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ORIGINAL INVESTIGATION

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Genetic variations in the cholesteryl ester transfer protein gene and high density lipoprotein cholesterol levels in Taiwanese Chinese

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Abstract This study analyzed the association of the I14A mutation, the D442G mutation, and the *Taq*IB polymorphism of the cholesteryl ester transfer protein (CETP) gene in 718 Chinese individuals with high-density lipoprotein cholesterol levels (HDL-C) living in Taiwan. The analysis revealed that the I14A mutation was not present in any of the 110 subjects with HDL-C levels above 60 mg/dl. By contrast, the D442G mutation was present in 48 of the 718 (6.7%) subjects tested. Significantly higher HDL-C levels were noted for bearers of the D442G mutation compared with non-bearers; however, this association was weaker for males and for subjects carrying the *Taq*IB1 allele. The *Taq*IB2 allele was also associated with higher HDL-C levels. From multivariate analysis, independent associations were demonstrated for the *Taq*IB2 polymorphism and the D442G mutation, and elevated HDL-C levels. For obese subjects, however, the presence of the *Taq*IB2 or D442G allele was not associated with increased HDL-C levels. For subjects with triglycerides at a concentration greater than 150 mg/dl, the association of both alleles with HDL-C levels was also diminished. Thus, genetic variation at the CETP gene locus may account for a significant proportion of the difference in HDL-C levels; however, it seems reasonable to suggest that the effects of the allele interact with genetic variations expressed within the sample population, and with sex, obesity, and plasma triglyceride levels.

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Introduction

Serum high-density lipoprotein cholesterol (HDL-C) levels may be altered by a variety of environmental factors including smoking, obesity, alcohol consumption, and exercise (Tall 1990). Twin studies also indicate that there is a strong genetic component for predicting HDL-C levels (Kuusi et al. 1987). Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that functions to transfer cholesterol ester from high-density lipoproteins (HDL) to triglyceride-rich lipoproteins and thus acts to regulate plasma HDL-C levels. A genetic deficiency of CETP activity, first described by Koizumi et al. (1985), is characterized by marked hyperalphalipoproteinemia and abnormalities of both the low-density lipoproteins (LDL) and HDL. Two CETP gene mutations, an intron 14 G(+1)-to-A mutation (I14A) and a missense mutation, Asp442 to Gly within exon 15 (D442G), were first described for Japanese populations and were associated with CETP deficiency and increased HDL-C levels (Brown et al. 1989; Takahashi et al. 1993). In addition, several common CETP gene polymorphisms have been described (Drayna and Lawn 1987; Freeman et al. 1989; Zuliani and Hobbs 1990). The most frequently reported polymorphism of the CETP gene, *Taq*IB, is associated with plasma CETP activity and HDL-C levels (Kondo et al. 1989; Freeman et al. 1990), with higher HDL-C levels being associated with the carrying of the B2 allele. Although studies of Caucasian sample populations have yielded conflicting evidence, revealing inconsistent linkages for CETP genetic variants and HDL levels (Cohen et al. 1994; Bu et al. 1994), studies of Japanese populations have established that CETP-gene mutations are important determinants of HDL-C levels for this ethnicity (Inazu et al. 1990; Hirano et al. 1993; Sakai et al. 1995). Thus, in order to determine the importance of genetic variants of the CETP gene for predicting HDL-C levels for Taiwanese Chinese and to delineate the effects of environmental factors on this influence, 718 Chinese were enrolled in this study to test the association for these genetic variations and HDL-C levels.

Materials and methods

Nomenclature

Gene symbols used in this article follow the recommendations of the HUGO Gene Nomenclature Committee (Povey et al. 2001).

Study population

A total of 1103 individuals was recruited for analysis of the genetic determinants of HDL-C levels during routine health examinations, after informed consent had been obtained. Of these, 385 subjects were excluded because of the diagnosis of major systemic or cardiovascular disease or a history of lipid-lowering medication. Of the original recruitment, a total of 718 individuals was enrolled in this study (392 males and 326 females; mean age: 55.4±9.9 years). The clinical and biometrical features of the study population are summarized in Table 1. Obesity was defined if the body mass index (BMI) was 26 kg/m^2 or more. This investigation was approved by the Ethics Committee of the Chang-Gung Memorial Hospital.

