Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity

Helena Gylling,[1,*] Maarit Hallikainen,* Jussi Pihlajamäki,[†] Jyrki Ågren,[§] Markku Laakso,[†] Radhakrishnan A. Rajaratnam,[**] Rainer Rauramaa,[††] and Tatu A. Miettinen[**]

Departments of Clinical Nutrition,* Medicine,[†] and Physiology,[§] University of Kuopio, and Kuopio University Hospital, Kuopio, Finland; Kuopio Research Institute of Exercise Medicine,[††] and Department of Clinical Physiology and Nuclear Medicine, Kuopio University Hospital, Kuopio, Finland; and Division of Internal Medicine,[**] Department of Medicine, University of Helsinki, Helsinki, Finland

Abstract **The roles of polymorphisms of the sitosterolemia genes ABCG5 and ABCG8 in the regulation of cholesterol metabolism and insulin sensitivity were studied in mildly hypercholesterolemic noncoronary subjects (n = 263, 144 men and 119 women) divided into tertiles by baseline serum cholestanol-to-cholesterol ratio (≤118.3 and ≥147.7 10^2 × mmol/mol cholesterol), a surrogate marker of cholesterol absorption efficiency. The lowest cholestanol tertile was associated with high body mass index (BMI), plasma glucose, serum insulin and triglycerides, and cholesterol synthesis markers (cholestenol, desmosterol, lathosterol) and low HDL cholesterol and cholesterol absorption markers (campesterol, sitosterol) ($P < 0.01$ for all). The 19H allele of the ABCG8 gene accumulated in the lowest cholestanol tertile ($P < 0.001$) and was associated with low total and LDL cholesterol and absorption markers and with high synthesis markers ($P < 0.05$ for all). The 604E allele of the ABCG5 gene in men was associated with high BMI, plasma insulin, low serum sitosterol, and high serum cholestenol levels ($P < 0.05$ for all). In a subgroup of 71 men, the 604E allele was associated with insulin resistance measured with the hyperinsulinemic euglycemic clamp. In conclusion, low cholesterol absorption efficiency was associated with characteristics of the metabolic syndrome. Low serum cholesterol and cholesterol absorption were linked to the D19H polymorphism of the ABCG8 gene, and characteristics of the insulin resistance syndrome in men were linked with the Q604E polymorphism of the ABCG5 gene.**—Gylling, H., M. Hallikainen, J. Pihlajamäki, J. Ågren, M. Laakso, R. A. Rajaratnam, R. Rauramaa, and T. A. Miettinen. **Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. *J. Lipid Res.* 2004.** 45: **1660–1665.**

Supplementary key words cholesterol synthesis • phytosterols • lathosterol

Serum cholesterol level is regulated by cholesterol absorption and synthesis. In different study populations (1–3), serum total and LDL cholesterol levels are associated positively with cholesterol absorption efficiency and negatively with cholesterol synthesis, suggesting that the higher the cholesterol absorption level, the higher the serum cholesterol level and the lower cholesterol synthesis. Cholesterol absorption efficiency and cholesterol synthesis are inversely related, and they can reliably be depicted by serum noncholesterol sterol levels (2). However, it is not known which of these variables, cholesterol absorption or synthesis, is the one primarily regulated. It has been shown that both absorption efficiency and synthesis of cholesterol are genetically determined (4, 5), such that in coronary families, the heredity of cholesterol metabolism can be predicted by serum cholestanol-to-cholesterol ratio, a surrogate marker of cholesterol absorption efficiency (4). We have recently shown that cholesterol absorption correlates positively and cholesterol synthesis correlates negatively with insulin sensitivity (6), but the link between insulin action and cholesterol metabolism has remained unknown. Phytosterolemia, an inherited disease with high absorption and low biliary secretion of cholesterol and plant sterols, is caused by a mutation in genes regulating ABCG5 (G5) or ABCG8 (G8) transporter proteins (7, 8). Two sequence variations (D19H and T400K) in the G8 gene were shown to be associated with lower serum plant sterol levels in a normolipidemic family study (5). In addition, in mice the overexpression of the human G5 and G8 genes reduced plasma plant sterol levels and cholesterol absorption and increased hepatic cholesterol synthesis and biliary cholesterol secretion (9). Two questions now arise. First, do healthy subjects

Manuscript received 22 December 2003, in revised form 19 April 2004, and in re-revised form 20 May 2004.

