review

Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences

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Abstract **The molecular basis of the processes that control two closely related traits, the absorption of cholesterol from the intestines and plasma plant sterol levels, are only partially understood. The discovery that mutations in two novel hemitransporters, ATP binding cassette transporter G5 (ABCG5) and ABCG8, underlie a rare inborn error in plant sterol metabolism, β-sitosterolemia, represents a major breakthrough in this field. More recently, genetic studies in the mouse that mapped loci in linkage with cholesterol absorption and plasma plant sterol levels and studies in humans that examined the relationship of plasma plant sterol levels to sequence variation in the ABCG5/ABCG8 locus suggested the involvement of other genes. Moreover, studies in β-sitosterolemic patients, in ABCG5/ABCG8-targeted animals, and on a newly developed cholesterol absorption inhibitor, ezetimibe, suggest commonalities and differences in the regulation of the two traits. This review summarizes the evidence for genetic control of cholesterol absorption and plasma plant sterol levels, presents the evidence for commonalities and differences between the two traits, and discusses recent developments and future perspectives in this field.**—E. Sehayek. **Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences.** ***J. Lipid Res.*** **2003.** 44: **2030–2038.**

Supplementary key words dietary sterols • phytosterolemia • quantitative trait loci

The intestinal absorption of cholesterol and plant sterols is a complex process that is regulated at multiple levels. To be absorbed, dietary sterols must first be presented to the enterocyte brush border membrane in a solubilized form. This is achieved through transfer, within the intestinal lumen, into the micellar compartment, a complex mixture of bile acids and glycerolipids [for detailed reviews see Turley and Dietschy (1), Dawson and Rudel (2), Wilson and Rudel (3), and Carey and Hernell (4)]. As shown in **Fig. 1**, the transfer of cholesterol and plant sterols across the enterocytic brush border membrane may follow one of two routes: sterols can be either excreted back into the intestinal lumen or, alternatively, they can be transported, packed, and delivered to the plasma compartment in the form of chylomicrons. Therefore, net cholesterol absorption is a balance between the transfer across the enterocytic brush border membrane and the excretion back into the intestinal lumen. Once in plasma, cholesterol and plant sterols are rapidly and efficiently disposed of by the liver in the form of chylomicron remnants. As shown in Fig. 1, within hepatocytes, cholesterol and plant sterols can be excreted through the canalicular membrane into bile. In addition, at least some of these sterols are transported, packed, and secreted back into plasma in the form of VLDLs or HDLs. Therefore, as illustrated in Fig. 1, the uptake of cholesterol and plant sterols by enterocytes and hepatocytes is followed by bidirectional transport: excretion through the apical membrane back into the intestinal lumen and the bile, respectively; and plasma secretion through the basolateral membrane. Furthermore, the processes of excretion into the bile and absorption from the intestines are interrelated. Studies in recent years have shown that the excretion of cholesterol into the bile may exert regulatory effects at the intestinal level (5, 6). Although the physiological aspects of the processes that are illustrated in Fig. 1 have been partially characterized, the regulation of these processes at the molecular level, and the commonalities and differences in the processing of cholesterol and plant sterols at each of these steps, are only partially understood. The main purpose of this review is to summarize the genetics of these processes, with special attention to both common and distinct aspects of cholesterol and plant sterol metabolism.

Twenty-nine years ago, Ashim K. Bhattacharyya and William E. Connor described two sisters with increased intestinal absorption and high plasma levels of plant sterols and named the disorder β-sitosterolemia, after β-sitosterol, the plant sterol that is abundant in the plasma of these patients (7). In this original report, the authors concluded that, "The occurrence of β-sitosterolemia in these

Manuscript received 23 June 2003 and in revised form 14 July 2003.

Published, JLR Papers in Press, August 1, 2003.
DOI 10.1194/jlr.R300008-JLR200

Abbreviations: ABCA1, ATP binding cassette transporter A1; LOD, logarithm of odds.