Genomic DNA extraction

Blood samples of approximately 10 ml were drawn into heparinized tubes, and white blood cells were separated. The genomic DNA was extracted from the peripheral blood leukocytes by using a standard method after proteinase K digestion of the nuclei. Phenol and chloroform extraction was followed by isopropanol precipitation of the DNA.

I14A mutation detection by polymerase chain reaction followed by *Nde*I restriction digestion

A 225-bp DNA fragment, encompassing the exon 14/intron 14 junction of the CETP gene, was amplified by using the polymerase chain reaction (PCR) with an F-14IA primer, 5'-CACGGATGGGCATGAGGATG-3', and an R-14IA primer, 5'-AAGCTCTGTCAGCCTCGGCACCCAGTTTCCCCGCCAGCCCACACATA-3' (Akita et al. 1994). The R14IA primer was designed to create a novel *Nde*I site, only from the G→A mutated allele. An amplified PCR product was digested with *Nde*I, with resulting fragments of 225 bp for the I14A normal type, 178 bp for the I14A homozygote, and 225 and 178 bp for the I14A heterozygote.

D442G mutation detection with PCR followed by *Sal*I-restriction digestion

To yield a 246-bp product, PCR was performed by utilizing the primers F-D442G 5'- ACAGCCCTCATGAACAGCAAAGGCGTGAGCCTCGTCG-3' and R-D442G 5'-AAGGGAGGGGCAGTAGGAGA-3' (Akita et al. 1994). Primer F-D442G was modified to generate a *Sal*I-endonuclease cleavage site for detection of D442G. The amplified PCR product was digested with *Sal*I, resulting in fragments of 246 bp for DD, 209 bp for GG, and 246/209 bp for the D442G heterozygote.

*Taq*IB polymorphism of the CETP gene

The primers were designed as previously reported (Fumeron et al. 1995). A 535-bp fragment in intron 1 of the CETP gene was amplified by using PCR and the forward and reverse primers, 5'-CACTAGCCCAGAGAGAGGAGTGCC-3' and 5'-CTGAGCCCAGCCGCACACTAAC-3'. The amplification product was digested with *Taq*I, resulting in fragments of 174 and 361 bp for the B1 allele, and 535 bp for the uncut B2 allele.

Statistical analysis

The chi-square test was used to examine differences in distributions for allelic and genotype frequencies. The clinical characteristics of continuous variables were expressed as mean ± SD and were tested by a two-sample *t* test or by an analysis of variance. A general linear model was applied to analyze HDL-C levels with respect to predictors of the investigated genotypes and other confounders. Stratified analyses of the genetic variants of the CETP genotypes and HDL-C levels were performed to explore further the interactive effects, while controlling for other variables. Variables were logarithmically transformed before statistical analysis to meet a normality assumption. All *P*-values were calculated based on two-sided tests, with statistical significance being defined as when the *P*-value was less than 0.05. The haplotype frequencies were estimated by using the Arlequin program (Version 2.0; Genetics and Biometry Laboratory, University of Geneva, Switzerland). The extent of disequilibrium was expressed in terms of D'=D/Dmax (Thompson et al. 1988).

Results

CETP-gene frequencies for the I14A and D442G mutations and the *Taq*IB polymorphism

A group of 110 individuals with HDL-C levels of 60 mg/dl or more was initially screened for the I14A and D442G mutations: none of these individuals carried the I14A mutation. By contrast, the D442G mutation was detected in 15 of this subgroup. Of the 718 enrolled subjects, the

Table 1 Clinical and lipid profiles. Continuous variables are presented as mean ± SD. Triglyceride and cholesterol/HDL values were logarithmically transformed before statistical testing to meet the assumption of the normal distribution. The untransformed data is however shown here

