# Association of ABCG5 and ABCG8 Transporters with Sitosterolemia

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**Abstract**

Sitosterolemia is a rare genetic lipid disorder, mainly characterized by the accumulation of dietary xenosterols in plasma and tissues. It is caused by inactivating mutations in either ABCG5 or ABCG8 subunits, a subfamily-G ATP-binding cassette (ABCG) transporters. ABCG5/G8 encodes a pair of ABC half transporters that form a heterodimer (G5G8). This heterodimeric ATP-binding cassette (ABC) sterol transporter, ABCG5/G8, is responsible for the hepatobiliary and transintestinal secretion of cholesterol and dietary plant sterols to the surface of hepatocytes and enterocytes, promoting the secretion of cholesterol and xenosterols into the bile and the intestinal lumen. In this way, ABCG5/G8 function in the reverse cholesterol transport pathway and mediate the efflux of cholesterol and xenosterols to high-density lipoprotein and bile salt micelles, respectively. Here, we review the biological characteristics and function of ABCG5/G8, and how the mutations of ABCG5/G8 can cause sitosterolemia, a loss-of-function disorder characterized by plant sterol accumulation and premature atherosclerosis, among other features.

**Keywords**

Sitosterolemia · ABC transporter · Sterol · ABCG5 · ABCG8

## Abbreviations

| | |
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| ABC | ATP-binding cassette |
| ABCB1 | ATP-binding cassette subfamily B1 |
| ABCG5 | ATP-binding cassette subfamily G5 |
| ABCG8 | ATP-binding cassette subfamily G8 |
| ATP | Adenosine triphosphate |
| BSEP | Bile salt exporter protein |
| KO mice | Knock out mice |
| MDR | Multidrug resistance |
| NBD | Nucleotide-binding domain |
| P-gp | P-glycoprotein |
| TMD | Transmembrane domain |

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## 2.1 Introduction

Sitosterolemia is a rare genetic recessive disease in which an individual is unable to excrete xenosterols.

In 1974, Bhattacharyya and Conner first described a new syndrome, a lipid storage disorder, in two sisters who presented with tendon and tuberous xanthomas with normal plasma cholesterol levels and elevated plasma levels of plant sterols (phytosterols), such as sitosterol, campesterol, and stigmasterol. The disease was named—sitosterolemia—after the most abundant dietary xenosterol, sitosterol. In fact, some think that perhaps a more appropriate name for the disease would be xenosterolemia (Maguire et al. 2001). Anyway, this discovery initiated fundamental studies on how dietary sterols traffic and are eliminated by the body (Williams et al. 2021).

It took another 26 years before the sitosterolemia locus was mapped to chromosome 2p21 and the discovery that the genetic locus whose dysfunction leads to sitosterolemia encodes two genes, abcg5 and abcg8, whose proteins (ABCG5 and ABCG8) function as obligate heterodimers (Berge et al. 2000; Lu et al. 2001).

ABCG5 and ABCG8 are expressed only in hepatocytes, gallbladder epithelium, and enterocytes and are responsible for excretion of sterols, with xenosterols preferred over cholesterol (Patel et al. 2018). In fact, the naming of the disease has led to a bias: a vast range of other sterols, not just sitosterol, are accumulating in the body (Gregg et al. 1986). According to some researchers, a better name should be xenosterolemia.

Several missense mutations on either genes are the causative gene defect that lead to loss of function of the ABCG5/G8 transporter, which is associated with lipid phenotypes (Miettinen 1980; Salen et al. 1985; Berge et al. 2000; Lee et al. 2001; Lu et al. 2001; Brown and Yu 2010; Williams et al. 2021). Subsequent case reports established the recessive genetics of the disease and greatly expanded its potential clinical presentation.

## 2.2 Sterol Transport

All living cells depend on their ability to transfer molecules such as nutrients, hormones, metabolites, and across their membranes. Cell membrane is the natural barrier for intracellular constituents and the checkpoint of molecules and signals from the extracellular milieu. Lipids are the primary component of mammalian cell membranes, with cholesterol being a key component. Cholesterol accounts for ~40–50% of the total lipid content in the plasma membrane (Steck and Lange 2018). Cholesterol serves as the precursor molecule for steroid hormones that modulate gene regulation, for bile acids that enable for nutrient absorption, and for vitamin D which are vital for body health (Rezaei et al. 2023).

