J Hum Genet (2008) 53:757–763
DOI 10.1007/s10038-008-0310-2

ORIGINAL ARTICLE

Significant association of *ABCG8*:D19H gene polymorphism with hypercholesterolemia and insulin resistance

Zhih-Cherng Chen · Shyi-Jang Shin · Kung-Kai Kuo · Kun-Der Lin · Ming-Lung Yu · Pi-Jung Hsiao

Received: 6 April 2008 / Accepted: 25 May 2008 / Published online: 26 June 2008

Abstract The absorption efficiency of cholesterol is closely correlated to dietary phytosterol content and determined by genetic factors. The ATP-binding cassette (ABC) transporters ABCG5 and ABCG8 act as a sterol efflux pump to regulate the absorption of cholesterol and phytosterol. The levels of cholesterol and phytosterol associated with a Chinese diet are very different from those associated with a Western diet. This study aims to explore the association between serum total cholesterol/LDL-C levels and *ABCG5*/*ABCG8* polymorphisms in a Taiwanese population consuming an ordinary Chinese diet. A total of 1,046 subjects (894 men and 152 women) were recruited in a hospital-based health check-up center in Kaohsiung Medical University Hospital. Five nonsynonymous polymorphisms of Q604E (*ABCG5*), D19H, C54Y, T400 K and A632 V (*ABCG8*) were analyzed by TaqMan genotyping assay. Analysis showed that the D19H polymorphism of the *ABCG8* gene was significantly associated with serum total cholesterol, LDL-C levels and HOMA-IR index. Adjusting for sex and age, subjects with the D19H (GC) genotype were significantly associated with a threefold higher risk of high cholesterol and LDL-C levels than subjects with D19 (GG). These results suggest that the D19H polymorphism of *ABCG8* could be considered a susceptible gene marker indicating an increased likelihood of developing high cholesterol and LDL-C levels in Taiwanese consuming an ordinary Chinese diet. It is supposed that the coexistence of higher insulin resistance and hypercholesterolemia for carriers of the D19H polymorphism may result in a greater risk of cardiovascular disease.

Keywords Cholesterol · LDL cholesterol · Insulin resistance · Single nucleotide polymorphism · *ABCG5* · *ABCG8*

Z.-C. Chen
Division of Cardiology and General Medicine,
Chi-Mei Medical Center, Chung Hwa University of Medical Technology, Tainan, Taiwan

S.-J. Shin · K.-D. Lin · P.-J. Hsiao (✉)
Division of Endocrinology and Metabolism,
Internal Medicine, Kaohsiung Medical University,
100 Tzyou 1st Rd, Kaohsiung 807, Taiwan
e-mail: pjhsiao@cc.kmu.edu.tw

K.-K. Kuo
Hepatobiliary Division, Department of Surgery,
Kaohsiung Medical University, Kaohsiung, Taiwan

M.-L. Yu
Department of Preventive Medicine,
Kaohsiung Medical University, Kaohsiung, Taiwan

Introduction

Cholesterol homeostasis is a very complex process. The serum total cholesterol level is a reflection of the balance between intestinal absorption, endogenous cholesterol biosynthesis, LDL receptor uptake and biliary excretion. In healthy individuals, the average Western diet contains 250–500 mg of cholesterol and 200–400 mg of non-cholesterol sterols (e.g., plant sterol) per day. Typically more than 50% of the cholesterol is absorbed and retained in the body, in contrast to only 1–5% of plant sterols (Lammert and Wang 2005; Wang 2007; Oram and Vaughan 2006). Plant sterol (phytosterol) acts at the enterocytes to reduce cholesterol absorption by 40–60% by binding with cholesterol esterase, cocrystallizing with cholesterol and competing with cholesterol for adenosine triphophate-binding cassette proteins (Kassis et al. 2008). In humans, intestinal

cholesterol absorption efficiency varies over a broad inter-individual range (20–80%). This wide range implies that dietary and genetic factors may play a crucial role in the cholesterol absorption process (Lammert and Wang 2005; Oram and Vaughan 2006; Sehayek 2003).

