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INVITED REVIEW

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Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols

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Abstract ABCG5 and ABCG8 are two ATP-binding cassette half-transporters that belong to the G family members. They were identified as proteins that are mutated in a rare human disorder, sitosterolemia, and their identification led to the completion of the physiological pathways by which dietary cholesterol, as well as noncholesterol sterols, traffics in the mammalian body. These proteins are likely to function as heterodimers, and current evidence suggests that these proteins are responsible for the majority of sterol secretions into bile, thus may define the long sought-after biliary sterol transporters. This review will focus on some of the backgrounds of this physiology, the genetics and regulation of these genes, as well as our current understanding of their functions. This review will also highlight the current limitations in our knowledge gap.

Keywords Cholesterol · Sitosterol · Sterols · Intestinal transport · Biliary secretion · Atherosclerosis · Diet

Historical

The history of how mammals can distinguish between dietary noncholesterol sterols and cholesterol is intertwined with the history of cholesterol itself; whether cholesterol could be synthesized by the body or was wholly absorbed from the diet, whether the body degraded cholesterol, what determines its absorption and biliary secretion, and whether cholesterol was involved in the process of atherosclerosis are all questions that have involved or continue to involve noncholesterol sterols [53, 54]. Cows, for example, eat only foods that contain plant sterols, and yet their bodies contain cholesterol but not plant sterols. Investigations of such observations led to the discovery that plant sterols were excluded by the body, but could compete with bulk cholesterol for entry into the micelles formed during digestion, thus preventing dietary absorption of cholesterol [6, 14, 21, 34, 50, 55, 59]. Into this milieu of understanding, two key landmark observations led to a revolution in our current knowledge of how whole-body sterol balance may be achieved. The context of the first landmark event was a knowledge that plant sterols were poorly absorbed relative to cholesterol [14], and that very high plasma cholesterol levels in humans were caused by a dominant genetic defect in the low-density lipoprotein receptor (and was associated with patients who developed accumulations of sterols in their tendons, called xanthomas). In a classic paper that should epitomize clinical investigation, Bhattacharyya and Connor described a new disease, named β-sitosterolemia, after identification of two sisters who had tendon xanthomas but did not have elevated plasma cholesterol, and who had very high amounts of plasma plant sterols, the major species of which was sitosterol, hence the name [3]. The ∝ conformer of sitosterol is not normally present in nature, and thus it is probably not necessary to use the term 'β' preceding sitosterol. In one single publication, these authors showed that a likely single gene defect led to the disruption of the intestinal processes that keeps noncholesterol sterols out, led to tendon xanthomas, an ominous sign of systemic atherosclerosis, and was key to understanding how dietary noncholesterol sterols were (not) absorbed. The second key observation was the identification of Niemann-Pick C1 Like 1 (NPC1L1), the 'cholesterol receptor', as the key molecule in determining entry of sterols into enterocytes [1, 31]. The latter will not be discussed in this review, although an overview is provided for better physiological context.

Once the clinical description of sitosterolemia had been reported (clinical and physiological features reviewed in

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[4]), the next breakthrough was the localization of the sitosterolemia locus, *STSL*, to chromosome 2p21 to a very narrow region which led to the identification of the genetic defect in sitosterolemia [43]. The surprise was that the *STSL* locus comprised of not one but two highly homologous genes; complete mutations in either of the two genes are necessary to cause sitosterolemia [2, 37, 39]. These genes encode for the two ABC 'G' family half-transporters, ABCG5 and ABCG8, also known as sterolin-1 and sterolin-2, respectively.

