A complex plasma plant sterol locus on mouse chromosome 14 has at least two genes regulating intestinal sterol absorption

Ephraim Sehayek,[1,*] Yee Yan Fung,[*] Hannah J. Yu,[*] Jan Lembcke,[†] Uta Ceglarek,[†] Daniel Teupser,[†] Joachim Thiery,[†] Dieter Lutjohann,[§] Klaus von Bergmann,[§] and Jan L. Breslow[*]

Laboratory of Biochemical Genetics and Metabolism,* The Rockefeller University, New York, NY; Institute of Laboratory Medicine, Clinical Chemistry, and Molecular Diagnostics,† University Hospital, Leipzig, Germany; and Department of Clinical Pharmacology,§ University of Bonn, Bonn, Germany

Abstract We previously identified two inbred mouse strains, C57BL/6J and CASA/Rk, with different plasma plant sterol levels. An intercross between these strains revealed a broad plasma plant sterol locus on chromosome 14, which peaked at 17 centimorgan (cM) with a maximum logarithm of the odds score of 9.9. Studies in a chromosome 14 congenic strain, 14KK, with a 4–60 cM CASA/Rk interval on the C57BL/6J background revealed that males, but not females, had decreased plasma plant sterol levels and intestinal cholesterol absorption. In two subcongenic strains, 14PKK and 14DKK, with 4–19.5 and 19.5–60 cM CASA/Rk intervals, respectively, both males and females had decreased plasma plant sterol levels and decreased intestinal cholesterol absorption. Compatible with the decreased plasma plant sterol phenotype, 14PKK mice had increased biliary plant sterol excretion, whereas 14DKK mice did not. Therefore, gender-dependent interactions of genes at the 14PKK and 14DKK intervals are likely to underlie the 14KK interval effect on plasma plant sterol levels and sterol absorption from the intestine. These studies confirm the plasma plant sterol locus on mouse chromosome 14 and provide evidence that there are at least two sets of genes operating: one set affecting intestinal sterol absorption and biliary excretion, and the other set mainly affecting intestinal sterol absorption.—Sehayek, E., Y. Y. Fung, H. J. Yu, J. Lembcke, U. Ceglarek, D. Teupser, J. Thiery, D. Lutjohann, K. von Bergmann, and J. L. Breslow. **A complex plasma plant sterol locus on mouse chromosome 14 has at least two genes regulating intestinal sterol absorption.** ***J. Lipid Res.*** **2006.** 47: **2291–2296.**

Supplementary key words cholesterol absorption • bile • genetics

The absorption of cholesterol from the intestine plays an important role in controlling plasma cholesterol levels, which are a well-established risk factor for atherosclerotic cardiovascular diseases. The diet of an average Western adult contains 250–500 mg of cholesterol and 100–250 mg of plant sterols, a specific class of sterols that are synthesized exclusively by plants, poorly absorbed from the diet, and whose plasma levels correlate directly with the intestinal cholesterol absorption rate (1, 2). Studies in humans, under strict metabolic-ward conditions, have found very large individual differences in intestinal cholesterol absorption rates, with some subjects absorbing only 20–30% of their daily cholesterol intake and others absorbing >70% (3). It is likely that genetic factors contribute to this variability. To identify genes controlling cholesterol absorption, we identified two inbred mouse strains, C57BL/6J and CASA/Rk, with different plasma plant sterol levels and through an intercross mapped a broad locus to chromosome 14, which peaks at 17 centimorgan (cM) with a maximum logarithm of the odds (LOD) score of 9.9, and two additional loci on chromosome 2, which peak at 18 and 65 cM with maximum LOD scores of 4.1 and 3.65, respectively (4).

This study was designed to confirm the effect of the chromosome 14 locus on plasma plant sterol levels through the creation and study of congenic and subcongenic mice. We generated one chromosome 14 congenic strain, designated 14KK, with a 4–60 cM CASA/Rk interval, introgressed onto the C57BL/6J background, and two subcongenic strains, designated 14PKK and 14DKK, with 4–19.5 and 19.5–60 cM chromosome 14 CASA/Rk intervals, respectively. Studies in congenic and subcongenic strains revealed that the chromosome 14 locus affects plasma plant sterol levels predominantly through effects on sterol absorption from the intestine and that this locus is complex, with at least two genes controlling the level of plasma plant sterols and cholesterol absorption rates.

Manuscript received 10 May 2006 and in revised form 21 June 2006 and in re-revised form 13 July 2006.

Published, JLR Papers in Press, July 13, 2006.
DOI 10.1194/jlr.M600202-JLR200

Abbreviations: cM, centimorgan; LOD, logarithm of the odds.

[1] To whom correspondence should be addressed.
e-mail: sehayee@rockefeller.edu (E.S.)