Niemann-Pick C1-like 1 (NPC1L1) protein resides in the apical surface of enterocytes and hepatocytes and regulates the absorption of phytosterol and cholesterol. It is regarded as a key modulator of cholesterol influx (Davis et al. 2004; Temel et al. 2007). On the other hand, two proteins from the ATP-binding cassette subfamily, ABCG5 and ABCG8, form a functional heterodimer that is localized at the apical membrane of enterocytes and the canalicular membrane of hepatocytes. ABCG5 and ABCG8 act as an efficient exporter, pumping dietary sterols back into the gut lumen and biliary excretions from the liver. In this way, the heterodimer of ABCG5/ABCG8 reduces intestinal absorption and promotes biliary excretion of phytosterols and cholesterol (Hazard and Patel 2006; Ordovas and Tai 2002; Klett et al. 2004). In humans, the efficiency of cholesterol absorption is counterbalanced by the NPC1L1 and the ABCG5/ABCG8 system in order to adjust dietary sterol absorption and biliary excretion (Oram and Vaughan 2006; Temel et al. 2007; Klett et al. 2004; Štefková et al. 2004).

In reports on Caucasians, no association has been observed between *NPC1L1* polymorphism and baseline total cholesterol (Simon et al. 2005; Cohen et al. 2006). Besides, nonsynonymous polymorphisms of *NPC1L1* are very rare in both Caucasian and Han populations, so theoretically the genetic influence of *NPC1L1* could not make a significant contribution to cholesterol homeostasis in our population. However, gene mutations in *ABCG5/ABCG8* transporters lead to abnormal absorption and excretion of sterols and result in sitosterolemia, which manifests clinically as hypercholesterolemia. This has been associated with premature coronary atherosclerosis and death (Hubáček et al. 2001). Common sequence variations in the *ABCG5* and *ABCG8* genes have been reported to account for the variability in cholesterol and phytosterol absorption (Gylling et al. 2004; Plat et al. 2005; Hubáček et al. 2004; Miwa et al. 2005; Weggemans et al. 2002). Although a clear link has been established between *ABCG5/ABCG8* genes and modification of the absorption and/or synthesis of plant sterols, there is still no consensus on the association of plasma total cholesterol and *ABCG5/ABCG8* gene polymorphisms (Sehayek et al. 2004; Berge et al. 2002). Large-scale study data on the genetic effect on this association and cholesterol homeostasis are scarce. The levels of plant sterol and cholesterol in the Chinese diet also differ greatly from those in the Western diet (Zhou et al. 2003). Therefore, the current study was undertaken to assess the effect of *ABCG5/8* polymorphisms on the serum total cholesterol and low-density lipoprotein cholesterol (LDL-C) levels in a Taiwanese population consuming an ordinary Chinese diet.

Materials and methods

The study subjects were recruited from a general population that attended the Department of Preventive Medicine at the Kaohsiung Medical University Hospital for health check-ups from July to December in 2005. Participants with recognized secondary dyslipidemia due to diabetes, nephrotic syndrome, chronic liver disease, alcoholism, Cushing's syndrome, hypothyroidism, or users of lipid-lowering agents were evaluated by an experienced physician and excluded by a detailed medical history review as well as biochemistry analysis. When the exclusion criteria were applied, 1,046 subjects (894 men and 152 women) consuming the ordinary Chinese diet were included in this study. According to the investigation performed during the INTERMAP study, the "ordinary" Chinese diet is composed of carbohydrate (68 vs. 62% of total calories), fat (20.5 vs. 19.5% of total calories), protein (12.6 vs. 12.2% of total calories), cholesterol (218 vs. 146 mg/day), and fiber (30.5 vs. 26.1 g/day) for men vs. women, respectively (Zhou et al. 2003).

Under the supervision and approval of the Institutional Review Board in Kaohsiung Medical University (KMUH-IRB-940175), a blood sample was taken from each subject following an overnight fast. DNA was isolated from the EDTA-treated blood samples by the standard method. A multichannel autoanalyzer recorded a full lipid profile for each participant, including serum cholesterol, triglyceride, LDL-C, and high-density lipoprotein cholesterol (HDL-C). Plasma glucose (dehydrogenase method) was measured by autoanalyzer, and serum insulin was quantified with commercial kits (radioimmunoassay; Diagnostic Products Corporation, Los Angeles, CA, USA). Insulin sensitivity was indicated by the homeostatic model assessment insulin resistance (HOMA-IR) index, calculated using [fasting glucose (mmol/l) × fasting insulin (uU/ml)]/22.5.