Genetics of ABCG5 and ABCG8

ABCG5 and ABCG8 contain 13 exons and are organized in a head-to-head organization spanning ~80 kb of the locus [39]. These genes are likely to have evolved from a common ancestral gene, with a tandem duplication and inversion event. The intergenic region between ABCG5 and ABCG8 is very small (<160 bases) and does not contain a conserved TATA motif or motifs that would indicate which transcriptional factors may be involved in regulating this locus (see below). The strongest evidence that ABCG5 and ABCG8 may act as obligate heterodimers comes from the genetic evidence that patients with sitosterolemia are completely mutated for ABCG5 or ABCG8, and mutations in either of these seem to result in an identical phenotype [2, 37, 39]. In addition, in vitro evidence supports their heterodimerization with each other but not other ABC G family members [15, 16]. A compilation of mutations and identified polymorphisms is shown in Fig. 1. There are some notable features. Firstly, founder effects for mutations underlie many of the cases, suggesting that this disease has been present for many generations, perhaps more than 4,000 years [36, 39]. Secondly, mutations in ABCG5 seem to be the cause of sitosterolemia in peoples who have oriental lineage (Chinese, Japanese and Indian) [39]. Currently, this group also represents ~20% of the cases, though this may be due to under-diagnoses. Thirdly, despite the close proximity and homology between *ABCG5* and *ABCG8*, considerably more polymorphisms are present in *ABCG8* compared to *ABCG5*. This seems to be a phenomenon confined to man, as analyses of the rodent *STSL* loci indicate an almost equal level of variability [40, 63]. It is not clear if this is an indication that ABCG5 and ABCG8 may also have a function, independent of their role as heterodimers. Other members of the ABCG family are known to function as homodimers (see this issue). Alternatively, it may be that there is a selection for variations at the *STSL* locus. A number of studies have implicated this locus in disease (or physiological) processes ranging from lipoprotein kinetics, cholesterol absorption, obesity to response to drug therapy [8, 20, 23–25, 42, 44]. At present, it is difficult to envisage a selective advantage for any of these traits. Indeed, the remarkable conservation of the *STSL* locus between species as diverse as fish, amphibians, rodents, and humans seems to indicate that the polymorphic changes may have a more dramatic effect on function (Fig. 2). Note that almost all of the cSNPs that are nonsynonymous seem to affect amino acid residues that are highly conserved (Fig. 2a,b). One of these is part of the consensus Walker B motif (E238L in ABCG8, Fig. 2b) and

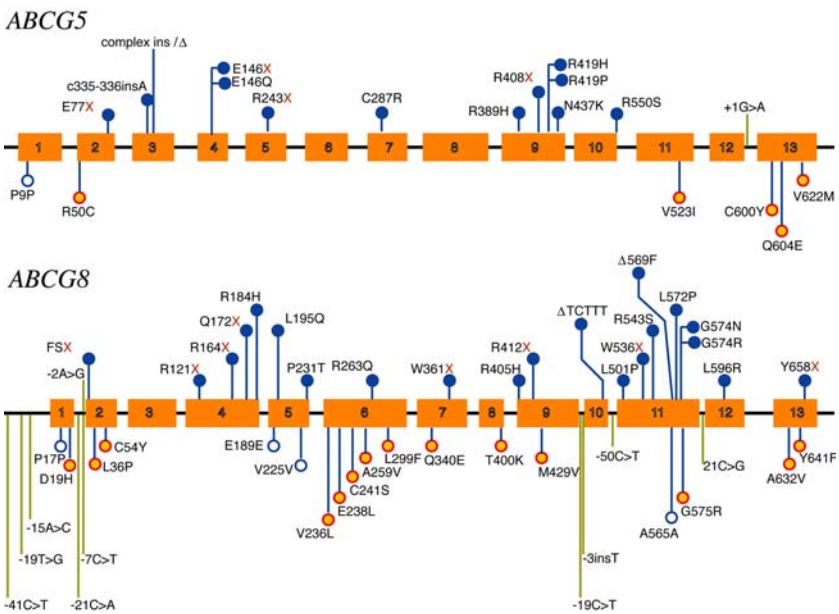

Fig. 1 A compilation of reported mutations and polymorphisms affecting the *STSL* locus. A *cartoon* representation of *ABCG5* (*top A*) and *ABCG8* (*bottom B*) is shown, each comprising of 13 exons. The sizes of exons and introns are not to scale. Note that the *STSL* locus comprises of both genes arranged in a head-to-head orientation, with less than 160 bases separating their start transcription sites. Above each gene are shown in *filled blue circles* all known mutations that cause sitosterolemia. Below each gene are shown polymorphisms. The *filled yellow circles* depict nonsynonymous changes, whereas the *open circles* depict synonymous changes. Note that many more polymorphisms have been reported for *ABCG8* than for *ABCG5*, despite extensive resequencing of this locus but a number of different groups

