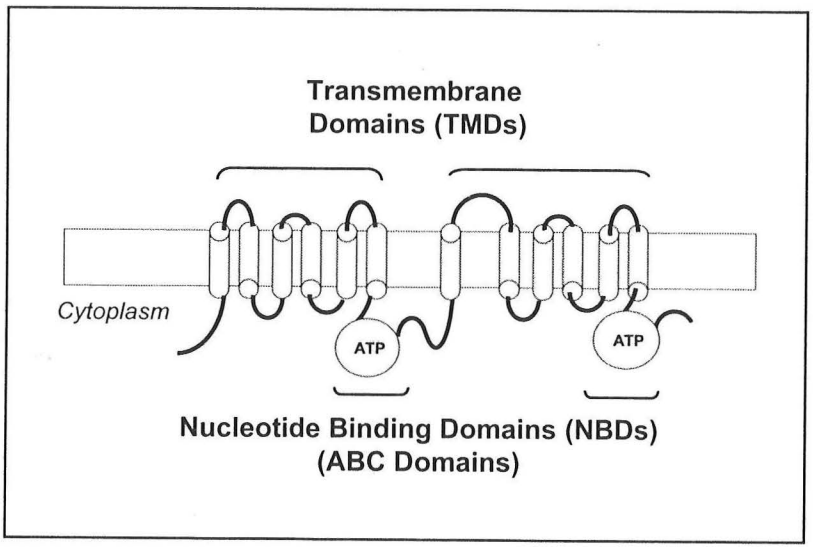
# ABC Transporters and Human Disease: From Cholestasis to Heart Disease

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# PATIENTS WITH MUTATIONS IN ABC TRANSPORTERS

## PATIENT 1

The patient was a healthy 30-year old woman who developed jaundice in the third trimester of her pregnancy. Her bilirubin was 10 mg/dl and her other liver function tests were normal. Her urine was positive for bile. Her gallbladder was not visible on oral cholecystogram. On biopsy, her liver was grossly black.

## PATIENT 2

M.H. had neonatal hepatitis that resolved spontaneously. She presented again at age 5 with cholestasis and a serum bile acid level that was increased 50-fold. Serum levels of bilirubin and alkaline phosphatase were both elevated 2-fold, but her other LFTs, including $\gamma$-glutamyl transpeptidase (GGT), were normal. She was lost to follow-up and died suddenly at age 14.

## PATIENT 3

The patient was a 13-year old adolescent found dead in his bed. On autopsy he had severe coronary atherosclerosis. Evaluation of his family revealed five siblings who were hypercholesterolemic and had large tendon xanthomas. Both parents were normocholesterolemic. One of the hypercholesterolemic sisters was placed on a low-cholesterol diet and her plasma LDL-cholesterol fell from 450 mg/dl to 120 mg/dl.

## PATIENT 4

T.M. presented at age 18 for evaluation of weakness in his right foot and hypocholesterolemia. On neurological exam he had normal reflexes and 4/5 strength in the flexors of his right foot with no associated sensory deficit; the remainder of his neurological exam was normal. He previously had a neurological work-up, including a myelogram, which was completely negative. He was referred to this medical center when he was incidentally noted to have a plasma cholesterol of 42 mg/dl with an LDL-C of 16 mg/dl and an HDL-C of 3 mg/dl.

## GENERAL FEATURES OF ABC TRANSPORTERS

The plasma membrane, just 7-10 nanometers in thickness, provides a barrier separating the contents of the cell and the external milieu. Steep concentration gradients must be maintained to partition chemicals and proteins on either side of the plasma membrane. Over the past 20 years, it has been established that almost all molecules traverse membranes by means of a dedicated transport mechanism. For instance, water and urea, which intrinsically have high degrees of membrane permeability in artificial lipid bilayers, are now known to move across cellular membranes through selective transporters. The focus today will be on the ATP binding cassette (ABC) transporters, an extremely versatile family of membrane proteins that arose early in evolution to selectively transport a wide range of substrates across cellular membranes. These transporters play critical roles in survival by segregating those essential cellular components that must be retained in the cell from those that must be exported. In humans, these membrane proteins are required for the elimination of environmental toxins and potentially harmful by-products of cellular metabolism from cells. The central role these transporters play in maintaining cellular homeostasis in humans is revealed by the many and varied diseases that result from mutations in members of the ABC transporter gene family.

All ABC transporters contain two functional domains – *the transmembrane domain* (TMD) and the *nucleotide binding domain* (NBD) (**Figure 1A**). The TMD contains ~12 membrane-spanning regions clustered in two groups of six. Each NBD consists of a ~215 amino acid motif containing three highly conserved sequence motifs (a Walker A and Walker B motif separated by a signature sequence), which are required for ATP hydrolysis to drive transport of substrates across cell membranes.

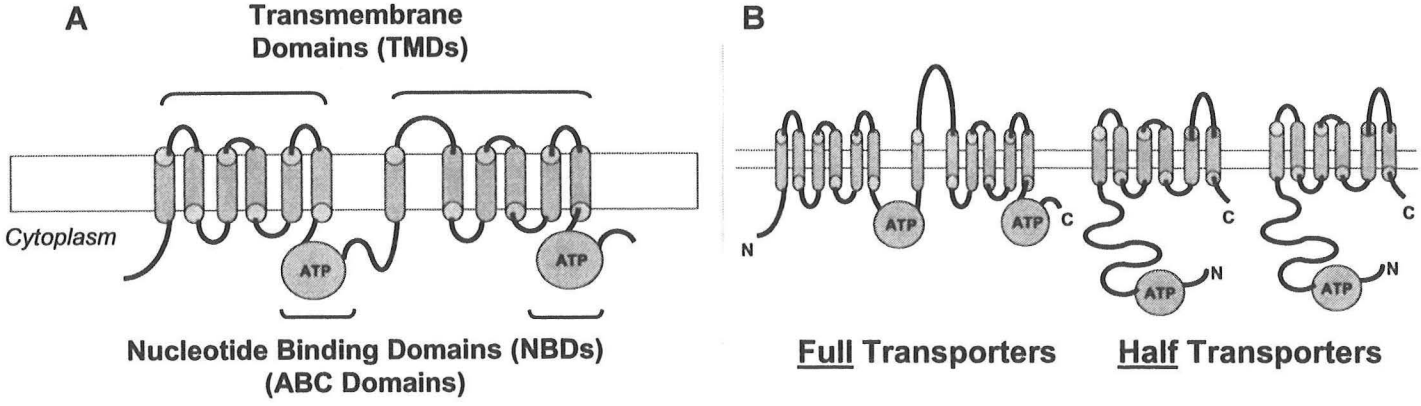


**Figure 1.** (**A**) The two major domains of ABC transporters and (**B**) the classification of ABC transporters as full and as half transporters.

ABC transporters are classified as either full transporters, which contain all four domains (two TMDs and two NBDs) within the same polypeptide chain or as half transporters, which contain only a single TMD and a single NBD (**Figure 1B**). Half transporters must homodimerize or heterodimerize with another half transporter to function.

## ABC TRANSPORTERS THROUGH EVOLUTION

In bacteria, ABC transporters can either import or export substrates. The import transporters move essential nutrients from the periplasmic space into cells (**Figure 2**). Bacteria overcome the problem of low substrate concentrations in the extracellular milieu by secreting substrate binding proteins, which bind substrates and present them to the transporter on the cell surface. Bacteria also have ABC transporters that export substances from the cell.