METHODS

Animals and diets

We first generated a chromosome 14 congenic strain with a 56 cM CASA/Rk interval, from 4–60 cM, introgressed onto the C57BL/6J background. This congenic strain was then used to generate two subcongenic strains, 14PKK and 14DKK, with 4–19.5 and 19.5–60 cM CASA/Rk intervals, respectively (**Fig. 1**). To generate the 14KK congenic strain, a speed congenic approach was used as described previously for other loci (5). Congenic mice with the C57BL/6J interval on the CASA/Rk background were not generated because the difficulty of breeding mice on this background precluded generating sufficient animals for the speed congenic technique (6). To generate 14PKK and 14DKK subcongenic strains, congenic 14KK males were first backcrossed onto C57BL/6J females to generate animals that are heterozygous at the 14KK interval. 14KK heterozygous males were then backcrossed onto C57BL/6J females, the progeny were genotyped for 11 chromosome 14 markers, and recombinant males and females heterozygous for the CASA/Rk alleles at the 4–19.5 cM interval (between D14Mit251 at 4 cM and D14Mit233 at 19.5 cM) or the 19.5–60 cM interval (between D14Mit259 at 19.5 cM and D14Mit267 at 60 cM) were selected for further breeding. In a last step, heterozygous males and females were intercrossed to generate 14PKK and 14DKK subcongenic animals homozygous for the CASA/Rk genotype at the 4–19.5 and 19.5–60 cM intervals, respectively. It is of note that both congenic strains were homozygous for the C57BL/6J allele at D14Mit19, which maps between D14Mit233 and D14Mit259, confirming that 14PKK and 14DKK are nonoverlapping congenic strains. The experimental design compared either 14KK, 14PKK, or 14DKK animals with wild-type C57BL/6J mice that were age- and sex-matched. The logistical difficulties of breeding the numbers of mice required precluded comparing 14KK, 14PKK, 14DKK, and wild-type mice in the same experiment. All animals were bred and housed in a single humidity- and temperature-controlled room with a 12 h light/dark cycle (6 AM–6 PM light) at the Laboratory Animal Research Center at The Rockefeller University. Animals were fed Picolab Rodent Chow 20 pellets (catalog number 5053) containing 0.02% (w/w) cholesterol. All experiments were approved by the Institutional Animal Care and Research Advisory Committee.

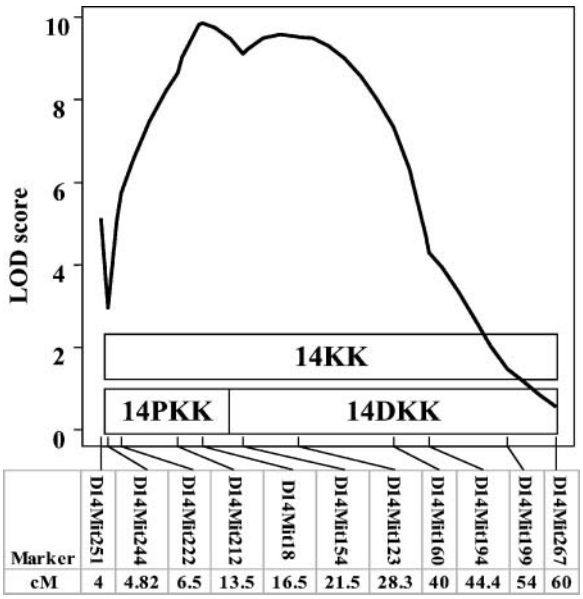

Fig. 1. Interval maps of 14KK congenic and 14PKK and 14DKK subcongenic strains. Shown is the chromosome 14 linkage map for plasma campesterol-to-cholesterol ratio in F2 animals of the original cross (4). Boxed are the intervals covered by 14KK, 14PKK, and 14DKK strains. Congenic and subcongenic strains are homozygous for the CASA/Rk genotype at the chromosome 14 intervals on the C57BL/6J background. cM, centimorgan; LOD, logarithm of the odds.

Genotyping

Animal tail tips were digested with proteinase K, and the extracts were ethanol-precipitated for DNA isolation. Animals were genotyped for microsatellite markers polymorphic between C57BL/6J and CASA/Rk using fluorescently labeled primers for PCR amplification, and allele size was analyzed as described previously (4).

