Common sequence variations in ABCG8 are related to plant sterol metabolism in healthy volunteers

Jogchum Plat,[1] Marjolijn C. E. Bragt, and Ronald P. Mensink

Department of Human Biology, Maastricht University, Maastricht, The Netherlands

Abstract **Polymorphisms in the ATP binding cassette (ABC) transporters ABCG5 and ABCG8 are related to plasma plant sterol concentrations. It is not known whether these polymorphisms are also associated with variations in serum plant sterol concentrations during interventions affecting plant sterol metabolism. We therefore decided to study changes in serum plant sterol concentrations with ABCG5/G8 polymorphisms after consumption of plant stanol esters, which decrease plasma plant sterol concentrations. Cholesterol-standardized serum campesterol and sitosterol concentrations were significantly associated with the ABCG8 T400K genotype, as were changes in serum plant sterol concentrations after consumption of plant stanols. The reduction of -57.1 ± 38.3 $10^2 \times$ μmol/mmol cholesterol for sitosterol in TT subjects was significantly greater compared with the -36.0 ± 18.7 reduction in subjects with the TK genotype ($P = 0.021$) and the -16.9 ± 13.0 reduction in subjects with the KK genotype ($P = 0.047$). Changes in serum campesterol concentrations showed a comparable association. No association with serum LDL cholesterol was found. Genetic variation in ABCG8 not only explains cross-sectional differences in serum plant sterol concentrations but also determines a subject's responsiveness to changes in serum plant sterols during interventions known to affect plant sterol metabolism.**—Plat, J., M. C. E. Bragt, and R. P. Mensink. **Common sequence variations in ABCG8 are related to plant sterol metabolism in healthy volunteers.** ***J. Lipid Res.*** **2005.** 46: **68–75.**

Supplementary key words atherosclerosis • genetics • nutrition

Despite the fact that Western diets provide approximately the same amounts of plant sterols and cholesterol, plasma concentrations of cholesterol are much higher. This is partly attributable to the very low absorption rates of plant sterols, which are less than 2% for campesterol and less than 1% for sitosterol, the two most abundant plant sterols in nature (1). In contrast, cholesterol absorption varies between 30% and 80% (2).

Two recently discovered ATP binding cassette (ABC) transporters, ABCG5 and ABCG8, play an important role in the regulation of intestinal plant sterol absorption by excreting plant sterols that have already been taken up from the enterocyte back into the intestinal lumen (3). ABCG5 and ABCG8 are half-transporters that function together as a heterodimer. Formation of a heterodimer is an absolute necessity to direct the ABCG5/G8 heterodimer from the endoplasmic reticulum to the apical membrane (4). This feature explains earlier observations that mutations in only one of the half-transporters already causes the rare inheritable autosomal recessive disease sitosterolemia (5–7). Sitosterolemic patients are characterized by severely increased serum plant sterol concentrations, normal to moderately increased serum cholesterol concentrations, and a high risk to develop coronary heart disease at a very young age (8, 9). Although not generally accepted, several studies have suggested that increased concentrations of plant sterols are a risk factor for premature atherosclerosis in sitosterolemic patients (10–12) and even in nonsitosterolemic subjects (13, 14).

Besides the various rare mutations in ABCG5 or ABCG8 as observed in sitosterolemic patients (15), more common sequence variations in both half-transporters, without the sitosterolemic phenotype, have been described. These polymorphisms in ABCG5 and ABCG8 are related to serum plant sterol concentrations (16). It is not known, however, whether these genetic variations are also associated with variations in serum plant sterol concentrations during interventions known to affect plant sterol metabolism. We therefore decided to examine the relationships between changes in serum plant sterol concentrations with ABCG5 and ABCG8 polymorphisms after consumption of plant stanol esters, which are known to decrease plasma plant sterol concentrations. Despite the clear association between ABCG5 and ABCG8 and intestinal plant sterol absorption, the role of ABCG5 and ABCG8 in cholesterol absorption is controversial (3, 17–19). Therefore, a second aim of the

Manuscript received 4 June 2004 and in revised form 8 October 2004.

Published, JLR Papers in Press, November 1, 2004.
DOI 10.1194/jlr.M400210-JLR200

[1] To whom correspondence should be addressed.
e-mail: j.plat@hb.unimaas.nl

present study was to look for associations between ABCG5 and ABCG8 polymorphisms with changes in LDL cholesterol after consumption of plant stanol esters.

MATERIALS AND METHODS

Subjects, diets, and design

Details of the study have been described before (20, 21). In brief, 112 healthy nonhypercholesterolemic volunteers (**Table 1**) were asked to replace during a 4 week run-in period their habitual margarines and baking fats for a low-erucic acid rapeseed oil-based margarine and shortening. For the next 8 weeks, subjects were randomly allocated, stratified for gender and age, to one of three intervention groups. The control group (N = 42) continued to use the rapeseed oil-based margarine and shortening, and the second and third groups used the same margarine and shortening to which a vegetable oil-based (N = 36) or a wood-based (N = 34) plant stanol ester mixture was added. The compositions of the experimental products have been described in detail elsewhere (22). The margarine was used at breakfast and lunch, and the shortening was used at dinner. During the intervention period, daily intake of plant stanols in the vegetable oil-based group was 3.8 ± 0.6 g (mean ± SD) and in the wood-based group was 4.0 ± 1.8 g. Plant stanols were esterified with fatty acids from rapeseed oil. All experimental products were prepared by the Raisio Group (Raisio, Finland). Energy intake and the proportions of energy from carbohydrates, fatty acids, and protein, as well as cholesterol and fiber intakes, did not change during the study (20).