Translocation of cholesterol molecules on biological membranes plays an essential role in maintaining cholesterol homeostasis. However, relatively little is known about the mechanisms that control the sterol shuttling across lipid-bilayer membranes. Anyway, lipid-transport membrane proteins have been shown to be essential for the translocation of sterols and phospholipids to maintain lipid homeostasis, cellular functions, and the structural integrity of mosaic lipid bilayers (Abumrad et al. 2000; Sharom 2011; López-Marqués et al. 2015).

Cholesterol can be obtained by de novo cell biosynthesis or via dietary uptake in the intestine. In normal diets, the levels of cholesterol and non-cholesterol sterols from plants (xenosterols) or other dietary sources are usually equal. However, 50–60% of dietary cholesterol is absorbed, while xenosterols exhibit poor bioavailability, with <5% absorption (Salen et al. 1970). When more plant sterols are ingested, they compete with the bulk cholesterol for solubilization, thereby reducing dietary absorption of cholesterol and lowering plasma cholesterol.

Elimination of excess cholesterol is vital for life. Abnormal elevations in plasma cholesterol contribute to hyperlipidemia, a critical factor leading to cardiovascular diseases and other metabolic disorders (Salen et al. 1970). However, not all imported cholesterol is metabolized in the

cells. In fact, few cells have this capacity. Therefore, elimination of the excess amount of cholesterol has to be cleared from cells and tissues via two metabolic pathways that are essential to maintain homeostasis: reverse cholesterol transport through sterol acceptors in the circulation or direct cholesterol excretion through biliary and intestinal secretion (Vrins et al. 2012; Ouimet et al. 2019). Xenosterols have efficient biliary elimination (Salen et al. 1970).

In more advanced life forms, functions such as nutrient intake and the exchange of compounds between cellular organelles or tissues often take place against concentration gradients across cellular membranes (Schumacher and Benndorf 2017). It is therefore not surprising that in simple life forms like bacteria, almost 10% of the entire genome is dedicated to proteins that are involved in transport processes in the form of membrane-bound or soluble proteins (Blattner et al. 1997). Transport processes against chemical gradients always require free energy which is derived from either by simultaneous use of an opposing electrochemical potential difference (secondary active transport) or a coupled enzymatic reaction exploiting the chemical energy of adenosine triphosphate (ATP) hydrolysis (primary active transport) (Blattner et al. 1997; Schumacher and Benndorf 2017). This ATP-driven transport, which act through the activity of energy-dependent unidirectional, membrane-bound, compound-efflux transporter proteins, comprise a large superfamily, the ABC (ATP-binding cassette) transporters.

## 2.3 ABC Transporters

The ABC transporter superfamily comprises one of the largest families of evolutionarily conserved membrane proteins and is ubiquitously expressed in all domains of life, from nearly all prokaryotes to virtually all types of eukaryotic cells (Higgins 1992; Dean et al. 2001a, b; Dean and Annilo 2005; Locher 2016; Bilsing et al. 2023). ABC transporters are most abundantly expressed in organs with high metabolic rates and in endothelial cells that isolate organs like the blood–brain barrier and blood–testis barrier (Schumacher and Benndorf 2017).

These transporters are involved in a broad range of cellular processes, therein actively transporting a wide range of different substrates across the plasma membrane (Schumacher and Benndorf 2017). ABC transporters use the energy from ATP hydrolysis to drive the passage or flipping of various moieties across the bilayer membrane, from small inorganic and organic molecules to larger organic compounds (Schumacher and Benndorf 2017), including both hydrophilic and hydrophobic molecules such as sugars, peptides, drugs, phospholipids, and sterols (Dean and Allikmets 1995; Linton and Higgins 1998; Dean et al. 2001a, b; Hwang et al. 2016; Plummer et al. 2021).