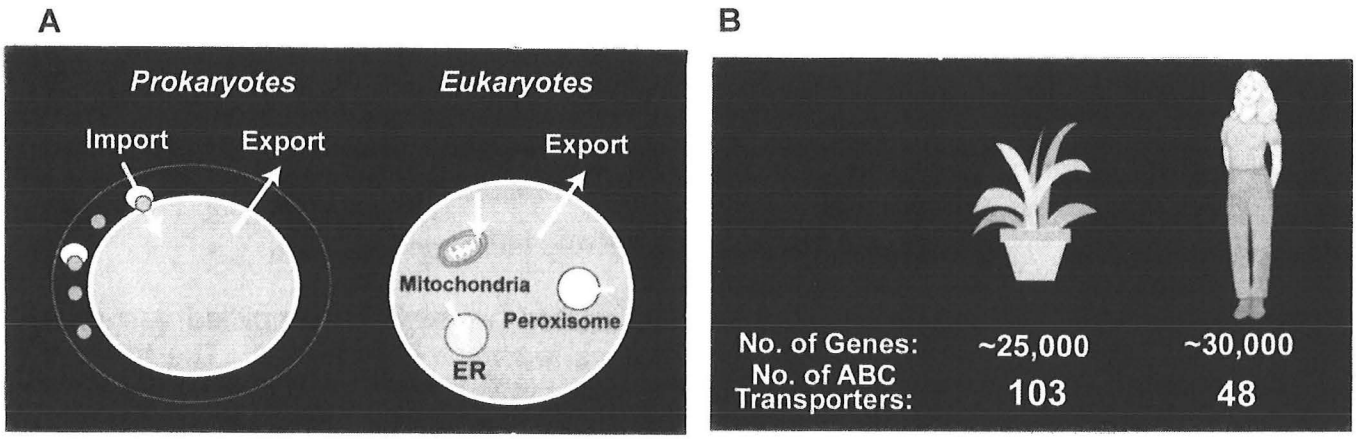


**Figure 2.** (**A**) ABC transporters in prokaryotes and eukaryotes. (**B**) Number of ABC transporters in plants is twice that of humans.

In contrast to bacteria, all mammalian ABC transporters identified to date are *export* pumps. Substrates are transported across the plasma membrane from the cytoplasm to the extracellular space, or alternatively, from the cytoplasm into a cellular organelle such as the endoplasmic reticulum, Golgi complex, mitochondria or peroxisome, which topographically represent extracellular space.

ABC transporters are particularly plentiful in plants. Plants have more than twice as many ABC transporters as do humans, despite having fewer total genes (**Figure 2B**). Possible reasons for the higher number of ABC transporters in plants include i) the inability to physically escape from various environmental toxins, ii) the absence of a specialized excretory system to remove wastes and iii) the metabolic complexity of plants. In this respect, plants produce more than 50,000 different metabolic products, which must be partitioned and compartmentalized within plant cells, or selectively excreted to avoid toxicities associated with their intracellular accumulation.

## THE ABC TRANSPORTER FAMILY

Sequencing of the human genome revealed 48 members of the ABC transporter gene family (**Figure 3**). Of these 48 members, only 16 have been assigned functions. Mutations in at least 14 of these transporters cause human disease. The best known disease due to mutations in an ABC transporter is cystic fibrosis, which is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR).

Ironically, despite CFTR being one of the first ABC transporters to be molecularly characterized, the mechanism by which mutations in this transporter cause defective chloride transport has yet to be defined.

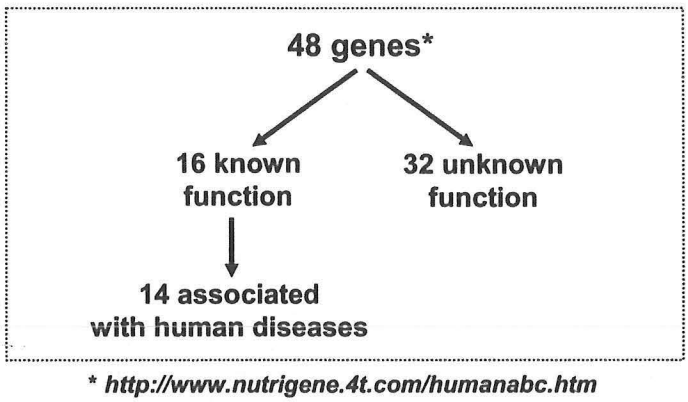


**Figure 3.** The numerology of ABC transporters.

Mammalian ABC transporters are structurally related proteins. A simplified version of the phylogenetic tree of human ABC transporters is shown in **Figure 4**. The tree was developed by performing a systematic comparison between the sequences of the NBD-encoding regions of the different genes. The sequences of the NBDs, rather than the sequences of the TMDs, provide the best indicator of relatedness between family members.

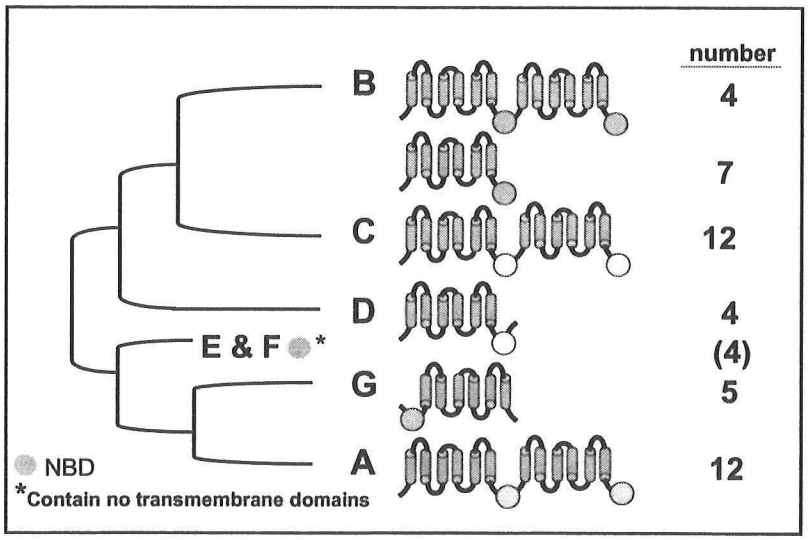


**Figure 4.** A simplified phylogenetic tree for human ABC transporter family.

The ABC transporter gene family has seven major subfamilies that are designated by the letters A through G. All ABC transporters contain both NBDs and TMDs, except those in subfamilies E and F, which have no TMDs; the four members of the E and F subfamilies will not be discussed further in this review since these family membranes do

not support transport across membranes. A complete list of the human diseases known to be due to mutations in ABC transporters is provided in **Table 1**.

**Table 1.** Genetic disorders due to mutations in ABC transporters.

| ABC Transporter | Substrate(s) | Disease |
|---|---|---|
| ABCA1 | Cholesterol/phospholipids | Tangier disease<br>Familial hypoalphalipoproteinemia |
| ABCA4 | Vitamin A derivatives | Stargardt macular dystrophy |
| ABCB4 (MDR3) | Phospholipids | Familial persistent intrahepatic cholestasis – Type 3<br>Cholestasis of pregnancy |
| ABCB11 | Bile acids | Familial persistent intrahepatic cholestasis – Type 2 |
| ABCB2 (TAP1) | Peptides | Immunodeficiency |
| ABCB3 (TAP2) | " " | " " |
| ABCB7 | Iron (?) | X-linked sideroblastic anemia and ataxia |
| ABCC2 (MRP2) | Bilirubin | Dubin-Johnson syndrome |
| ABCC6 | ? | Pseudoxanthoma elasticum |
| ABCC7 (CFTR) | ($Cl^-$) | Cystic fibrosis |
| ABCC8 (SUR1) | ($K^+$) | Persistent hyperinsulinemic hypoglycemia |
| ABCD2 (ALDR) | Very long chain fatty acids | Adrenoleukodystrophy |
| ABCG5 | Cholesterol | Sitosterolemia |
| ABCG8 | " " | " " |

In this review, I will focus on those diseases resulting from ABC transporter mutations that perturb lipid metabolism and cause either liver disease or premature atherosclerosis. Although these disorders are rare, they are important to the general internist for two reasons. First, studies of patients with these disorders have elucidated fundamental pathways in lipid homeostasis by providing entrees for the identification of proteins that play critical roles in these pathways. Second, derangements in the function of these ABC transporters provide insights into the pathophysiology of common diseases. Understanding the molecular roles of these proteins also provides opportunities to develop diagnostic tests and therapeutic agents for the detection and treatment of human diseases.