Blood sampling and DNA isolation

At the start of the study (day 1), blood was sampled in a 10 ml EDTA tube (Monoject sterile; Sherwood Medical, Ballymoney, Northern Ireland), which was used for DNA isolation (23). At the end of the run-in period (weeks 3 and 4) and at the end of the experimental period (weeks 11 and 12), blood was sampled in a 10 ml clotting tube (CORVAC integrated serum separator tube; Sherwood Medical Co., St. Louis, MO) after an overnight fast. Subjects abstained from drinking alcohol on the day preceding, and from smoking on the morning of, blood sampling. Serum was prepared by centrifugation at 2,000 *g* for 30 min at 4°C, minimally 1 h after venipuncture, and aliquots were stored directly at −80°C for analysis of serum lipids, lipoproteins, plant sterols, plant stanols, and cholesterol precursor concentrations at the end of the study (20, 21).

Analysis of genetic variation in ABCG5 and ABCG8

For this study, we decided to evaluate only associations between serum plant sterols, lipids, and lipoproteins with single nucleotide polymorphisms (SNPs) in ABCG5/G8 with a published population frequency of >10%. Otherwise, the number of subjects in a genotype group could become too low for meaningful statistical analyses. In addition, we have chosen to use only those SNPs that showed a significant cross-sectional association with serum plant sterol or LDL cholesterol concentrations in earlier studies. These criteria resulted in the selection of three SNPs: ABCG8 T400K, ABCG8 A632V, and ABCG5 Q604E.

Genotyping of exons 8 and 13 from ABCG8 and of exon 13 from ABCG5 was performed by analysis of restriction fragment length polymorphisms, as described (15). In brief, PCR amplifications were performed in 20 μl volumes containing 350 ng of genomic DNA, 25 pmol of each nucleotide primer (Sigma Genosys, Cambridge, UK), 1 unit of Taq polymerase (Pharmacia Biotech, Roosendaal, The Netherlands), 0.2 mM of each deoxynucleoside triphosphate (Pharmacia Biotech, Roosendaal, The Netherlands), and 1.5 mM $MgCl_2$. Before amplification, each sample was denatured for 5 min at 95°C. For ABCG8 genotyping, each of the following 30 cycles consisted of 15 s at 96°C (denaturation), 15 s at 60°C (primer annealing), and 30 s at 72°C (extension), followed by 10 min at 72°C (elongation). For ABCG5 genotyping, the program was slightly modified in that annealing occurred for 30 s at 60°C and no elongation step was used. PCR products were digested with specific restriction enzymes, and the DNA fragments obtained were electrophoresed for 1.5 h at 125 V on a 2.5% agarose gel containing Gelstar (Sanvertech, Heerhugowaard, The Netherlands). DNA fragments were visualized by ultraviolet light at 312 nm using a Wratten gelatin filter on a VDS Imagemaster (Pharmacia Biotech, San Francisco, CA). Sequences of the primers have been described (15). The restriction enzymes (New England Biolabs, Beverly MA) used were *Mse*I and *Nco*I for ABCG8 exons 8 and 13, respectively, and *Xmn*I for ABCG5 exon 13.

Statistics

Because of the limited number of subjects, and because plant sterol concentrations were not significantly different, heterozygous and homozygous carriers of the genetic variants [ABCG8 T400K (TK+KK), ABCG8 A632V (VV+VA), and ABCG5 Q604E (QE+EE)] were combined before data analysis. Excluding the homozygous carriers did not affect the conclusions. Linkage disequilibrium was evaluated according to standard procedures and reported as D′ values.

At the end of the run-in period, cross-sectional differences in metabolic parameters between genotype groups were compared with an unpaired *t*-test. When significant, the three genotype groups were also compared using ANOVA with Bonferroni correction to

TABLE 1. Population characteristics before the start of the study

Variable	All	Control Group	Experimental Group
Number of males	41	16	25
Number of females	71	26	45
Age (years)	33 ± 16	33 ± 16	33 ± 15
Body mass index (kg/m^2)	22.9 ± 3.6	23.0 ± 2.8	22.8 ± 4.0
Total cholesterol (mmol/l)	5.02 ± 0.81	4.97 ± 0.73	5.04 ± 0.86
Triacylglycerol (mmol/l)	0.99 ± 0.54	0.88 ± 0.37	1.05 ± 0.62

Values shown are means ± SD.

TABLE 2. Frequency distribution of the different genotypes in exons 8 (T400K) and 13 (A632V) of ABCG8 and in exon 13 (Q604E) of ABCG5

Subjects	All	Control Group	Experimental Group
All	112	42	70
ABCG8 T400K			
TT	77 (68.7)	30 (71.4)	47 (67.1)
TK	31 (27.7)	11 (26.2)	20 (28.6)
KK	4 (3.6)	1 (2.4)	3 (4.3)
ABCG8 A632V			
AA	70 (62.5)	23 (54.8)	47 (67.1)
VA	37 (33.0)	17 (40.5)	20 (28.6)
VV	5 (4.5)	2 (4.8)	3 (4.3)
ABCG5 Q604E			
QQ	81 (72.3)	33 (78.6)	48 (68.6)
QE	29 (25.9)	9 (21.4)	20 (28.6)
EE	2 (1.8)	0 (0)	2 (2.9)

Values shown are absolute frequencies (with relative frequencies in parentheses).

TABLE 3. Linkage disequilibrium between genotypes in exons 8 (T400K) and 13 (A632V) of ABCG8 and in exon 13 (Q604E) of ABCG5

Locus 1	Locus 2	D′	95% Confidence Interval
ABCG8 T400K	ABCG8 A632V	0.29	0.03–0.76
	ABCG5 Q604E	0.81	0.06–0.97
ABCG8 A632V	ABCG5 Q604E	0.0	−0.01–0.22

D′ and the corresponding 95% confidence intervals were calculated by using all 112 subjects included in the study.

determine if relations were dose allele-dependent. In view of their well-known relationships with lipid metabolism, body mass index, gender, and age were considered as potential confounders. However, these parameters were not significantly different between the various genotype groups (data not shown) and were therefore not included into the models.