From the National Centre for Biotechnology Information (NCBI) website, we selected the common (minor allele frequence >5%) nonsynonymous polymorphisms in the Han population for this study. However, D19H of the *ABCG8* gene, which has a frequency of less than 5%, was also chosen because of its strong correlation to cholesterol homeostasis as reported in the literature (Gylling et al. 2004; Hubáček et al. 2004; Kajinami et al. 2004a, 2004b). Five nonsynonymous polymorphisms, including Q604E (rs6720173) of the *ABCG5* gene and D19H (rs11887534), C54Y (rs4148211), T400K (rs4148217) and A632V (rs6544718) of the *ABCG8* gene, were chosen for

genotyping (http://www.ncbi.nlm.nih.gov/). Genotyping was carried out using the TaqMan technology. Briefly, PCR primers and TaqMan Minor Groove Binder (MGB) probes were designed and reactions were performed in 96-well microplates with ABI 9700 thermal cyclers (Applied Biosystems, Foster City, CA, USA). Fluorescence was measured with an ABI 7500 Real Time PCR system and analyzed with its System SDS software, version 1.2.3.

Serum total cholesterol levels from the population sample were divided into three groups in accordance with guidance from the National Cholesterol Education Program—Adult Treatment Panel III (NCEP-ATP III): desirable (<200 mg/dl), moderately high (200–239 mg/dl) and high (≥240 mg/dl). Similarly, the serum LDL-C level was classified as desirable (<130 mg/dl), moderately high (130–159 mg/dl) and high (≥160 mg/dl) (Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults 2001).

Statistical analyses

All statistical analyses were performed with SPSS 12.0 for Windows (SPSS Inc., Chicago, IL, USA). Results for continuous variables were expressed as mean ± standard deviation (SD). Allele frequencies were estimated by direct gene counting. Each genotype was tested for Hardy–Weinberg equilibrium by using the chi-square test. Haploview™ software was used to reconstruct the haplotype blocks and haplotype analysis was done using the Hapclustering software (Tzeng et al. 2006). Comparisons of categorical variables among groups were assessed using the chi-square test. Continuous variables with normal distributions were compared between groups using one-way ANOVA or *t* test. Variables that were not normally distributed were compared between groups (e.g., HOMA-IR) using the Mann–Whitney *U* test. Logistic regression was used to estimate the genetic effect (expressed by the odds ratio) on the trichotomized total cholesterol and LDL-C levels using the desirable level as the reference. Therefore, we obtain odds ratios for high and moderately high levels. Adjusted odds ratios were estimated using multivariable logistic regressions and additional adjustments were made for age and sex. Statistical significance was defined as a *P* value of <0.05 using a two-tailed test.

Results

Our population included 1,046 subjects (894 men and 152 women) with a mean age of 47.0 ± 9.3 years (ranging from 20 to 77 years). Their serum cholesterol levels ranged from 114 to 350 mg/dl, and their serum LDL-C levels were distributed from 50 to 245 mg/dl. Five nonsynonymous polymorphisms (*ABCG5*: Q604E; *ABCG8*:D19H, C54Y, T400K, A632V) were genotyped in our population. In our initial genotyping of 500 subjects, A632V was found to be monomorphic and it was removed for further investigation. The minor allele frequencies (MAFs) of Q604E (C:G); D19H (G:C), C54Y (G:A) and T400K (C:A) were 10.5, 1.4, 9.7 and 8.0%, respectively. The genotype distribution was in Hardy–Weinberg equilibrium for all polymorphisms. These allele frequencies were different to those reported previously in European-American populations (http://www.ncbi.nlm.nih.gov/SNP/). Our results showed that the minor C allele of D19H occurred with a lower frequency in Chinese than in Caucasians.

Table 1 shows the association between the serum total cholesterol/LDL-C levels and the genotypes of the *ABCG5* and *ABCG8* genes. Subjects with genotype Q604 (CC) had significantly higher serum total cholesterol levels than those of genotypes Q604E (CG) or 604E (GG). The serum total cholesterol and the LDL-C level were significantly higher in subjects with the D19H (GC) genotype than those with D19 (GG). There was a significant difference in the genotype frequencies between the three categories of cholesterol level (Table 2). This exhibited a significant trend with an increasing frequency of the C allele in Q604 (*ABCG5*) and D19H (*ABCG8*) genotypes from the