Characteristic	Total	Male	Female	*P*-value
n	718	392	326	–
Age (years)	55.4±9.9	55.0±10.1	55.8±9.7	0.280
Cholesterol (mg/dl)	204±39	201±39	207±40	0.039
HDL-cholesterol (mg/dl)	52±15	48±13	57±15	0.000
LDL-cholesterol (mg/dl)	123±34	122±32	124±36	0.460
Triglycerides (mg/dl)	146±133	160±162	128±80	0.002
Cholesterol/HDL	4.17±1.51	4.45±1.68	3.84±1.19	0.000
Body mass index (kg/m^2)	24.8±3.6	24.8±3.5	24.9±3.5	0.682
Diabetes mellitus (%)	7.2	9.2	4.9	0.028
Smokers (%)	37.5	64.8	4.6	0.001
CETP 442G allele (%)	3.5	3.2	3.5	0.336
*Taq*I B2 allele (%)	42.3	42.1	42.6	0.644

Table 2 Lipid profiles in genotypes of genetic variants of the CETP gene (*C* cholesterol, *TG* triglycerides). Continuous variables are shown as mean ± SD. Triglycerides and total C/HDL-C were logarithmically transformed before statistical testing to meet the assumption of the normal distribution; untransformed data is however shown

Characteristic	CETP	D442G		CETP	*Taq*I B		
	DG/GG	DD	*P*-value	B1B1	B1B2	B2B2	*P*-value
n	48	670	–	231	366	121	–
Total C (mg/dl)	201±33	204±40	0.633	205±39	203±39	205±43	0.655
HDL-C (mg/dl)	58±18	52±14	0.028	50±13	53±15	56±17	0.003
LDL-C (mg/dl)	120±29	123±34	0.518	126±35	123±14	119±31	0.173
TG (mg/dl)	119±83	148±135	0.015	154±142	138±92	154±201	0.357
Total C/HDL-C	3.76±1.15	4.20±1.53	0.048	4.33±1.34	4.11±1.31	4.07±2.20	0.142

D442G mutation was heterozygous in 46 individuals (6.4%), with only two individuals (0.28%) being homozygous. On analyzing the *Taq*IB polymorphism, the frequencies for the B1B1, B1B2, and B2B2 variants were 32.2%, 51%, and 16.8%, respectively.

Associations between the CETP-gene D442G mutation and lipid parameters

A dominant mode of action was demonstrated for the presence of the D442G heterozygotes and homozygotes on increasing HDL-C levels (*n*=46, 57±17 mg/dl and *n*=2, 89±22 mg/dl, respectively). Significantly higher HDL-C levels were noted when comparing individuals with the D442G mutation (58±18 mg/dl) and those without (52±14 mg/dl; *P*-value=0.028; Table 2). A significant trend toward lower triglyceride levels (TG) and a lower total cholesterol/HDL-C ratio were also demonstrated for bearers of the D442G mutation (*P*-values=0.015 and 0.048, respectively).

Associations between the CETP-gene *Taq*IB polymorphism and lipid parameters

A statistically significant association was also demonstrated for the B2 variant of the *Taq*IB polymorphism and elevated HDL-C, with higher levels (56±17 mg/dl) revealed for subjects homozygous for the B2 allele compared with the B1B2 (53±15 mg/dl) and B1B1 (50±13 mg/dl) analogs (*P*-value=0.003; Table 2). Furthermore, plasma total cholesterol, LDL, and TG levels were similar on comparing the different genotypes.

Multivariate analysis for parameters influencing HDL-C

On univariate analysis, obesity, maleness, smoking, and diabetes were also significantly associated with low HDL-C. Multiple linear regression analysis of HDL-C levels, however, revealed that sex, BMI, *Taq*IB (B2B2 and B1B2), and D442G (DG and GG) were independent predictive variables. In a gender comparison of HDL-C (Table 3), female levels were 0.287 mg/dl higher (*P*-value=0.000). Overall, for every 1 kg/m^2 increase in BMI, the HDL-C was decreased by 0.282 mg/dl (*P*-value=0.000), with a 0.081 mg/dl reduction for the *Taq*IB B1B1 genotype compared with the B1B2 or B2B2 genotypes (*P*-value=0.018). Furthermore, the HDL-C level was 0.073 mg/dl lower for subjects bearing the D442G DD genotype compared with those with the DG or GG genotypes (*P*-value=0.034).