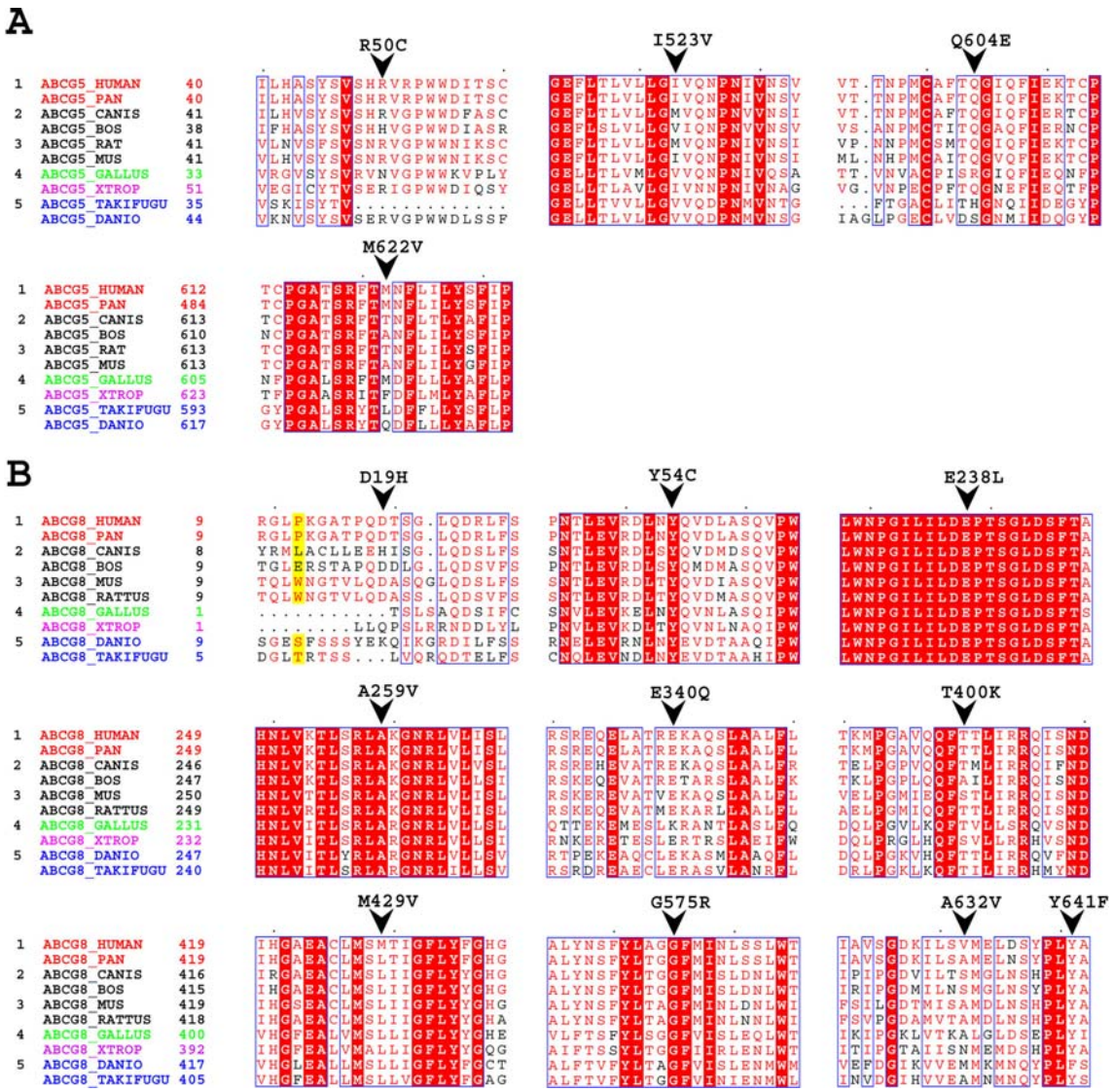

Fig. 2 Conservation of ABCG5 and ABCG8 polypeptide sequences in the animal kingdom. Polypeptide sequences for man, chimpanzee, dog, cow, rat, mouse, chicken, toad, and two fishes, Zebra fish and Fugu, were compiled from the publicly available databases. *Panel* **a** shows the homology for ABCG5 and *panel* **b** that for ABCG8. Homologies around the nonsynonymous changes in humans are shown. It should be noted that the sequences for all but man, mouse, and rat are considered preliminary and need to be parsed for accuracy. For example, the exact start translation sites for some of these have not been established and in some cases, the protein translation is based upon electronically predicted exons, which seem to be incorrect. Despite these reservations, mapping the nonsynonymous changes seen at the *STSL* locus in humans onto these homology comparisons shows that many of these polymorphisms affect highly conserved residues. It is therefore remarkable that these 'polymorphisms' are not 'disease'-causing (see text for discussion). Note that the variable amino acids not conserved in fish and chicken may turn out to be conserved once the genome sequences for these organisms have been 'cleaned up'. A complete homology comparison for both genes is available on request from the authors

thus would be expected to have a dramatic effect on function, though none has been reported thus far. This raises the possibility that each of these cSNPs should alter function in some manner. The key to what these subtle alterations may be may come from the study of individuals who are homozygous for these cSNPs. At present, we do not understand how ABCG5/ABCG8 alter function other than play a role in keeping noncholesterol sterols out of our bodies. These polymorphisms may remain mysterious in the manner they affect physiology for the foreseeable future.

Regulation of ABCG5 and ABCG8

The major regulation of expression of sterolins appears to be at the transcriptional level, although there is a paucity of data on whether regulation at the post-transcriptional levels occurs. There are robust data to support the role of Lxr-mediated increased mRNA expression of Abcg5 and Abcg8, both in vivo (Table 1) as well as in vitro [2, 11, 47, 48, 65, 68]. LXR ligands have been shown to increase expression of the *STSL* locus in mouse liver and intestine and in rat hepatoma cells; cholesterol feeding upregulates