Cholesterol absorption and plasma plant sterol measurements

Eleven week old chromosome 14 congenic or subcongenic mice and age- and sex-matched control C57BL/6J mice were individually placed in metabolic cages. Food consumption was measured and percentage cholesterol absorption determined using a modified form of the dual-isotope method described previously (7). Briefly, animals received an intragastric bolus of 100 μl of olive oil containing 1.67 μCi of [^{14}C]cholesterol (Amersham Biosciences) and 0.67 μCi of [^{3}H]β-sitostanol (American Radiolabeled Chemicals, St. Louis, MO). The animals were returned to metabolic cages, and feces were collected for 24 h. Collected feces were dried by overnight incubation at 55°C, homogenized, and extracted with chloroform-methanol (2:1, v/v), and fecal sterols were counted for ^{14}C and ^{3}H labels. Cholesterol absorption was calculated as described previously (7). For plasma plant sterol measurements, food was removed from the cages at 10 AM and the animals allowed access to water. At 3 PM, mice were anesthetized with an intramuscular injection of ketamine/xylazine, and the abdominal cavity was exposed through a ventral excision. The gallbladder bile was aspirated, and blood was drawn through heart puncture. Plasma was immediately separated by centrifugation, and plasma and bile samples were stored at −80°C until analysis. Cholesterol, campesterol, sitosterol, stigmasterol, and brassicasterol concentrations in plasma and bile were determined by a recently described liquid chromatography tandem mass spectrometry method (8). In this article, plasma plant sterol "levels" are expressed as the ratio of absolute plasma plant sterol concentration (in μg/dl) to absolute total plasma cholesterol concentration (in mg/dl), and absolute biliary plant sterol concentrations are expressed in mg/l. The biliary-to-plasma ratio for each plant sterol is the ratio of absolute bile concentration to the level in plasma.

Statistical analyses

Differences in plasma plant sterol levels, cholesterol absorption rates, biliary plant sterol concentrations, and ratio of bile-to-plasma plant sterol levels between 14KK, 14PKK, or 14DKK animals and their age- and sex-matched C57BL/6J controls were analyzed using an unpaired Student's *t*-test. Values are expressed as means ± SD.

RESULTS

To determine whether the locus on chromosome 14 affects the plasma concentration of plant sterols and the

absorption of cholesterol from the intestine, we generated a chromosome 14 congenic strain, designated 14KK, homozygous for a 56 cM CASA/Rk interval from 4 to 60 cM, that was introgressed onto the C57BL/6J background (Fig. 1). In our original study, the genotypic mean for total plasma campesterol level in F2 mice homozygous for the CASA/Rk allele marker at the peak of the chromosome 14 locus (D14Mit18) was 33% lower than that for F2 mice homozygous for the C57BL/6J allele at this marker (4). To confirm this finding, plasma plant sterol levels were measured in 14KK congenic mice and age- and sex-matched C57BL/6J controls. As shown in **Table 1** for 14KK congenic males, plasma campesterol, sitosterol, stigmasterol, and brassicasterol levels were significantly lower than in age- and sex-matched controls. Furthermore, in 14KK males, plasma levels of campesterol and sitosterol, the two major plasma plant sterols, were 31% and 37% lower than in controls, respectively. Thus, the magnitude of the effect in 14KK males was approximately equal to that seen for the genotypic means of the chromosome 14 locus in F2 mice in the original cross. In contrast to what was seen for congenic males, and as shown in Table 1, 14KK congenic females had plasma plant sterol levels that did not differ from those of age- and sex-matched controls, indicating that the 14KK interval displayed a gender-dependent effect on plasma plant sterol levels.

In 14KK congenic males, lower plasma plant sterol levels could result from a decrease in sterol absorption from the intestine and/or an increase in liver biliary excretion. As a measure of sterol absorption from the intestine, we measured the absorption of cholesterol in 14KK congenic animals. As shown in **Fig. 2** for 14KK congenic males, cholesterol absorption rates were significantly lower than in age- and sex-matched controls (68.8 ± 4.9% vs. 46.4 ± 9.5% in C57BL/6J and 14KK males, respectively; $P < 0.003$). In contrast, in 14KK females, absorption rates did not differ from those of controls (69.4 ± 5.4% vs. 72.4 ± 14.7% in C57BL/6J and 14KK females, respectively; $P = 0.68$). As an index of the biliary excretion of plant sterols, absolute biliary plant sterol levels were measured and the ratio of absolute biliary plant sterol levels to plasma plant sterol levels was calculated. As shown in Table 1, in 14KK male and female congenic mice, biliary plant sterol levels did not differ significantly from those in age- and sex-matched controls, and there was no significant difference for the ratios in congenic 14KK animals and age- and sex-matched controls. Finally, the 14KK interval had no effect on food consumption (data not shown). Therefore, in 14KK males, a decrease in sterol absorption from the intestine is responsible for the decrease in plasma plant sterol levels.