Published, JLR Papers in Press, June 1, 2004.
DOI 10.1194/jlr.M300522-JLR200

[1] To whom correspondence should be addressed.
e-mail: helena.gylling@uku.fi

with high cholesterol absorption efficiency have a sequence variation in the G5 or G8 gene resulting in increased intestinal absorption of sterols? Second, could polymorphisms in these genes regulate insulin action? To answer these questions, we recruited noncoronary subjects with mild to moderate hypercholesterolemia and with cholesterol absorption efficiency ranging from low to high assayed with serum cholestanol-to-cholesterol ratio and evaluated the effect of common sequence variants of the G5 and G8 genes (5, 10) on cholesterol absorption and insulin action in these subjects.

MATERIALS AND METHODS

Study population

Two-hundred sixty-three mildly to moderately hypercholesterolemic subjects (serum cholesterol < 7.5 mmol/l), 144 men and 119 women with a mean age of 53.1 ± 0.5 years (mean ± SEM) were recruited to the study from former studies carried out at the Departments of Clinical Nutrition and Medicine, University of Kuopio (11), the Department of Medicine, University of Helsinki (12), and the Kuopio Research Institute of Exercise Medicine (**Table 1**). Thirty-six subjects had hypertension, seven subjects were on thyroid hormone therapy from 15 to 26 years, and they all were euthyroid. Two subjects had symptomless coeliacia under good control. None of the subjects had diabetes, hepatic or malignant disease, or lipid-lowering therapy. Thirty-nine women had hormone replacement therapy, and seven used oral contraceptives or had a hormone-releasing intrauterine device. Sixteen subjects used β-blocking agents, 10 used calcium channel blockers, 21 used angiotensin-converting enzyme or receptor blocking agents, and 13 used diuretics for hypertension. Fifty-four were smokers. The subjects were advised to keep their normal habitual diet and daily drug treatment unchanged. Seventy-one healthy men participated in a hyperinsulinemic euglycemic clamp study to evaluate insulin metabolism (11).

A blood sample was drawn after an overnight fast. All participants volunteered for the study, and the subjects gave their informed consent. The study protocol was approved by the ethics committees of the Department of Medicine, University of Helsinki, University of Kuopio, and the Kuopio University Hospital.

Chemical analyses

Serum and lipoprotein cholesterol and triglycerides were analyzed with commercial kits (CHOD-PAP and GPO-PAP; Roche Diagnostics, Mannheim, Germany). Plasma glucose (dehydrogenase method; Granutest 500, Merck, Darmstadt, Germany) and serum insulin (radioimmunoassay; Phadeseph Insulin RIA, Pharmacia, Uppsala, Sweden) were quantified with commercial kits. Blood hemoglobin and thrombocytes and serum thyroid-stimulating hormone, alanine aminotransferase, and creatinine were analyzed by routine hospital laboratory methods.

Serum cholesterol and its precursors squalene, cholestenol, desmosterol, and lathosterol, reflecting cholesterol synthesis (2), and the plant sterols campesterol and sitosterol as well as cholestanol (a metabolite of cholesterol), sterols reflecting cholesterol absorption efficiency (2, 4), were quantitated with gas-liquid chromatography (GLC) on a 50 m long capillary column (Ultra 1; Hewlett-Packard, Wilmington, DE) using 5α-cholestane as an internal standard (13). The squalene and noncholesterol sterol values were expressed in terms of $10^2 \times$ mmol/mol cholesterol (called ratio in the text), dividing the squalene and sterol values by the cholesterol value of the same GLC run to eliminate the effects of different serum cholesterol concentrations.

Determination of insulin sensitivity

The degree of insulin sensitivity was evaluated by the hyperinsulinemic euglycemic clamp and indirect calorimetry in 71 healthy men. Euglycemic clamp (14) was performed after a 12 h fast as previously described (11). After baseline blood drawing, a priming dose of insulin (Actrapid 100 IU/ml, Novo Nordisk, Gentofte, Denmark) was administered during the initial 10 min

TABLE 1. Demographic data, plasma glucose and serum insulin, serum lipids, squalene, and noncholesterol sterols in tertiles by serum cholestanol-to-cholesterol ratio in the study population (n = 263)