this locus in part by increased activation of the Lxr pathway [48]. Unfortunately, it is still not clear if this is a direct effect or an indirect effect. To date, scanning for classical *cis*-acting DNA motifs for LXR recognition in the *STSL* locus have been negative. The strongest evidence that this is a direct effect is that the changes in RNA expression can be seen with as little as 12 h exposure to the ligands, both in vivo and in vitro [48]. The only direct evidence for both binding sites and an action on transcription has come from the investigation of the role of liver receptor homolog-1 (LRH-1), an orphan receptor, in the pathway that classically involves the FXR-SHP-LRH-1 [12]. The human intergenic sequence contains a LRH-1 binding site, this sequence has been shown to bind directly LRH-1 and activate expression of ABCG5 and ABCG8 in vitro [12]. It is interesting to note that this motif is not conserved in the rodent intergenic region. This is one of the differences between murine and human 'promoter' sequences in that the mouse not only has an identifiable TATA box, it also contains sequences that may bind HNF4 (both of which are absent from the human sequence) [12, 40]. This may suggest that the human *STSL* locus may be fundamentally differently regulated relative to the murine locus, although most of the evidences are based upon the experimental data derived from murine models. Table 1 summarizes data gleaned from the literature, both directly and indirectly summarizing the involvement (or not) of some of the transcriptional factors. Of note is that not only are there species differences that are evident but also the differential effects between liver and intestine for the same transcriptional pathway which deserve some attention. Furthermore, there are some evidences for a post-translational level of regulation. In canine gall bladder epithelial cells, Lxr activation, or exposure to model bile, led to a translocation of Abcg5 and Abcg8 from an intracellular compartment to the apical surface, suggesting a post-translational level of regulation [58]. No changes in RNA expression levels were seen.