ABC transporters are divided into three subclasses, two groups of importers and one group of exporters, according to their functional and architectural characteristics (Schumacher and Benndorf 2017). ABC importers are predominantly found in prokaryotes where they manage the nutrient and ion intake (Ferreira and de S-á-Nogueira 2010; Gisin et al. 2010); they only sparsely occur in eukaryotes. The vast majority of ABC transporters expressed in eukaryotes are ABC exporters, promoting functions such as secreting dietary substances and metabolites or even transport signaling molecules (Schumacher and Benndorf 2017). Also, ABC transporters are major sterol exporters responsible for both cholesterol efflux from peripheral cells and the elimination of excess cholesterol and dietary sterols such as sitosterols (Borst and Elferink 2002; Xavier et al. 2019).

There are currently 49 different genes known to encode ABC transporters in humans, which categorize subfamilies of ABC transporter proteins based on sequence similarity, sequence divergence and structural arrangement (Plummer et al. 2021; Huang and Ecker 2023). Since several of those genes are alternatively spliced during transcription, each of those 49 genes not only encode one single protein, but instead often a multiple of different ABC protein variants (Dean et al. 2001a, b). The subfamilies are named ABCA–ABCG. Five distinct families (A, B,

C, D, and G) display a wide array of substrate specificities and functionalities (Alam and Locher 2023). Of all ABC transporters that have been described so far, the three members ABCB1, ABCC1, and ABCG2 are less organ specific (Zhang et al. 2015).

The first human ABC transporter described, P-glycoprotein (ABCB1) (170 kDa) was discovered in 1976 by Juliano and Ling in Chinese hamster ovary (CHO) cells, selected for resistance to colchicine. These cells displayed pleiotropic cross-resistance to a wide range of amphiphilic drugs. Because the glycoprotein altered the membrane permeability (P), it was called P-glycoprotein (P-gp, encoded by the MDR-1/abcb1 gene) (Huang and Ecker 2023). ABCB1 is the first mammalian member of the large family of ABC transporters present in prokaryote (Davidson et al. 2008) and eukaryotes, from plants (Theodoulou 2000) to humans (Gottesman and Ambudkar 2001).

The division of ABC transportes in subfamilies is based mainly on similarity in gene structure, e.g., half vs. full transporters, and on sequence homology in the nucleotide-binding domains (NBDs) and transmembrane domains (TMD) (Schumacher and Benndorf 2017).

ABC transporters are organized as two symmetric halves that are expressed either (*a*) as separate subunits (half-transporters) that assemble as homodimers or heterodimers or (*b*) as monomers containing two nonidentical halves within a single polypeptide (full transporter) (Alam and Locher 2023). Each half comprises, at minimum, a NBD that is responsible for ATP binding and hydrolysis and a TMD that facilitates substrate export (away from the NBDs) or import (toward the NBDs) (Alam and Locher 2023). The human ABC transporters can be either full or half-transporters. In principle, full ABC transporter transcripts, such as members in the A- and C-subfamilies, comprise four domains within one polypeptide chain, namely 2 TMDs embedded in the lipid bilayer, and 2 NBDs facing the cytoplasmatic space (Plummer et al. 2021; Huang and Ecker 2023). The motifs are arranged as N-TMD-NBD-TMD-NBD-C, whereas the half ones have only one TMD and one NBD (Plummer et al. 2021). Hence, the half-transporters should form homodimers or heterodimers to perform their function (Huang and Ecker 2023). Among ABC transporter families, NBDs exhibit high sequence homology, with several canonical motifs. The transport function is generally believed to be driven by the NBD dimerization, in which ATP is bound and hydrolyzed. TMDs are structurally highly diverse, suggesting distinctive transport mechanisms for individual transporters (Ford and Beis 2019).

## 2.4 Main Diseases Related to ABC Transporters

Active in nearly all cells and tissues, ABC transporters play vital physiological roles ranging from lipid homeostasis to transport of diverse endogenous and exogenous compounds (Alam and Locher 2023). Several diseases result directly from dysfunction of these transporters, making them important targets for therapeutic intervention. Therefore, human ABC transporters hold tremendous biomedical and pharmacological relevance (Dean et al. 2001a, b; Borst and Elferink 2002; Leonard et al. 2003).