## MULTI-DRUG RESISTANCE PROTEINS: ABCB1, ABCG2, and ABCC1

The first mammalian ABC transporter to be isolated was ABCB1, also referred to as P-glycoprotein or multi-drug resistance protein 1 (MDR1). This protein was isolated from tumor cells chronically exposed to chemotherapeutic agents. Resistance to cell death was found to be associated with amplification in the number of copies of MDR1 in the cell membrane. Cells expressing high levels of MDR1 were shown to be resistant not only to the drug to which the cells were chronically exposed, but also to a wide range of other drugs. Subsequently, a whole series of ABC transporters with overlapping but nonidentical substrate specificities were identified that confer resistance to various compounds. This family of proteins is of clinical significance because they are expressed in many tumors and confer resistance to many of the chemotherapeutic agents used to treat malignancies.

Multi-drug resistance proteins are expressed in specific membrane domains on polarized cells. Some ABC transporters are expressed exclusively on the apical membrane, whereas others are expressed only on the basolateral surface. In general, transporters located on apical surfaces either transport substances from the cytoplasm of the cell into the blood or from inside the cell to outside the body (e.g. into the bile or the gut lumen). Other types of ABC transporters reside on the basolateral surfaces of epithelial cells and protect internal collections of fluids (e.g. the cerebrospinal fluid, the urine, the seminal fluid) from the accumulation of toxic substances.

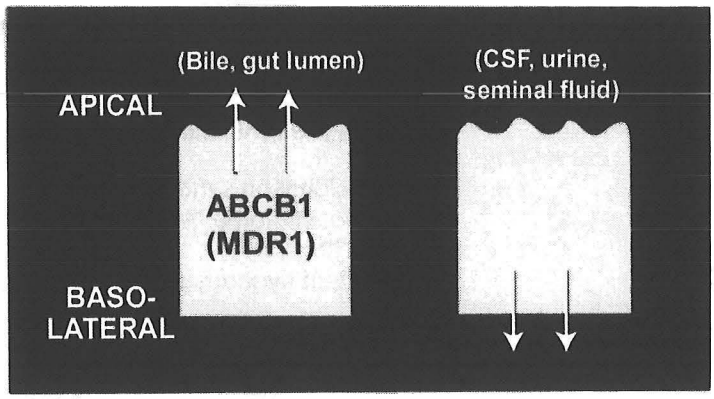


**Figure 5.** Localized expression of ABC transporters to apical or basolateral membranes of polarized cells.

MDR1 is expressed ubiquitously and resides on the apical surface of cells. Insights into the physiological role of this transporter came from the characterization of mice expressing no MDR1a. Humans have just one MDR1 gene whereas mice have two closely related MDR1 genes, *Mdr1a* and *Mdr1b.* Mice expressing no MDR1a initially appeared to be phenotypically normal. It was only after the mouse colony was sprayed with ivermectin for treatment of mites (ivermectin is used in humans to treat filariasis) that a phenotypic difference between the mice of different genotypes became evident. Soon after the spraying, some mice developed a movement disorder and died. Genotyping the dead mice revealed that the mice that had died were all homozygous for the inactivated *Mdr1a* allele. None of the heterozygous mice or wild type animals died. Normally, ivermectin fails to enter the brain because MDR1 promptly exports any drug entering endothelial cells that line the blood brain barrier. In the absence of MDR1, ivermectin accumulates in the brain, causing neurotoxicity and death.

MDR1 also limits the transit of a wide range of other chemicals into multiple other tissues (**Figure 6**).

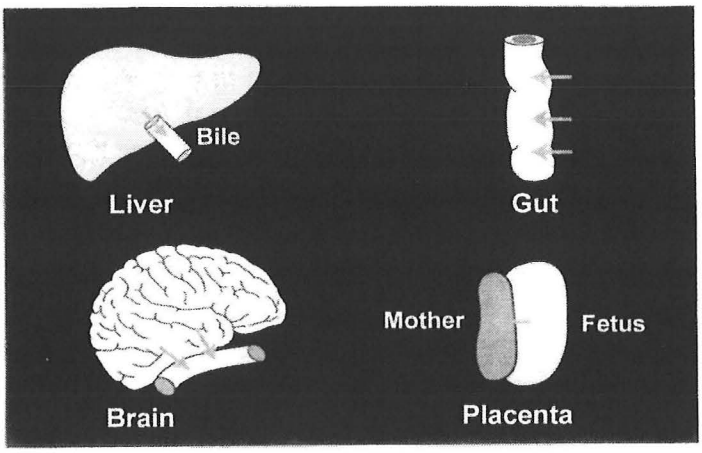


**Figure 6.** Role of MDR1 in the brain and other tissues.

MDR1 is but one of a large number of multi-drug resistance genes in the ABC transporter gene family. The unifying characteristic of this class of transporters is the vast array of structurally different classes of compounds they efficiently transport. Shown in **Figure 7** are some of the multi-drug resistance proteins and the locations of these proteins in hepatocytes. Some ABC transporters, such as MDR1, are located on the apical (or bile canalicular) surface. Other transporters, such as ABCC1 (MRP1), are found exclusively on the basolateral (or sinusoidal) surface. Despite significant differences in the sequences of many of these proteins, they have overlapping substrate specificities. ABCB1 and ABCG2 prefer neutral and cationic compounds, whereas another family member, ABCC1, transports both nonpolar and polar compounds. Glutathione-linked proteins, glutathione itself, and leukotriene 4 are all transported out of cells via MRP1.

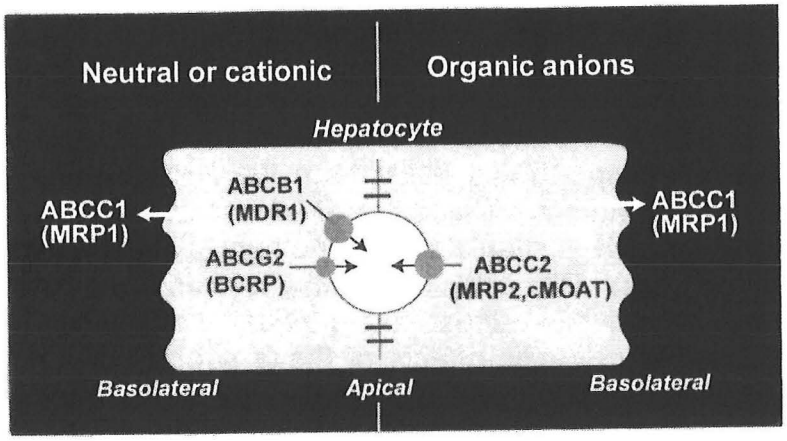


**Figure 7.** The location and general substrate specificity of selected multi-drug resistance proteins expressed in hepatocytes.

## ABCC2 AND DUBIN-JOHNSON SYNDROME

A major pathway used to secrete amphipathic compounds into bile involves, as a first step, the conjugation of these compounds to glucuronide, sulfate or glutathione to increase their solubility. Many of these organic anions, including bilirubin, are then transported into the bile by ABCC2 (MRP2). Bilirubin, a byproduct of heme breakdown,

undergoes conjugation to glucuronide prior to secretion into the bile via ABCC2 (**Figure 8**). **Patient 1** has Dubin-Johnson syndrome, which is caused by inactivating mutations in *ABCC2*. As a consequence of having no functional ABCC2, bilirubin-diglucuronide conjugates accumulate in the liver and blood, accounting for the hyperbilirubinemia and the black discoloration of the liver of this patient. The contrast dye used to visualize the gallbladder also enters the bile via ABCC1, so the gallbladders of patients with Dubin-Johnson syndrome fail to visualize on cholecystogram.