Function of ABCG5 and ABCG8

Sterolins are critically involved in regulating the whole-body retention of noncholesterol plant sterols, and that these proteins are also key to secreting cholesterol into the biliary lumen (and likely the intestinal lumen) [30, 32, 35, 46, 64–66]. The genetic disease of sitosterolemia attests to their role in keeping noncholesterol sterols (plant sterols as well as shellfish sterols, etc.) out of the human body [17].

In the intestine, it is clear that the entry of all sterols, but particularly cholesterol, is primarily regulated by a step involving NPC1L1 (see Fig. 3). Based upon the response of patients with sitosterolemia to the drug ezetimibe, blocking NPC1L1 seems to lower plasma plant sterol levels and seems to improve symptoms and signs [52]. From studies involving mice as well as humans, the intestine can secrete cholesterol back into the lumen of the intestine [33, 55]. It is possible that it may also be able to

Table 1 Factors involved in regulation of the *STSL* locus

Agent	Mode of action	Man		Mouse		Rat		Reference
		Liver	Intestine	Liver	Intestine	Liver	Intestine	
Dietary cholesterol	Lxr			↑	↑	↓		[2, 9, 11, 48]
T0901317, GW3965	Lxr			↑	↑			[2, 11, 33, 48]
Phytosterols*	Lxr, LXR		↑	↔	↑, ↔			[7, 11, 29, 45, 62]
Srebps	?Lxr			↑				[22]
Chenodoxycholic acid	Fxr	↓		↑	↔	↑, ↔	↔	[11, 12, 48]
Cholic acid	?Pxr			↑	↑, ↔			[19, 64]
	?Fxr							
Cholesterol/Cholic acid	Pxr			↔				[57]
Fenofibrate	Pparα	↔		↔	↔			[48, 49]
Bezafibrate	PPARα	↑						[49]
Gemfibrozil	PPARα	↔						[49]
Troglitazone	Pparγ			↔	↔			[48]
GW610742	Pparδ			↔	↔			[60]
Pregnenolone α carbonitrile	Pxr			↔	↔	↑		[9, 61]
Spironolactone	?Pxr					↓		[9]
	?GR							
Phenobarbital	Car			↔				[61]
Streptozotocin-induced DM	?					↓	↓	[5]
Streptozotocin-induced DM + insulin	?					Not restored	Restored	[5]
Bile duct ligation	?					↓↓	↓, ↔	[26, 28]
Estrogen	?				↑	↓↓	↔	[10, 27]
Diosgenin	?			↑	↔	↑	↔	[27, 28, 64]