Table 3 Multiple linear regression analysis for predictors of HDL-C level by using a general linear model procedure with the backward method (*BMI* body mass index)

Parameter	Estimate	T for H0	*P*-value
Sex (female vs male)	0.287	6.638	0.000
BMI (each increment)	–0.282	–8.307	0.000
CETP *Taq*I B1B1 vs B1B2/B2B2	–0.081	–2.376	0.018
CETP 442 DD vs DG/GG	–0.073	–2.126	0.034
Diabetes mellitus (yes vs no)	–0.047	–1.385	0.166
Smoking (yes vs no)	–0.027	–0.631	0.529
Age (each increment)	–0.021	–0.628	0.530

Linkage disequilibrium for the D442G mutation and the *Taq*IB polymorphism

The relationships of the D442G-mutation genotypes and the *Taq*IB polymorphism were examined to determine whether the effect of the two genetic variants on HDL-C could be explained by linkage disequilibrium. The haplotype frequencies were estimated by using the Arlequin program (Table 4), with the extent of linkage disequilibrium between the polymorphisms being calculated based on these frequencies. Statistical significance was demonstrated for linkage disequilibrium (D/Dmax=0.70, *P*-value <0.001) between the two less-common alleles. As presented in Table 4, 31.3% (15/48) of subjects who carried

Table 4 Association of the D442G mutation and the *Taq*IB polymorphism. Estimated haplotype frequencies for the *Taq*IB and D442G mutation: B1/D=57.1, B1/G=0.6, B2D=39.4, B2G=2.9

	*Taq*IB polymorphism			
D442G	B1B1	B1B2	B2B2	Total
DD	226	338	106	670
DG	5	28	13	46
GG	0	0	2	2
Total	231	366	121	718

Table 5 Interaction of *Taq*IB and D442G on HDL-C level (mg/dl). The number of cases is given in *brackets*

Variable	*Taq*IB			P_1[a]	P_2
	B1B1 Mean ± SD (*n*)	B1B2 Mean ± SD (*n*)	B2B2 Mean ± SD (*n*)		
DD	50±12 (226)	53±15 (338)	54±16 (106)	0.0097	0.0259*
DG/GG	49±23 (5)	53±14 (28)	70±18 (15)	0.0012	0.0019**
P_3	0.8704	0.8446	0.0001	–	–

[a]Trend test with the multiple general linear model adjusted for age, sex, BMI, smoking, and diabetes mellitus
*Significant difference comparing B1B1 and B2B2, by using multiple comparisons with the Duncan procedure
**Significant difference comparing B1B1 and B2B2, and B1B2 and B2B2, by using multiple comparisons with the Duncan procedure

the D442G mutation also bore the B2B2 genotype of the *Taq*IB polymorphism, compared with 15.8% bearing the 442DD genotype (106/670).

Interaction of the D442G mutation and the *Taq*IB polymorphism on HDL-C levels

The trend for the association of the *Taq*IB B2 allele and elevated HDL-C levels remained strongly significant even when the subjects carrying the D442G mutation were excluded from the analysis (Table 5; *P*-value=0.0097). In the subgroup of 442DD carriers, subjects homozygous for the B2 allele of *Taq*IB had significantly higher HDL-C levels than B1B1 analogs (54±16 mg/dl vs 50±12 mg/dl; *P*-value=0.0259). For the subjects bearing the D442G mutation, the significance of the association for the presence of the *Taq*IB B2 allele and elevated HDL-C was strengthened (*P*=0.0012). In the 442DG/GG carrier subgroup, significantly higher HDL-C levels were demonstrated for subjects homozygous for the *Taq*IB B2 allele (70±18 mg/dl) than for both the B1B1 (49±23 mg/dl) and B1B2 (53± 14 mg/dl; *P*-value=0.0019) analogs. This can be interpreted as evidence of an additive effect for the *Taq*IB B2 allele and the D442G mutation with respect to HDL-C levels. Significant association for D442G mutation and elevated HDL-C levels was only observed for subjects carrying the B2B2 genotype of the *Taq*IB polymorphism, however, and not for the B1B1 and B1B2 genotypes (*P*-value=0.0001 vs *P*-values=0.8704 and 0.8446, respectively). This means that the strength of the association for the D442G mutation and HDL-C level is dependent on the genotype of the *Taq*IB polymorphism. We further determined the effects of haplotype on HDL-C levels after exclusion of the double heterozygotes (*n*=28) in whom the haplotype could not be determined unambiguously. As revealed in Table 6, the HDL-C effect was still largely confined to individuals with a *Taq*IB2 allele (β=+0.086, *P*-value=0.014), with the greatest HDL elevation being observed for subjects who bore both of the less common alleles (β=+0.290, *P*-value=0.000).

