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Effects of hepatic lipase gene promoter nucleotide variations on serum HDL cholesterol concentration in the general Japanese population

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Abstract Genetic factors may play a major role in determining serum high-density lipoprotein (HDL) cholesterol (HDL-C) levels in the general population. Cholesteryl ester transfer protein (CETP) is a strong genetic factor as a determinant of HDL-C levels in Japanese, whereas hepatic lipase (HL) plays a predominant role in Caucasian populations. We investigated the effects of *HL* gene promoter polymorphisms on HDL-C levels in a general population of Japanese men ($n = 299$). An *HL* promoter polymorphism of $-514C/T$ explained a considerable variance of HDL-C (2.9%), as compared with *CETP* mutations of D442G and IVS14 + 1G > A (3.6% and 1.9%). *HL* promoter variation of the $-514C/C$ genotype, reported to have high HL activity, had significant effects on decreasing HDL-C levels (-3.8mg/dl), but $-514T$ allele carriers had a weak effect on increasing HDL-C levels. The frequency of the $-514T$ allele was three times higher (0.50) in the Japanese than in Caucasian populations (0.15–0.19). Thus, the higher frequency of the *HL* $-514T$ allele, along with *CETP* gene mutations, could explain about 9% of phenotypic variability of HDL-C. These genetic attributes may be among the many factors that contribute to the relatively higher serum HDL-C levels in Japanese subjects.

Key words Hepatic lipase · Single-nucleotide polymorphism · HDL · Promoter · Cholesteryl ester transfer protein · Lipoprotein lipase

Introduction

High-density lipoprotein (HDL) cholesterol (HDL-C) is a potent anti-atherogenic particle, as shown in numerous epidemiological studies (Gordon and Rifkind 1989). However, it is unclear how HDL exerts its protective actions. Both genetic and environmental factors affect serum levels of HDL-C (Heller et al. 1993; Funke 1997). Hepatic lipase (HL) activity was previously shown to be a genetic determinant of HDL-C levels in a twin study (Kuusi et al. 1987), mediated by triglyceride (TG) lipase activity, resulting in the conversion of HDL2 to HDL3. The *HL* gene (also abbreviated as *LIPC*) is located on chromosome 15q21 and is composed of nine exons coding a 476-amino acid mature protein (Datta et al. 1988; Cai et al. 1989; Ameis et al. 1990). In the promoter, *cis*-acting negative elements and hepatocyte nuclear factor binding sites were found (Hadzopoulou-Cladaras and Cardot 1993; Oka et al. 1996; Chang et al. 1997). Cohen et al. (1994) initially reported that *HL* gene variation determined HDL-C levels in Caucasian families, and subsequently found that nucleotide diversity and the haplotype of the *HL* promoter was associated with low HL activity and high HDL-C levels in Caucasians (Guerra et al. 1997). Interestingly, the $-514T$ allele, which is associated with low HL activity (70%–80% of normal), is more frequent in African Americans than in Caucasians (Vega et al. 1998; Nie et al. 1998). Also, the polymorphism influences HDL subclass distribution, with higher HDL2b and lower HDL3a (Grundy et al. 1999; Couture et al. 2000). Consistently, the $-514T$ allele, also denoted as $-480T$ by others, has shown an association with the elevated HDL-C levels in Europeans (Murtomaki et al. 1997; Jansen et al. 1997), Finns (Tahvanainen et al. 1998), and Americans (Zambon et al. 1998; Couture et al. 2000).

The lipoprotein lipase (*LPL*) gene is located on chromosome 8p22 and is composed of ten exons coding a 448-amino acid mature protein (Wion et al. 1987; Kirchgesner et al. 1989). *LPL* catabolizes chylomicron- or very low density lipoprotein (VLDL)-TG, thereby transferring excess lipoprotein surface lipids of phospholipid and free

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cholesterol into HDL. Ser447Term (S447X), resulting in two amino acids lacking at the C-terminus, was associated with increased LPL activity (+4%) and HDL-C and decreased coronary artery disease (CAD) risk in Caucasian populations (Wittrup et al. 1999).

CETP deficiency, caused by two prevalent mutations, of exon 15 D442G (+1376A > G), and intron 14 splice donor site G(+1)-to-A (IVS14 + 1G > A), is found in 9% of the general population. The heterozygotes have decreased CETP activity (60%–70% of normal) and a mild-to-moderate increase in HDL-C, with a considerable impact on plasma HDL-C distribution (Inazu et al. 1994).

In this study, we examined whether or not *HL* gene promoter polymorphisms influenced HDL-C levels in the Japanese population, in whom CETP deficiency is frequently found, and in whom serum HDL-C levels are higher than in other ethnic groups.