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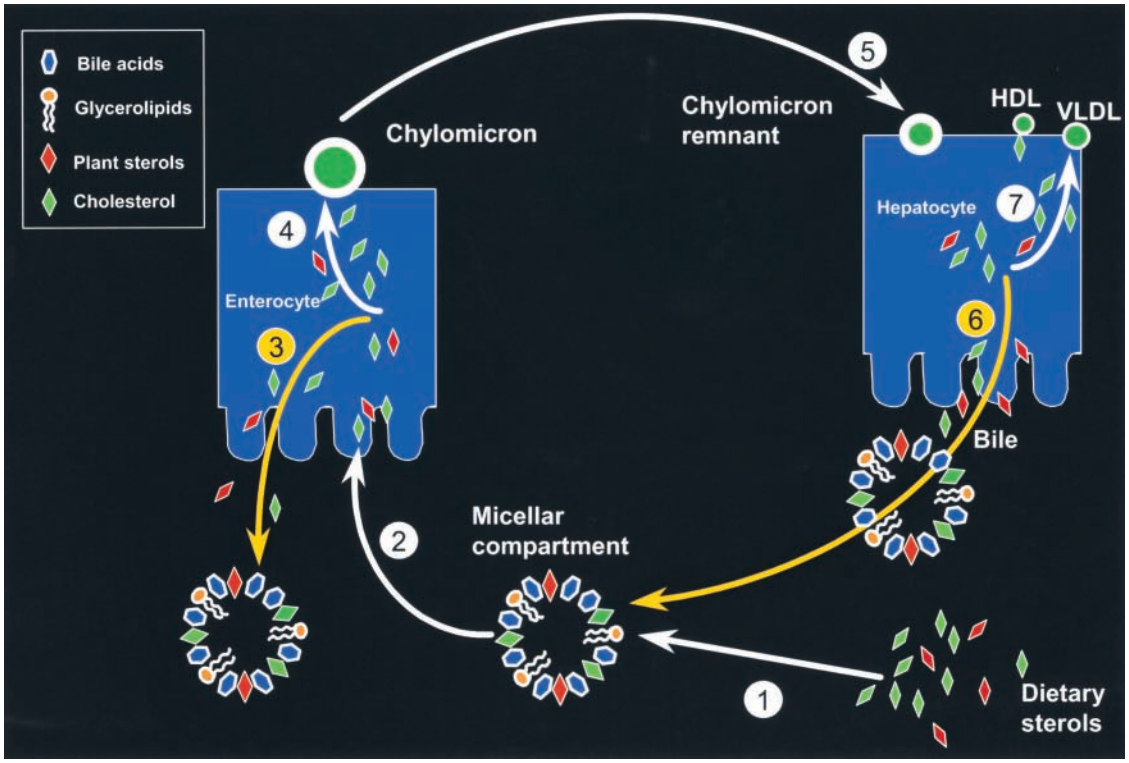

Fig. 1. The enterohepatic metabolism of cholesterol and plant sterols. Within the intestinal lumen, dietary cholesterol and plant sterols are transferred into the micellar compartment (1), taken up across the enterocytic brush border membrane (2), partly excreted back into the intestinal lumen (3), transported and secreted into plasma in the form of chylomicrons (4), disposed off by the liver in the form of chylomicron remnants (5), excreted through the hepatocytic canalicular membrane into the bile (6), and partly transported, packed, and secreted into plasma in the form of VLDLs and HDLs (7).

two sisters with unaffected parents suggests an inherited recessive trait." [page 1033 in ref. (7)] This description suggested for the first time that sterol absorption from the intestine is subject to genetic control and provoked research that focused on the genetics of this process. This research suggested that several genes, e.g., apolipoprotein E, apolipoprotein A-IV, ACAT, scavenger receptor class B type 1, carboxyl ester lipase, ATP binding cassette transporter A1 (ABCA1), and others, are modifiers of cholesterol absorption. The emergence of ABCA1 as a candidate gene deserves special attention. The observation that liver X receptor (LXR) agonists decrease the absorption of cholesterol, in conjunction with the notion that ABCA1 is: *i*) involved in cholesterol transport, *ii*) expressed in the intestines, and *iii*) induced by LXR agonists, leads Mangelsdorf and colleagues (8) to suggest that ABCA1 is involved in the regulation of cholesterol absorption. However, later studies that localized ABCA1 to the enterocytic basolateral membrane, as well as studies in a chicken model with a mutation in the ABCA1 gene, have challenged this view (9, 10). Nevertheless, as detailed below, the observation that LXR agonists decrease the absorption of cholesterol was of paramount importance in deciphering the molecular basis of β-sitosterolemia. Indeed, recent efforts in two laboratories that applied a purely genetic approach and a challenge with LXR agonists led to the discovery that mutations in two genes that encode for the novel hemitransporters ABCG5 and ABCG8 are the cause of β-sitosterolemia (11–13). Furthermore, studies in humans that looked into the relation of sequence variation in the ABCG5/ABCG8 locus to plasma plant sterol levels, and studies that examined the linkage of cholesterol absorption and plasma plant sterol levels in the mouse, provided support for the involvement of genes other than ABCG5/ABCG8 in controlling the absorption of cholesterol and the plasma levels of plant sterols. These observations set new challenges in solving the genetics of cholesterol absorption and plasma plant sterol levels.