To better understand the effect of the chromosome 14 locus, we generated two subcongenic strains, 14PKK and 14DKK, with different chromosome 14 CASA/Rk intervals, which together cover the entire 14KK interval. As shown in Fig. 1, 14PKK subcongenic mice are homozygous for a 15.5 cM CASA/Rk interval from 4–19.5 cM and contain the peak for plasma plant sterol linkage at 17 cM (4). As shown in **Table 2**, in subcongenic 14PKK males and females, plasma plant sterol levels were significantly lower than in age- and sex-matched controls, with brassicasterol showing only a trend toward lower levels in males. Furthermore, as shown in Fig. 2, in subcongenic 14PKK males and females, cholesterol absorption rates were significantly lower than in age- and sex-matched controls [80.7 ± 3.7% vs. 46.3 ± 13.8% in C57BL/6J and 14PKK males, respectively ($P < 0.0002$) and 75.9 ± 4.4% vs. 44.6 ± 18.9% in C57BL/6J and 14PKK females, respectively ($P < 0.003$)]. Interestingly, as shown in Table 2, although in

TABLE 1. Plasma plant sterol levels, absolute biliary plant sterol concentrations, and ratios of bile-to-plasma plant sterol concentrations in 14KK congenic and age- and sex-matched C57BL/6J males and females

	Males				Females			
Variable	C57BL/6J (5)	14KK (5)	Percentage Difference[a]	*P*	C57BL/6J (5)	14KK (5)	Percentage Difference[b]	*P*
Plasma plant sterol levels[c]								
Campesterol	11.95 ± 0.80	8.30 ± 1.86	−31	<0.004	11.35 ± 0.91	10.19 ± 1.23	−10	0.13
Sitosterol	3.67 ± 0.30	2.32 ± 0.47	−37	<0.0007	2.22 ± 0.33	2.28 ± 0.41	+3	0.80
Stigmasterol	0.23 ± 0.02	0.15 ± 0.02	−35	<0.0003	0.16 ± 0.03	0.16 ± 0.03	−2	0.86
Brassicasterol	0.60 ± 0.05	0.38 ± 0.08	−37	<0.0007	0.67 ± 0.08	0.58 ± 0.10	−13	0.17
Absolute biliary plant sterol concentrations[d]								
Campesterol	49.1 ± 16.2	37.5 ± 17.8	−24	0.31	59.1 ± 10.5	62.5 ± 18.7	+6	0.73
Sitosterol	20.2 ± 7.4	13.7 ± 5.15	−32	0.15	16.9 ± 2.7	20.1 ± 6.1	+19	0.32
Stigmasterol	5.3 ± 2.0	3.8 ± 1.5	−30	0.20	4.1 ± 0.5	4.5 ± 1.3	+10	0.53
Brassicasterol	6.4 ± 2.1	4.6 ± 2.2	−27	0.24	7.5 ± 1.2	7.8 ± 1.9	+3	0.83
Ratios of bile-to-plasma plant sterol concentrations[e]								
Campesterol	4.1 ± 1.5	4.4 ± 1.1	+6	0.78	5.2 ± 1.1	6.1 ± 1.5	+16	0.33
Sitosterol	5.6 ± 2.4	5.8 ± 1.1	+2	0.92	7.8 ± 2.0	8.8 ± 2.2	+12	0.49
Stigmasterol	23.1 ± 9.7	24.1 ± 7.8	+4	0.86	26.9 ± 6.7	28.2 ± 5.1	+5	0.74
Brassicasterol	10.7 ± 3.8	11.8 ± 3.0	+10	0.64	11.5 ± 2.8	13.5 ± 2.7	+17	0.29

Genotype names are shown for each gender with number of animals in parentheses.
[a] Congenic versus age-matched C57BL/6J males.
[b] Congenic versus age-matched C57BL/6J females.
[c] Values are ratios of plasma plant sterol (μg/dl) to plasma total cholesterol (mg/dl).
[d] Values are mg/l of biliary free plant sterol.
[e] Values are ratios of absolute biliary free plant sterol to plasma plant sterol levels.

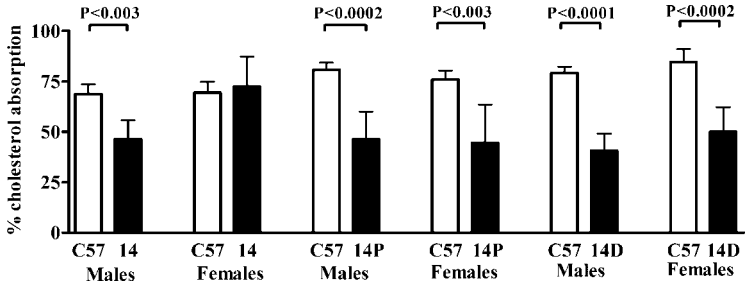

Fig. 2. Cholesterol absorption in congenic 14KK and subcongenic 14PKK and 14DKK males and females. Chow-fed animals were placed in metabolic cages, and cholesterol absorption was measured using a modified form of the dual-isotope method as described in Methods. Congenic (14 = 14KK) and subcongenic (14P = 14PKK, 14D = 14DKK) animals are represented by closed bars, and age- and sex-matched C57BL/6J (C57) males and females are represented by open bars. Values are expressed as means ± SD (n = 5–6 animals per group).