In a search for gene-diet interactions, changes in metabolic parameters were calculated for each subject as differences between values of the experimental period and the run-in period. Because responses in serum lipoprotein and plant sterol and stanol concentrations were not different between the two experimental groups (20, 21), results of the vegetable oil-based and the wood-based plant stanol ester groups were combined (N = 70). Effects of genotype on these responses were examined with an unpaired *t*-test. Again, allele dose-dependent relationships were examined by ANOVA plus Bonferroni correction.

Because cross-sectional studies (16) have reported that individuals with the TT genotype have higher cholesterol-standardized plasma plant sterol concentrations, we chose not to use analysis of covariance with values from the experimental period as dependent variables and genotype and baseline plant sterol concentrations as independent variables. The two latter parameters are mutually dependent, which creates the possibility of introducing collinearity into the statistical model. All statistical analyses were performed with Statview 4.5 (24).

RESULTS

Frequency distributions for the genotypes in exons 8 (T400K) and 13 (A632V) of ABCG8 and in exon 13 (Q604E) of ABCG5 are shown in **Table 2**. All genotype frequency distributions were in Hardy-Weinberg equilibrium. Significant linkage disequilibrium was found for two of three pairs of the three polymorphisms analyzed (**Table 3**). At the end of the 4 week run-in period, serum concentrations of LDL cholesterol, HDL cholesterol, and triacylglycerol in all subjects (N = 112) were 2.95 ± 0.78, 1.59 ± 0.38, and 0.92 ± 0.52 mmol/l, respectively. Serum concentrations of plant sterols, plant stanols, and lathosterol at the end of the run-in period are shown in **Table 4**.

Next, associations between the different genotypes and serum plant sterol concentrations, both absolute (μmol/l) and cholesterol-standardized (10^2 × μmol/mmol cholesterol), were evaluated. Analysis of absolute and cholesterol-standardized ratios gave similar results, so in the following analysis only serum cholesterol-standardized ratios were used. Both cholesterol-standardized serum campesterol and sitosterol concentrations were associated with the ABCG8

TABLE 4. Relationships between genetic polymorphisms in ABCG8 and ABCG5 with absolute and cholesterol-standardized serum noncholesterol sterol concentrations

Subjects	Campesterol	Sitosterol	Lathosterol	Campestanol	Sitostanol
	Absolute concentrations (μmol/l)				
All	15.8 ± 5.3	6.0 ± 2.4	5.2 ± 2.1	0.5 ± 0.3	0.4 ± 0.2
ABCG8 T400K					
TT	16.9 ± 5.5	6.6 ± 2.6	5.0 ± 2.1	0.5 ± 0.4	0.4 ± 0.2
TK/KK	13.5 ± 3.8	4.8 ± 1.4	5.7 ± 2.1	0.5 ± 0.3	0.4 ± 0.2
P value	<0.001	<0.001	0.070	0.887	0.675
ABCG8 A632V					
AA	16.3 ± 5.6	6.2 ± 2.6	5.1 ± 2.0	0.5 ± 0.3	0.4 ± 0.2
VV/VA	15.1 ± 4.8	5.7 ± 2.1	5.4 ± 2.4	0.6 ± 0.4	0.4 ± 0.2
P value	0.280	0.266	0.516	0.349	0.759
ABCG5 Q604E					
QQ	16.3 ± 5.4	6.3 ± 2.5	5.1 ± 2.1	0.5 ± 0.3	0.4 ± 0.2
QE/EE	14.6 ± 5.0	5.4 ± 2.2	5.4 ± 2.3	0.6 ± 0.4	0.4 ± 0.2
P value	0.125	0.093	0.517	0.043	0.768
	Cholesterol-standardized concentrations (10^2 × μmol/mmol cholesterol)				
All	303.4 ± 98.0	115.0 ± 44.5	97.3 ± 33.5	9.8 ± 6.6	7.6 ± 4.3
ABCG8 T400K					
TT	324.2 ± 98.5	125.2 ± 45.8	93.2 ± 35.0	9.6 ± 6.8	7.5 ± 4.4
TK/KK	257.7 ± 80.8	92.4 ± 31.9	106.4 ± 28.4	10.2 ± 6.1	7.7 ± 3.9
P value	<0.001	<0.001	0.053	0.636	0.862
ABCG8 A632V					
AA	305.8 ± 95.3	117.3 ± 47.3	95.2 ± 32.2	9.3 ± 6.1	7.6 ± 4.2
VV/VA	299.3 ± 103.3	111.0 ± 39.8	101.0 ± 35.8	10.6 ± 7.2	7.6 ± 4.4
P value	0.736	0.467	0.377	0.332	0.938
ABCG5 Q604E					
QQ	310.5 ± 98.8	118.3 ± 43.7	95.3 ± 34.8	8.9 ± 5.7	7.6 ± 4.3
QE/EE	284.8 ± 94.9	106.2 ± 46.2	102.7 ± 29.9	11.9 ± 8.1	7.6 ± 4.2
P value	0.216	0.198	0.298	0.029	0.987

Values shown are means ± SD and were analyzed after a 4-week period of consumption of rapeseed oil-based margarine and shortening.