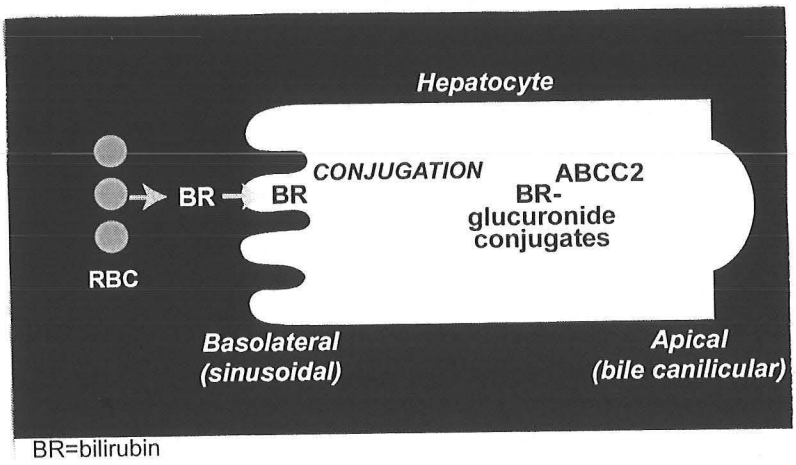


**Figure 8.** Mutations in ABCC2 (MRP2) cause Dubin-Johnson syndrome.

## ROLE OF ABC TRANSPORTERS IN HEPATOBILIARY TRANSPORT

The two main functions of the biliary system are 1) to deliver potent detergents (i.e., bile acids) to the intestine for the solubilization of dietary fats and 2) to provide a conduit for the exit of cholesterol from the body. Each of the three major lipids of bile – bile acids, cholesterol, and phosphatidylcholine – are each transported from the hepatocyte into bile via its own designated ABC transporter (**Figure 9**). ABCB11, also called the bile salt export protein (BSEP), transports bile acids from the hepatocyte into bile against a >1000-fold concentration gradient. The entry of bile acids promotes the secretion of phospholipids into bile, which is mediated by another ABC transporter, ABCB4, also called MDR3 (MDR2 in mice). Bile acids and phospholipids form mixed micelles, which serve to solubilize the bile acids and thus protect the bile canaliculus and the epithelium of the biliary tree from the cytotoxic effects of these strong detergents. These mixed micelles trap amphipathic compounds in the bile to keep them from reentering hepatocytes. Bile salt-phospholipid vesicles also promote secretion of cholesterol from hepatocytes, which requires a pair of ABC half transporters, ABCG5 and ABCG8.

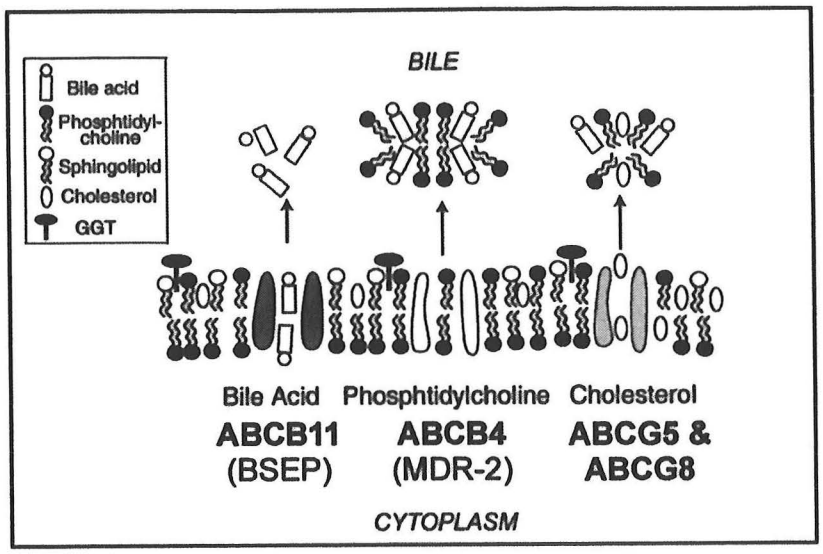


**Figure 9.** The triad of biliary ATP cassette transporters.

The bile canalicular membrane itself is a very specialized structure derived from microvillar extensions of the apical membranes of two or three adjacent hepatocytes. The outer leaflet of the canalicular membrane is unusually rich in sphingomyelin and cholesterol. These two lipids are able to pack very closely together and provide a surface that is resistant to the detergent effects of bile acids.

## EXTRAHEPATIC AND INTRAHEPATIC CHOLESTASIS

Genetic defects in both ABCB11 and ABCB4 cause cholestasis, which is defined as an interruption of bile flow. There are two major forms of cholestasis - extrahepatic cholestasis and intrahepatic cholestasis (**Figure 10**). *Extrahepatic cholestasis*, which is much more common, results from a structural lesion in the biliary tree that compromises biliary flow from the liver to the intestines (e.g. gallstones or mass lesions). Clinical manifestations of extrahepatic cholestasis include pruritis and jaundice, due to the accumulation of bile acids and bilirubin in the blood. Elevations in serum alkaline phosphatase, γ-glutamyltranspeptidase (GGT), bilirubin and cholesterol are also clinical hallmarks of extrahepatic cholestasis.

Less frequently, cholestasis occurs without any evidence of an extrahepatic obstruction; this condition was given the misnomer *intrahepatic cholestasis*, since it was originally presumed that individuals with this disorder had microscopic intrahepatic lesions obstructing biliary outflow from the liver. The pathological lesion in this form of cholestasis is characterized by less inflammation and less bile duct hyperplasia than is seen in association with extrahepatic obstructive lesions.

Progressive familial intrahepatic cholestasis (PFIC) is a form of intrahepatic cholestasis that usually presents in the neonatal period and has an autosomal recessive inheritance pattern. The disease progresses to liver failure and death if the patient does not obtain a liver transplant. Defects in three different genes cause PFIC and two of these genes encode ABC transporters.

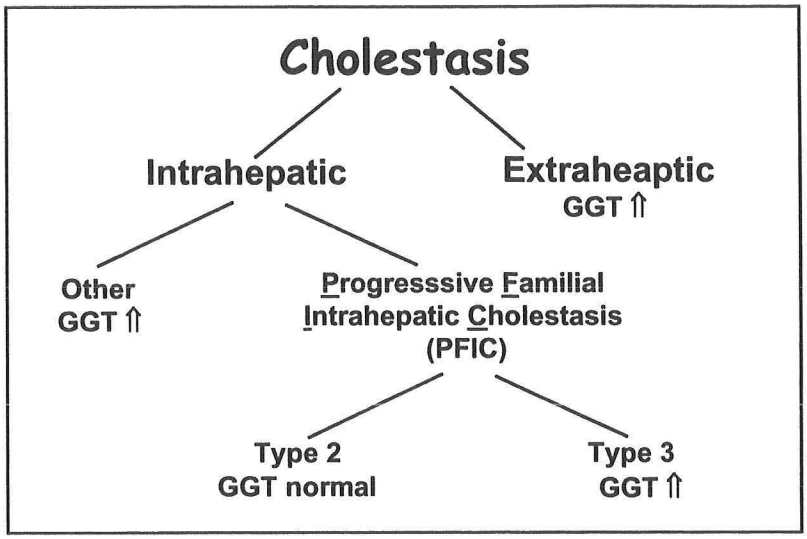


**Figure 10.** Extrahepatic and intrahepatic cholestasis

## PROGRESSIVE FAMILIAL INTRAHEPATIC CHOLESTASIS: BYLER'S SYNDROME

In the 1980's, pediatric hepatologists noted that children with intrahepatic cholestasis who have low serum levels of GGT have a much worse prognosis than do those having an elevated GGT. One of the first well characterized families with intrahepatic cholestasis and a normal serum level of GGT was the Byler family; thus, this disease was originally called Byler's syndrome. Mutations in *ATP8B1* (also called *FIC1*) have been identified as the molecular cause of Byler's syndrome. ATP8B1 is a member of another large family of transporters called the P-Type ATPases (the disease-causing gene for Wilson's disease is also a member of this family of transporters), and is not an ABC transporter. Patients will Byler's syndrome have very high levels of bile acids in the serum but low levels of bile acids in the bile. Since ATP8B1 cannot transport bile acids, mutations in *ATP8B1* must adversely affect entrance of bile acids into bile by an indirect mechanism.

Serum levels of GTT are uniformly low in Byler's syndrome. GGT is a GPI-anchored protein synthesized in hepatocytes and transported to the bile canalicular membrane, where it is thought to be attached by to the membrane by its "greasy foot." GGT is released into the circulation if biliary outflow is compromised, likely due to its solubilization by bile acids. Since patients with Byler's syndrome have little to no bile acids in their bile, no GGT is released from the liver into the circulation.