Moreover, the discovery that β-sitosterolemia is characterized by increased absorption of plant sterols provoked an intriguing hypothesis suggesting the existence of discriminative processes that distinguish plant sterols from cholesterol at the intestinal level. According to this hypothesis, the uptake by enterocytes (Fig. 1, step 2) is followed by discriminative excretion of plant sterols, but not of cholesterol, back into the intestinal lumen (Fig. 1, step 3). Indeed, further studies in β-sitosterolemic patients found that a 3- to 12-fold increase in plant sterol absorption, a 30- to 100-fold increase in plasma plant sterol levels, and 13- to 100-fold increase in body plant sterol pool size due to prolonged sterol retention are associated with only a moderate increase in intestinal cholesterol absorption (14–19). These studies suggested that in subjects with β-sitosterolemia, the genetic defect primarily affects the metabolism of plant sterols. In contrast, studies in normal subjects disclosed a significant correlation between cho-

lesterol absorption rates and plasma plant sterol levels and provided the basis for using plasma plant sterol levels as a surrogate measure of cholesterol absorption (20, 21). These studies suggested that in the general population, processes controlling cholesterol absorption and plasma plant sterol levels are interrelated. Furthermore, recent studies in Abcg5/Abcg8-targeted animals and studies on a newly developed cholesterol absorption blocking agent, ezetimibe, provided further support for commonalities and differences in the regulation of cholesterol absorption and plasma plant sterol levels (22–24).

This review will focus on the evidence for genetic control of cholesterol absorption and plasma plant sterol levels, will examine the commonalities and differences in the regulation of these two traits, and will discuss recent developments and future perspectives in this field.

EVIDENCE FOR GENETIC CONTROL OF CHOLESTEROL ABSORPTION

Mouse studies

Studies in laboratory animals and, in particular, studies in the mouse largely expanded the understanding of the genetics of cholesterol absorption. The availability of a large number of inbred mouse strains, a continuous expansion of the mouse databases, and the accessibility of mouse genetic resources are only some of the factors that led to the mouse becoming a valuable mammalian genetic model for this purpose. As detailed in **Table 1**, four different studies have utilized at least 14 different inbred mouse strains to examine the role of genetic determinants in controlling the absorption of cholesterol. Carter, Howles, and Hui (25) studied the absorption of cholesterol in six different strains and found that when mice were fed a low-fat low-cholesterol diet (Teklad LM485, no cholesterol, 5.7% fat), the strains displayed only moderate differences in cholesterol absorption (from 65% to 76%). However, when mice were fed a high-fat high-cholesterol diet (Purina Mouse Chow 5015 supplemented with cocoa butter and cholesterol to a final content of 1.25% cholesterol and 15.8% fat), clear strain differences were observed in the absorption rates, ranging from 11% to 31.8%. Jolly, Dietschy, and Turley (26) fed two mouse strains, 129sV and C57BL/6J, with Teklad cereal-based diet (Teklad 8604, 0.02% cholesterol) and found a 2-fold difference in cholesterol absorption rates. Wang, Paigen, and Carey (27) fed 12 different mouse strains with a Purina Chow

TABLE 1. Strain differences in cholesterol absorption and plasma plant sterol levels in the mouse

Strains	Diet	Method	End-Point and Findings	Reference
C57L/J	Teklad basal nonpurified diet and HFHC diet	Fecal dual isotope ratio	% Cholesterol absorption: Teklad diet 65–76%: DBA/2 ≅ C57BL/6 ≅ C3H/He ≅ BALB/c ≅ AKR/J>C57L/J HFHC diet 11–31.8%: C57BL/6≅C3H/He≅BALB/c> DBA/2≅AKR/J>C57L/J	Carter et al. (25)
BALB/c				
C57BL/6				
C3H/He				
DBA/2				
AKR/J				
C57BL/6J	Teklad cereal-based diet	Fecal dual isotope ratio	% Cholesterol absorption: 20 ± 3	Jolly et al. (26)
129/Sv			% Cholesterol absorption: 44 ± 3	
C57BL/6J	Purina Chow	Plasma dual isotope ratio and fecal dual isotope ratio	% Cholesterol absorption: 39 ± 5	Wang et al. (27)
C57L/J			% Cholesterol absorption: 37 ± 5	
FVB/J			% Cholesterol absorption: 37 ± 5	
129/SvJ			% Cholesterol absorption: 37 ± 5	
SM/J			% Cholesterol absorption: 31 ± 4	
SWR/J			% Cholesterol absorption: 29 ± 4	
BALB/cJ			% Cholesterol absorption: 28 ± 4	
DBA/2J			% Cholesterol absorption: 27 ± 4	
SJL/J			% Cholesterol absorption: 27 ± 3	
AKR/J			% Cholesterol absorption: 6 ± 3	
A/J			% Cholesterol absorption: 24 ± 4	
C3H/J			% Cholesterol absorption: 24 ± 5	
			% Cholesterol absorption: 22 ± 5	
129P3/J	Teklad cereal-based diet	Fecal dual isotope ratio	% Cholesterol absorption: 129 > AKR > C3H > BALB/c > C57BL/6 > DBA/2 > SJL	Schwarz et al. (28)
AKR/J				
C3H/HeJ				
BALB/cJ				
C57BL/6J				
DBA/2J				
SJL/J				
C57BL/6J	Purina Chow	GC-MS	Plasma campesterol to 1.39 ± 0.18	Sehayek et al. (33)
CASA/Rk			Total cholesterol ratio: 0.71 ± 0.09	