Table 1 Serum cholesterol and LDL levels for the genotypes of *ABCG5*/*ABCG8*

	Cholesterol (mg/dl)	*P*	LDL-C (mg/dl)	*P*
ABCG5: Q604E (C1810G)				
Genotype				
CC ($n = 9$)	225.9 ± 48.5	0.008	133.7 ± 40.0	0.733
CG ($n = 203$)	189.6 ± 37.6		130.1 ± 32.7	
GG ($n = 833$)	187.2 ± 38.1		127.9 ± 35.9	
ABCG8:D19H (G55C)				
Genotype				
GG ($n = 1{,}016$)	187.2 ± 37.9	0.005	127.8 ± 35.2	0.023
GC ($n = 30$)	207.1 ± 45.1		145.1 ± 40.6	
CC ($n = 0$)	–		–	
ABCG8:C54Y (G161A)				
Genotype				
GG ($n = 853$)	187.3 ± 37.8	0.297	127.8 ± 35.4	0.671
GA ($n = 189$)	190.4 ± 40.0		130.0 ± 35.2	
AA ($n = 8$)	171.6 ± 24.0		118.0 ± 23.8	
ABCG8:T400 K (C1199A)				
Genotype				
CC ($n = 885$)	188.0 ± 38.2	0.869	128.3 ± 35.4	0.997
CA ($n = 164$)	186.9 ± 38.2		128.3 ± 34.3	
AA ($n = 2$)	194.0 ± 22.6		131.0 ± 10.2	

P values were obtained from a one-way ANOVA and the *t* test

Table 2 Genotype frequency of *ABCG5*/*ABCG8* versus categorized cholesterol level

Serum cholesterol (mg/dl)	Desirable (<200)	Moderately high (200–239)	High (≥240)	*P*
ABCG5: Q604E (C1810G)				
Genotype				
CC	3 (0.43%)	2 (0.84%)	4 (3.5%)	0.004
CG+GG	692 (99.6%)	235 (99.2%)	109 (96.5%)	
ABCG8:D19H (G55C)				
Genotype				
GG	682 (98.0%)	227 (96.6%)	104 (92.9%)	0.009
GC	14 (2.0%)	8 (3.4%)	8 (7.1%)	
ABCG8:C54Y (G161A)				
Genotype				
GG	576 (82.1%)	190 (80.5%)	87 (77.7%)	0.517
GA+AA	126 (17.9%)	46 (19.5%)	25 (22.3%)	
ABCG8:T400 K (C1199A)				
Genotype				
CC	592 (84.3%)	197 (83.5%)	96 (85.0%)	0.927
CA+AA	110 (15.7%)	39 (16.5%)	17 (15.0%)	

P values were obtained from the chi-square test

Table 3 Comparison of the biochemical characteristics of subjects with D19 (GG) and D19H (GC) genotypes

	GG	GC	*P*
Number	1,016	30	
Sex (M:F)	870:146	24:6	0.39
Age (years)	49.1 ± 9.2	49.8 ± 11.4	0.09
BMI (kg/m^2)	24.4 ± 3.3	25.4 ± 4.2	0.09
SBP (mmHg)	125.4 ± 16.5	124.3 ± 13.8	0.76
DBP (mmHg)	77.6 ± 11.1	78.6 ± 7.0	0.64
CHOL (mg/dl)	187.2 ± 37.9	207.1 ± 45.1	0.005
TG (mg/dl)	139.8 ± 146.3	124.5 ± 46.8	0.57
LDL-C (mg/dl)	127.8 ± 35.2	145.1 ± 40.6	0.023
HDL-C (mg/dl)	52.2 ± 13.2	53.1 ± 10.1	0.77
F-glucose (mg/dl)	96.7 ± 28.7	98.4 ± 25.2	0.76
HOMA-IR	1.12 ± 2.00	3.21 ± 7.84	0.026

Data are shown as as mean ± SD

Student's *t* test was used for statistical analysis and the Mann–Whitney *U* test was used for HOMA-IR

BMI, body mass index; *SBP*, systolic blood pressure; *DBP*, diastolic blood pressure; *CHOL*, cholesterol; *TG*, triglyceride; *LDL-C*, low-density lipoprotein; *HDL-C*, high-density lipoprotein; *F-glucose*, fasting glucose; *HOMA-IR*, homeostatic model assessment insulin resistance

Table 4 Risk stratification for the *ABCG8* genotypes (D19H vs. D19) in terms of serum cholesterol and LDL-C levels