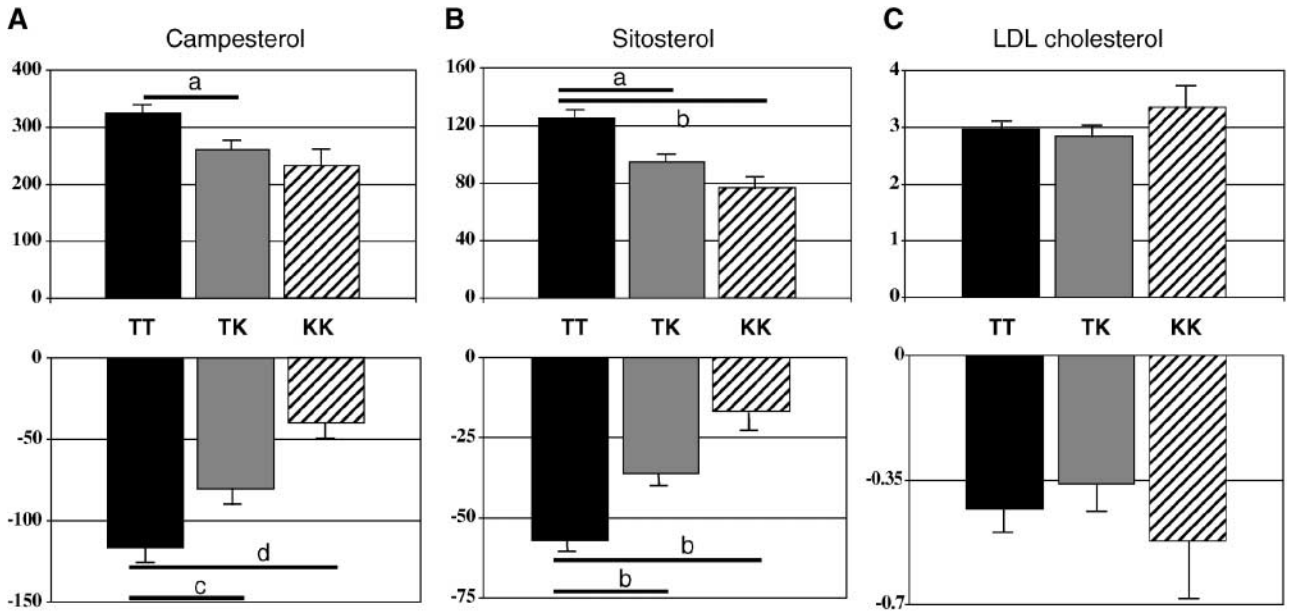

Fig. 1. Cholesterol-standardized campesterol (A), sitosterol (B), and LDL cholesterol (C) concentrations at the end of the run-in period (upper panels) and the change during the stanol ester feeding period (lower panels) in the different genotypes of the ABCG8 T400K polymorphism. Changes in sitosterol and campesterol concentrations are expressed per $10^2 \times$ μmol/mmol cholesterol, and those of LDL cholesterol are expressed in mmol/l. Values shown are means ± SEM. [a] $P < 0.017$; [b] $P < 0.05$; [c] $P = 0.053$; [d] $P = 0.063$.

T400K genotype (Table 4). Moreover, this relationship was allele-dependent (**Fig. 1**). The association with cholesterol-standardized serum lathosterol concentrations nearly reached statistical significance ($P = 0.053$). Differences for cholesterol-standardized serum plant stanol (sitostanol and campestanol) concentrations between the different genotypes never reached statistical significance (Table 4). No associations were found between the polymorphisms in the ABCG8 gene with serum lipids and lipoproteins. Subjects with the QQ genotype (ABCG5 Q604E) showed significantly higher serum LDL cholesterol concentrations compared with QE/EE subjects (**Table 5**).

In the 70 subjects who consumed the plant stanol ester-enriched margarines, cholesterol-standardized serum campesterol and sitosterol concentrations decreased, whereas those of lathosterol increased, compared with the control group (**Table 6**). These changes indicated that plant stanol ester consumption decreased cholesterol absorption and increased cholesterol synthesis, which is in agreement with the known underlying mechanism. As a consequence, LDL cholesterol concentrations were significantly decreased. HDL and triacylglycerol concentrations were not affected (**Table 7**). Changes in cholesterol-standardized serum campesterol and sitosterol concentrations were significantly associated with the ABCG8 T400K polymorphism (Table 6, Fig. 1). The decrease of -116.7 ± 76.5 $10^2 \times$ μmol/mmol cholesterol for campesterol in TT subjects was significantly different from the change of -75.4 ± 46.9 $10^2 \times$ μmol/mmol in subjects with the TK/KK genotype [$P < 0.05$; 95% confidence interval (CI), -76.0 to -6.6 $10^2 \times$ μmol/mmol cholesterol]. The reduction of -57.1 ± 38.3 $10^2 \times$ μmol/mmol cholesterol for sitosterol in TT subjects was also larger compared with the reduction of -33.5 ± 19.0 $10^2 \times$ μmol/mmol cholesterol in subjects with the TK/KK genotypes ($P < 0.01$; 95% CI for the difference in changes, -40.5 to -6.7 $10^2 \times$ μmol/mmol cholesterol). Additional analysis to evaluate the association between the three different genotype groups of the ABCG8 T400K polymorphism (TT, TK, and KK) with changes in serum plant sterol and lipoprotein concentrations suggested an allele-dependent relation for both cholesterol-standardized serum campesterol and sitosterol concentrations (Fig. 1). The reduction of -57.1 ± 38.3 10^2 μmol/mmol cholesterol for sitosterol in TT subjects was significantly greater compared with the -36.0 ± 18.7 in subjects with the TK genotype ($P = 0.021$; 95% CI,

TABLE 5. Relationships between genetic polymorphisms in ABCG8 and ABCG5 with serum lipid and lipoprotein concentrations

Subjects	LDL Cholesterol	HDL Cholesterol	Triacylglycerol
		mmol/l	
All	2.95 ± 0.78	1.59 ± 0.38	0.92 ± 0.52
ABCG8 T400K			
TT	2.97 ± 0.74	1.59 ± 0.37	0.85 ± 0.47
TK/KK	2.89 ± 0.86	1.60 ± 0.40	1.08 ± 0.58
P value	0.622	0.976	0.025
ABCG8 A632V			
AA	3.03 ± 0.78	1.58 ± 0.39	0.91 ± 0.48
VV/VA	2.81 ± 0.77	1.61 ± 0.36	0.93 ± 0.58
P value	0.137	0.666	0.883
ABCG5 Q604E			
QQ	3.04 ± 0.75	1.56 ± 0.35	0.89 ± 0.45
QE/EE	2.70 ± 0.81	1.68 ± 0.43	0.99 ± 0.65
P value	0.039	0.145	0.342

Values shown are means ± SD and were analyzed after a 4-week period of consumption of rapeseed oil-based margarine and shortening.