A major obstacle in cancer treatment is the development of cancer resistance to several structurally dissimilar cytotoxic substances (Huang and Ecker 2023). This phenomenon is termed as multidrug resistance (MDR), which renders the cancer cells ineffective in accumulating drugs, preventing their death. ABCB1, ABCC1, and ABCG2 were frequently observed with enhanced overexpression in multiple cancer types (Zhang et al. 2015). In fact, ABCB1 transporter is the most studied ABC regarding chemotherapy against cancer (Hwang et al. 2016; Seelig and Li-Blatter 2023). Subsequent to the discovery of P-gp, studies of cancer cells revealed other phenotypes, which showed multidrug resistance related characteristics. These multidrug resistance related proteins (MRPs) were later classified as the ABCC subfamily (Cole et al. 1992). Simultaneously, a novel half transporter member of the ABC superfamily was identified from a resistant

breast cancer cell line (Doyle et al. 1998), hence named as breast cancer resistance protein (BCRP), encoded by the ABCG2 gene. Additionally, other members of ABC transporter were reported to export at least one anticancer agent. For instance, ABCA2, ABCC2, ABCC3, ABCC4, ABCC5, ABCC6, and ABCC11 (Hwang et al. 2016). Others, such as ABCB11, also known as Spgp (sister of P-glycoprotein) or BSEP (bile salt exporter protein), which is predominantly expressed in liver, has the capacity to confer resistance to cytotoxic substrates like taxol and vinblastine (Childs et al. 1995, 1998).

With at least 20 human ABC transporters being related to the transport of lipids or lipid-like compounds, it is not surprising that some of these transporters have been linked to the pathogenesis of atherosclerotic vascular diseases (Schumacher and Benndorf 2017). Moreover, ABC transporters have also been associated with vascular endothelial homeostasis and blood pressure regulation, as well as platelet production and aggregation (Schumacher and Benndorf 2017).

Dysfunction of ABCA1 can lead to Tangier's disease (Alam and Locher 2023). ABCA2 dysfunction has been associated with intellectual and developmental deficiency, and also to amyloid homeostasis, thereby pointing to a potential role in Alzheimer's disease (Alam and Locher 2023). Genetic variations in the ABCA3 gene, involved in phospholipid transport from the cytoplasm to the lumen of lamellar bodies, can cause pulmonary surfactant metabolism dysfunction 3, a severe respiratory disorder (Alam and Locher 2023).

## 2.5 ABCG5/G8 Transporter

ATP-binding cassette subfamily G (ABCG) sterol transporters maintain the homeostasis of endogenous and exogenous sterol. A substantial part of exogenous sterols are undigestible phytosterols, which can lead to complications when accumulated. ABCG5/G8 is the main functioning protein to remove ingested plant sterols providing protection from their toxic effects, although the structural features behind substrate binding in ABCG5/G8 remain relatively poorly resolved.

### 2.5.1 ABCG5/G8 Transporter Genes

The ABCG subfamily comprises five genes encoding half-transporters. Both of the two genes *abcg5 and abcg8* are located on chromosome 2p21, adjacent to each other in a head-to-head fashion, on opposite strands, and are separated by only 374 base pairs (Berge et al. 2000).

Due to their proximity and opposite orientation, these two genes are regulated by a bidirectional intergenic promotor, which contains binding sites for hepatocyte nuclear factor 4α, GATA 4/6 (Sumi et al. 2007), and a liver receptor homolog 1 (Freeman et al. 2004). In addition, agonists for either liver X receptor or farnesoid X receptor regulate ABCG5/G8 mRNA levels (Repa et al. 2002).

Each ABC transporter (ABCG5/G8) comprises two nucleotide-binding composite sites, where the Walker A motif of one NBD is paired with the ABC signature motif of the other NBD. Therefore, one of the ATP-binding sites presents a degenerate motif, while the other presents a conserved motif, which is the only one able to support ATP hydrolysis.

### 2.5.2 Characteristics of the ABCG5/G8 Transporter Structure

The members of the G-subfamily are half transporters with only one NBD and one TMD. The ABCG transporters are also characteristic. They are the only human ABC transporters with their inverted domain topology that contain an N-terminal NBD followed by a C-terminal TMD (Alam and Locher 2023).