	Proband			
Variable	Low Absorbers (n = 87)	Intermediate Absorbers (n = 89)	High Absorbers (n = 87)	P^a
Male/female	48/39	53/36	43/44	
Age (years)	54.4 ± 0.7	53.1 ± 0.8	51.7 ± 1.2	0.117
Body mass index (kg/m^2)	28.1 ± 0.4	26.6 ± 0.4	24.6 ± 0.3	<0.001
Plasma glucose (mmol/l)	5.79 ± 0.07	5.61 ± 0.08	5.38 ± 0.07	<0.001
Serum insulin (mU/l)	11.03 ± 0.75	8.01 ± 0.46	6.84 ± 0.39	<0.001
Serum cholesterol (mmol/l)	5.71 ± 0.11	5.80 ± 0.11	5.75 ± 0.12	0.874
LDL cholesterol (mmol/l)	3.64 ± 0.09	3.79 ± 0.11	3.67 ± 0.12	0.549
HDL cholesterol (mmol/l)	1.36 ± 0.04	1.45 ± 0.04	1.56 ± 0.04	0.002
Serum triglycerides (mmol/l)	1.61 ± 0.14	1.23 ± 0.06	1.12 ± 0.07	<0.001
Squalene[b]	32 ± 1	31 ± 1	30 ± 1	0.500
Cholestenol[b]	18 ± 1	15 ± 1	14 ± 1	<0.001
Desmosterol[b]	82 ± 2	78 ± 2	73 ± 3	0.002
Lathosterol[b]	217 ± 5	170 ± 5	138 ± 5	<0.001
Campesterol[b]	174 ± 6	232 ± 8	330 ± 13	<0.001
Sitosterol[b]	101 ± 3	123 ± 4	172 ± 6	<0.001
Cholestanol[b]	101 ± 1	132 ± 1	177 ± 3	<0.001
Apolipoproteins E2/E3/E4[c]	5/48/29	6/55/25	4/52/24	

Values shown are means ± SEM. For cholesterol, to obtain mg/dl, multiply by 38.6; for triglycerides, multiply by 88.2. For insulin, to obtain pmol/l, multiply by 6.

[a] Significance between the probands of different tertiles analyzed with one-way ANOVA.

[b] $10^2 \times$ mmol/mol cholesterol.

[c] Apolipoprotein E genotypes E2 (2/3, 2/4), E3 (3/3), and E4 (4/3, 4/4).

to increase plasma insulin concentration quickly to the desired level, where it was maintained by a continuous insulin infusion of 480 pmol/m^2/min. Blood glucose was clamped at 5.0 mmol/l for the next 180 min by the infusion of 20% glucose at varying rates according to blood glucose measurements performed at 5 min intervals. The mean rates of glucose infusion during the last hour of the clamp procedure were used to calculate the rates of insulin-stimulated whole-body glucose uptake. The coefficient for variation of blood glucose was <4% during the clamp procedure.

DNA sequence variants in the ABCG5, ABCG8, and apoE genes

Five previously identified common polymorphisms of the G5 and G8 genes were assayed by PCR amplification and restriction fragment length polymorphism analysis (5, 9) in 262 subjects. The restriction enzymes used were *Tru*1I (Thr400→Lys, exon 8, G8), *Nco*I (Ala632→Val, exon 13, G8), and *Pdm*I (Gln604→Glu, exon 13, G5). The polymorphic sites Asp19→His in exon 1 and Tyr54→Cys in exon 2 of the G8 gene were analyzed with the single-strand conformation polymorphism analysis. The apoE genotype was analyzed by PCR amplification and restriction fragment length polymorphism (15) analysis in 248 subjects.

Statistical analyses

All statistical analyses were performed with the SPSS for Windows 11.5 statistics program (SPSS, Chicago, IL). The results are given as means ± SEM. Normal distribution and homogeneity of variance were checked before further analyses. Logarithmic, square root, or inverse transformation was performed for variables that were not normally distributed or homogenous in variances. One-way ANOVA and Scheffe's test or Student's *t*-test were used for between-group comparisons. When BMI was included as a covariate, analysis of covariance was used for between-group comparisons with Bonferroni adjustment. Those variables that were not normally distributed even after different transformations, or were not homogenous in variances, or were noncontinuous were tested with the Kruskal-Wallis test, the Mann-Whitney test, or the Chi-square test, when appropriate.

RESULTS

Variables of glucose and cholesterol metabolism in serum cholestanol tertiles

The subjects were divided into tertiles by baseline serum cholestanol-to-cholesterol ratio (lowest and highest cholestanol tertiles ≤118.30 and ≥147.73 10^2 × mmol/mol cholesterol), so that the tertiles illustrated subjects with low to high absorption efficiency of cholesterol. The results in the different tertiles shown in Table 1 were similar on or off β-blockers, diuretics, and other blood pressure medications, hormone replacement therapy, or oral contraceptives.