TABLE 6. Relationships between genetic polymorphisms in ABCG8 and ABCG5 with changes in absolute and cholesterol-standardized serum noncholesterol sterol concentrations after consumption of plant stanol esters

Subjects	Campesterol	Sitosterol	Lathosterol	Campestanol	Sitostanol
			Absolute concentrations (μmol/l)		
Experimental group					
Control	−0.5 ± 2.2	−0.5 ± 1.2	0.0 ± 1.1	−0.0 ± 0.4	0.0 ± 0.2
Stanol	−6.3 ± 3.9	−3.0 ± 2.1	0.2 ± 1.4	0.3 ± 0.4	0.3 ± 0.2
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype group					
ABCG8 T400K					
TT	−7.0 ± 4.4	−3.4 ± 2.3	0.2 ± 1.2	0.2 ± 0.4	0.3 ± 0.2
TK/KK	−4.9 ± 2.4	−2.1 ± 0.9	0.2 ± 1.7	0.3 ± 0.4	0.3 ± 0.2
P value	0.042	0.017	0.989	0.526	0.967
ABCG8 A632V					
AA	−6.5 ± 4.5	−2.9 ± 2.3	0.3 ± 1.1	0.3 ± 0.4	0.3 ± 0.2
VV/VA	−6.0 ± 2.7	−3.0 ± 1.6	0.2 ± 1.3	0.2 ± 0.4	0.3 ± 0.2
P value	0.598	0.831	0.836	0.832	0.395
ABCG5 Q604E					
QQ	−6.6 ± 4.3	−3.1 ± 2.3	0.1 ± 1.5	0.2 ± 0.4	0.3 ± 0.2
QE/EE	−5.8 ± 3.1	−2.7 ± 1.6	0.6 ± 1.2	0.2 ± 0.4	0.4 ± 0.2
P value	0.448	0.413	0.185	0.956	0.097
			Cholesterol-standardized concentrations (10^2 × μmol/mmol cholesterol)		
Experimental group					
Control	−5.9 ± 39.2	−7.8 ± 22.4	0.5 ± 19.5	−0.6 ± 7.2	0.4 ± 4.0
Stanol	−103.1 ± 70.6	−49.3 ± 34.9	17.4 ± 22.2	6.3 ± 8.1	8.2 ± 4.5
P value	<0.001	<0.001	<0.001	<0.001	<0.001
Genotype group					
ABCG8 T400K					
TT	−116.7 ± 76.5	−57.1 ± 38.3	16.5 ± 19.8	6.1 ± 8.3	8.4 ± 4.5
TK/KK	−75.4 ± 46.9	−33.5 ± 19.0	19.2 ± 26.7	6.8 ± 7.6	7.7 ± 4.6
P value	0.020	0.007	0.624	0.737	0.541
ABCG8 A632V					
AA	−107.3 ± 75.4	−49.3 ± 38.8	16.0 ± 23.9	6.1 ± 8.3	8.3 ± 4.4
VV/VA	−94.6 ± 60.3	−49.4 ± 25.9	20.2 ± 18.3	6.7 ± 7.8	8.0 ± 4.9
P value	0.483	0.998	0.465	0.756	0.780
ABCG5 Q604E					
QQ	−106.3 ± 75.6	−50.9 ± 36.9	14.7 ± 21.3	6.3 ± 8.2	7.6 ± 4.2
QE/EE	−96.1 ± 59.2	−46.0 ± 30.6	23.2 ± 23.5	6.3 ± 7.9	9.5 ± 4.9
P value	0.579	0.596	0.140	0.997	0.087

Values shown are means ± SD. Changes in all parameters were calculated as the difference between values at the end of the run-in period (weeks 3 and 4) and the experimental period (weeks 11 and 12).

−39.1 to −3.1 10^2 × μmol/mmol cholesterol) and the −16.9 ± 13.0 in subjects with the KK genotype ($P = 0.047$; 95% CI, −85.2 to −4.8 10^2 × μmol/mmol cholesterol). Changes in serum campesterol concentrations showed a comparable association pattern with the ABCG8 T400K genotype. No statistically significant relationships between the ABCG8 and ABCG5 polymorphisms with changes in serum plant stanol (Table 6), lipid, and lipoprotein (Table 7) concentrations were found.