HFHC, high-fat, high-cholesterol.

diet (0.02% cholesterol and 4.5% fat) and found absorption rates that ranged from 22% to 39%. Schwarz et al. (28) fed seven different strains with a Teklad cereal-based diet (Teklad 7001, 0.016% cholesterol and 4% fat) and found that the percent absorption spanned between 22% and 66%. It is of note that some of these studies reported remarkably different absorption rates for the same strain and, in some cases, there are disagreements between studies regarding the ranking of the absorption rates among the same strains. The reason for these disagreements is not entirely clear, but it is possible that differences in study design, including diet composition, gender, age, and details in cholesterol absorption assays, may explain some of these differences. The differences related to cholesterol absorption assays deserve special attention. It is also of note that although a recent study in the mouse reported a large agreement between different methods (29), there are considerable laboratory-to-laboratory differences in assay protocols, even when the same method is applied. This complicates the comparison of reports from different laboratories. Nevertheless, regardless of these disagreements and difficulties, it is noteworthy that all of the above-described studies, with no exception, have shown that there is a large strain-to-strain variability in percent cholesterol absorption under given conditions.

Human studies

Several studies have examined the variability of cholesterol absorption in humans. One study measured cholesterol absorption rates in a randomly selected group of 63 normal volunteers and found that the absorption rates ranged between 25% and 75% (21). In another study, 18 normal volunteers were kept under strict metabolic ward conditions, and each individual was fed for three different periods with three different diets that differed in fat and cholesterol content. Interestingly, regardless of the diet composition, there was a remarkable individual-to-individual variability in cholesterol absorption rates. Whereas some subjects displayed absorption rates of only 25%, others absorbed over 70% of their daily cholesterol intake (30). In still another study, cholesterol absorption rates were measured in outpatient normal volunteers that consumed their own diet. Again, the percent cholesterol absorption in different individuals spanned between 25% and 85% (31). Finally, in a more recent study, Sudhop et al. (24) examined the absorption of cholesterol in 18 moderately hypercholesterolemic outpatients and reported absorption rates that ranged from 25% to 75%. These studies clearly show that in humans, there is a large interindividual variability in cholesterol absorption rates.

A most relevant question is, therefore: how much of the variability in cholesterol absorption rates can be ascribed to genetic factors? It should be noted that unlike the solid evidence for genetic control of plasma plant sterol levels (see below), the literature is deficient in cholesterol absorption studies that carefully examine the absorption in nuclear families and in monozygotic and dizygotic twins. Nevertheless, studies in humans support a role for genetic determinants. Gylling and Miettinen (32) recently examined the inheritance of cholesterol metabolism in siblings of probands with high and low cholesterol absorption rates. In a screening step, the investigators measured plasma cholestanol level (a sterol that correlates with cholesterol absorption) in 330 subjects with coronary heart disease and identified probands that ranked at the upper and lower ends of the cholestanol-to-cholesterol ratio distribution. Next, these investigators used cholesterol balance studies in an outpatient setting to examine the absorption of cholesterol in the siblings of these probands. The authors reported that, compared with siblings of probands with low cholesterol absorption, the siblings of high absorbing probands displayed significantly higher absorption rates (mean ± SEM 48.7 ± 2.4% vs. 36.9 ± 2.6% absorption in siblings of high- and low-absorbing probands, respectively).

EVIDENCE FOR GENETIC CONTROL OF PLASMA PLANT STEROL LEVELS

The genetics of plasma plant sterol levels in the mouse has so far been addressed by one study. As shown in Table 1, our group identified two inbred mouse strains that, when fed a Purina chow diet, displayed a 2-fold difference in plasma plant sterol levels (33). A similar variability was also found by studies that examined the heritability of plasma plant sterols in humans. Berge et al. (34) measured plasma plant sterol levels in 502 individuals in 74 nuclear families and found interindividual differences in plasma sitosterol and campesterol concentrations of 3- to 5-fold.