Table 6 Significance of haplotype effects on HDL levels by reference to B1/D

Halplotype	Estimated frequency (%)	HDL β	*P*-value
B1D	57.1	–	–
B1G	0.6	–0.015	0.806
B2D	39.4	+0.086	0.014
B2G	2.9	+0.290	0.000

Interactions of obesity, triglycerides, and sex, and the genetic variants of the CETP gene on HDL-C levels

Subjects were divided into groups according to their *Taq*IB and D442G genotype and with respect to sex, obesity, smoking, and hypertriglyceridemia. The associations for the *Taq*IB2 allele and the D442G mutation and elevated HDL-C levels were only observed for the non-obese subjects (Table 7). These patterns were maintained after plasma HDL-C level was controlled for the effects of sex, age, smoking, diabetes, and triglyceride levels. Interestingly, these results revealed that the associations for the *Taq*IB2 allele and the D442G mutation, and elevated HDL-C levels were only observed for subjects with normal levels of TG (≤150 mg/dl). Although the HDL-C levels differed markedly on comparing the DD and DG/GG genotypes of the D442G mutation, the differences between the HDL-C levels were smaller for males than for females (*P*-value=0.0701 vs *P*-value=0.0126) after adjustment for the covariates. No interaction was determined for the CETP *Taq*IB polymorphism and smoking, as reflected in the HDL-C levels. This finding was repeated for the D442G mutation (data not shown).

Discussion

This study has focused on the analysis of the genetic determinants for HDL-C levels in a sample of Taiwanese Chinese. Both univariate and multivariate analyses have shown that, in addition to obesity and sex, genetic variants of the CETP gene, such as the D442G mutation and the *Taq*IB polymorphism, also affect HDL-C levels. The sample size was adequate to ensure good estimates of the

Table 7 Interaction of BMI (kg/m^2), triglycerides (mg/dl), and sex with *Taq*IB, and D442G on HDL-C (mg/dl). The number of cases is given in *brackets*

Variable		*Taq*IB				D442G		
		B1B1 Mean ± SD (*n*)	B1B2 Mean ± SD (*n*)	B2B2 Mean ± SD (*n*)	*P*-value[a]	DD Mean ± SD (*n*)	DG/GG Mean ± SD (*n*)	*P*-value[a]
BMI	BMI ≤26	51±12 (148)	55±16 (244)	61±16 (77)	0.0001	54±15 (435)	62±19 (34)	0.0015
	BMI >26	48±14 (83)	47±11 (122)	46±11 (44)	0.2338	47±13 (235)	49±12 (14)	0.5735
TG	TG ≤150	53±13 (156)	56±15 (248)	60±16 (90)	0.0001	56±14 (457)	62±18 (37)	0.0054
	TG >150	43± 9 (75)	46±12 (118)	42±11 (31)	0.9568	44±11 (213)	45±11 (11)	0.8399
Sex	Female	55±13 (102)	58±16 (170)	61±16 (54)	0.0038	57±15 (301)	62±18 (25)	0.0126
	Male	46±11 (129)	48±13 (196)	51±16 (67)	0.0031	48±12 (369)	54±18 (23)	0.0701

[a]Performed by using the general linear model adjusted for the effects of sex, age, BMI, smoking, diabetes mellitus, and triglycerides (other than sex in testing with main effect of sex models)

prevalence of genetic variants in terms of the general population and the efficacy of further analysis of gene-environment interaction. Selection bias associated with enrollment of subjects from routine health examinations could not be avoided, however, given the organizational constraints of the study.