DISCUSSION

Serum plant sterol concentrations vary widely between individuals (14, 25) but are rather stable over time for a given individual (16, 26). This suggests a strong effect of genetic background on plant sterol metabolism and heritability; indeed, it explains more than 80% of the variability in serum plant sterol concentrations between individuals (16). In agreement, we also found that genetic variation in ABCG8 explained a part of this variability. Our study further showed that subjects with the highest plant sterol concentrations (ABCG8 exon 8, TT genotype) had the largest reduction in serum plant sterol concentrations during consumption of plant stanol esters (Fig. 1). These findings indicate that the functionality of the ABCG5/G8 heterodimer is reduced in subjects with the ABCG8 TT genotype. This may result in decreased transport of plant sterols out of the enterocytes back into the intestinal lumen or from the hepatocytes into bile, and ultimately in increased serum plant sterol concentrations. Why these subjects also showed the largest decrease in serum plant sterol concentrations after consumption of plant stanols is more difficult to explain. It is known that serum plant sterol concentrations are decreased by consumption of plant stanol esters (27–29), probably because plant stanols compete with sterols for incorporation into mixed micelles and hence for uptake into the enterocytes. As plant sterol uptake is not mediated by ABCG5/G8, the lower flux of plant sterols into the enterocyte during plant stanol ester consumption does not depend on the ABCG8 genotype. However, if ABCG8 transports the same proportion of plant sterols out of the enterocytes and hepatocytes into the lumen and bile, respectively, as before plant stanol ester

TABLE 7. Relationships between genetic polymorphisms in ABCG8 and ABCG5 with changes in lipid and lipoprotein concentrations after consumption of plant stanol esters

Subjects	LDL Cholesterol	HDL Cholesterol	Triacylglycerol
Control	−0.06 ± 0.36	0.01 ± 0.16	0.02 ± 0.23
Stanols	−0.42 ± 0.31	0.01 ± 0.12	−0.04 ± 0.30
P value	<0.001	0.903	0.272
ABCG8 T400K			
TT	−0.43 ± 0.32	0.02 ± 0.11	−0.01 ± 0.22
TK/KK	−0.38 ± 0.30	−0.01 ± 0.13	−0.09 ± 0.42
P value	0.531	0.345	0.304
ABCG8 A632V			
AA	−0.42 ± 0.32	0.01 ± 0.12	−0.00 ± 0.25
VV/VA	−0.41 ± 0.29	−0.00 ± 0.13	−0.10 ± 0.38
P value	0.935	0.588	0.199
ABCG5 Q604E			
QQ	−0.44 ± 0.30	0.01 ± 0.10	−0.01 ± 0.34
QE/EE	−0.36 ± 0.34	−0.01 ± 0.16	−0.09 ± 0.18
P value	0.297	0.368	0.357

Values shown are means ± SD. Changes in all parameters were calculated as the difference between values at the end of the run-in period (weeks 3 and 4) and the experimental period (weeks 11 and 12).

consumption, it can be calculated that the circulating concentrations of plant sterols in subjects with the TT genotype (with a lower ABCG8 activity) may decrease to a greater degree than in other subjects. More detailed studies on the effects of the functional consequences of the T400K polymorphism on plant sterol metabolism are needed, which can be obtained through the development of specifically designed cell or transgenic animal models carrying this genetic variation. Concerning the potential functionality of the different genotypes, it should be remarked that the polymorphic site of the ABCG8 T400K polymorphisms is located in a coding region but is predicted not to contain a transmembrane domain, a signature, or a Walker motif (15).

As none of the polymorphisms showed any relationship with (changes in) serum lipid or lipoprotein concentrations, our data suggest that ABCG8 and ABCG5 do not determine the serum lipoprotein profile. This agrees with observations in mice fed chow diets containing 2% cholesterol. In these animals, it was found that disruption of the ABCG8 and ABCG5 genes increased the fractional absorption of dietary plant sterols but not of cholesterol (3, 18, 19). As a result, serum and hepatic plant sterol concentrations were dramatically increased in ABCG5$^{-/-}$/G8$^{-/-}$ (3, 18) and ABCG5$^{-/-}$ (19) mice, whereas serum cholesterol concentrations were not changed. Cholesterol concentrations in the liver were increased, however, which was attributable to a disturbed biliary cholesterol secretion. Unfortunately, biliary plant sterol secretion was not measured in that study, but in view of the increased hepatic plant sterol levels it is likely that biliary plant sterol secretion was disturbed as well. In agreement with these findings, Yu et al. (17) reported that overexpression of ABCG8 and ABCG5 significantly decreased serum plant sterol concentrations, whereas serum cholesterol concentrations were again not different between transgenic and wild-type mice. In contrast to what could be expected from the studies with the ABCG5$^{-/-}$/G8$^{-/-}$ mice, it was reported that these transgenic mice showed a 50% reduction in fractional cholesterol absorption. As discussed by the authors, however, the reduced fractional cholesterol absorption may be secondary to the increased delivery of biliary cholesterol to the intestinal lumen from the liver. Therefore, the question remains whether total intestinal cholesterol absorption is also decreased. In addition, biliary cholesterol levels increased 5- to 7-fold, whereas hepatic cholesterol concentration remained unchanged. The unchanged hepatic and serum cholesterol concentrations could be explained by a counteractive 2- to 4-fold increase in hepatic cholesterol synthesis. Therefore, results in ABCG5/G8 knockout and overexpressing mice showed that ABCG5 and ABCG8 affect serum plant sterol concentrations but not those of cholesterol. In addition, it seems that ABCG8 and ABCG5 play a role in the intestinal absorption of plant sterols, whereas hepatic ABCG8 and ABCG5 are involved in the excretion of cholesterol into bile and possibly also of plant sterols.

Weggemans et al. (30) found that subjects with the ABCG5 Q604E EE genotype had higher serum cholesterol concentrations than carriers with the Q allele. Responses to dietary treatments, however, were not related to this genotype. Other studies did not observe any associations between these polymorphisms with serum lipid concentrations (16, 31, 32), although we found a significantly higher serum LDL cholesterol concentration in subjects with the QQ genotype compared with carriers of the E allele. In a recent study, a statistically significant association was observed between the ABCG8 D19H polymorphism and the proportional reduction in LDL cholesterol during atorvastatin treatment (31). In this respect, Gylling et al. (32) recently showed that the higher efficacy of atorvastatin treatment in DH/HH subjects found earlier (31) might be related to the higher endogenous cholesterol synthesis and lower cholesterol absorption in these subjects compared with DD wild types. As statin treatment may increase plasma plant sterol concentrations (10–12), it would have been interesting to know if changes in serum plant sterol concentrations during atorvastatin treatment were also related to this polymorphism in the ABCG8 gene. Taken together, these studies have not revealed a consistent relationship between ABCG5 or ABCG8 genotypes with serum lipid or lipoprotein concentrations. Furthermore, the observation that the T400K polymorphism in ABCG8 is associated with changes in plant sterol levels with stanol ester treatment, but not with changes in the cholesterol levels, suggests that individual variation in the cholesterol-lowering efficacy of plant stanols is not strongly determined by the magnitude of the reduction in intestinal cholesterol absorption achieved.