Again, a most relevant question is: how much of the variability in plasma plant sterol levels can be ascribed to genetic factors? This critical question has been carefully addressed by Berge et al. (34). These investigators measured the plasma levels of sitosterol and campesterol in parents and their offspring and reported that 81% to 84% of the variability in plasma plant sterol levels can be attributed to hereditary factors. Furthermore, the same study compared the correlation of plasma plant sterol levels in 12 pairs of monozygotic twins and 12 pairs of dizygotic twins and found a significantly higher correlation in the former. This important study provided strong support for the role of heredity in controlling the plasma levels of plant sterols.

THE ROLE OF ABCG5 AND ABCG8 IN CONTROLLING PLASMA PLANT STEROL LEVELS

A breakthrough in understanding the molecular basis of sterol absorption has recently been achieved by the discovery of two ABC cassette hemitransporters, ABCG5 and ABCG8. Mapping studies in β-sitosterolemic pedigrees enabled Sheilandra Patel and his colleagues to map the locus of this disorder to the short arm of human chromosome 2 and subsequently to narrow the interval to less

than 2 centimorgans (cM) (35, 36). Shortly thereafter, studies in the mouse showed that agonists of the orphan nuclear hormone receptor LXR suppress the absorption of cholesterol from the intestine (8). Helen Hobbs and her colleagues (11) used mouse liver and intestinal microarrays to search for transcripts that were regulated by an LXR agonist. The search revealed a mouse expressed sequence tag (EST) that was induced ~2.5-fold and resembled three different *Drosophila* genes that encode for three ABC half-transporters. Because other ABC transporters have been implicated in cholesterol transport, these investigators postulated that the novel hemitransporter designated ABCG5 is involved in liver and intestinal sterol transport. Moreover, the cloning of the human ortholog allowed the identification of a human EST clone that maps directly to the β-sitosterolemia locus on chromosome 2. Furthermore, genome clustering of ABC transporters guided the investigators to search for another ABC transporter in the vicinity of ABCG5. This search identified another novel ABC hemitransporter, ABCG8, that is separated by only 374 nucleotides from the translational start site of ABCG5. Finally, sequencing of the ABCG5 and ABCG8 genes in individuals with β-sitosterolemia identified nonsense and missense mutations and established a causative role for these genes in this disorder. In parallel, Patel and his colleagues (12, 13) postulated that the β-sitosterolemic gene is expressed in the intestine and the liver, and searched for ESTs that are expressed in these organs and map to their 2 cM interval on chromosome 2. This search revealed three ESTs, of which one was found to encode a 'half ABC transporter.' This gene turned out to be ABCG5. Again, sequencing the ABCG5 gene in individuals with β-sitosterolemia revealed missense mutations and deletions that established the causative role of this gene in this disease. Therefore, combined efforts in two laboratories successfully solved the molecular basis of β-sitosterolemia. Further studies could show that the expression of these transporters is induced by cholesterol feeding, that their expression is largely restricted to liver and intestine, that coexpression of the two hemitransporters is necessary for their apical localization, and that ABCG5/ABCG8 play a critical role in the excretion of cholesterol into bile (6, 11, 22, 37). Furthermore, overexpression and disruption of these genes in the mouse caused a decrease and an increase in plasma plant sterols, respectively (6, 22). These findings suggest that in the intestine, ABCG5 and ABCG8 are involved in the apical excretion of sterols back into the intestinal lumen (Fig. 1, step 3) and, in the liver, into the bile (Fig. 1, step 6). It is important to note, however, that this suggestion is entirely speculative, as the exact mechanism(s) whereby ABCG5/ABCG8 exert their effects on sterol metabolism has not yet been clarified. Elucidating the function of these transporters at the molecular level represents a considerable challenge, as no specific activity-function test for these transporters is currently available.

A relevant question is whether common variations in the ABCG5/ABCG8 genes affect interindividual differences in plant sterol levels in non-β-sitosterolemic subjects. Berge and colleagues (34), who studied the heritability of plant sterols in humans, directly addressed this question. Examining the association of polymorphism in the ABCG5/ABCG8 locus and plasma plant sterol levels, these investigators found that two sequence variations in ABCG8 were associated with lower plasma plant sterol levels in parents and their offspring. Carriers of ABCG8 D19H in 10% of the population had a 31% lower genotypic mean in absolute campesterol level, and carriers of ABCG8 T400K in 36% of the population had a 2% lower genotypic mean. Thus, these account for approximately 5.5% and 0.1% of the variation in absolute plasma campesterol levels. In the same study, the authors calculated that the genetic contribution to the variation in absolute plasma campesterol concentrations is 59%. Thus, it is probable that variations at other loci, in addition to ABCG5/ABCG8, contribute to the variation in plasma plant sterol levels in the general population. Therefore, although the discovery of ABCG5/ABCG8 solved the molecular basis of β-sitosterolemia, these studies suggest that other genes are involved in the regulation of plasma plant sterol levels.