## PFIC TYPE 2: MUTATIONS IN *ABCB11* (BSEP)

In some families with a clinical picture resembling Byler's syndrome, such as **Patient 2**, the disease did not map to the *FIC1* locus. The disease in these families was found to be due to mutations in *ABCB11*, which encodes the bile salt export protein (BSEP); this disease was designated PFIC Type 2, to distinguish it from Byler's syndrome, which

was designated PFIC Type 1. BSEP mediates the transport of bile acids into the bile, therefore defects in this protein cause a marked reduction in the levels of bile acids in the bile and consequently, in the amount of bile produced by the liver. The only bile acids present in bile are those that traverse the bile canalicular membrane independent of BSEP. The low biliary levels of bile acids allow GGT to remain anchored to the biliary membrane; therefore, serum levels of GGT are normal in PFIC Type 2, as they are in PFIC Type 1 (**Figure 11**).

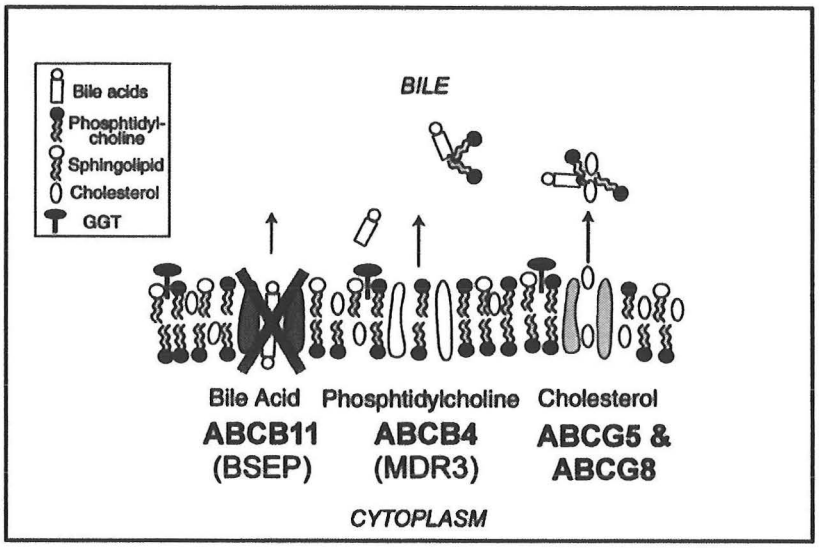


**Figure 11.** Mutations in ABCB11 marked reduce bile salt secretion into bile.

## PFIC TYPE 3: MUTATIONS IN ABCB4

A subset of patients with a clinical picture resembling PFIC Type 2 were found to have elevated serum levels of GGT. These patients also had a different pathological lesion than patients with either Type 1 or Type 2 PFIC. These patients had a more prominent biliary ductal hyperplasia and associated inflammation. The disease-causing gene in families with this form of PFIC (now called PFIC Type 3) was not linked to either *ATP8B1* or the ABCB11, resulting in the identification of a third gene defect causing of PFIC. The defective gene in these patients was discovered to be *ABCB4* (also called MDR3). Since the *Abcb4* gene had already been inactivated in mice, the physiological role of this transporter was well characterized prior to the discovery that naturally occurring mutations in this gene caused PFIC in humans. Mice with no ABCB4 have a marked reduction in the biliary levels of phosphatidylcholine since ABCB4 is responsible for transporting phospholipids into bile (**Figure 12**). In the absence of biliary phospholipids, the bile acids remain poorly solubilized and incite a profound inflammatory response and promote ductal hyperplasia. As expected, patients with PFIC Type 3 have elevated serum levels of GGT due to the increased levels of free bile acids in the bile. Biliary phospholipid micelles are also required for the efficient secretion of cholesterol into bile, so in the absence of MDR2 there are only trace amounts of cholesterol in the bile.

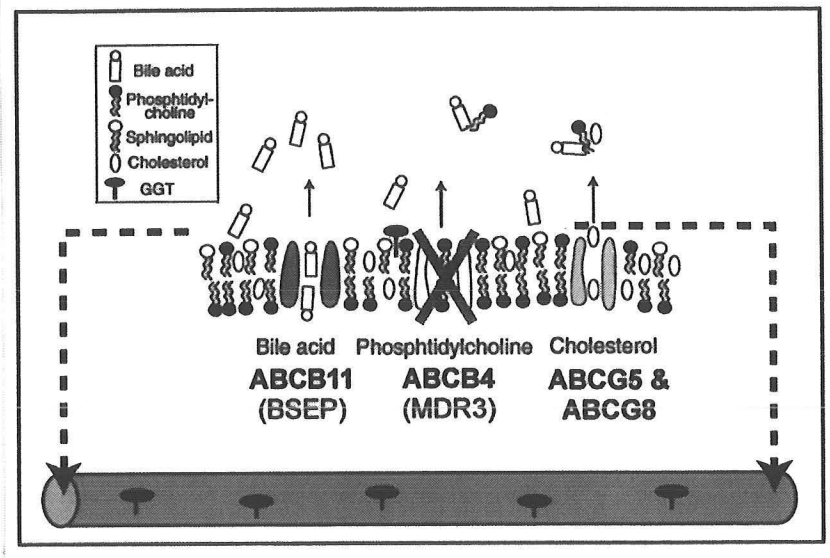


**Figure 12.** Mutations in ABCB4 reduce the phosphatidylcholine and cholesterol content of bile.

Unlike patients with extrahepatic cholestasis, patients with PFIC Type 3 do not develop hypercholesterolemia. Extrahepatic cholestasis is almost invariably associated with elevated levels of plasma cholesterol level due to the formation and circulation of the lipoprotein Lp(X). Phospholipid vesicles continue to form in the livers of patients with cholestasis, but the vesicles cannot be eliminated in the bile (**Figure 13A**). Consequently, the vesicles diffuse into the circulation, probably by traversing the tight junctions between adjacent hepatocytes. Lp(X) consists of disc-like structures containing cholesterol and phospholipids (**Figure 13B**). The particles acquire apoC's and small amounts of albumin as they circulate. The particles have a density similar to LDL so are reported as elevations in LDL-cholesterol on a routine lipid panel. Rouleau formation occurs when levels of Lp(X) are high in plasma. Since ABCB4 is required for the formation of phospholipid vesicles, Lp(X) is not present in the plasma of patients with PFIC Type 2.

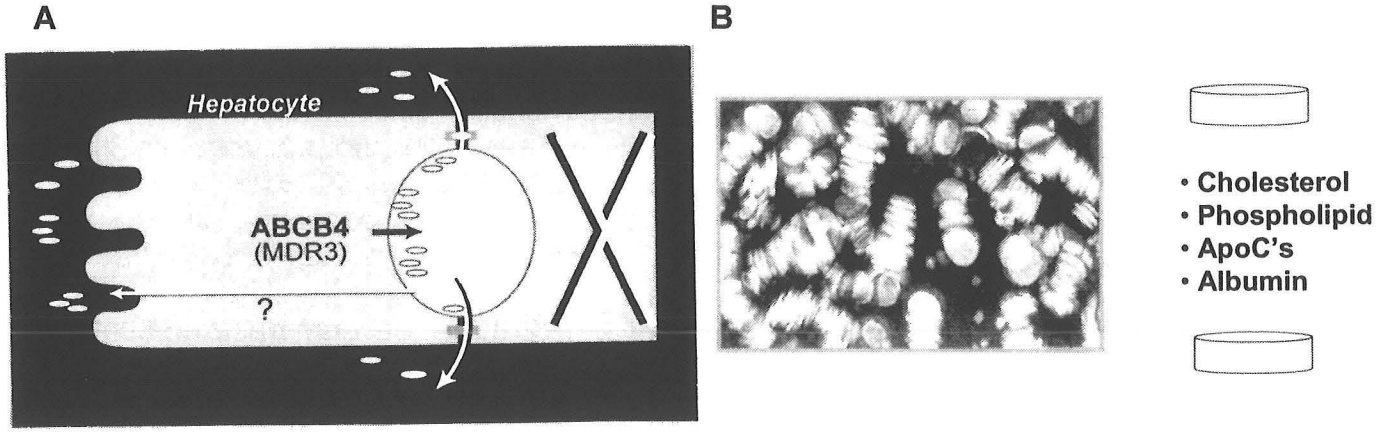


**Figure 13. (A)** Formation of Lp(X) in extrahepatic cholestasis and (**B**) Dark field picture of Lp(X).

Finally, heterozygosity for mutations in ABCB4 has been implicated in other disease processes, including cholestasis of pregnancy, gallstones and recurrent symptomatic cholestasis after gallstone removal. The most convincing of these three associations is with cholestasis of pregnancy. Mothers of patients with PFIC Type 3, who are obligate heterozygotes, frequently report the development of pruritis during their pregnancies.