*Activation by stigmasterol in an adrenal cell line has also been demonstrated

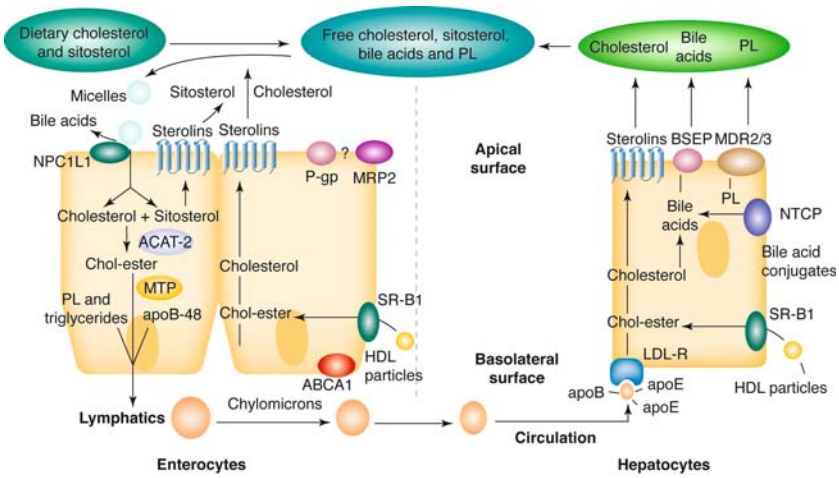

Fig. 3 Physiological pathways involved in dietary sterol trafficking. Upon ingestion, dietary sterols are unesterified, mixed with biliary sterols, phospholipids, and bile salts to form mixed micelles, a necessary step for absorption. These interact with the apical surface of the proximal small intestine enterocyte (process as yet undefined), whereby the sterols are allowed to enter the enterocyte. Although the exact molecular processes remain to be defined, it is clear that this process involves the multipass transmembrane protein NPC1L1; blocking this protein with the drug ezetimibe blocks entry of all sterols. In a process not understood, noncholesterol sterols seem to be excluded from progressing further and may be pumped back into the lumen by sterolins, ABCG5, and ABCG8. Cholesterol is allowed to progress to the ER where it is esterified, packaged with triglyceride and apolipoprotein B48 into chylomicrons to be secreted at the basolateral surface into the lymphatic channels for transport to the venous circulation. The enterocyte also synthesizes ApoA1 and may play a role in the maturation of HDL via ABCA1. HDL is a particle involved in the removal of cholesterol (and noncholesterol sterols) from the body. Thus, intestinal uptake of HDL may result in the net secretion of sterols. Although other ABC transporters are also expressed at the apical membrane, their role in cholesterol trafficking is not defined (indicated by the *question mark*). In addition, although bile acids are a necessary constituent of the micelles, these are not absorbed in the proximal jejunum but are transported to the terminal ileum where they are specifically taken up via interscapular brown adipose tissue. It is not clear if bile acids enter the jejunal enterocyte at all, or entered and are pumped back out by a specific transporter. At the hepatocyte level, entry of sterols adds to the pool of sterols that are rapidly channeled for secretion via ABC transporters ABCG5/ABCG8, with bile salt and phospholipid secretion mediated by bile salt export pump (BSEP) (ABCB11) and MDR2/3 (ABCB4). It has been demonstrated in humans that the liver preferentially excretes noncholesterol sterols and that HDL, via SR-B1-mediated sterol entry, is important in this channeling process. Note that cholesterol can be secreted directly into bile (via sterolins), as well as broken down to a molecule of bile acid and secreted into bile (via BSEP). Furthermore, although sterol secretion is greatly attenuated in sitosterolemia, as well as in knockout mice, some sterols are still secreted, suggesting that there are other pathways for sterol secretion. *ACAT-2* acyl-CoA: cholesterol acyltransferase-2, *Chol* cholesterol, *MTP* microsomal triglyceride transfer protein, *MRP2* multidrug resistance-associated protein 2 (ABCC2), *NTCP* sodium taurocholate cotransporting polypeptide, *PL* phospholipids, *SR-B1* scavenger receptor B1. Adapted from Lu et al. [38], copyright 2001 with permission from Elsevier

secrete noncholesterol sterols. At present, this has not been tested in animal models, though evidence for this has recently been presented in a mouse model. In humans, it would appear that the liver may be a more important organ for keeping the levels of plant sterols low; a patient with sitosterolemia underwent a liver transplantation, and following this, his plant sterol levels almost normalized despite his genetic defect in the intestine [41]. Thus, ABCG5 and ABCG8 may selectively 'pump' sterols out of the intestinal cells as a first-line defense to dietary input, but in the fasting state, the liver can continually pump sterols (cholesterol and noncholesterol sterols) into bile and thus maintain a low noncholesterol sterol level in the body. In the fasting state, the intestine may also be able to reduce whole body sterol pools by continuing to pump sterols (presumably delivered to the intestinal enterocytes via the high-density lipoprotein pathway) [55]. It should be noted that a formal demonstration that these proteins carry out this function actively and utilize adenosine triphosphate (ATP) has not been reported. This is assumed as the ATP-binding domain sequences and both the highly conserved and invariant from the consensus ABC motifs.