14PKK subcongenic animals absolute biliary plant sterol levels did not differ significantly from those in age- and sex-matched controls, both male and female subcongenic mice displayed differences in bile-to-plasma ratios. Therefore, as shown in Table 2 for 14PKK animals, there were significant increases in the ratios for campesterol and sitosterol, with the ratios for stigmasterol and brassicasterol being nearly significant in males and increased significantly in females. Finally, the 14PKK interval had no effect on food consumption (data not shown). Therefore, in 14PKK males and females, decreased sterol absorption from the intestine in conjunction with increased biliary sterol excretion appears to be responsible for the decrease in plasma plant sterol levels.

As shown in Fig. 1, 14DKK subcongenic animals are homozygous for a 40.5 cM CASA/Rk interval from 19.5–60 cM that covers the distal end of the chromosome 14 locus. As shown in **Table 3** and Fig. 2, subcongenic 14DKK males and females displayed plasma plant sterol levels and cholesterol absorption rates that were significantly lower than in age- and sex-matched controls [cholesterol absorption rates of 79.1 ± 3.2% vs. 40.6 ± 8.5% in C57BL/6J and 14DKK males, respectively ($P < 0.0001$) and 84.6 ± 6.4% vs. 50.1 ± 12.1% in C57BL/6J and 14DKK females, respectively ($P < 0.0002$)]. As shown in Table 3, in 14DKK subcongenic mice, there was a gender-dependent effect on absolute biliary concentrations and the ratio of biliary to plasma plant sterol levels, with no effect in males and both decreased in females (45–61% and 33–47%, respectively). The apparent decreased excretion in females should act to increase plasma plant sterol levels and therefore cannot explain the decreased levels actually observed. Finally, the 14DKK interval had no effect on food consumption (data not shown). Therefore, in 14DKK mice, the decrease in plasma plant sterol levels is attributable primarily to decreased intestinal sterol absorption.

DISCUSSION

This study confirms and extends our previous QTL analysis suggesting a strong locus for plasma plant sterol levels on mouse chromosome 14. Studies in one congenic and two subcongenic strains indicate that the locus is complex, with at least two sets of genes. In the proximal part of the locus, one set of genes operates to decrease intestinal sterol

TABLE 2. Plasma plant sterol levels, absolute biliary plant sterol concentrations, and ratios of bile-to-plasma plant sterol concentrations in 14PKK subcongenic and age- and sex-matched C57BL/6J males and females

	Males				Females			
Variable	C57BL/6J (5)	14PKK (5)	Percentage Difference[a]	*P*	C57BL/6J (6)	14PKK (6)	Percentage Difference[b]	*P*
Plasma plant sterol levels[c]								
Campesterol	27.72 ± 2.84	21.02 ± 1.96	−24	<0.005	24.31 ± 2.86	15.04 ± 1.67	−38	<0.0001
Sitosterol	9.10 ± 0.95	6.68 ± 1.19	−27	<0.01	5.98 ± 0.96	3.84 ± 0.30	−36	<0.0001
Stigmasterol	1.37 ± 0.15	0.85 ± 0.11	−38	<0.0002	0.42 ± 0.05	0.29 ± 0.04	−32	<0.0001
Brassicasterol	1.16 ± 0.11	1.01 ± 0.16	−13	0.12	1.45 ± 0.16	0.78 ± 0.11	−46	<0.0001
Absolute biliary plant sterol concentrations[d]								
Campesterol	85.6 ± 23.2	111.4 ± 32.1	+30	0.16	96.9 ± 9.5	82.1 ± 17.3	−15	0.10
Sitosterol	44.0 ± 11.3	51.5 ± 11.0	+17	0.32	32.0 ± 5.0	30.1 ± 7.0	−4	0.69
Stigmasterol	7.2 ± 2.0	7.8 ± 1.1	+8	0.59	4.0 ± 0.5	3.7 ± 0.8	−8	0.39
Brassicasterol	7.5 ± 2.0	9.8 ± 2.7	+31	0.16	12.5 ± 1.1	10.6 ± 2.7	−15	0.14
Ratios of bile-to-plasma plant sterol concentrations[e]								
Campesterol	3.1 ± 0.7	5.6 ± 1.7	+83	<0.02	4.0 ± 0.4	5.5 ± 1.2	+37	<0.02
Sitosterol	4.8 ± 1.0	8.1 ± 3.3	+70	<0.004	5.4 ± 0.6	7.9 ± 1.6	+48	<0.004
Stigmasterol	5.2 ± 1.2	9.3 ± 2.0	+79	0.06	9.5 ± 1.5	12.9 ± 3.1	+36	<0.04
Brassicasterol	6.4 ± 1.5	10.0 ± 3.5	+57	0.07	8.7 ± 1.5	13.8 ± 3.8	+58	<0.02

Genotype names are shown for each gender with number of animals in parentheses.
[a] Subcongenic versus age-matched C57BL/6J males.
[b] Subcongenic versus age-matched C57BL/6J females.
[c] Values are ratios of plasma plant sterol (μg/dl) to plasma total cholesterol (mg/dl).
[d] Values are mg/l of biliary free plant sterol.
[e] Values are ratios of absolute biliary free plant sterol to plasma plant sterol levels.