LOCI CONTROLLING CHOLESTEROL ABSORPTION AND PLASMA PLANT STEROL LEVELS

Cholesterol absorption

Genetic studies in the mouse mapped loci in linkage with cholesterol absorption and plasma plant sterols. In one study, David Russell and colleagues (28) identified two inbred mouse strains, 129P3/J and AKR/J, with high cholesterol absorption rates and two inbred mouse strains, DBA/2J and SJL/J, with low cholesterol absorption rates and conducted three different experiments to map loci in linkage with cholesterol absorption. In one experiment, the F1 progeny of AKR/J and DBA/2J were backcrossed onto the low-absorption DBA/2J progenitor strain and cholesterol absorption was measured in 132 N2 males. Genome scan and linkage analysis in 20 N2 males with the highest and 20 N2 males with the lowest percentage cholesterol absorption revealed a locus on chromosome 2 at 64 cM with a logarithm of odds (LOD) score of 3.5 (designated cholesterol absorption 1, *Chab* 1) and a second locus on chromosome 10 at 24 cM with a LOD score of 1.9 (*Chab* 2). In both loci, animals heterozygous for the AKR/J allele displayed increased cholesterol absorption rates. In another approach, these investigators measured the absorption and analyzed the linkage in 21 different DBA × AKR recombinant inbred strains and identified three additional loci: the first on chromosome 6 at 51 cM with a LOD score of 2.0 (*Chab* 3), the second on chromosome 15 at 58 cM with a LOD score of 2.0 (*Chab* 4), and the third on chromosome 19 at 16 cM with an LOD score of 1.6 (*Chab* 5). Homozygosity for the AKR/J allele at *Chab* 3 increased the absorption, whereas homozygosity for the AKR/J allele at *Chab* 4 and *Chab* 5 decreased the absorption of cholesterol. It is of note that the

mapping in N2 animals and in recombinant inbred strains resulted in the identification of different loci. The reason is not entirely clear, but, as pointed out by the authors, this may have occurred due to a combination of insufficient power and hindrance of allele effects as a result of the breeding strategy. In a third experiment, F1 progeny of 129P3/J and SJL/J were backcrossed onto the high-absorption 129P3/J progenitor strain and cholesterol absorption was measured in 168 N2 progeny animals. Linkage analysis in 40 N2 animals with the highest and 40 N2 animals with the lowest percentage cholesterol absorption revealed a locus on chromosome 1 at 57 cM with a LOD score of 2.1 (*Chab* 6) and another locus on chromosome 5 at 57 cM with a LOD score of 3.3 (*Chab* 7). In *Chab* 6 and *Chab* 7, heterozygosity for the SLJ/J allele increased and decreased, respectively, the absorption of cholesterol. Therefore, as summarized in **Table 2**, this study identified seven loci on seven chromosomes in linkage with cholesterol absorption.

Plasma plant sterols

Our group identified two inbred mouse strains with distinctively different concentrations of plasma plant sterols: C57BL/6J, with high plasma plant sterols and castaneus (CASA/Rk), with low plasma plant sterol levels (33). In this experiment, the F1 progeny of C57BL/6J and CASA/Rk were intercrossed, and plasma plant sterols and total cholesterol levels were measured in 201 F2 animals. Linkage analysis of the ratio of plasma campesterol to plasma total cholesterol concentration in the F2s revealed a locus on chromosome 14 at 17 cM with a LOD score of 9.9 (designated plasma plant sterol, *Plast*14) and two loci on chromosome 2, the first at 18 cM with a LOD score of 4.1 (*Plast*2a) and the second at 65 cM with a LOD score of 3.7 (*Plast*2b). No linkage was found of plasma total cholesterol to any of these loci, suggesting that the linkage of campesterol-to-cholesterol ratio can be entirely attributed to variation in plasma campesterol levels. For *Plast*14, *Plast*2a, and *Plast*2b, homozygosity for the C57BL/6J allele increased the plasma levels of plant sterols. In addition, four significant locus interactions with predominantly additive effects were identified. Therefore, as summarized in Table 2, this study identified three loci on two chromosomes in linkage with plasma plant sterol levels.

The results of these two mapping studies deserve further consideration. First, although these studies used different mouse strains and focused on two different measurements, i.e., cholesterol absorption and plasma plant sterol levels, they identified a common locus on chromosome 2 that peaked at 64–65 cM (*Chab* 1 and *Plast*2b). Second, both studies failed to find linkage for cholesterol absorption or plasma plant sterol levels to mouse ABCG5/ABCG8 locus on chromosome 17 (38). Thus, when considered together with the association studies of Berge and his colleagues in humans (34), these studies provide compelling evidence that genes other than ABCG5/ABCG8 control the absorption of cholesterol and the levels of plasma plant sterols. Third, so far no linkage studies have been published on either cholesterol absorption or plasma plant sterol levels in humans. It is expected that studies addressing this gap will increase our understanding of the role of the ABCG5/ABCG8 locus and the relevance of the mouse mapping findings to the human case. Moreover, a combination of linkage analyses in the two species together with genetic manipulation in the mouse are expected to accelerate the identification and increase the understanding of the function of these genes.