In both genders, BMI, plasma glucose, serum insulin, and serum triglycerides were related negatively and HDL cholesterol was related positively to increasing cholestanol tertiles (Table 1) even when BMI was used as a covariate (except for HDL cholesterol; $P = 0.086$). Ratios of the synthesis marker sterols (cholestenol, desmosterol, and lathosterol), but not of squalene, were related negatively, and those of the absorption markers (campesterol and sitosterol) were related positively to an increase in serum cholestanol level, and except for cholestenol and desmosterol, all associations remained significant after adjustment for BMI. Thus, the findings in the first tertile resemble those seen in the metabolic syndrome. ApoE genotype distribution did not differ among the tertiles.

Polymorphisms of the ABCG5 and ABCG8 genes

The genotype frequencies of the different polymorphisms were in Hardy-Weinberg equilibrium in the whole study group and also in subgroups of different cholesterol absorption tertiles. All five polymorphisms were in linkage disequilibrium with each other (density = 0.63–0.95; $P < 0.05$). The D19H polymorphism of the G8 gene was unevenly distributed in the cholestanol tertiles (Chi square = 15.292; $P < 0.001$), so that the 19H allele was more common in the first tertile compared with the other tertiles (**Table 2**). ApoE genotype distribution did not differ among the ABCG5 and ABCG8 genotype groups.

The 19H allele of the G8 gene was associated with lower serum total cholesterol (5.4 ± 0.2 vs. 5.8 ± 0.1 mmol/l; $P < 0.05$) and LDL cholesterol (3.3 ± 0.1 vs. 3.8 ± 0.1 mmol/l; $P < 0.05$) levels, higher serum cholestenol and lathosterol ratios, and lower serum campesterol, sitosterol, and cholestanol ratios than in subjects without the allele (**Table 3**). Compared with the wild type, other alleles of the G8 gene were associated with lower serum triglyceride level (400K) and lower plasma glucose level (632V). There were no differences in age or gender between the genotypes. The results were similar in men and women.

The 604E allele of the G5 gene was associated with high BMI, serum insulin level, and serum cholestenol ratio and with low serum campesterol and sitosterol ratios (Table 3). In men, the following variables were significantly different between the groups with or without the 604E allele: BMI (28.4 ± 0.6 vs. 26.3 ± 0.3 kg/m^2; $P = 0.002$), serum

TABLE 2. Frequencies of five common polymorphisms of the ABCG8 and ABCG5 genes in subjects with mild to moderate hypercholesterolemia in the tertiles of cholestanol-to-cholesterol ratio (n = 262)

Polymorphism	Low Absorbers (n = 87)	Intermediate Absorbers (n = 88)	High Absorbers (n = 87)
ABCG8:D19H			
Wild type	64	77	82[a]
Heterozygotes plus homozygotes	23	11	5
ABCG8:Y54C			
Wild type	22	27	26
Heterozygotes plus homozygotes	65	61	61
ABCG8:T400K			
Wild type	49	52	53
Heterozygotes plus homozygotes	38	36	34
ABCG8:A632V			
Wild type	55	55	55
Heterozygotes plus homozygotes	32	33	32
ABCG5:Q604E			
Wild type	65	68	72
Heterozygotes plus homozygotes	22	20	15

[a] Chi-square = 15.292 ($P < 0.001$).

TABLE 3. Five common polymorphisms of the ABCG8 genes and variables of glucose and cholesterol metabolism in probands (n = 262) with mild to moderate hypercholesterolemia