Our data indicate that the ABCG8 genotype can predict to what extent serum plant sterol concentrations change after interventions. In our study, plasma plant sterol concentrations decreased, although increases in cholesterol-standardized serum plant sterol concentrations are possible after treatment with statins (10) and consumption of functional foods enriched with plant sterol esters (27, 33).

Whether these increases also relate to the ABCG8 genotype may be relevant to know. In the Scandinavian Simvastatin Survival Study, for example, it was found that changes in plasma plant sterol concentrations after 5 years of treatment were positively related to baseline values. More importantly, however, recurrence of coronary events during simvastatin treatment was not reduced in subjects from the highest quartile of plasma sterol concentrations at baseline, despite the fact that reductions in serum total cholesterol concentrations were comparable in all quartiles (10, 12). These observations do not prove that increased plant sterol concentrations counteract the beneficial effects of statins on cardiovascular risk. However, it would be interesting to know if subjects with the ABCG8 TT genotype were overrepresented in this quartile. If so, then more research is needed to examine whether this drug-genotype interaction should have consequences for optimizing individual-based treatment of hypercholesterolemia.

In conclusion, serum cholesterol-standardized campesterol and sitosterol concentrations, as well as their changes after consumption of plant stanol-enriched foods, are related to a variation in the ABCG8 gene that is present in ~70% of the population. No relationship with serum lipid and lipoprotein concentrations was observed. This suggests that changes in the functionality of the ABCG5/G8 heterodimer mainly affects plasma sterol concentrations, but not those of cholesterol. However, these conclusions need to be confirmed in other studies with other population groups. Whether these findings should have consequences for a patient's optimal cholesterol-lowering drug treatment warrants further investigation.

The authors are indebted to F. Cox for analyzing serum plant sterol concentrations and to M. M. A. van Heugten for dietary counseling throughout the study. This study was supported by the Netherlands Organization for Scientific Research (Project 014-12-010) and by the Raisio Group, Raisio, Finland.