EZETIMIBE AND GENETIC CONTROL OF CHOLESTEROL ABSORPTION AND PLASMA PLANT STEROL LEVELS

Studies on a recently developed novel cholesterol absorption-blocking agent, ezetimibe, provide additional insight into the genetics of cholesterol absorption and plasma plant sterol levels. Studies in a number of species including the rat, hamster, mouse, and rhesus monkey, and also in humans have shown that ezetimibe decreases the absorption of cholesterol by 50% to 90% (24, 39, 40). Moreover, the inhibitory effects of ezetimibe are characterized by: *i*) a high potency, as reflected by an inhibition dose 50% of 0.0005 and 0.0015 mg/kg in the rat and rhesus monkey, respectively (40); *ii*) independence of changes in bile acid pool size and composition or hydrolysis and processing of dietary fats within the intestinal lumen (41, 42); and most importantly, *iii*) efficient concentration and retention of the drug at the intestinal villus

TABLE 2. Loci controlling cholesterol absorption and plasma plant sterol levels in the mouse

Strains and Cross	Locus	Chromosome (cM)	LOD	Reference
AKR/J × DBA/2J backcross	*Chab* 1	2 (64)	3.5	Schwarz et al. 2001 (28)
	Chab 2	10 (24)	1.9	
AKR/J × DBA recombinant inbred strains	*Chab* 3	6 (51)	2	Schwarz et al. 2001 (28)
	Chab 4	15 (58)	2	
	Chab 5	19 (16)	1.6	
129P3/J × SJL/J backcross	*Chab* 6	1 (57)	2.1	Schwarz et al. 2001 (28)
	Chab 7	5 (57)	3.3	
C57BL/6J × CASA/Rk intercross	*Plast*14	14 (17)	9.9	Sehayek et al. 2002 (33)
	*Plast*2a	2 (18)	4.1	
	*Plast*2b	2 (65)	3.7	

Chab, cholesterol absorption; cM, centimorgan; LOD, logarithm of odds; *Plast*, plasma plant sterol.

tip (40). Thus, these findings raise the possibility that ezetimibe may exert its effect through interaction(s) with specific enterocytic protein(s). Although at the present time this possibility is entirely speculative, a relevant question is whether ezetimibe exerts its inhibitory effects through ABCG5/ABCG8. Two studies do not support such a possibility. In one study, the effect of an ezetimibe analog on intestinal expression of ABCG5/ABCG8 was examined in the mouse (41). If ezetimibe decreases the absorption of cholesterol through induction of these genes, it is expected that the analog will increase the expression of ABCG5/ABCG8 in the intestine. In contrast, the treatment resulted in a decreased expression of ABCG5/ABCG8. Another study examined the effect of ezetimibe in β-sitosterolemic patients (23). Again, if ezetimibe acts through ABCG5/ABCG8, it is expected that it will fail to affect the plasma levels of plant sterols in these patients. In contrast, the treatment with ezetimibe significantly decreased the concentrations of plasma plant sterol levels in these patients. There is no report yet on the effect of ezetimibe on cholesterol absorption and plasma plant sterol levels in Abcg5/Abcg8 knockout animals or in a recently described rat model with mutation in Abcg5 (43). Therefore, when taken together, these studies indicate that: *i*) the ezetimibe inhibitory effects are independent of ABCG5/ABCG8; and *ii*) if ezetemibe indeed exerts its cholesterol absorption-blocking effect through interaction with enterocytic proteins, these are expected to be different from the ABCG5/ABCG8 hemitransporters. It remains to be seen whether the speculation regarding the interaction of ezetimibe with specific enterocytic proteins will be substantiated.