	ABCG8:D19H		ABCG8:Y54C		ABCG8:T400K		ABCG8:A632V		ABCG:5Q604E	
Variable	Wild Type	Heterozygotes plus Homozygotes	Wild Type	Heterozygotes plus Homozygotes	Wild Type	Heterozygotes plus Homozygotes	Wild Type	Heterozygotes plus Homozygotes	Wild Type	Heterozygotes plus Homozygotes
Male/female	119/104	24/15	37/38	106/81	91/63	52/56	91/74	52/45	110/95	33/24
Age (years)	53.1 ± 0.6	52.8 ± 1.5	53.9 ± 0.9	52.7 ± 0.7	52.6 ± 0.7	53.6 ± 0.7	53.6 ± 0.6	52.1 ± 1.0	53.2 ± 0.6	52.6 ± 1.2
Body mass index (kg/m^2)	26.3 ± 0.3	26.8 ± 0.5	26.2 ± 0.4	26.5 ± 0.3	26.6 ± 0.3	26.1 ± 0.4	26.4 ± 0.3	26.3 ± 0.4	26.2 ± 0.3	27.3 ± 0.5[a]
Plasma glucose (mmol/l)	5.40 ± 0.05	5.66 ± 0.12	5.54 ± 0.08	5.62 ± 0.05	5.65 ± 0.06	5.52 ± 0.06	5.65 ± 0.06	5.50 ± 0.06[a]	5.58 ± 0.05	5.64 ± 0.09
Serum insulin (mU/l)	8.53 ± 0.35	9.06 ± 1.06	8.49 ± 0.68	8.66 ± 0.39	8.79 ± 0.43	8.35 ± 0.55	8.49 ± 0.39	8.81 ± 0.62	8.01 ± 0.33	10.74 ± 0.95[b]
Serum cholesterol (mmol/l)	5.82 ± 0.07	5.37 ± 0.15[a]	5.63 ± 0.12	5.81 ± 0.08	5.81 ± 0.09	5.67 ± 0.10	5.83 ± 0.08	5.63 ± 0.10	5.75 ± 0.07	5.78 ± 0.16
LDL cholesterol (mmol/l)	3.78 ± 0.07	3.29 ± 0.14[a]	3.59 ± 0.11	3.75 ± 0.07	3.74 ± 0.08	3.65 ± 0.10	3.78 ± 0.08	3.57 ± 0.10	3.71 ± 0.07	3.67 ± 0.14
HDL cholesterol (mmol/l)	1.45 ± 0.03	1.49 ± 0.05	1.50 ± 0.05	1.44 ± 0.03	1.43 ± 0.03	1.49 ± 0.04	1.45 ± 0.03	1.47 ± 0.03	1.46 ± 0.03	1.44 ± 0.05
Serum triglycerides (mmol/l)	1.32 ± 0.06	1.30 ± 0.10	1.20 ± 0.07	1.37 ± 0.07	1.42 ± 0.09	1.17 ± 0.06[a]	1.31 ± 0.06	1.34 ± 0.11	1.27 ± 0.05	1.50 ± 0.18
Squalene[c]	31 ± 1	31 ± 2	30 ± 1	31 ± 1	31 ± 1	31 ± 1	31 ± 1	30 ± 1	31 ± 1	30 ± 1
Cholestenol[c]	15 ± 1	19 ± 1[b]	15 ± 1	16 ± 1	16 ± 1	15 ± 1	16 ± 1	16 ± 1	15 ± 1	18 ± 1[b]
Desmosterol[c]	77 ± 1	84 ± 3	78 ± 2	78 ± 1	79 ± 2	77 ± 2	78 ± 2	78 ± 2	77 ± 1	81 ± 2
Lathosterol[c]	169 ± 4	202 ± 9[b]	176 ± 6	174 ± 4	176 ± 5	172 ± 5	175 ± 5	174 ± 5	171 ± 4	188 ± 9
Campesterol[c]	258 ± 7	174 ± 12[d]	249 ± 13	244 ± 8	244 ± 9	247 ± 10	243 ± 9	250 ± 11	252 ± 8	223 ± 15[b]
Sitosterol[c]	138 ± 3	99 ± 5[d]	138 ± 7	129 ± 4	131 ± 4	133 ± 5	132 ± 4	132 ± 5	136 ± 4	117 ± 7[b]
Cholestanol[c]	141 ± 2	116 ± 4[d]	137 ± 4	137 ± 3	138 ± 3	135 ± 3	135 ± 3	141 ± 4	138 ± 2	132 ± 6

Values shown are means ± SEM. For cholesterol, to obtain mg/dl, multiply by 38.6; for triglycerides, multiply by 88.2. For insulin, to obtain pmol/l, multiply by 6.

[a,b,d] Significant difference between the wild types and the heterozygotes plus homozygotes analyzed with the *t*-test or the Mann-Whitney test: [a] $P < 0.05$; [b] $P < 0.01$; [d] $P < 0.001$.

[c] $10^2 \times$ mmol/mol cholesterol.

insulin (11.8 ± 1.1 vs. 8.2 ± 0.5 mU/l; $P < 0.001$), serum cholestenol (18 ± 1 vs. 15 ± 1; $P = 0.014$), serum lathosterol (198 ± 11 vs. 169 ± 5; $P = 0.020$), serum campesterol (202 ± 18 vs. 252 ± 11; $P = 0.016$), serum sitosterol (105 ± 7 vs. 135 ± 5; $P = 0.004$), and serum cholestanol (121 ± 5 vs. 138 ± 3; $P = 0.010$) ratios (all serum sterols are in terms of $10^2 \times$ mmol/mol cholesterol). However, after recalculation with BMI as a covariate, the higher serum insulin and cholestenol ratios and the lower serum sitosterol ratio in men with but not without the 604E allele remained significant. In women, only serum cholestenol ratio was significantly higher in the subjects with than without the allele. In the subgroup of 71 men volunteering in the hyperinsulinemic euglycemic clamp study, 17 had the 604E allele. The whole-body glucose uptake was 52 ± 3 μmol/kg/min in the subjects with the allele and 60 ± 2 μmol/kg/min in the subjects without the allele ($P = 0.041$).