In summary, the clinical features of the three forms of PFIC are compared in Figure 14.

| | Type 1 *(Bylers)* | Type 2 | Type 3 |
|---|---|---|---|
| **Gene Defect** | **FIC1** | **ABCB11** | **ABCB4** |
| **Onset** | **Neonatal** | **Neonatal** | **Childhood-adolescence** |
| **Bile salts in bile** | ↓↓ | ↓↓ | + |
| **Serum GGT** | **normal** | **normal** | ⇑ |
| **Hepatic fibrosis** | + | + | ++ |
| **Ductal proliferation** | + | + | ++ |
| **Hypercholesterolemia** | +/- | +/- | - |

**Figure 14.** Comparison of the clinical features of the three difference forms of PFIC.

## SITOSTEROLEMIA; MUTATIONS IN EITHER *ABCG5* and *ABCG8*

The final biliary ABC transporter that I will discuss is a heterodimer of two half transporters of the G family, ABCG5 and ABCG8, that plays a critical role in the secretion of cholesterol into bile. These half transporters, which are encoded by two coordinately regulated genes that reside next to each other in the genome (**Figure 15**), complex together in the endoplasmic reticulum before being transported to the apical surface of hepatocytes. Mutations in either ABCG5 or ABCG8 cause sitosterolemia, an autosomal recessive disease characterized by hypercholesterolemia, cutaneous and tendon xanthomas and premature coronary artery disease. **Patient 3** is a member of the second family identified with this disorder and remains the youngest sitosterolemic patient to have died from coronary atherosclerosis.

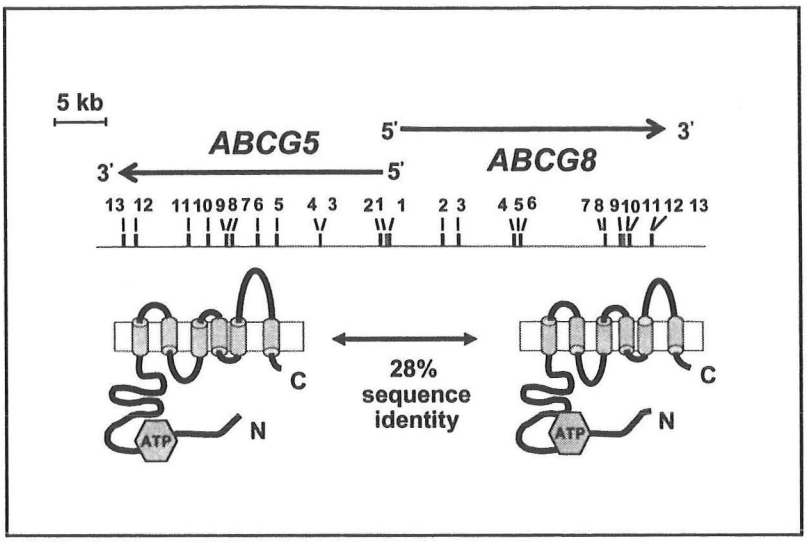


**Figure 15.** ABCG5 and ABCG8 are oppositely oriented genes that encode for two half transporters that share 28% sequence identity.

A comparison of the clinical features of sitosterolemia and homozygous familial hypercholesterolemia (FH) is provided in **Figure 16**. A major difference between these two disorders (other than their inheritance pattern) is that the plasma cholesterol level in sitosterolemia varies over a much broader range than does the cholesterol levels in FH homozygotes, which are invariably markedly elevated. Some patients with sitosterolemia have plasma cholesterol levels that are within the normal range. Unlike patients with homozygous FH, the hypercholesterolemia associated with sitosterolemia responds well to the cholesterol-lowering effects of bile acid resins or to reductions in dietary cholesterol intake.

The pathognomonic feature of sitosterolemia is a 30 to 100 fold increase in plasma levels of the plant sterol sitosterol, which is the most plentiful noncholesterol dietary sterol. Incorporation of sitosterol in the membranes of red blood cells results in a low grade hemolysis in patients with sitosterolemia.

| | *Homozygous FH* | *Sitosterolemia (Pseudohomo. FH)* |
|---|---|---|
| **Inheritance** | **Dominant** | **Recessive** |
| **Sitosterolemia (mg/dL)** | **< 1** | **15-30** |
| **Cholesterol (mg/dL)** | **650-1000** | **100-800** |
| **Xanthomas** | **++** | **++** |
| **Premature CAD** | **++** | **+** |
| **Hemolysis** | **–** | **+** |
| **Diet-responsiveness** | **–** | **+++** |

**Figure 16.** Comparison of the clinical characteristics of homozygous familial hypercholesterolemia and sitosterolemia.

Although sitosterol differs from cholesterol only by a single ethyl group in the C24 position of the side chain, the sterol is handled very differently from cholesterol by cells in the liver and in the small intestine (**Figure 17A**). Normally, <5% of dietary sitosterol and ~40% of cholesterol is delivered from the proximal small intestine to the liver. In sitosterolemia, ~15% of dietary sitosterol and ~55% of dietary cholesterol is absorbed. In normal individuals, there is a preferential secretion of sitosterol into the bile. In sitosterolemia, there is a generalized defect in the biliary excretion of neutral sterols (**Figure 17B**). The hyperabsorption of dietary sterols coupled with the decrease in biliary secretion of these sterol results in plasma level of cholesterol being much more responsive to decreases in dietary cholesterol intake. Since the major route of exodus of dietary cholesterol is via secretion into the bile and this pathway is severely impaired in sitosterolemia, the only pathway by which patients can get rid of cholesterol is to convert it to bile acids and secrete the bile acids into bile. Since treatment with bile acid binding resins results in an increase in the conversion of cholesterol into bile acids, these drugs are particularly effective cholesterol-lowering agents in sitosterolemia.