Subjects and methods

The study population consisted of a sample of 299 Japanese men from the general population, aged 47 years $\pm$ 10 (SD) who attended annual health checkups. Information on cigarette smoking and alcohol consumption was obtained through questionnaires and interviews. None of these men were taking any lipid-lowering agents. Antihypertensive drugs were used by 3.3% of this population (10/299), but beta-blockers were used by only 2 of the subjects. None of the subjects used corticosteroids. Blood samples were obtained after a 12-h overnight fast. Serum cholesterol, TG, and HDL-C were determined enzymatically. HDL-C was assayed by a precipitation method (dextran sulfate / phosphotungstate Mg^{2+} , HDL-C2; Daiichi Chemical, Tokyo, Japan). Low-density lipoprotein-cholesterol (LDL-C) was calculated using the formula of Friedewald et al. (1972). Informed consent was obtained for all studies.

Single-nucleotide polymorphism (SNP) genotyping

Genomic DNA was isolated from the buffy coat of 5 ml ethylenediaminetetraacetic acid (EDTA)-2Na blood according to a standard method. D442G and IVS14 + 1G > A of the *CETP* gene were determined as described elsewhere (Inazu et al. 1994). The S447X polymorphism of the *LPL* gene was determined using *MnII* (Daiichi Chemicals) digestion, as described by Kuivenhoven et al. (1997). The *HL* promoter –250G/A polymorphism was determined using *DraI* (Toyobo, Tokyo, Japan) digestion, as described by Zambon et al. (1998). The *HL* promoter –514C/T polymorphism was determined by a mismatch primer-mediated polymerase chain reaction (PCR)-restriction fragment length polymorphism (RFLP), using *NcoI* (Wako, Osaka, Japan) digestion, which recognizes the sequence of ccattg (T allele) instead of ccacgg (C allele). Oligonucleotide primers for –514 polymorphism detection were as follows: forward mismatch primer HL514A 5'-TTC TTC GTC

AGC TCC TTT TGc C-3' (A > C transversion –2 position from the 3' end of primer), reverse primer HL514B 5'-GGT GGC TTC CAC GTG GCT GCC TAA G-3'. PCR was performed with 35 cycles of 94°C for 30s, 55°C for 60s, and 72°C for 90s after 95°C 2-min denaturation. Digested PCR products were run on 3% NuSieve 3:1 agarose gel (EMC Bioproducts, Rockland, ME, USA) or 10%–20% polyacrylamide gel in 1 $\times$ Tris-borate-EDTA (TBE), and stained with ethidium bromide.

Statistical analyses

The observed genotype frequency was compared with those expected under Hardy-Weinberg equilibrium by the χ^2 test. Linkage disequilibrium between two loci was assessed by the χ^2 test, D value, and $D' = D/D_{\max}$ or $D/D_{\min}$ (Hill 1974). One-way analysis of variance (ANOVA) was performed, then multiple comparisons were made by Fisher's protected least significant difference. Mean levels between two groups were compared by *t*-test. Serum HDL-C was adjusted by multiple linear regression analysis. The amount of alcohol consumed and number of cigarettes smoked were transformed by the square root of the values. Two-way ANOVA was used for the assessment of gene-environment interactions. The genetic variance component was determined as described previously (Boerwinkle and Sing 1986). Statistical analysis was performed using StatView 5.0 (SAS Institute Japan, Tokyo, Japan).

Results

D442G missense and IVS14 + 1G > A splice donor site mutations of the *CETP* gene, S447X nonsense polymorphism of the *LPL* gene, and two *HL* promoter variations (–250 and –514) were genotyped in 299 Japanese men. The baseline clinical and biochemical characteristics of this general population are shown in Table 1. In this cohort, the frequencies of alcohol drinkers and cigarette smokers were high. However, alcohol consumption was moderate.

Table 1. Baseline clinical and biochemical characteristics of 299 men

	Mean ($\pm$ SD)	Range
Age (years)	46 $\pm$ 10	18–69
CHOL (mg/dl)	194 $\pm$ 32	116–276
TG (mg/dl)	137 $\pm$ 91	26–762
LDL-C (mg/dl)	119 $\pm$ 31	47–204
HDL-C (mg/dl)	48.3 $\pm$ 12.4	24–95
BMI (kg/m ²)	23.5 $\pm$ 2.6	13.2–36.0
Alcohol drinker (%)	82	
Alcohol (units/week) ^a	7 $\pm$ 5	0–21
Current smoker (%)	56	
Number of cigarettes/day	13 $\pm$ 14	0–60
Use of antihypertensive drug (%)	3.3	
Diabetes mellitus (%)	2.7	