The metabolic variables were examined in the cholestanol tertiles in subjects with and without the D19H polymorphism of the G8 gene (**Fig. 1**). In subjects with the 19H allele, serum sitosterol ratio was not increased with increasing cholestanol tertiles, whereas in subjects with the wild-type gene, there was an increasing trend. Serum cholestenol ratio was increased with the cholestanol tertiles in subjects with the 19H allele, and the difference in the third tertile between subjects with and without the allele was significant ($P < 0.05$).

Because the D19H polymorphism of the ABCG8 gene and the Q604E polymorphism of the ABCG5 gene, the polymorphisms most strongly associated with cholesterol absorption and insulin action, were in linkage disequilibrium, we analyzed the effect of combined genotypes of these two polymorphisms on the levels of cholestanol (a marker of cholesterol absorption) and fasting insulin (a marker of insulin action). **Figure 2** demonstrates that the D19H polymorphism of the G8 gene was associated more strongly with cholesterol absorption and the Q604E polymorphism of the G5 gene was associated with fasting insulin.

DISCUSSION

The new findings in the present study were, first, that in a mildly to moderately hypercholesterolemic noncoronary population, subjects with the lowest tertile of serum cholestanol-to-cholesterol ratio, a surrogate marker of low cholesterol absorption efficiency, had characteristics of the metabolic syndrome, including overweight, high plasma glucose and serum triglyceride and insulin levels, high levels of the cholesterol synthesis markers in serum, and low HDL cholesterol level. This complex association was found earlier in coronary families (4). Second, polymorphisms of the G8 and G5 genes were associated with different variables of glucose and cholesterol metabolism. Although some of the associations could be considered sporadic, two of the polymorphisms seemed to be related to glucose and cholesterol metabolism. The D19H polymorphism of the G8 gene was most strongly associated with cholesterol absorption, and the Q604E polymorphism of the G5 gene was associated with insulin action.

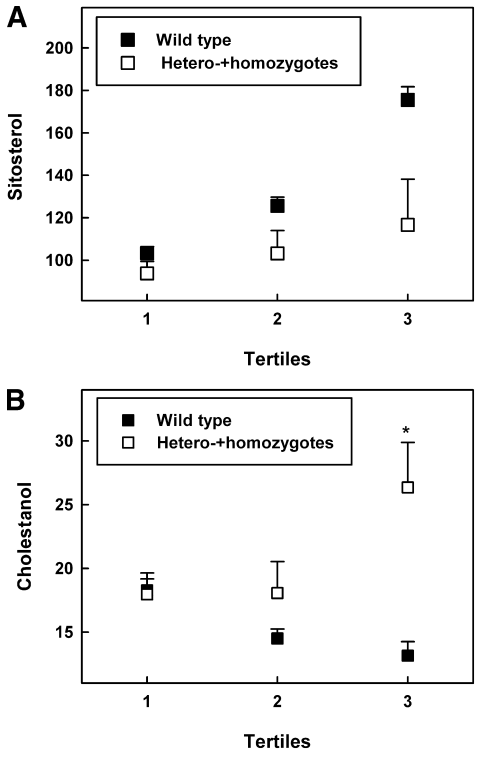

Fig. 1. Serum sitosterol (upper panel) and cholestenol (lower panel) ratios ($10^2 \times$ mmol/mol cholesterol) in tertiles by cholestanol ratio ($10^2 \times$ mmol/mol cholesterol) in subjects with (open squares) and without (closed squares) the 19H allele of the ABCG8 gene. Error bars represent mean ± SEM. * $P < 0.05$ from the wild type.

