Available online at www.sciencedirect.com
ScienceDirect
Metabolism Clinical and Experimental 58 (2009) 1178 – 1184
Metabolism
Clinical and Experimental
www.metabolismjournal.com

Two novel mutations and functional analyses of the *CETP* and *LIPC* genes underlying severe hyperalphalipoproteinemia

Wanee Plengpanich[a,b], Satapakorn Siriwong[b], Weerapan Khovidhunkit[a,b,*]

[a]*Biomedical Sciences Program, Graduate School, Chulalongkorn University, Bangkok 10330, Thailand*
[b]*Endocrinology and Metabolism Unit, Faculty of Medicine, Department of Medicine, Chulalongkorn University, and King Chulalongkorn Memorial Hospital, Thai Red Cross Society, Patumwan, Bangkok 10330, Thailand*

Received 5 August 2008; accepted 16 March 2009

Abstract

Previous studies have shown that *CETP* and *LIPC* mutations contribute to hyperalphalipoproteinemia (HALP) in some populations. We investigated whether activities in cholesteryl ester transfer protein (CETP) and hepatic lipase (HL) contribute to HALP in the Thai population and performed genetic analyses of the *CETP* and *LIPC* genes. We recruited 38 individuals with high-density lipoprotein cholesterol (HDL-C) levels of at least 2.59 mmol/L (100 mg/dL) (HALP group) and an equal number of individuals with normal serum HDL-C levels (control group). The CETP and HL activities were determined in both groups. Genetic analyses covering all the coding regions and exon-intron junctions of the *CETP* and *LIPC* genes were performed in subjects who had low CETP activity and HL activity, respectively. The mean CETP and HL activities were significantly lower in the HALP group than in the control group (34 ± 4 vs 44 ± 3 pmol/[μL h], $P = .04$ and 150 ± 17 vs 227 ± 16 nmol free fatty acid/[mL min] $P = .002$, respectively). Of the 38 individuals with HALP, 19 and 16 were found to have low CETP activity and HL activity, respectively. Of the 19 subjects with low CETP activity, 6 subjects were found to be heterozygous for a known functionally relevant c.1325A>G (D442G) mutation. The other subject was found to be heterozygous for a novel deletion mutation, c.734_737delTCCC mutation. Of the 16 subjects with low HL activity, 8 and 2 subjects were found to be heterozygous for known variants, c.283 G>A (V73M) and c.1068A>C (L334F), respectively. These variants have previously been shown not to be associated with HALP. Another subject was found to be heterozygous for a novel missense mutation, c.421G>A (G119S). Its amino acid change, absence in controls, evolutionary conservation, occurrence in functionally important domain, and predicted damaging function suggested that the G119S mutation is functionally relevant. Two novel mutations in the *CETP* and *LIPC* genes found in this study are likely to be the causes of low enzyme activities and elevated HDL-C levels.

1. Introduction

High-density lipoprotein (HDL) is a group of lipoproteins that play an important role in the development of atherosclerosis. An inverse relationship between plasma HDL cholesterol (HDL-C) levels and the risk of cardiovascular diseases suggests that HDL protects against atherogenesis [1-3]. High-density lipoprotein metabolism is complex and requires a number of plasma enzymes, transfer proteins, and cell surface receptors. One of the main functions of HDL is to transport excess cholesterol from peripheral tissues to the liver for elimination from the body in a pathway known as *reverse cholesterol transport* [3,4]. Alterations of several proteins involved in reverse cholesterol transport have been shown to affect both metabolism and plasma concentrations of HDL in humans [5]. For example, deficiency in adenosine triphosphate–binding cassette A1 or lecithin-cholesterol acyltransferase results in low levels of HDL-C [6], whereas deficiency in cholesteryl ester transfer protein (CETP) or hepatic lipase (HL) leads to high levels of HDL-C [7].

In Japan, hyperalphalipoproteinemia (HALP) is prevalent and is mainly caused by the genetic mutations in the *CETP* gene resulting in decreased or no activity of CETP [8]. Outside Japan, however, information on the cause of HALP is limited. A few studies performed on white subjects have demonstrated that HALP due to genetic CETP deficiency is rare and that the cause of HALP remains unknown [9-13].

* Corresponding author. Tel.: +66 011 662 256 4101; fax: +66 011 662 652 5347.
E-mail address: wkhovid@gmail.com (W. Khovidhunkit).

0026-0495/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.metabol.2009.03.020

Besides CETP, lower HL activity has also been found in some subjects with HALP [14-19]. The objective of our study was to determine the functional activities of CETP and HL in Thai subjects with severe HALP. We found that both CETP and HL activities were significantly lower in subjects with HALP compared with controls. Furthermore, we identified the genetic mutations in the *CETP* and *LIPC* genes in subjects who have low enzyme activities.

2. Methods

2.1. Materials

The CETP activity kit for measuring plasma CETP activity was purchased from Roar Biomedical (New York, NY). Glyceryl-tri (1-^{14}C) oleate (100 μCi/mL) and ExoSAP-IT kit were purchased from Amersham Biosciences (Buckinghamshire, England). *Taq* DNA polymerase and restriction enzymes were purchased from Fermentas (Vilnius, Lithuania). Phusion DNA polymerase was purchased from New England Biolabs (Beverly, MA). Chemical reagents were obtained from Sigma (Steinheim, Germany) or Merck (Damstadt, Germany).