TABLE 3. Plasma plant sterol levels, absolute biliary plant sterol concentrations, and ratios of bile-to-plasma plant sterol concentrations in 14DKK subcongenic and age- and sex-matched C57BL/6J males and females

	Males				Females			
Variable	C57BL/6J (5)	14DKK (5)	Percentage Difference[a]	*P*	C57BL/6J (6)	14DKK (6)	Percentage Difference[b]	*P*
Plasma plant sterol levels[c]								
Campesterol	25.9 ± 3.93	13.85 ± 1.83	−45	<0.0005	22.64 ± 1.80	18.68 ± 1.37	−17	<0.002
Sitosterol	7.28 ± 1.01	4.33 ± 0.56	−41	<0.0005	5.21 ± 0.57	3.80 ± 0.36	−27	<0.0001
Stigmasterol	1.25 ± 0.12	0.67 ± 0.12	−46	<0.0001	0.39 ± 0.07	0.30 ± 0.05	−25	<0.02
Brassicasterol	1.15 ± 0.17	0.76 ± 0.09	−34	<0.003	1.40 ± 0.15	1.00 ± 0.10	−28	<0.0003
Absolute biliary plant sterol concentrations[d]								
Campesterol	79.1 ± 30.6	49.0 ± 18.7	−38	0.10	88.8 ± 28.4	41.4 ± 18.1	−53	<0.003
Sitosterol	36.5 ± 13.8	24.9 ± 9.4	−32	0.32	30.2 ± 10.6	12.9 ± 6.0	−57	<0.003
Stigmasterol	6.6 ± 2.6	4.0 ± 1.8	−40	0.40	3.5 ± 1.0	1.9 ± 0.9	−45	<0.009
Brassicasterol	7.6 ± 3.0	5.4 ± 2.1	−29	0.21	13.2 ± 4.4	5.2 ± 2.0	−61	<0.001
Ratios of bile-to-plasma plant sterol concentrations[e]								
Campesterol	3.1 ± 0.9	3.6 ± 1.2	+16	0.49	3.9 ± 1.3	2.2 ± 1.0	−44	<0.01
Sitosterol	4.9 ± 1.5	5.8 ± 2.1	+19	0.45	5.9 ± 2.1	3.3 ± 1.4	−44	<0.02
Stigmasterol	5.3 ± 2.0	6.0 ± 2.4	+14	0.60	9.3 ± 3.7	6.3 ± 2.5	−33	0.06
Brassicasterol	6.5 ± 2.1	7.1 ± 2.4	+10	0.68	9.6 ± 3.3	5.1 ± 1.8	−47	<0.02

Genotype names are shown for each gender with number of animals in parentheses.
[a] Subcongenic versus age-matched C57BL/6J males.
[b] Subcongenic versus age-matched C57BL/6J females.
[c] Values are ratios of plasma plant sterol (μg/dl) to plasma total cholesterol (mg/dl).
[d] Values are mg/l of biliary free plant sterol.
[e] Values are ratios of absolute biliary free plant sterol to plasma plant sterol levels.

absorption and increase biliary sterol excretion, whereas in the distal part of the locus, one or more genes acts mainly to decrease intestinal sterol absorption.

Breakthroughs have occurred in the last few years that have broadened our understanding of the molecular basis of intestinal sterol absorption and biliary sterol excretion. A rare inborn error in plant sterol metabolism, phytosterolemia, was found to be attributable to homozygosity or compound heterozygosity for mutations in two adjacent ATP cassette binding hemitransporters, which map to the short arm of human chromosome 2, ABCG5, and ABCG8 (9, 10). Additional studies have suggested that these hemitransporters must heterodimerize to successfully efflux plant sterols and cholesterol from enterocytes back into the intestinal lumen and from the liver into bile (11). In addition, studies in a relatively homogeneous Micronesian population on the Pacific island of Kosrae have shown that heterozygosity for a dysfunctional ABCG8 allele is sufficient to increase plasma plant sterol levels and suppress the synthesis of cholesterol. This suggests that even carriers of a dysfunctional mutant ABCG8 allele have increased sterol absorption and/or decreased sterol excretion (12). In other studies, a search for transcripts homologous to the human Niemann-Pick C1 gene led to the discovery of the Niemann-Pick C1-Like1 (NPC1L1) gene, which maps to human chromosome 7 and when knocked out in mice results in nearly 70% diminished sterol absorption (13–15). Because studies in cell cultures transfected with NPC1L1 disclosed a complex intracellular trafficking with relation to cellular cholesterol content (16), it appears that NPC1L1 acts in concert with other genes in an intestinal cholesterol absorption pathway not yet fully revealed.