	D19	D19H	*P*
Moderately high versus desirable cholesterol			
Crude odds ratio	1	1.72 (0.71–4.14)	0.23
Adjusted odds ratio	1	1.54 (0.62–3.83)	0.35
High versus desirable cholesterol			
Crude odds ratio	1	3.75 (1.53–9.15)	0.004
Adjusted odds ratio	1	3.44 (1.32–8.97)	0.012
Moderately high versus desirable LDL-C			
Crude odds ratio	1	1.92 (0.67–5.54)	0.227
Adjusted odds ratio	1	1.65 (0.55–4.89)	0.37
High versus desirable LDL-C			
Crude odds ratio	1	3.51 (1.25–9.85)	0.017
Adjusted odds ratio	1	3.29 (1.10–9.82)	0.033

Desirable cholesterol indicates <200 mg/dl; moderately high cholesterol indicates 200–239 mg/dl; high cholesterol indicates ≥240 mg/dl

Desirable LDL-C indicates <130 mg/dl; moderately high LDL-C indicates 130–159 mg/dl; high LDL-C indicates ≥160 mg/dl

Logistic regression analysis was used to estimate the odds ratio, by adjusting for age and sex. ($P < 0.05$ is significant)

desirable to the moderately high and then to the high cholesterol category. As there were only a few with the genotype of Q604 (CC), further analysis of this was limited. Haplotype analysis using the three SNPs of *ABCG8* did not demonstrate a significant association with serum cholesterol ($P = 0.18$) or LDL-C level ($P = 0.81$). The relationships of the biochemical parameters to the genotypes of the D19 and D19H polymorphisms are shown in Table 3. Subjects with genotype D19H (GC) have significantly higher serum cholesterol and LDL-C levels than those with D19 (GG). Although there was no difference in metabolic biomarkers (BMI, blood pressure, fasting glucose) between these two genotypes, subjects with genotype D19H (GC) had a significantly higher HOMA-IR index than those with D19 (GG).

The genotypic effect on serum cholesterol and LDL-C levels was tested for D19H (Table 4). The results showed that the subjects with the D19H genotype (i.e., the GC genotype) were significantly associated with a higher risk of raised cholesterol and LDL-C levels than subjects with D19 (GG). This risk remained significant after adjustment for age and sex.

Discussion

In the present study, we investigated the relationship between five nonsynonymous polymorphisms of *ABCG5*/*ABCG8* and serum total cholesterol and LDL-C levels. Our results demonstrated that subjects with the C allele of the D19H polymorphism had significantly higher total cholesterol and LDL-C levels compared with subjects that did not carry the C allele. In addition, subjects with the D19H variant were associated with an almost threefold higher risk of developing high total cholesterol and LDL-C levels

compared with those with D19 in this large population. Our finding implicated that the D19H polymorphism may have a functional consequence due to the substitution of aspartic acid by histidine, which results in a conformational change that alters the ability to export sterol and cholesterol homeostasis.

It has been reported that the D19H polymorphism is linked to a lower plant sterol and cholesterol absorption efficiency. However, there has been no consistent conclusion about the changes in the serum lipid profiles (including serum cholesterol and LDL-C levels) in subjects with this polymorphism so far (Gylling et al. 2004; Plat et al. 2005; Hubáček et al. 2004; Kajinami et al. 2004a, b). Berge and colleagues identified significantly reduced plant sterol absorption in carriers with the D19H polymorphism in a family study of a healthy normolipidemic population, but there was no association of the serum lipid level with this polymorphism (Berge et al. 2002). Gylling et al. conducted another study with a population of subjects with mild to moderate hypercholesterolemia (serum total cholesterol <7.5 mmol/l, equal to 285 mg/dl). They found that D19H/19H (GC or CC) variants were more frequent in the lowest cholesterol absorption tertile. Serum total cholesterol (5.37 ± 0.15 mmol/l) and LDL-C levels (3.29 ± 0.14 mmol/l) were lower in subjects with D19H/19H (GC or CC) variants than in those (5.82 ± 0.07 mmol/l; 3.78 ± 0.07 mmol/l) with the D19 genotype (Gylling et al. 2004). Kajinami et al. found that hypercholesterolemic patients (LDL-C ≥160 mg/dl) with D19H/19H variants had a lower total cholesterol level at baseline. However, subjects with D19H/19H variants were noted to exhibit greater reductions in LDL-C levels with statin treatment under the NCEP step 1 diet throughout the study. The greater response of LDL-C to statin treatment could be explained by a compensatory increase in endogenous cholesterol synthesis in subjects with D19H/19H variants compared to the D19 variant (Kajinami et al. 2004a, 2004b). The discordance between our results for the serum total cholesterol and LDL-C levels in our subjects with D19H and previous results may be attributed to the following reasons: (1) the populations studied had different recruitment criteria for serum lipid levels; (2) dietary habits of different races; (3) different dietary interventions within the study period.