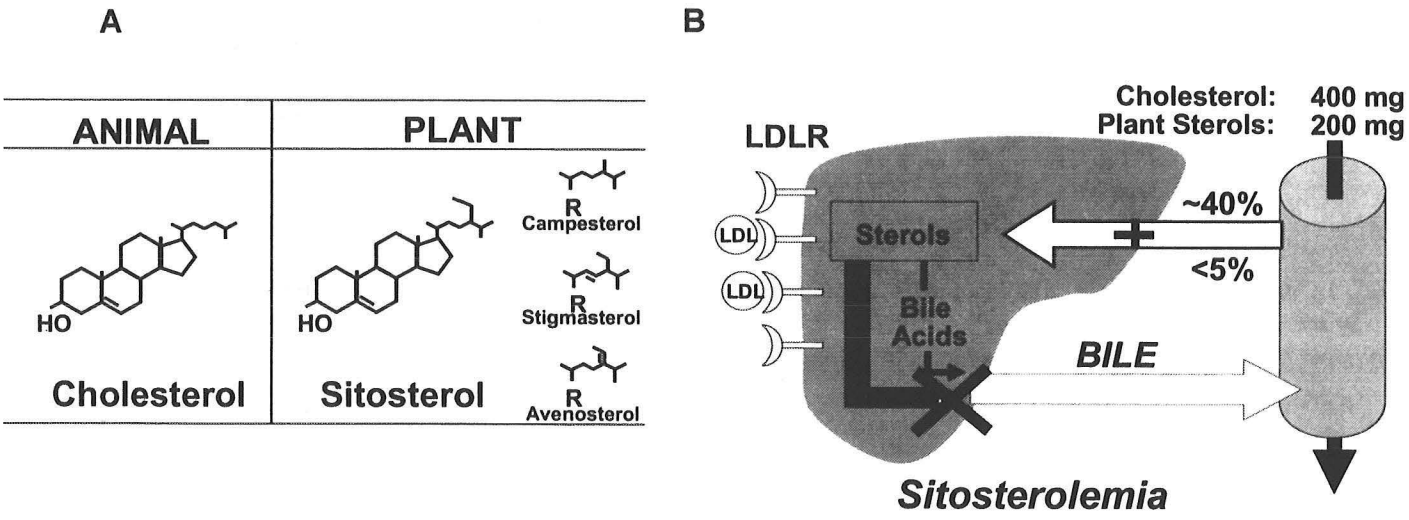


**Figure 17.** (**A**) Chemical structure of cholesterol and sitosterol. (**B**) Defective trafficking of dietary sterols in sitosterolemia.

ABCG5 and ABCG8 are both expressed almost exclusively in the enterocytes of the small intestine and in hepatocytes. Both sitosterol and cholesterol enter enterocytes by a yet-to-be-defined mechanism (**Figure 18**). Cholesterol is esterified and packaged with apolipoproteins to form chylomicrons, which are secreted into the lymphatic system. Sitosterol has a very low affinity for acetyl CoA acyltransferase-2, which is the enzyme that catalyzes the esterification of cholesterol; consequently, only small amounts of sitosterol are absorbed as part of chylomicrons. Thus, more free sitosterol than cholesterol is available in the enterocyte for export via ABCG5 and ABCG8 into the gut lumen. As a consequence, a much lower proportion of sitosterol is absorbed from the diet of normal individuals, and mutations in either ABCG5 or ABCG8 are associated with an increase in the fractional absorption of dietary sterols.

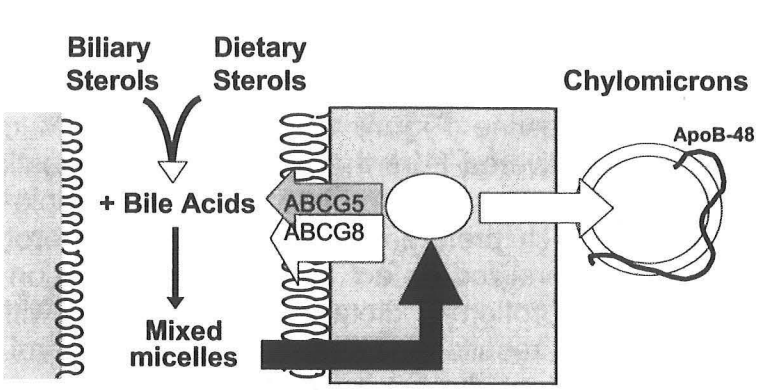


**Figure 18.** Role of ABCG5 and ABCG8 in dietary sterol absorption the duodenum and jejunum.

Patents with sitosterolemia are treated with a low-cholesterol, low-plant sterol diet. If the hypercholesterolemia persists, bile acid resins are added to their drug regimen. A new alternative treatment for sitosterolemia has recently became available with the development of the grug Ezetimibe (Zetia), which interferes with the up-take of dietary sitosterol as well as cholesterol into enterocytes.

We have developed mice expressing large amounts of ABCG5 and ABCG8 in the liver and intestine. These mice have a reduction in the fractional absorption of dietary cholesterol and have a 4-5 fold increase in cholesterol in the bile. We have also developed mice lacking both ABCG5 or ABCG8 and these mice have the opposite phenotype; the mice have increased fractional absorption of cholesterol and very low levels of cholesterol in the bile. These studies in genetically modified mice are consistent with ABCG5 and ABCG8 playing a critical role in limiting the accumulation of both plant sterols and cholesterol in the blood (**Figure 19**).

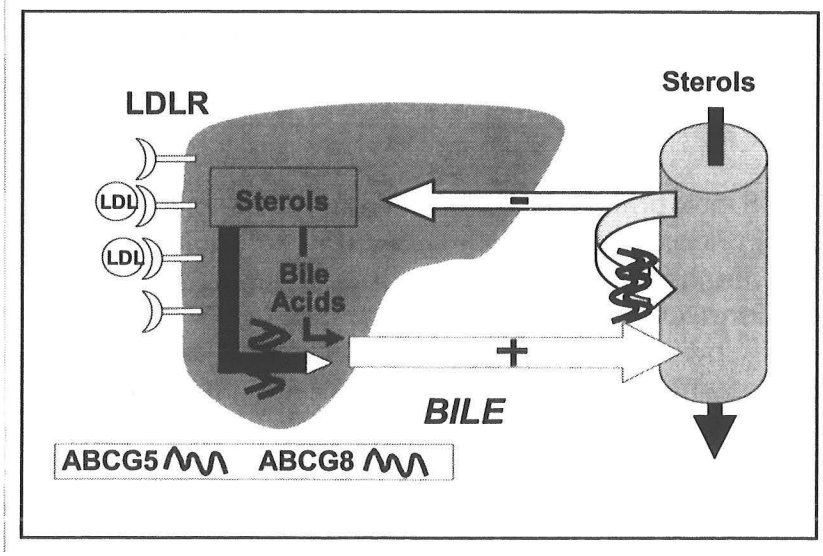


**Figure 19.** ABCG5 and ABCG8 protect against sterol accumulation by reducing the fraction of cholesterol absorbed from the diet and by promoting secretion of sterols into bile.

ABCA1, participates in the first step of this pathway, which is the transport of cholesterol from the membranes of peripheral cells to lipoproteins.

**Tangier Disease: Mutations in ABCA1**

The isolation of ABCA1 came from the identification of the genetic defect causing Tangier disease, the disease responsible for the extremely low plasma level of HDL-cholesterol and for the peripheral neuropathy in **Patient 4**. ABCA1 is a widely expressed plasma membrane protein that plays a critical role in the formation of HDL particles. ABCA1 participates in the formation of nascent HDL by mediating transport of phospholipids and cholesterol from cells to lipid-poor apoA1 (**Figure 20A**). This pathway is a major mechanism by which cholesterol is removed from lipid-laden macrophages, and in the absence of ABCA1, cholesterol esters accumulate in scavenger cells. **Patient 4**, as well as his brother (**Figure 20B**), who also has Tangier disease, had their tonsils surgically removed as young children. If the tonsils had not been removed, the disease would probably have been diagnosed earlier, since a well-known physical feature of this disease is enlarged yellow or orange tonsils, resulting from the accumulation of sterols in phagocytic cells.