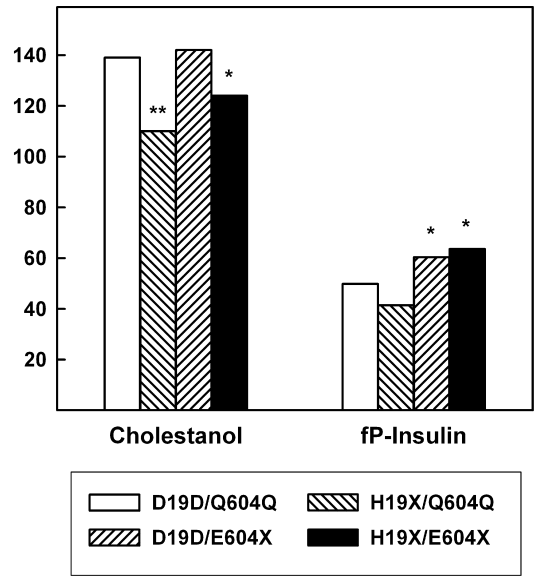

Fig. 2. Effect of the combined genotypes of the D19H and Q604E polymorphisms of the G8 and G5 genes on serum cholestanol-to-cholesterol ratio ($10^2 \times$ mmol/mol cholesterol) and serum insulin level (mU/l) in the study population (n = 262). * $P < 0.05$ and *** $P < 0.001$ from the wild type. fP-insulin, fasting plasma insulin.

The 19H allele of the G8 gene separated subjects with lower serum total and LDL cholesterol level, lower cholesterol absorption marker sterols, and higher cholesterol synthesis marker sterols. The Q604K polymorphism of the G5 gene was associated with obesity, low cholesterol absorption marker level, high serum fasting insulin level, and impaired whole-body glucose uptake, suggesting insulin resistance in men.

We have shown earlier that coronary subjects with low cholesterol absorption markers are more overweight and have high cholesterol synthesis marker sterols (16) and high serum triglycerides and low HDL cholesterol levels (17). The present study confirms these findings in non-coronary subjects and shows in addition that high plasma glucose, even within the normal range, and high serum insulin levels are found in subjects with low absorption and high synthesis of cholesterol. Low cholesterol absorption and high synthesis has been observed in nondiabetic subjects with high-normal blood glucose levels (18) and in patients with type 2 diabetes (19), in whom cholesterol absorption was increased after weight reduction, together with improved variables of insulin resistance (20). Accordingly, it seems quite evident from studies in different populations that low cholesterol absorption efficiency and high cholesterol synthesis are part of the insulin resistance syndrome. This has been confirmed in normoglycemic men in insulin clamp studies (6).

The question then arises of whether the regulation of cholesterol absorption efficiency or cholesterol synthesis is genetically regulated. In mice, disruption of G5 and G8 results in a 2- to 3-fold increase in the absorption of dietary plant sterols, an increase in plasma phytosterol levels, and a decrease in biliary cholesterol secretion (21). In addition, loci on chromosomes 14 and 2 in mice, which are distinct from the G5 and G8 genes, have been shown to regulate plasma phytosterol levels (22). In humans, mutations in the G5 and G8 genes result in pathologically high intestinal absorption of cholesterol and phytosterols. In a family study of a normal population by Berge et al. (5), the D19H substitution of the G8 gene resulted in a completely different situation: low serum cholestanol, sitosterol, and campesterol levels, suggesting limited sterol absorption. However, no association with serum lipid levels was found, nor any compensatory upregulation of cholesterol synthesis, which, according to cholesterol homeostasis, could be expected to follow suppressed cholesterol absorption. The authors also described the following two associations: the T400K polymorphism of the G8 gene was associated with high serum desmosterol and lathosterol ratios to cholesterol and low sitosterol ratios, and the A632V polymorphism of the G8 gene was associated with high serum total cholesterol value (5), none of which could be observed in the present study.

Concerning the D19H polymorphism of the G8 gene, the present results confirmed the findings by Berge et al. (5) that serum absorption marker sterols were lower in subjects with this polymorphism. In addition, a novel finding in the present study was that the D19H polymorphism was more frequent in the first tertile than in the other cholestanol tertiles. In addition, subjects with this poly-

morphism had low serum total and LDL cholesterol levels, supporting the hypothesis presented above that serum cholesterol level is also regulated by cholesterol absorption efficiency through the D19H polymorphism in noncoronary subjects with primary mild to moderate hypercholesterolemia. Furthermore, the increased serum lathosterol ratio suggested that cholesterol synthesis was compensatorily increased. This was a logical result regarding cholesterol homeostasis. The upregulated cholesterol synthesis in these subjects explains the efficacy of atorvastatin therapy (23). In the present study, the presence of the D19H polymorphic site seemed to prevent the increase of serum sitosterol ratio differently from the wild type in increasing cholestanol tertiles, suggesting an inhibitory effect on cholesterol absorption, and the compensatory increase in cholesterol synthesis in these subjects could be seen. It can be concluded that the G8 gene regulates cholesterol absorption also in nonsitosterolemic subjects and that this polymorphism results in low cholesterol absorption efficiency, high synthesis of cholesterol, and low serum total and LDL cholesterol levels.