BMI, Body mass index

^a1 unit = 20–25 g ethanol

Table 2. Effects of *CETP* genotype on serum HDL-C levels

	Number of subjects	HDL-C ($\pm$ SD) (mg/dl)	Δ HDL-C (mg/dl) ^a	Variance of HDL-C (%)
IVS14 + 1G/A	4	63.0 $\pm$ 11.2	14.7	1.9
442D/G	18	57.9 $\pm$ 15.3	9.6	3.6
Wild-type	277	47.4 $\pm$ 11.8	-0.9	0.5

^aDeviation from population mean of HDL-C (48.3mg/dl)

Table 3. Effects of *HL* genotype on serum HDL-C levels

	Number of subjects	HDL-C ($\pm$ SD) (mg/dl)	Δ HDL-C (mg/dl) ^a	Variance of HDL-C (%)
-514T/T	72	49.3 $\pm$ 11.7	1.0	0.2
-514T/C	150	49.4 $\pm$ 12.4	1.1	0.4
-514C/C	74	44.5 $\pm$ 11.8	-3.8	2.3

^aSee Table 2 footnote

Table 4. Comparison of genotypic effects on serum HDL-C levels

	HDL-C (mg/dl)		Adjusted HDL-C (mg/dl) ^a	
	Reg Coeff ($\pm$ SE)	F value	Reg Coeff ($\pm$ SE)	F value
<i>CETP</i> 442G carriers vs noncarriers	10.6 $\pm$ 2.9	13.5	11.6 $\pm$ 2.6	20.0
<i>CETP</i> IVS14 + 1A carriers vs noncarriers	15.5 $\pm$ 6.0	6.8	14.4 $\pm$ 5.4	7.2
<i>HL</i> -514T carriers vs noncarriers	5.2 $\pm$ 1.6	11.2	3.4 $\pm$ 1.4	5.8
<i>LPL</i> 447X carriers vs noncarriers	—	1.6	—	0.4

^aAdjusted by multiple linear regression analysis including variables (age, BMI, alcohol, and smoking)
Reg Coeff, regression coefficient

Effects of the *CETP* genotype on serum HDL-C levels are shown in Table 2. The effect of IVS14 + 1A carriers was stronger than that of 442G carriers, as judged from the deviation from the population mean of serum HDL-C. No homozygote of IVS14 + 1A or 442G was found. The attributable fraction of variance of HDL-C was larger in the 442D/G genotype than in IVS14 + 1G/A because of the higher frequency of D442G.

We analyzed two polymorphic sites in the *HL* promoter, but there was complete linkage disequilibrium between C/T (-514) and G/A (-250), indicating haplotype C-G and T-A ($\chi^2 = 343.0$; $P = 0.0001$; $D = -0.243$, $D' = -0.90$). Therefore, we have shown only the -514C/T genotype data in this report. The genotype frequencies of *HL* and *LPL* were compatible with Hardy-Weinberg equilibrium. The effects of the *HL* promoter polymorphism of -514 are shown in Table 3. The T allele appeared to have dominant effects on plasma HDL-C levels. As compared with the population mean of serum HDL-C levels, which has been reported to be higher in the Japanese than in other ethnic groups (Dwyer et al. 1997; Ueshima et al. 1982; Yano et al. 1980); the deviation was minimal. The attributable fraction explained by this genotype was largely due to lower HDL-C levels in the -514C/C genotype in this cohort. Serum TG levels in the C/C genotype (159 $\pm$ 133mg/dl, SD) were significantly increased as compared with those in the C/T genotype (127 $\pm$ 74mg/dl; $P = 0.014$).

Multiple regression analysis by stepwise forward selection was performed for comparisons of genotypic effects on serum HDL-C (Table 4). Regression coefficients for serum

HDL-C levels were higher in IVS14 + 1G/A > 442D/G > *HL* -514. Effects on adjusted HDL-C levels were similar. Low statistical power in analyses of *LPL* genotype precluded meaningful results.

Effects on serum HDL-C levels were compared among genetic and environmental factors (Table 5). By one-way ANOVA, *CETP* heterozygous mutations, *HL* -514T allele carriers, and alcohol consumption increased HDL-C levels. In contrast, smoking and increased body mass index (BMI) decreased HDL-C levels. By two-way interaction, probable gene-environment interaction was found between *CETP* genotype and smoking or BMI. Higher HDL-C levels in subjects heterozygous for *CETP* mutations were evident in intermediate- to high-BMI groups (BMI, more than 22), but not in the low-BMI group. Also, higher HDL-C levels in subjects heterozygous for *CETP* mutations were marked in nonsmokers. Because cigarette smoking can increase HL activity (Moriguchi et al. 1990), it could interact with the *HL* genotype in exerting effects on serum HDL levels. However, no interaction between *HL* genotype and environmental factors, such as alcohol, smoking, and obesity, was found, indicating that these effects are additive.