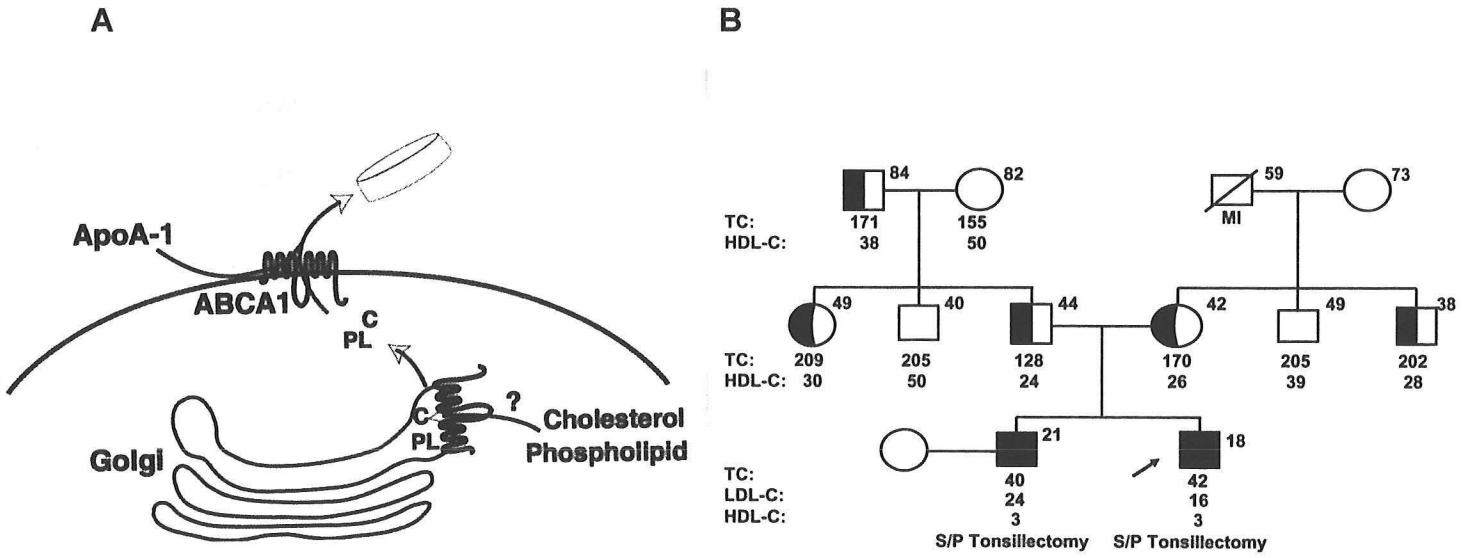


**Figure 20. (A)** Role of ABCA1 in HDL formation and (**B**) Pedigree of T.M. family.

T.M. presented with one of the most troubling and poorly understood manifestations of Tangier disease, which is the development of peripheral neuropathies. Patients with Tangier disease can develop either a syringomyelia-like syndrome or a mononeuritis multiplex pattern of neuronal involvement, as was the case in **Patient 4**. Interestingly, no patients have been identified who developed both syringomyelia and mononeuritis multiplex. The reason why patients with Tangier disease develop neurological

symptoms is not known but is presumed to be due to the accumulation of a neurotoxic substance, either within the Schwann cells or the peripheral neurons.

Patients with Tangier disease have a statistically significant increase in the incidence of coronary artery disease, as might be expected given their very low plasma levels of HDL-cholesterol, but clinically significant disease occurs much later in life and is much less pronounced than in patients with genetic forms of severe hypercholesterolemia. The very low plasma levels of LDL-cholesterol in Tangier disease protect somewhat from the atherogenic consequences of having a reduced ability to export cholesterol out of lipid-laden macrophages in these patients.

The relatives of T.M. who are heterozygous for a mutant *ABCA1* allele have significantly lower plasma levels of HDL-cholesterol (**Figure 20**), which is typical ABCA1 heterozygotes. The proportion of individuals with low plasma levels of HDL-cholesterol in the general population who have mutations in *ABCA1* is not known. Nor is it known whether the relative risk of individuals who have a low plasma HDL-cholesterol due to mutations in *ABCA*I is at any higher risk for the development of coronary atherosclerosis than individuals who have hypoalphalipoproteinemica due to another molecular defect.

## THE CRITICAL ROLE OF ABC TRANSPORTERS IN CHOLESTEROL TRANSPORT

Thus, the so-called reverse cholesterol transport pathway is bracketed by the action of two ABC transporters (**Figure 21**). ABCA1 initiates the process by transporting cholesterol from cells to HDL. Cholesterol is then delivered to the liver either directly as part of HDL or indirectly after transfer to apoB-containing lipoproteins. Once in the liver, the cholesterol is transported via ABCG5 and ABCG8 into bile and excreted. ABCA1, ABCG5 and ABCG8, as well as many of the other genes involved in the centripetal trafficking of cholesterol from peripheral tissues to the liver (e.g. ApoE, cholesterol ester transfer protein) are coordinately regulated by the nuclear hormone receptor LXR, which acts activates genes as a heterodimer with RXR.

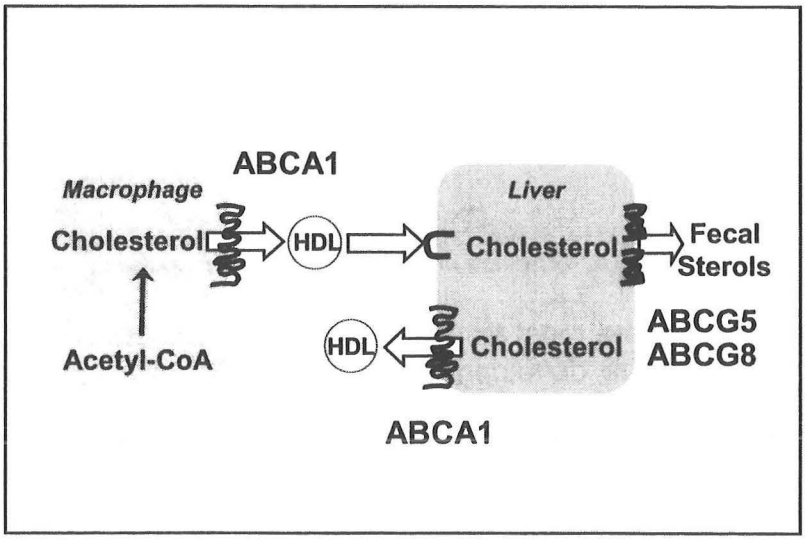


**Figure 21.** ABCA1 and ABCG5/ABCG8 in centripetal cholesterol transport.

## OTHER HUMAN DISEASES DUE TO MUTATIONS IN ABC TRANSPORTERS.

**Table 2** lists the other human diseases not mentioned previously in this review that are caused by mutations in ABC transporters. Note the wide range of organelles and tissues in which these transporters function and the wide variety of clinical manifestations resulting from defects in this family of genes.

**Table 2.** Other defects in ABC transporters that cause human disease.

| Name | Substrate | Location | Disease |
|---|---|---|---|
| ABCA4 | Vitamin A derivatives | *Discs: rods and cones* | Stargardt's macular dystrophy |
| ABCB2/3 (TAP1/2) | Peptides | *ER* | Immunodeficiency |
| ABCB7 | Iron (?) | *Mitochondria* | X-linked sideroblastic anemia and ataxia |
| ABCC6 | ? | ? | Pseudoxanthoma elasticum |
| ABCC8 (SUR1) | (K+) | *Cell membrane* | Persistent hyperinsulinemic hypoglycemia |
| ABCD2 (ALDR) | Very long chain fatty acids | *Peroxisomes* | Adrenoleukodystrophy |

The extent of our knowledge regarding most human ABC transporters is extremely limited. It can be anticipated that defects in the 32 yet-to-be-characterized members of the ABC transporter family will contribute to other human diseases. Haploinsufficiency of some ATP transporters (e.g., *ABCB4* and *ABCA1*) has been convincingly shown to be associated with specific clinical phenotypes. Numerous claims have been made for associations between sequence polymorphisms in ABC transporter genes and disease susceptibility phenotypes, although to date none of these reports are particularly compelling. Additional studies to assess these relationships in other populations will be required to adequately assess how common sequence variations in these genes contribute significantly to disease or to disease susceptibility.

Better understanding of the biochemistry and regulation of ABC transporters will undoubtedly lead to the development of new therapeutic agents altering the expression and function of selected members of this ancient, large and diverse family of membrane proteins for the prevention and treatment of disease.

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## X. Other ABC Transporter Diseases

### A. ABCA4 – Stargardt Macular Dystrophy

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### C. ABCB2 and ABCB3 (TAP1 and TAP2) – Immunodeficiency

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## D. ABCB7– X-linked Siderablastic Anemia and Cerebellar Ataxia

Allikmets R, Raskind WH, Hutchinson A, Schueck ND, Dean M, Koeller DM. 1999. Mutation of a putative mitochondrial iron transporter gene (ABC7) in X-linked sideroblastic anemia and ataxia (XLSA/A). Hum Mol Genet 8:743-749.

## E. ABCC8 – Persistent Hyperinsulinemic Hypoglycemia of Infancy

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