Although our study design was not a hyperlipidemic cohort study, it essentially enrolled a large, normal, healthy population for routine physical examination. These results clearly demonstrated that the D19H polymorphism, even though it was rare, significantly contributed to an independent genetic risk of developing high total cholesterol and LDL-C levels. As reports have shown previously, this points to the fact that subjects with D19H/19H (GC or CC) variants are disposed to having a lower cholesterol absorption efficiency (Gylling et al. 2004; Hubáček et al. 2004; Kajinami et al. 2004a, 2004b). However, the cholesterol absorption efficiency of the intestine is inversely proportional to the rate of hepatic cholesterol biosynthesis. Since hepatic cholesterol biosynthesis has the greatest effect on cholesterol homeostasis (Lammert and Wang 2005; Wang 2007; Sehayek 2003; Temel et al. 2007), we can plausibly speculate that the compensatory hepatic cholesterol biosynthesis in subjects with D19H (GC) caused their higher serum total cholesterol and LDL-C levels than those observed with the D19 (GG) genotype in our results.

It has been recognized that dietary plant sterols can lower the serum total cholesterol level with a dose–response effect by competing with and inhibiting intestinal cholesterol absorption. Also, the withdrawal of dietary plant sterol attenuates the overall cholesterol-lowering effects of lipid-lowering agents (Jenkins et al. 2008). Although dietary intake of phytosterol results in a lower absorption efficiency of cholesterol, it may also upregulate the de novo biosynthesis of cholesterol somewhat (Lammert and Wang 2005; Wang 2007; Lichtenstein and Deckelbaum 2001; von Bergmann et al. 2005). Based on the INTERMAP study performed at the end of twentieth century, it is clear that there is still a significant difference in dietary composition between East Asian (Chinese) and Western (UK and USA) diets. The Western diet has significantly higher cholesterol and lower fiber contents than the Chinese diet, in both men and women (Zhou et al. 2003). Following a survey of the consumption of plant sterol throughout the world, it has been shown that different populations consume very different amounts of plant sterol. It appears that plant sterol intake is high in Asian populations and much lower in Western populations (Chan et al. 2006). It is known that both plant sterol and cholesterol absorption vary widely among individuals and are determined by genetic influences (Lammert and Wang 2005; Wang 2007; Sehayek 2003). At the physiological level, the serum cholesterol level is maintained mainly by de novo synthesis in the liver and partially by the absorption efficiency of the intestine (Wang 2007; Davis et al. 2004; Temel et al. 2007). Based on the findings of Gylling et al. (2004) and Kajinami et al. (2004a), subjects with D19H/19H have a lower cholesterol absorption efficiency but respond more to statin treatment than D19 subjects. Upon comparing our results with those in previous reports, we rationally assumed that consuming a higher ratio of plant sterols to cholesterol in the diet may influence the relative absorption efficiency of cholesterol in subjects with D19H variants, causing a much lower cholesterol absorption and therefore increasing hepatic cholesterol biosynthesis, thus resulting in higher serum total cholesterol and LDL-C levels than those of D19 variants.

Our results indicated that subjects with D19H variants had greater insulin resistance (higher HOMA-IR index)

than those with D19 variants, although they did not differ in terms of other metabolic biomarkers (BMI, blood pressure, serum triglyceride, plasma glucose). Upon reviewing the literature on Caucasians, the polymorphisms of *ABCG5* and *ABCG8* genes did not differ between cases of metabolic syndrome and matched control (Gylling et al. 2007). In a study of hypercholesterolemic subjects, Q604E polymorphism was linked to insulin resistance in men (Gylling et al. 2004). It has been demonstrated there is a complex interrelation between cholesterol, glucose and insulin response. The insulin resistance status is significantly associated with increased cholesterol synthesis and decreased cholesterol absorption (Pihlajamäki et al. 2004, Gylling et al. 2004). High-fiber diets such as those based on modified carbohydrates from rye bread and pasta significantly decrease cholesterol absorption but enhance cholesterol synthesis (Hallikainen et al. 2006). Therefore, the higher insulin resistance status of our subjects with the D19H polymorphism is reasonably explained by the effects of lower cholesterol absorption and higher cholesterol synthesis compared to those with D19 variants. Their higher serum total cholesterol and HOMA-IR may lead to a higher risk of progressive atherosclerosis and cardiovascular disease in people with the D19H polymorphism. It seems wise to start preventive intervention strategies for atherosclerosis earlier in carriers of the D19H polymorphism.