Although it has been demonstrated that genetic factors play an important role in determining inter-individual variation for plasma HDL-C levels, the attribution of specific genetic determinants remains controversial. Using a Caucasian sample, Cohen et al. (1994) have demonstrated that the hepatic lipase and apolipoprotein AI/CIII/AIV gene loci, but not the CETP gene, are the major genetic determinants of plasma HDL-C levels. By contrast, Bu et al. (1994) have reported that the CETP gene is linked to a locus that is involved in the determination of HDL-C levels. Moreover, in a Japanese sample, 10% of the total variance in HDL-C levels has been shown to be attributable to CETP mutations (Inazu et al. 1994). In this study of Taiwanese Chinese, a significant proportion of the variation in HDL-C levels can be explained in terms of associations for genetic variants of the CETP gene. In previous studies of other ethnicities, several CETP polymorphisms and mutations have been reported; however, these have not been investigated for Chinese living in Taiwan. The polymorphism most studied to date is *Taq*IB, which is a silent base change affecting the 277th nucleotide in the first intron. Confirming the results of previous groups (Kondo et al. 1989; Freeman et al. 1990, 1994; Hannuksela et al. 1994), this study has demonstrated a significant association for the *Taq*IB polymorphism at the CETP gene locus and plasma HDL-C levels. It has been suggested that this association is population-specific or gender-dependent and highly influenced by environmental factors such as smoking, alcohol consumption, and obesity (Freeman et al. 1994; Hannuksela et al. 1994; Fumeron et al. 1995; Kauma et al. 1996). The B2-allele frequency for our sample population is similar to that reported for Caucasian populations. However, the strong association demonstrated for the *Taq*IB allele and HDL-C levels is not influenced by sex or smoking in our study. Since this polymorphism is located in an intron, it may not be a functional mutation. Moreover, previous studies of the association between the *Taq*IB polymorphism and CETP activity are not consistent (Freeman et al. 1994). This suggests that there may be an unknown functional mutation at, or adjacent to, the CETP gene locus, which is in linkage disequilibrium with the *Taq*IB polymorphism, and which gives rise to the inter-individual HDL-C variation. Linkage disequilibrium has been reported between a recently discovered polymorphism of the CETP gene promoter at position –629 (C to A), which affects transcription in vitro and associated with plasma CETP mass and HDL-C levels in vivo, and the *Taq*IB polymorphism in a Caucasian population (Dachet et al. 2000). In our study, the CETP I14A and D442G mutations have been chosen for analysis because previous studies of these mutations have been confined to Asian populations, and studies of Japanese populations have established that these mutations are important determinants of HDL-C levels (Inazu et al. 1990; Hirano et al. 1993; Sakai et al. 1995). Therefore, we have sought to determine whether the association between the *Taq*IB polymorphism and elevated HDL-C in our sample population can be explained through linkage disequilibrium with the D442G or I14A mutation. The frequency of the D442G mutation in our sample of Taiwanese Chinese is similar to that reported for Japanese in various regions, suggesting that this mutation originated from common ancestors of the Chinese and Japanese populations. Our results are similar to those from Japanese and Korean studies (Song et al. 1997) in that the D442G mutation is significantly associated with elevated HDL-C levels. In addition, sex has some effect on how the D442G mutation regulates HDL-C levels, with a more significant effect being observed for women. This gender effect needs to be confirmed, however, since the association demonstrated for males is similar to the female analog, although not statistically significant, probably because of the small size of the D442G-mutation sample (23 males, 25 females). Alternatively, sex-hormone effects or gender-specific differences for CETP affinity for apolipoprotein AI, as proposed by Moulin (1994), cannot be ruled out. Although, a statistically significant but not complete linkage disequilibrium has been demonstrated for the two less-common alleles in our sample population, the association for the *Taq*IB polymorphism and HDL-C is independent, how-

ever, and is strengthened by the D442G mutation. By contrast, the association for the D442G mutation and HDL-C is influenced by the *Taq*IB genotype. This finding has also been confirmed by the results of multivariate analysis, which indicate that *Taq*IB accounts for more of the variation in plasma HDL-C levels than does D442G. On the other hand, based on the results for our sample population, the I14A mutation appears to be rare in Taiwanese Chinese. The frequencies of the I14A mutation also vary when comparing different regions in Japan. In Omagari, the mutation is 20 times more frequent than in other areas. These results suggest that the I14A mutation may be unique to Japan, and that perhaps it arose in the Omagari area. Alternatively, it may be speculated that a Japanese ancestor carrying the CETP mutation moved to the Omagari area, and that the geographical isolation resulted in an accumulation of the genetic defect in the CETP gene (Hirano et al. 1997).