COMMONALITIES AND DIFFERENCES IN THE GENETICS OF CHOLESTEROL ABSORPTION AND PLASMA PLANT STEROL LEVELS

Several studies suggest that the absorption of cholesterol is related to plant sterol metabolism. Human studies have found that plasma plant sterol levels are correlated with cholesterol absorption rates. One study examined the correlation of cholesterol absorption and plasma plant sterol levels in 17 subjects from two families and found that the ratio of serum campesterol to cholesterol correlates with percent cholesterol absorption ($r = 0.73$) (20). In another study, serum plant sterols and cholesterol absorption were measured in a sample of 63 randomly selected male volunteers of the Finnish population. Again, the serum campesterol-to-cholesterol ratio was significantly correlated with percent cholesterol absorption ($r = 0.42$) (21). These correlations raise the possibility that common processes are involved in the regulation of cholesterol absorption and plasma plant sterol levels. This possibility is further supported by a recent study that examined the effect of ezetimibe on cholesterol absorption and plasma plant sterol levels in humans (24). In this study, treatment with ezetimibe not only decreased the absorption of cholesterol by 54%, but also decreased the plasma level of plant sterols by 41% to 48%. Moreover, there was a large subject-to-subject variability in the response of cholesterol absorption rates and plasma plant sterol levels to ezetimibe treatment. Unfortunately, the correlation of changes in percent absorption and plasma plant sterol levels has not been reported in this study. Therefore, if ezetimibe indeed interacts with specific protein(s) at the intestinal villus, it is reasonable to speculate that the genes that encode for these proteins will be involved in the regulation of plant sterol levels and cholesterol absorption from the intestine. When taken together, these studies point toward commonalities in the processes that regulate cholesterol absorption and plasma plant sterol levels.

In contrast to the above-described evidence, other studies suggest that there are genes that are largely involved in plant sterol metabolism but that have only a moderate effect on cholesterol absorption. For example, in β-sitosterolemic patients, a dramatic increase in the absorption (3- to 12-fold), plasma levels (30- to 100-fold), and body pool size (13- to 100-fold) of plant sterols is associated with cholesterol absorption rates that range in the upper limits of the normal levels (15–19). Moreover, compared with cholesterol, plant sterols are efficiently excreted into the bile, and this has been suggested to protect sitosterolemic heterozygotes, who have increased plant sterol absorption, from plant sterol accumulation (17). Furthermore, targeting the ABCG5/ABCG8 genes in the mouse revealed markedly different effects on plant sterols and cholesterol metabolism (22). For example, when compared with control animals, cereal-based rodent chow-fed Abcg5/Abcg8 knockouts were characterized by a 2- to 3-fold increase in plant sterol absorption rates, a nearly 30-fold increase in plasma plant sterol levels, and a dramatic increase in liver plant sterol content. In contrast, knockout animals displayed no differences in cholesterol absorption rates, a significant decrease in plasma cholesterol levels, and a significant decrease in liver cholesterol content. It should be noted that the studies on cholesterol absorption in the Abcg5/Abcg8 knockouts applied the fecal dual isotope method that, in this particular case, may underestimate the absorption rates. Applying the plasma dual isotope method could solve this problem. Nevertheless, studies in β-sitosterolemic patients that applied the plasma dual isotope methods reported cholesterol absorption rates that rank at the higher end of the normal distribution (16). Therefore, when combined, the findings in β-sitosterolemic subjects and those of studies in the mouse suggest that the ABCG5/ABCG8 locus is primarily involved in the process of plant sterol absorption, whereas the intestinal absorption of cholesterol is largely dependent on other genes. If this is indeed the case, how can ABCG5/ABCG8 play a critical role in the excretion of cholesterol into bile? The answer to this question is unclear, but it is possible that a promiscuous affinity of ABCG5/ABCG8 heterodimers to different sterols may allow efficient cholesterol excretion under conditions in which these transporters are operating in membranes that are particularly enriched

with cholesterol, such as the hepatocytic canalicular membrane.

Therefore, it is possible that genes involved in cholesterol absorption and plant sterol metabolism are characterized by a spectrum of specificity and affinity to different sterols, and that some genes, such as ABCG5/ABCG8, are primarily involved in plant sterol metabolism whereas others are involved in cholesterol absorption. It is expected that the identification of novel genes in these metabolic pathways will enable us to examine the validity of this hypothesis.

FUTURE DIRECTIONS AND SIGNIFICANCE

The 29 years that have passed since the original description of β-sitosterolemia have brought about the discovery of ABCG5 and ABCG8. The current literature strongly suggests that genes other than ABCG5/ABCG8 are involved in the processes of cholesterol absorption and the regulation of plasma plant sterol levels. Furthermore, mapping studies in the mouse suggest that these processes are regulated by a number of genes. The discovery of these genes is expected to provide gains at multiple levels. For example, identification of common variants in genes that affect the absorption of cholesterol may enable: *i*) the screening of young individuals in families at a high risk for cardiovascular diseases with a resulting focus on education and fostering of appropriate dietary habits at an early age; *ii*) the identification of patients who will benefit the most from restricted intake of dietary cholesterol; and *iii*) the selection of appropriate drug intervention in individual patients. Finally, a better understanding of the absorption processes at the molecular level is expected to accelerate the development of drugs that will specifically modulate the absorption process at multiple levels.

The author would like to thank Dr. Jan L. Breslow at The Rockefeller University for a critical reading, thorough discussions, and helpful suggestions in preparing this manuscript. This publication was supported by the American Heart Association grant 0335222N.

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