In conclusion, there is a significant association of the *ABCG8*:D19H polymorphism with hypercholesterolemia and higher HOMA-IR index. D19H polymorphism could be regarded as a susceptible gene marker for developing high total cholesterol, high LDL-C levels and insulin resistance in people consuming a Chinese diet. Because of the limited number of D19H subjects in our population, this result can be regarded as intriguing but tentative, and will therefore require more study before any further application of it is made.

Acknowledgments This study was supported by grants from the cooperative research program at Chi-Mei Medical Center and Kaohsiung Medical University, 94-CM-KMU-13. We would like to sincerely thank Yi-Hsin Connie Yang, Ph.D. in Biostatistics, for the statistical analysis, and Shu-Ting Lee for technical assistance.

References

Berge KE, von Bergmann K, Lutjohann D, Guerra R, Grundy SM, Hobbs HH, Cohen JC (2002) Heritability of plasma noncholesterol sterols and relationship to DNA sequence polymorphism in ABCG5 and ABCG8. J Lipid Res 43:486–494

Chan YM, Varady KA, Lin Y, Trautwein E, Mensink RP, Plat J, Jones PJ (2006) Plasma concentrations of plant sterols: physiology and relationship with coronary heart disease. Nutr Rev 64(9):385–402

Cohen JC, Pertsemlidis A, Fahmi S, Esmail S, Vega GL, Grundy SM, Hobbs HH (2006) Multiple rare variants in *NPC1L1* associated with reduced sterol absorption and plasma low-density lipoprotein levels. Proc Natl Acad Sci USA 103:1810–1815

Davis HR, Zhu JLJ, Hoos LM, Tetzloff G, Maguire M, Liu J, Yao X, Iyer SPN, Lam MH, Lund EG, Detmers PA, Graziano MP, Altmann SW (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

Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (2001) Executive summary of the Third Report of the National Cholesterol Education Program (NCEP) Expert Panel on Detection, Evaluation, and Treatment of High Blood Cholesterol in Adults (Adult Treatment Panel III). JAMA 285(19):2486–2497

Gylling H, Hallikainen M, Pihlajamäki J, Argen J, Laakso M, Rajaratnam RA, Rauramaa R, Miettinen TA (2004) Polymorphisms in the ABCG5 and ABCG8 genes associate with cholesterol absorption and insulin sensitivity. J Lipid Res 45:1660–1665

Gylling H, Hallikainen M, Kolehmainen M, Toppinen L, Pihlajamäki J, Mykkänen H, Ågren JJ, Rauramaa R, Laakso M, Miettinen TA (2007) Cholesterol synthesis prevails over absorption in metabolic syndrome. Transl Res 149:310–316

Hallikainen M, Toppinen L, Mykkänen H, Ågren JJ, Laaksonen DE, Miettinen TA, Niskanen L, Poutanen KS, Gylling H (2006) Interaction between cholesterol and glucose metabolism during dietary carbohydrate modification in subjects with the metabolic syndrome. Am J Clin Nutr 84:1385–1392

Hazard SE, Patel SB (2006) Sterolins ABCG5 and ABCG8: regulators of whole body dietary sterols. Eur J Physiol 27:1–8

Hubáček JA, Berge KE, Cohen JC, Hobbs HH (2001) Mutations in ATP-cassette binding proteins G5 (ABCG5) and G8 (ABCG8) causing sitosterolemia. Hum Mutat 18(4):359–360

Hubáček JA, Berge KE, Štefková J, Pitha J, Skodová Z, Lánská V, Poledne R (2004) Polymorphism in ABCG5 and ABCG8 transporters and plasma cholesterol levels. Physiol Res 53:395–401

Jenkins DJA, Kendall CWC, Nguyen TH, Marchie A, Faulkner DA, Ireland C, Josse AR, Vidgen E, Trautwein EA, Lapsley KG, Homes C, Josse RG, Leiter LA, Connelly PW, Singer W (2008) Effect of plant sterols in combination with other cholesterol-lowering foods. Metabolism 57:130–139

Kajinami K, Brousseau ME, Nartsupha C, Ordovas JM, Schaefer EJ (2004a) ATP binding cassette transporter G5 and G8 genotypes and plasma lipoprotein levels before and after treatment with atorvastatin. J Lipid Res 45:653–656