Notwithstanding the discoveries of ABCG5/ABCG8 and NPC1L1, the pathway(s) resulting in net intestinal sterol absorption are complex and probably involve many other genes. This concept is supported by mouse mapping studies of loci that regulate cholesterol absorption and/or plasma plant sterol levels (17). Cholesterol absorption mapping studies using the inbred mouse strains 129P3, AKR, BALB/c, C3H/He, C57BL/6J, DBA/2J, and SJL have identified the following seven loci: chromosome 1 at 57 cM (LOD = 2.1), chromosome 2 at 64 cM (LOD = 3.5), chromosome 5 at 57 cM (LOD = 3.3), chromosome 6 at 51 cM (LOD = 2.0), chromosome 10 at 24 cM (LOD = 1.9), chromosome 15 at 58 cM (LOD = 2.0), and chromosome 19 at 16 cM (LOD = 1.6) (18). Mapping studies for genes regulating plasma plant sterol levels (a surrogate for cholesterol absorption) by our group in an intercross between C57BL/6J and CASA/Rk mice revealed three loci: chromosome 14 at 17 cM (LOD = 9.9), chromosome 2 at 18 cM (LOD = 4.1), and chromosome 2 at 65 cM (LOD = 3.65) (4). It is noteworthy that these loci represent potentially novel genes, because the Abcg5/Abcg8 and Npc1l1 genes map to mouse chromosomes 17 and 11, respectively. The current study suggests at least two genes at the chromosome 14 locus, which predominantly regulate intestinal sterol absorption.

The main uses of the congenic and subcongenic strains reported here are to confirm the chromosome 14 locus for plasma plant sterol levels suggested in our previous quantitative trait locus analysis and to clarify the mechanism(s) whereby this locus affects plasma plant sterol levels. Indeed, studies in 14KK congenic males disclosed that a decrease in sterol absorption from the intestine is likely to explain the decrease in plasma plant sterol levels in this gender. Unexpectedly, however, studies in 14KK females found no effect on either sterol absorption or plasma plant sterol levels. Our original linkage studies on plasma plant sterol levels were done on pooled data of F2 males and females (4); therefore, we reanalyzed the

linkage in each gender separately. Interestingly, the analyses failed to disclose linkage differences that explain the observed gender effect at the 14KK interval (data not shown). Hence, we do not have a satisfactory answer for why our linkage studies failed to predict the gender-dependent effect at the 14KK interval. Therefore, it is likely that gender-dependent gene-gene interactions in a complex locus that harbors at least two genes that modify plasma plant sterol levels failed detection by our analytical tools. Support for the presence of such interactions is provided by our findings in 14PKK and 14DKK subcongenic strains. Our studies in 14PKK animals indicate that the 14PKK interval harbors one or more genes that decrease plasma levels of plant sterols through decreases in sterol absorption from the intestine and increases in liver-biliary excretion. These findings correlate with the Abcg5/Abcg8 effect on sterol metabolism in the intestine and liver, respectively. However, because Abcg5/Abcg8 do not map to the 14PKK interval, it is possible that the causative gene(s) at the 14PKK interval affects plasma plant sterol levels through effects on the activity of Abcg5/Abcg8. Moreover, our studies in 14DKK animals clearly show that the 14DKK interval harbors one or more additional genes that decrease the plasma level of plant sterols, predominantly through decreases in sterol absorption from the intestine. Therefore, gender-dependent interactions of genes at the 14PKK and 14DKK intervals are likely to underlie the 14KK interval effect on plasma plant sterol levels and sterol absorption from the intestine.

In summary, this study indicates that chromosome 14 harbors genes that modify plasma plant sterol levels and the intestinal absorption of cholesterol. Furthermore, the data strongly suggest that the predominant effect of these genes occurs at the intestine. It is expected that further narrowing of this interval through studies in additional subcongenic strains, in conjunction with studies of intestinal expression and sequence variation in genes at this locus, will lead to the discovery of novel genes in the cholesterol absorption pathway.

This work was supported by American Heart Association Grant 0335222N (to E.S.) and National Institutes of Health Grant HL-032435-22 (to J.L.B.).