G subfamily members must form homo- or heterodimers for functionality (Dean et al.

2001a, b). Although two transportes, ABCG1 and ABCG4, have been proposed to also function as heterodimers, most studies support the notion that both are homodimers (Cserepes et al. 2004; Hegyi and Homolya 2016). In contrast, ABCG5 and ABCG8 form obligate heterodimers (Lee et al. 2001; Wang et al. 2006; Brown and Yu 2010).

Each gene ABCG5 and ABCG8 encodes a "half-transporter" protein that is nonfunctional in the monomeric state (Brown and Yu 2010). However, assembly of an ABCG5/G8 heterodimer, driven by the adipocyte-derived hormone leptin, leads to the formation of the fully functional transporter ABCG5/G8 (Brown and Yu 2010).

Structural information of the ABCG5/G8 heterodimer was revealed by X-ray crystallography, establishing a new molecular framework toward a mechanistic understanding of ABC sterol transporters (Graf et al. 2002, 2003). This structure contained an asymmetric unit with two heterodimers that interact through their TMDs with NBDs at opposite sides of the membrane. Without bound nucleotide or lipid substrate inside the transporter, the ABCG5/G8 structure exhibits an inward-facing conformation (Plummer et al. 2021). Three helices from both ABCG5 and ABCG8, form a three-helix bundle, which bridges the TMD and NBD.

In addition, a sterol-binding site was postulated at the membrane-transporter interface based on the crystal structure of ABCG5/G8 (Farhat et al. 2022), solving the crystal structure of ABCG5/G8 in complex with cholesterol. The structure shows that an orthogonal cholesterol molecule fitting horizontally in front of A540, a conserved ABCG5 residue at this orthogonal sterol-binding site.

### 2.5.3 Observations on ABCG5/G8 Mutations

Mutations present in some sitosterolemia patients impair heterodimer trafficking (Graf et al. 2004), suggesting that these mutations disrupt ABCG5/G8 cellular localization rather than reducing ABCG5/G8 transport activity (Plummer et al. 2021). However, this seems not to be a rule. The Ala540Phe mutant in ABCG5, a residue that putatively binds cholesterol, resulted in reduced biliary cholesterol transport (Lee et al. 2016).

On the triple-helical bundle or the transmembrane polar relay, several residues have been shown to bear disease-causing missense mutations from patients with sitosterolemia or other lipid metabolic disorders. Notably, several disease-causing mutations are clustered in the membrane-spanning region or at the NBD–TMD interface. This suggests the roles of these structural motifs in regulating the ABCG5/G8 function.

ABCG5/G8 has also been studied through overexpression of either wild-type or mutant ABCG5/G8 in *abcg5*/*abcg8* KO mice (Plummer et al. 2021). Mice lacking *abcg5*, *abcg8*, or both show increased plasma levels of sterols and reduction of sterol secretion into the bile. In contrast, overexpression of ABCG5/G8 showed the opposite effect, with reduced sterol absorption and increased biliary sterol levels (Yu et al. 2002a, b). These effects are dependent on ABCG5/G8 expression levels. Moreover, using *mdr2* KO mice, a lack of secretion was observed, suggesting that the function of ABCG5/G8 is dependent on a functional ABCB4 transporter (Plummer et al. 2021).

Homodimers of either ABCG5 or ABCG8 are likely nonfunctional, evident from low ATPase activity relative to the native heterodimer (Wang et al. 2006), impaired trafficking (Graf et al. 2003), and low biliary cholesterol transfer in KO mice (Graf et al. 2003; Zhang et al. 2006). Surprisingly, *abcg8* KO in mice results in continued expression of ABCG5 at the apical membrane and secretion of sitosterol into the bile, suggesting that the ABCG5 homodimer may be functional or that an alternative pathway of sterol secretion into the bile exists (Klett et al. 2004).

### 2.5.4 Function and Regulation of ABCG5/G8

Physiologically, ABCG5/G8 plays an essential role in controlling sterol homeostasis in our bodies.