It seems reasonable to suggest that the strength of the relationships for the CETP gene mutations and polymorphisms and HDL-C levels are affected by environmental factors. For example, the significance of the association for the *Taq*IB polymorphism and HDL-C levels is reduced by obesity and smoking (Kondo et al. 1989; Freeman et al. 1994) and enhanced by alcohol consumption (Fumeron et al. 1995). In our study, obesity has been demonstrated to decrease the strength of the associations for both the *Taq*IB polymorphism and the D442G mutation, and HDL-C levels. No interaction for CETP genotype and smoking has however been observed. It was not possible to test the interaction for alcohol consumption and CETP genotype on HDL-C, because alcohol-consumption information for the subjects was not available.

Interestingly, this study has demonstrated that the strength of the association between *Taq*IB, D442G, and HDL-C levels is reduced for subjects with hypertriglyceridemia. These findings contradict previous reports of an interaction for hypertriglyceridemia and the CETP I405 V polymorphism with respect to HDL-C levels. In studies of Japanese and Danish populations (Bruce et al. 1998; Agerholm-Larsen et al. 2000), homozygosity for the V allele has borderline significantly higher HDL-C levels than subjects with the VI and II genotypes among men with TG≥165 mg/dl but not in those with TG<165 mg/dl. This interaction is explained by the proposition put forward in previous reports that, for hypertriglyceridemia, the rate-limiting factor in cholesteryl ester transfer may be the CETP (Mann et al. 1991). Our apparently contradictory results may be related either to genetic differences inherent to the sample populations, or to the variety of polymorphisms and/or mutations studied. Like the *Taq*IB variant, the CETP I405 V polymorphism may not be a functional mutation. Furthermore, its association with HDL-C levels may be the product of linkage disequilibrium with another mutation. Therefore, it is possible that the mutations with which *Taq*IB and I405 V are linked are different. This proposition has been supported by the reports of Gudnason et al. (1999) and Corbex et al. (2000), with both studies suggesting that the *Taq*IB and I405 V polymorphisms represent two functionally independent CETP gene variations that affect CETP activity or mass and, thus, plasma HDL-C levels. Moreover, these two different loci may act in functionally opposite ways with respect to the modification of TG levels. Another possibility is that hypertriglyceridemia per se is a more important predictor of HDL-C levels than CETP. When TG is above a certain level, it may exhibit an over-riding effect on the HDL-C levels compared with that of the genetic polymorphisms of CETP. As previous investigations have revealed, the mechanisms that cause low HDL-C in hypertriglyceridemic patients are complex. In addition to CETP, it has been reported that lecithin cholesterol acyl transferase, hepatic lipase, and lipoprotein lipase play important roles in the regulation of HDL-C levels in cases of this type (Tato et al. 1997). Therefore, reverse cholesterol transport through non-CETP pathways may have been more important for our hypertriglyceridemic subjects. Furthermore, Murakami et al. (1995) have demonstrated a positive correlation for the cholesterol esterification rate (CER) and the cholesteryl ester transfer rate (CETR), particularly for hypertriglyceridemic patients. When CETR is decreased because of a CETP mutation, less-efficient transport of cholesterol ester out of HDL can result in the accumulation of a large lipid-rich HDL_2 product. This accumulation has been reported to have an inhibitory effect on CER and the further rise in HDL-C level (Barter et al. 1984). This proposition is also supported by our results, with associations for D442G and HDL-C levels only being observed in subjects with normal TG levels.

In conclusion, our findings suggest that CETP genetic variation, particularly the *Taq*IB polymorphism and the D442G mutation, may explain a significant proportion of the variability in HDL-C levels for Taiwanese Chinese. Furthermore, obesity and TG appear to modulate the strength of these associations. Additional studies need to be carried out to demonstrate whether this polymorphism and this mutation play an important role in the risk of coronary artery disease among Chinese in Taiwan.

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