The Q604K polymorphism of the G5 gene was associated with insulin sensitivity, at least in men. This observation has not been reported earlier. Two important questions arise: how genes that regulate cholesterol absorption could regulate insulin action, and how this function interacts with gender. At present, we have no answers. Taken together, these results suggest that low absorption of cholesterol is at least partially regulated through the ABCG8 gene, whereas the ABCG5 gene may associate with insulin action. The limited number of subjects obliges us to consider these results with caution. However, if true, these observations may be of great significance, because they suggest a link at the genetic level between two major risk factors of cardiovascular diseases: hypercholesterolemia and insulin resistance.

This study was supported by grants from the Finnish Cultural Foundation of Northern Savo, the Finnish Heart Research Association, the Kuopio University Foundation, the Kuopio University Hospital, the Raisio Research Foundation, and the Yrjö Jahnsson Foundation. The expert technical assistance of Kaija Erola, Pia Hoffström, Anne Honkonen, Leena Kaipiainen, Päivi Kärkkäinen, Raija Miettinen, Ritva Nissilä, Päivi Turunen, and Leena Uschanoff is gratefully acknowledged.

REFERENCES

1. Kesäniemi, Y. A., and T. A. Miettinen. 1987. Cholesterol absorption efficiency regulates plasma cholesterol level in the Finnish population. *Eur. J. Clin. Invest.* **17:** 391–395.
2. Miettinen, T. A., R. S. Tilvis, and Y. A. Kesäniemi. 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. Gylling, H., and T. A. Miettinen. 1989. Absorption and metabolism of cholesterol in familial hypercholesterolaemia. *Clin. Sci.* **76:** 297–301.
4. Gylling, H., and T. A. Miettinen. 2002. Inheritance of cholesterol metabolism of probands with high or low cholesterol absorption. *J. Lipid Res.* **43:** 1472–1476.
5. 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.
6. Pihlajamäki, J., H. Gylling, T. A. Miettinen, and M. Laakso. 2004. Insulin resistance with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. *J. Lipid Res.* **45:** 507–512.
7. 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.
8. 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.
9. 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.
10. Hubacek, J. A., K. E. Berge, J. C. Cohen, and H. H. Hobbs. 2001. Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. *Hum. Mutat.* **18:** 359–360.
11. Haffner, S. M., P. Karhapää, L. Mykkänen, and M. Laakso. 1994. Insulin resistance, body fat distribution, and sex hormones in men. *Diabetes.* **43:** 212–219.
12. Rajaratnam, R. A., H. Gylling, and T. A. Miettinen. 2000. Independent association of serum squalene and noncholesterol sterols with coronary artery disease in postmenopausal women. *J. Am. Coll. Cardiol.* **35:** 1185–1191.
13. Miettinen, T. A., and P. Koivisto. 1983. Non-cholesterol sterols and bile acid production in hypercholesterolaemic patients with ileal bypass. *In* Bile Acid and Cholesterol in Health and Disease. G. Paumgartner, A. Stiehl, and W. Gerok, editors. MTP Press, Lancaster, MA. 183–187.
14. DeFronzo, R. A., J. D. Tobin, and R. Andres. 1979. Glucose clamp technique: a method for quantifying insulin secretion and resistance. *Am. J. Physiol.* **237:** E214–E223.
15. Tsukamoto, K., T. Watanabe, T. Matsushima, M. Kinoshita, H. Kato, Y. Hashimoto, K. Kurokawa, and T. Teramoto. 1993. Determination by PCR-RFLP of apo E genotype in a Japanese population. *J. Lab. Clin. Med.* **121:** 598–602.
16. Miettinen, T. A., T. E. Strandberg, and H. Gylling, for the Finnish Investigators of the Scandinavian Simvastatin Survival Study Group. 2002. 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.
17. 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 Study. Finnish 4S Investigators. *BMJ.* **316:** 1127–1130.
18. Strandberg, T. E., V. Salomaa, H. Vanhanen, and T. A. Miettinen. 1996. Associations of fasting blood glucose with cholesterol absorption and synthesis in nondiabetic middle-aged men. *Diabetes.* **45:** 755–761.
19. Simonen, P. P., H. K. Gylling, and T. A. Miettinen. 2002. Diabetes contributes to cholesterol metabolism regardless of obesity. *Diabetes Care.* **25:** 1511–1515.
20. Simonen, P., H. Gylling, A. N. Howard, and T. A. Miettinen. 2000. Introducing a new component of the metabolic syndrome: low cholesterol absorption. *Am. J. Clin. Nutr.* **72:** 82–88.
21. 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.
22. 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.
23. 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.