The ABCG5/G8 transporter is predominantly expressed on the apical surface of hepatocytes along the canalicular membranes of the bile ducts in the liver and on the brush-border membranes of enterocytes in the small intestines (Patel et al. 1998; Berge et al. 2000; Graf et al. 2002, 2003; Yu et al. 2002a, b; Hirata et al. 2009; Brown and Yu 2010; Zein et al. 2019).

ABCG5/G8 is the primary transporter and sterol-efflux pump that selectively exports excess cholesterol, noncholesterol sterols, and dietary plant sterols from hepatocytes into bile canaliculi and in the intestine back to the intestinal lumen (Lee et al. 2001, Wang et al. 2006; Rezaei et al. 2023), by translocating sterols within the plasma membrane and in endosomes (Sano et al. 2014; Pandzic et al. 2017; Xavier et al. 2020). Expression level of ABCG5 and ABCG8 is further modulated by bile acid levels in both the liver (Dean et al. 2001a, b) and intestine (Kamisako et al. 2007).

One proposed mechanistic mode for the sterol transfer to bile acid suggests that ABCG5/G8 translocates sterol across the bilayer membrane, functioning as a liftase (Small 2003; Lee et al. 2016). However, there is little experimental evidence to support this hypothesis.

Another model suggests that ABCG5/G8 functions only as a sterol floppase, increasing the sterol concentration in the outer leaflet and allowing for extraction of sterol by the bile salt micelle (Kosters et al. 2006). This hypothesis is supported by the observation that the function of ABCG5/G8 depends on ABCB4, a phosphatidylcholine transporter, and sterol is potentially extracted from the outer leaflet via sterol-phospholipid vesicles (Crawford et al. 1997). Multidrug-resistant protein 3 (MDR3), also known as ABCB4, is a phospholipid translocase embedded in the canalicular membrane. Although it actively flips inner leaflet phospholipids and sterols to the outer leaflet, there is evidence supporting its subsequent role in substrate secretion toward the bile. Furthermore, MDR3 is found to be essential for the proper function of ABCG5/G8, which believed to be caused by its involvement in the formation of mixed micelles.

Finally, ABCG5/G8 may directly transfer sterols to bile salt micelles following sterol flipping across the plasma membrane (Plummer et al. 2021). Spontaneous cholesterol flipping between leaflets is a common event in the plasma membrane, capable of undertaking intermediate horizontal orientation within the membrane core. It is thus possible that ABCG5/G8 catalyzes cholesterol flipping from inner to outer leaflets, peripherally through its exterior surface.

In addition, ABCG5/G8 exhibits cholesterol efflux activity in the presence of bile acid micelles (Vrins et al. 2007). Mixed micelles are very charged, small aggregates of phospholipids, cholesterol and bile salts, and these micelles form the basis of currently known ABCG5/G8 acceptor particles. Accordingly, it has been suggested that mixed micelles of bile acids dock onto ABCG5/G8 to induce conformational changes and stimulate ATPase for the transport of cholesterol (Johnson et al. 2010). Acceptor particles will then intake the exported lipids from ABCG5/G8.

Differently from homodimeric ABCG1, the heterodimeric ABCG5/G8 carries out selective sterol excretion. ABCG5/G8 is unique in its capability of preferential efflux for dietary plant sterols over cholesterol, preventing the abnormal accumulation of plant sterols in human body (Berge et al. 2000; Lee et al. 2001; Graf et al. 2003; Yang et al. 2004; Schumacher and Benndorf 2017). Intestinal ABCG5/G8 acts as a first-pass gate, pumping xenosterols back into the intestinal lumen, whereas liver ABCG5/G8 pumps xenosterols into the bile (Patel et al. 2018). There are numerous reports that ABCG5/G8 exhibits a preference for the transport of noncholesterol sterols over cholesterol (Yu et al. 2004; Plummer et al. 2021). The preference for noncholesterol sterols in either intestine- or liver-localized *abcg5/abcg8* KO mice was slightly reduced compared to whole-animal *abcg5/abcg8* KO models, suggesting that expression of ABCG5/G8 in both liver and intestine is required for full selectivity (Wang et al. 2015). The mechanisms that govern such substrate selectivity within this protein subfamily, however, remain elusive.