REFERENCES

1. Ostlund, R. E., J. B. McGill, C. M. Zeng, D. F. Covey, J. Stearns, W. F. Stenson, and C. A. Spilburg. 2002. Gastrointestinal absorption and plasma kinetics of soy Delta(5)-phytosterols and phytostanols in humans. *Am. J. Physiol. Endocrinol. Metab.* **282:** E911–E916.
2. Bosner, M. S., L. G. Lange, W. F. Stenson, and R. E. Ostlund, Jr. 1999. Percent cholesterol absorption in normal women and men quantified with dual stable isotopic tracers and negative ion mass spectrometry. *J. Lipid Res.* **40:** 302–308.
3. Yu, L., R. E. Hammer, J. Li-Hawkins, K. von Bergmann, D. Lutjohann, J. C. Cohen, and H. H. Hobbs. 2002. Disruption of ABCG5 and ABCG8 in mice reveals their crucial role in biliary cholesterol secretion. *Proc. Natl. Acad. Sci. USA.* **99:** 16237–16242.
4. 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.
5. Lee, M., K. Lu, and S. Patel. 2001. Genetic basis of sitosterolemia. *Curr. Opin. Lipidol.* **12:** 141–149.
6. 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.
7. Lee, M. H., K. Lu, S. Hazard, H. Yu, S. Shulenin, H. Hidaka, H. Kojima, R. Allikmets, N. Sakuma, R. Pegoraro, A. K. Srivastava, G. Salen, M. Dean, and S. B. Patel. 2001. Identification of a gene, ABCG5, important in the regulation of dietary cholesterol absorption. *Nat. Genet.* **27:** 79–83.
8. Bhattacharyya, A. K., and W. Connor. 1974. β-Sitosterolemia and xanthomatosis. *J. Clin. Invest.* **53:** 1033–1043.
9. Björkhem, I., and K. M. Boberg. 1994. Inborn errors in bile acid biosynthesis and storage of sterols other than cholesterol. *In* The Metabolic Basis of Inherited Diseases. C. R. Scriver, A. L. Beaudet, W. S. Sly, and D. Valle, editors. McGraw Hill, New York. 2073–2100.
10. Miettinen, T. A., T. E. Strandberg, and H. Gylling. 2000. Noncholesterol sterols and cholesterol lowering by long-term simvastatin treatment in coronary patients: relation to basal serum cholestanol. *Arterioscler. Thromb. Vasc. Biol.* **20:** 1340–1346.
11. Miettinen, T. A., H. Gylling, N. Lindbohm, T. E. Miettinen, A. Radhakrishnan, A. Rajaratnam, and H. Relas. 2003. Serum noncholesterol sterols during inhibition of cholesterol synthesis by statins. *J. Lab. Clin. Med.* **141:** 131–137.
12. Miettinen, T. A., H. Gylling, T. Strandberg, and S. Sarna. 1998. Baseline serum cholestanol as predictor of recurrent coronary events in subgroup of Scandinavian Simvastatin Survival Trial. *BMJ.* **316:** 1127–1130.
13. Sudhop, T., B. M. Gottwald, and K. Von Bergmann. 2002. Serum plant sterols as a potential risk factor for coronary heart disease. *Metabolism.* **51:** 1519–1521.
14. Glueck, C. J., J. Speirs, T. Tracey, P. Streicher, E. Illig, and J. Vandegrift. 1991. Relationships of serum plant sterols (phytosterols) and cholesterol in 595 hypercholesterolemic subjects, and familial aggregation of phytosterols, cholesterol, and premature coronary heart disease in hypercholesterolemic probands and their first degree relatives. *Metabolism.* **40:** 842–848.
15. Hubacek, J. A., K. E. Berge, J. C. Cohen, and H. H. Hobbs. 2001. Mutations in ATP-binding cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. *Hum. Mutat.* **452:** 1–5.
16. Berge, K. E., K. Von Bergmann, D. Lutjohann, R. Guerra, S. M. Grundy, H. H. Hobbs, and J. C. Cohen. 2002. Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. *J. Lipid Res.* **43:** 486–494.
17. Yu, L., J. Li-Hawkins, R. E. Hammer, K. E. Berge, J. D. Horton, J. C. Cohen, and H. H. Hobbs. 2002. Overexpression of ABCG5 and ABCG8 promotes biliary cholesterol secretion and reduces fractional absorption of dietary cholesterol. *J. Clin. Invest.* **110:** 671–680.
18. Yu, L., J. York, K. Von Bergmann, D. Lutjohann, J. C. Cohen, and H. H. Hobbs. 2003. Stimulation of cholesterol excretion by the liver X receptor agonist requires ATP-binding cassette transporters G5 and G8. *J. Biol. Chem.* **278:** 15565–15570.
19. Plosch, T., V. W. Bloks, Y. Terasawa, S. Berdy, K. Siegler, F. van der Sluijs, I. P. Kema, A. K. Groen, B. Shan, F. Kuipers, M. Schwarz, and M. Schwarz. 2004. Sitosterolemia in ABC-transporter G5 deficient mice is aggravated on activation of the liver-X receptor. *Gastroenterology.* **126:** 290–300.
20. Plat, J., and R. P. Mensink. 2000. Vegetable oil based versus wood based stanol ester mixtures: effects on serum lipids and hemostatic factors in non-hypercholesterolemic subjects. *Atherosclerosis.* **148:** 101–112.
21. Plat, J., and R. P. Mensink. 2001. Effects of diets enriched with two different plant stanol ester mixtures on plasma ubiquinol-10 and fat-soluble antioxidant concentrations. *Metabolism.* **50:** 520–529.
22. Plat, J., E. N. M. van Onselen, and R. P. Mensink. 1999. Dietary plant stanol ester mixtures: effects on safety parameters and erythrocyte membrane fatty acid composition in non hypercholesterolemic subjects. *Eur. Heart J.* **1 (Suppl.):** 58–63.
23. Plat, J., and R. P. Mensink. 2002. Relationship of genetic variation in genes encoding apolipoprotein A-IV, scavenger receptor BI, HMG-CoA reductase, CETP and apolipoprotein E with cholesterol metabolism and the response to plant stanol ester consumption. *Eur. J. Clin. Invest.* **32:** 242–250.
24. Statview manual (for the Macintosh). 1992. Statview Abacus Concepts, Inc: Berkeley, CA.
25. Kempen, H. J. M., J. F. C. Glatz, J. A. Gevers Leuven, H. A. van der Voort, and M. B. Katan. 1988. Serum lathosterol concentration is an indicator of whole-body cholesterol synthesis in humans. *J. Lipid Res.* **29:** 1149–1155.
26. Li, J. H., A. B. Awad, C. S. Fink, Y. Wu, M. Trevisan, and P. Muti.

2001. Measurement variability of plasma beta-sitosterol and campesterol, two new biomarkers for cancer prevention. *Eur. J. Cancer Prev.* **10:** 245–249.
27. Weststrate, J. A., and G. W. Meijer. 1998. Plant sterol-enriched margarines and reduction of plasma total- and LDL-cholesterol concentrations in normocholesterolaemic and mildly hypercholesterolaemic subjects. *Eur. J. Clin. Nutr.* **52:** 334–343.
28. Gylling, H., R. Radhakrishnan, and T. A. Miettinen. 1997. Reduction of serum cholesterol in postmenopausal women with previous myocardial infarction and cholesterol malabsorption induced by dietary sitostanol ester margarine: women and dietary sitostanol. *Circulation.* **96:** 4226–4231.
29. Miettinen, T. A., P. Puska, H. Gylling, H. Vanhanen, and E. Vartiainen. 1995. Reduction of serum cholesterol with sitostanol-ester margarine in a mildly hypercholesterolemic population. *N. Engl. J. Med.* **333:** 1308–1312.
30. Weggemans, R. M., P. L. Zock, E. S. Tai, J. M. Ordovas, H. O. F. Molhuizen, and M. B. Katan. 2002. ATP binding cassette G5 C1950G polymorphism may affect blood cholesterol concentrations in humans. *Clin. Genet.* **62:** 226–229.
31. Kajinami, K., M. E. Brousseau, C. Nartsupha, J. M. Ordovas, and E. J. Schaefer. 2004. ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. *J. Lipid Res.* **45:** 653–656.
32. Gylling, H., M. Hallikainen, J. Pihlajamaki, J. Agren, M. Laakso, R. A. Ratjaratnam, R. Rauramaa, and T. A. Miettinen. 2004. Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *J. Lipid Res.* **45:** 1660–1665.
33. Naumann, E., J. Plat, and R. P. Mensink. 2003. Changes in serum concentrations of non-cholesterol sterols and lipoproteins in healthy subjects do not depend on the ratio of plant stanols in the diet. *J. Nutr.* **133:** 2741–2747.