REFERENCES

1. Tilvis, R. S., and T. A. Miettinen. 1986. Serum plant sterols and their relation to cholesterol absorption. *Am. J. Clin. Nutr.* **43:** 92–97.
2. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesaniemi. 1990. Serum plant sterols and cholesterol precursors reflect cholesterol absorption and synthesis in volunteers of a randomly selected male population. *Am. J. Epidemiol.* **131:** 20–31.
3. Sehayek, E., C. Nath, T. Heinemann, M. McGee, C. E. Seidman, P. Samuel, and J. L. Breslow. 1998. U-shape relationship between change in dietary cholesterol absorption and plasma lipoprotein responsiveness and evidence for extreme interindividual variation in dietary cholesterol absorption in humans. *J. Lipid Res.* **39:** 2415–2422.
4. Sehayek, E., E. M. Duncan, D. Lutjohann, K. Von Bergmann, J. G. Ono, A. K. Batta, G. Salen, and J. L. Breslow. 2002. Loci on chromosomes 14 and 2, distinct from ABCG5/ABCG8, regulate plasma plant sterol levels in a C57BL/6J × CASA/Rk intercross. *Proc. Natl. Acad. Sci. USA.* **99:** 16215–16219.
5. Sehayek, E., L. R. Hagey, Y. Y. Fung, E. M. Duncan, H. J. Yu, G. Eggertsen, I. Bjorkhem, A. F. Hofmann, and J. L. Breslow. Two loci on chromosome 9 control bile acid composition in a C57BL/6J × CASA/Rk intercross: evidence that a strong candidate gene, Cyp8b1, is not the culprit. *J Lipid Res.* Epub ahead of print. June 8, 2006; doi:10.1194/jlr.M600176-JLR200.
6. Markel, P., P. Shu, C. Ebeling, G. A. Carlson, D. L. Nagle, J. S. Smutko, and K. J. Moore. 1997. Theoretical and empirical issues for marker-assisted breeding of congenic mouse strains. *Nat. Genet.* **17:** 280–284.
7. Sehayek, E., J. G. Ono, S. Shefer, L. B. Nguyen, N. Wang, A. K. Batta, G. Salen, J. D. Smith, A. R. Tall, and J. L. Breslow. 1998. Biliary cholesterol excretion: A novel mechanism that regulates dietary cholesterol absorption. *Proc. Natl. Acad. Sci. USA.* **95:** 10194–10199.
8. Lembcke, J., U. Ceglarek, G. M. Fiedler, S. Baumann, A. Leichtle, and J. Thiery. 2005. Rapid quantification of free and esterified phytosterols in human serum using APPI-LC-MS/MS. *J. Lipid Res.* **46:** 21–26.
9. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, et al. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
10. Berge, K. E., H. Tian, G. A. Graf, L. Yu, N. V. Grishin, J. Schultz, P. Kwiterovich, B. Shan, R. Barnes, and H. H. Hobbs. 2000. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science.* **290:** 1771–1775.
11. Graf, G. A., W. P. Li, R. D. Gerard, I. Gelissen, A. White, J. C. Cohen, and H. H. Hobbs. 2002. Coexpression of ATP-binding cassette proteins ABCG5 and ABCG8 permits their transport to the apical surface. *J. Clin. Invest.* **110:** 659–669.
12. Sehayek, E., H. J. Yu, K. von Bergmann, D. Lutjohann, M. Stoffel, E. M. Duncan, L. Garcia-Naveda, J. Salit, M. L. Blundell, J. M. Friedman, et al. 2004. Phytosterolemia on the island of Kosrae: founder effect for a novel ABCG8 mutation results in high carrier rate and increased plasma plant sterol levels. *J. Lipid Res.* **45:** 1608–1613.
13. Davies, J. P., B. Levy, and Y. A. Ioannou. 2000. Evidence for a Niemann-Pick C (NPC) gene family: identification and characterization of NPC1L1. *Genomics.* **65:** 137–145.
14. Altmann, S. W., H. R. Davis, Jr., L. J. Zhu, X. Yao, L. M. Hoos, G. Tetzloff, S. P. Iyer, M. Maguire, A. Golovko, M. Zeng, et al. 2004. Niemann-Pick C1 Like 1 protein is critical for intestinal cholesterol absorption. *Science.* **303:** 1201–1204.
15. Davis, H. R., L. J. Zhu, L. M. Hoos, G. Tetzloff, M. Maguire, J. Liu, X. Yao, S. P. Iyer, M. H. Lam, E. G. Lund, et al. 2004. Niemann-Pick C1 Like 1 (NPC1L1) is the intestinal phytosterol and cholesterol transporter and a key modulator of whole-body cholesterol homeostasis. *J. Biol. Chem* **279:** 33586–33592.
16. Yu, L., S. Bharadwaj, J. M. Brown, Y. Ma, W. Du, M. A. Davis, P. Michaely, P. Liu, M. C. Willingham, and L. L. Rudel. 2006. Cholesterol-regulated translocation of NPC1L1 to the cell surface facilitates free cholesterol uptake. *J. Biol. Chem.* **281:** 6616–6624.
17. Sehayek, E. 2003. Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences. *J. Lipid Res.* **44:** 2030–2038.
18. Schwarz, M., D. L. Davis, B. R. Vick, and D. W. Russell. 2001. Genetic analysis of intestinal cholesterol absorption in inbred mice. *J. Lipid Res.* **42:** 1801–1811.