## 2.6 Sitosterolemia

This very rare autosomal recessive disorder is characterized by drastically elevated plasma and tissue levels of plant sterols (Schumacher and Benndorf 2017). As stated before, missense mutations in both genes, ABCG5 and ABCG8, are related to sitosterolemia (Berge et al. 2000; Hubacek et al. 2001; Lu et al. 2001). ABCG5/G8 exhibits substrate specificity, particularly for plant sterols (e.g., sitosterol and campesterol) over cholesterol (Matsumura et al. 2007; Michaki et al. 2012). In fact, sitosterolemia patients exhibit increased absorption of numerous plant xenosterols, not just sitosterol (Morita and Imanaka 2012).

The clinical presentation may include elevated low-density lipoprotein (LDL) cholesterol and tuberous tendon xanthomas. Hypercholesterolemia-induced premature atherosclerosis was particularly observed to affect male patients at a young age, leading to CVD-like angina pectoris, myocardial infarctions, and sudden cardiac death (Kwiterovich et al. 1981; Brown and Yu 2010). Hematologic manifestations (hemolytic anemia, macrothrombocytopenia, splenomegaly, and bleeding disorders) can result from the accumulation of plant sterols in platelet membranes, producing hypertrophic and hyperplasic dysfunctional platelets. Adrenal dysfunction, arthritis, elevated liver function tests, and cirrhosis (in rare cases hepatic failure) are other features (Shulman et al. 1976; Miettinen 1980; Kwiterovich et al. 1981; Lin et al. 1983; Salen et al. 1985; Beaty et al. 1986; Nguyen et al. 1990; Bhattacharyya et al. 1991; Rees et al. 2005; Mushtaq et al. 2007; Wang et al. 2014; Bazerbachi et al. 2016). Clinical studies in individuals with sitosterolemia revealed reductions in cholesterol synthesis, biliary cholesterol secretion, plasma clearance, and fecal elimination of neutral sterols (Salen et al. 1989; Nguyen et al. 1990; Bhattacharyya et al. 1991; Cobb et al. 1997). Due to similar clinical presentations, sitosterolemia has been, in many cases, inaccurately diagnosed as familial hypercholesterolemia or idiopathic liver cirrhosis.

The ABCG5/G8 loss of function in animal models, as well as in humans, shows that accumulation of xenosterols leads to dramatic phenotypes, such as macrothrombocytopenia and platelet dysfunction, liver disease, appearance of gallstones, elevation of low-density lipoprotein cholesterol levels and cholesterol accumulation with xanthoma formation and atherosclerosis (Grass et al. 1995; Yu et al. 2002a, b, 2005; Kajinami et al. 2004; Acalovschi et al. 2006; Buch et al. 2007; Wang et al. 2007; Chen et al. 2008; Kuo et al. 2008; Katsika et al. 2010; Patel and Salen 2010; Srivastava et al. 2010; Renner et al. 2013; Von Kampen et al. 2013; Jiang et al. 2014). In mouse models (but not humans), infertility, immune dysfunction, and cardiomyopathy have been reported (McDaniel et al. 2013; Solca et al. 2013; Wilson et al. 2013). This begs the question whether a lifetime of low-level exposure to dietary bioactive xenosterols, whose levels of entry and retention may be altered by polymorphisms in ABCG5 and ABCG8, may have biological consequences.

Despite the absorptive phenotype and metabolism of phytosterols to bile acids, the clinical management of these patients with low sterol diets and bile acid-binding resins resulted in modest and inconsistent reductions in plasma phytosterols (Lin et al. 1983; Nguyen et al. 1991). Treatment of sitosterolemia involves a diet that is low in plant lipids and the administration of the drug ezetimibe, which acts as an inhibitor of Niemann-Pick C1-like protein 1 (NPC1L1), that functions in intestinal sterol absorption Morita et al. 2007).

In conclusion, although sitosterolemia is a rare genetic disease, this disorder should be considered in the differential diagnosis, due to its early clinical manifestations and relatively difficult treatment.

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