From genetic manipulations in mice, it is clear that these proteins are key to secreting cholesterol into bile. The liver is the dominant organ for whole-body sterol balance and uses the biliary system for sterol loss, whether as direct sterol secretion or by breakdown of cholesterol into bile acids and secretion into bile. Over-expression of human ABCG5 and ABCG8 in mice led to a supersaturation of cholesterol in bile [66], whereas knockout of either Abcg5/Abcg8 or Abcg8 genes led to a failure to secrete sterols [30, 65]. However, knockout of Abcg5 led to a sterol-poor bile, but sterol secretion seemed to be restored upon Lxr activation [46]. Lxr activation in Abcg5/Abcg8 double knockout did not lead to a stimulation of biliary sterol secretion [65, 68]. There are no data reported for Abcg8 knockout in response to Lxr agonists. From both genetic data, as well as in vitro experimental data, ABCG5 and ABCG8 are likely to function as obligate heterodimers. It is also unlikely that they can heterodimerize with other ABCG family members. Thus, this observation, as well as the difference in the accumulations of polymorphisms in *ABCG5* gene relative to *ABCG8*, is perplexing. Studies in humans have shown that there is preferential excretion of sitosterol compared to

cholesterol when these are directly infused into their veins [51]. Studies in knockout mice confirm that this response, at least for biliary sterol secretion, is mediated by Abcg5/Abcg8 and is increased by Lxr activation [67]. While it seems that sterols in general seem to be substrates for ABCG5/ABCG8, limited studies indicate that oxysterols are not substrates [67]. The full spectrum of substrates for ABCG5/ABCG8 is not known. Finally, despite their role in secreting sterols into the lumen, the exact mechanism of action remains a matter of controversy. One hypothesis argues that ABCG5/ABCG8 may act as 'extruders', exposing sterols in the outer leaflet of the membrane for facilitated extraction into the lumen by sterol acceptors [56], such as bile acid:phospholipid complexes, whereas others have proposed that sterolins may act as 'flippases', akin to the flipping of phospholipids from the inner to the outer leaflet of the apical membranes [18]. In the absence of robust in vitro assays, it is not possible to discern if either of these models is valid. Finally, it should be noted that some sterol secretions continue, both in man, as well as mice deficient in sterolins, suggesting that there are other pathways whereby sterols can gain entry into the biliary tract, albeit at very low levels. Moreover, the 'rate-limiting' step for biliary sterol secretion has not been identified. ABCG 5/ABCG8 may not be rate-limiting, because parents of sitosterolemic patients have no biochemical phenotype under physiological conditions, although they must have half-normal functioning sterolins. In this context, a recent study of ABCG5/ABCG8 expression in liver transplant patients found no correlation between mRNA expression of these transporters and biliary cholesterol secretion (sitosterol secretion was not reported) [13].

Conclusions

There are now robust data to support the contention that sterolins (ABCG5 and ABCG8) are the major sterol transporters active at the apical surface of hepatocytes and enterocytes. Evidence in support of this comes from the genetic basis of sitosterolemia, from the development of animal models that specifically manipulate their function, and from experiments that show to alter biliary sterol secretion. The *STSL* genetic locus is highly conserved from fish, toad, chicken, rodent, cow, dog, ape, and man, preserving not only the exon–intron organization but also shows a remarkably high degree of polypeptide conservation, raising the possibility that the true function of these proteins may still remain to be defined. A number of questions remain to be fully determined. One of these is defining fully the mode of transcriptional control; another is how much post-transcriptional regulation may occur as well as how these putative transporters 'pump' sterols out of the cell. Finally, the conservation of the polypeptides suggests a conservation in function. Thus, it is not clear what the selective advantage would be in this remarkable evolution, unless these proteins evolved to limit the entry of as yet unknown dietary sterol toxins.

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