Discussion

The low HL activity allele of -514 of the *HL* promoter is associated with increased HDL-C in all Caucasian populations but Canadians (Hegele et al. 1999). The *HL* promoter

Table 5. Two-way ANOVA of genetic and environmental factors on serum HDL-C levels

Factor	Stratification	Partial sum of squares	F value	P value
Main effects				
<i>CETP</i> mutations ^a	Carriers vs noncarriers	2660	18.4	0.0001
Smoking	No, Yes	2601	17.8	0.0001
<i>HL</i> -514	T carriers vs noncarriers	1550	10.4	0.0014
BMI (kg/m ²)	<22, 22–25, >25	2199	7.5	0.0007
Alcohol (units/week)	0, 1–13, 14–21	2082	7.0	0.0010
Two-way interactions				
Smoking* <i>CETP</i> mutations		598	4.4	0.0367
BMI* <i>CETP</i> mutations		661	2.4	0.0898

ANOVA, Analysis of variance

^aIVS14 + 1G > A or D442G

–514T allele frequency is three times higher in the Japanese than in Caucasian populations. *HL* –514C/T was a significant determinant of HDL-C; however, the increasing effects on serum HDL-C were smaller than those of *CETP* variants. Thus, *HL* promoter variation had significant effects on the variability of HDL-C levels, predominantly through the –514C/C genotype decreasing HDL-C levels in the Japanese population.

HL promoter genotypes have been well characterized in Caucasian populations (Guerra et al. 1997; Nie et al. 1998; Grundy et al. 1999; Murtomaki et al. 1997; Jansen et al. 1997; Tahvanainen et al. 1998; Shohet et al. 1999; Jansen et al. 1999). However, there are a few reports on other populations (Vega et al. 1998; Zambon et al. 1998). Interestingly, the –514T allele, rare in Caucasian Americans and the Dutch (allele frequency, 0.15–0.19), was more frequent in Japanese (0.50), as shown in this study, similar to findings in African-Americans (0.53) and Koreans (0.44) (Vega et al. 1998; Hong et al. 2000). This finding is compatible with the higher frequency of the –250A allele in Japanese-Americans (0.47) (Zambon et al. 1998). We did not measure post-heparin lipolytic activity in this population, but the lipid phenotype was compatible with those in Caucasians, in whom the –514T (or the –250A) allele was associated with lower HL activity and higher HDL-C levels. The –250A allele could account for 38% of the variation of HL activity in an American population (De Oliveira e Silva et al. 1999). Furthermore, the –514T allele was shown to have 30% lower promoter activity regardless of the genotype at –250 by a transfection study (Deeb and Peng 2000). However, this association was not confirmed by another study (van't Hooft et al. 2000).

The atherogenicity of low HL activity has been debated. Although hyperalphalipoproteinemia with *HL* deficiency was not associated with arteriosclerotic diseases by itself (Sich et al. 1998), hyperalphalipoproteinemia with low activity of both HL and *CETP* was suggested to be pro-atherogenic (Hirano et al. 1995). In *HL* promoter analysis, more –514T heterozygotes, but fewer –514T homozygotes, were found in CAD patients, suggesting the complex nature of atherogenicity (Jansen et al. 1997 and 1999; Cohen et al. 1999). However, that finding was not confirmed in Finns or American whites (Tahvanainen et al. 1998; Shohet et al. 1999).

The *LPL* S447X polymorphism has been associated with higher LPL activity, lower TG, and higher HDL-C levels in several studies (Wittrup et al. 1999; Kuivenhoven 1997), which is compatible with the higher LPL activity of S447X in a transfection study (Kozaki et al. 1993; Zhang et al. 1996). However, that association was not found in this study, probably because of the relatively small sample size. We identified four homozygotes for 447X in this study, who did not have increased HDL-C levels (48.3mg/dl $\pm$ 11.8, SD), but had higher LDL-C levels (152 $\pm$ 46mg/dl; P = 0.026 vs 447Ser/Ser (S/S), 118 $\pm$ 30mg/dl). This finding has not been reported previously, and needs to be confirmed in a larger study.

In conclusion, subjects who were *HL* –514T allele carriers had higher HDL-C levels, just above the population mean, but subjects with *HL* –514C/C had decreased HDL-C levels. Nucleotide variations in the *HL* promoter, and *CETP* gene mutations, explain the considerable variability of serum HDL-C levels through an increasing effect produced by *CETP* gene mutations and a decreasing effect produced by the *HL* –514C/C genotype in Japanese. Thus, the relatively low frequency of the *HL* –514C/C genotype, in addition to a high frequency of *CETP* deficiency, as well as substantial differences in environmental factors, may explain the relatively higher HDL-C levels in the Japanese (Dwyer et al. 1997; Ueshima et al. 1982; Yano et al. 1980). Thus, the Japanese population may be a suitable model for association studies of low HL activity and CAD, in contrast with Turks, who have elevated HL activity and low HDL (Mahley et al. 2000).

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