Kajinami K, Brousseau ME, Ordovas JM, Schaefer EJ (2004b) Interactions between common genetic polymorphism in *ABCG5*/*ABCG8* and *CYP 7A1* on LDL cholesterol-lowering response to atorvastatin. Atherosclerosis 175:287–293

Kassis AN, Vanstone CA, AbyNweus SS, Jones PJH (2008) Efficacy of plant sterols is not influenced by dietary cholesterol intake in hypercholesterolemic individuals. Metabolism 57:339–346

Klett EL, Lee MH, Adama DB, Chavin KD, Patel SB (2004) Localization of ABCG5 and ABCG8 proteins in human liver, gall bladder and intestine. BMC Gastroenterol 4(21):1–12

Lammert F, Wang DQH (2005) New insights into the genetic regulation of intestinal cholesterol absorption. Gastroenterology 129:718–734

Lichtenstein AH, Deckelbaum RJ (2001) Stanol/sterol ester-containing foods and blood cholesterol levels. A statement for healthcare professionals from the Nutrition Committee of the Council on Nutrition, Physical Activity, and Metabolism of the American Heart Association. Circulation 103:1177–1179

Miwa K, Inazu A, Kobayashi J, Higashikata T, Nohara A, Kawashiri M, Katsuda S, Takata M, Koizumi J, Mabuchi H (2005) ATP-

binding cassette transporter G8 M429V polymorphism as a novel genetic marker of higher cholesterol absorption in hypercholesterolaemic Japanese subjects. Clin Sci 109:183–188

Oram JF, Vaughan AM (2006) ATP-binding cassette cholesterol transporters and cardiovascular disease. Circ Res 99:1031–1043

Ordovas JM, Tai ES (2002) The babel of the ABCs: novel transporters involved in the regulation of sterol absorption and excretion. Nutr Rev 60(1):30–33

Plat J, Bragt MCE, Mensink RP (2005) Common sequence variations in ABCG8 are related to plant sterol metabolism in healthy volunteers. J Lipid Res 46:68–75

Pihlajamäki J, Gylling H, Miettinen TA, Laakso M (2004) Insulin resistance is associated with increased cholesterol synthesis and decreased cholesterol absorption in normoglycemic men. J Lipid Res 45:507–512

Sehayek E (2003) Genetic regulation of cholesterol absorption and plasma plant sterol levels: commonalities and differences. J Lipid Res 44:2030–2038

Sehayek E, Yu HJ, von Bergmann K, Lutjohann D, Stoffel M, Duncann EM, Garcia-Naveda L, Salit J, Blundell ML, Friedman JM, Breslow JL (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

Simon JS, Karnoub MC, Devlin DJ, Arreaza MG, Qiu P, Monks SA, Severino ME, Deutsch P, Palmisano J, Sachs AB, Bayne ML, Plump AS, Schadt EE (2005) Sequence variation in NPC1L1 and association with improved LDL-cholesterol lowering in response to ezetimibe treatment. Genomics 86:648–656

Štefková J, Poledne R, Hubáček JA (2004) ATP-binding cassette (ABC) transporters in human metabolism and disease. Physiol Res 53:235–243

Temel RE, Tang W, Ma Y, Rudel LL, Willinhan MC, Ioannou YA, Davies JP, Nilsson LM, Yu L (2007) Hepatic Niemman-Pick C1-like 1 regulates biliary cholesterol concentration and is a target of ezetimibe. J Clin Invest 117:1968–1978

Tzeng JY, Wang CH, Kao JT, Hsiao CK (2006) Regression-based association analysis with clustered haplotypes through use of genotypes. Am J Hum Genet 78:231–242

von Bergmann K, Sudhop T, Lütjohann D (2005) Cholesterol and plant sterol absorption: recent insights. Am J Cardiol 96(suppl):10–14

Wang DQH (2007) Regulation of intestinal cholesterol absorption. Annu Rev Physiol 69:221–248

Weggemans RM, Zock PL, Tai ES, Ordovas JM, Molhuizen HO, Katan MB (2002) ATP binding cassette G5 C1950G polymorphism may affect blood cholesterol concentrations in humans. Clin Genet 62:226–229

Zhou BF, Stamler J, Dennis B, Moag-Stahlberg A, Lkuda N, Robertson C, Zhao L, Chan Q, Elliott P (2003) Nutrient intakes of middle-aged men and women in China, Japan, United Kingdom and United States in the late 1990s: The INTERMAP study. J Hum Hypertens 17:623–630