2.2. Subjects

Ambulatory subjects with HDL-C levels of at least 2.59 mmol/L (100 mg/dL) on more than one occasion were recruited from the outpatient clinic of King Chulalongkorn Memorial Hospital. We chose a cutoff level of 2.59 mmol/L to represent extremely high levels of HDL-C or severe HALP because this level approximated 5 standard deviations (SDs) of mean HDL-C in our population [20]. Secondary causes of HALP, such as long-term use of alcohol, cirrhosis, thyrotoxicosis, nephrotic syndrome, hemodialysis, emphysema, and certain drugs (steroid, insulin, estrogen, fibrate, statin, nicotinic acid, and phenytoin) [7], were excluded in all subjects and controls. A total of 38 subjects were included in the HALP group, and an equal number of age-matched controls were also recruited (HDL-C levels <2.59 mmol/L). Medical history was obtained and physical examination was performed in all subjects. Genomic DNA from 50 unrelated healthy subjects (HDL-C levels <2.59 mmol/L) were also used for confirmation of novel mutations by polymerase chain reaction–restriction fragment length polymorphism (PCR-RFLP). Informed consent was obtained from each subject; and the study protocol was approved by the Ethics Committee of the Faculty of Medicine, Chulalongkorn University, Thailand. Procedures were performed in accordance with institutional guidelines.

2.3. Biochemical measurements

Venous blood samples were collected after a 12-hour overnight fast. Postheparin plasma samples were also obtained 15 minutes after intravenous injection of heparin (100 IU/kg body weight). Samples were immediately centrifuged at 3500*g* for 10 minutes, and plasma was frozen at −80 C until analyses. Total cholesterol, triglyceride, and HDL-C concentrations were determined using enzymatic methods in an automated system (Roche COBAS Integra 400 Plus, Laval, Quebec, Canada). Low-density lipoprotein cholesterol (LDL-C) concentrations were determined by the Friedewald formula. Cholesteryl ester transfer protein activity was determined using a CETP activity kit according to the manufacturer's instructions. Hepatic lipase activity was determined as previously described [21].

2.4. Genetic analyses of the CETP and LIPC genes

Because CETP and HL activities vary from assay to assay and there are no "normal" levels of CETP and HL, we chose the levels less than 1 SD of the mean of those in the control group to represent low activities. Genomic DNA of the HALP subjects with low CETP activity (n = 19) and low HL activity (n = 16) was isolated from the whole blood by phenol-chloroform extraction. Each exon and exon-intron junction of the *CETP* and *LIPC* genes were individually amplified by PCR. Exon 14 of the *CETP* gene was sequenced in all subjects with HALP (n = 35; DNA was not available in 3 subjects) for detecting an intron 14 splicing defect (Ivs14+1G>A) that was a hot spot in the Japanese. Data on the primers used are available upon request. In general, PCR was carried out using 0.1 μg of genomic DNA in a buffer containing 10 mmol/L Tris (pH 8.3), 50 mmol/L KCl, 0.2 mmol/L dNTP, 1.5 mmol/L $MgCl_2$, 0.2 mmol/L of each primer, and 0.5 U *Taq* DNA polymerase. The PCR conditions were denaturation at 95°C for 5 minutes, followed by 35 cycles of denaturation at 95° for 30 seconds, annealing at 56°C to 65°C for 30 seconds, and extension at 72°C for 1 minute, with final extension at 72°C for 10 minutes. The abundance and quality of DNA fragments were analyzed by electrophoresis on 1% agarose gels containing ethidium bromide and inspected under UV light. The PCR products were purified with the ExoSAP-IT kit and sent to Macrogen (Seoul, Korea) for sequencing. The standard mutation nomenclature was used according to the Human Genome Variation Society [22].

2.5. Mutation screening using PCR-RFLP analysis in both HALP and control groups

A known mutation of the *CETP* gene (D442G) and a novel deletion mutation (c.734_737delTCCC) were analyzed using PCR-RFLP. The PCR amplification was performed with primers 5′-CTG CCG CCA GCG AAA CTC TG-3′ (sense) and 5′-CCG TAC TCC TAA CCC AAC TT-3′ (antisense) for a D442G mutation and 5′-CTG CAC TCT GGG CTG AAT GC-3′ (sense) and 5′-TCG TCC TGC TAC ATC TCA GC-3′ (antisense) for a c.734_737delTCCC mutation. The amplified PCR products were digested at 37°C overnight with the restriction enzymes *Bgl*I and *NLa*IV for the D442G and the c.734_737delTCCC mutations, respectively.

Two known variants of the *LIPC* gene (V73M and L334F) and a novel missense mutation (c.421G>A or G119S) were analyzed using PCR-RFLP. Exon 3 of the *LIPC* gene was amplified using a pair of primers: 5′-GGA GCT GGA GAA GGA AGA AG-3′ (sense) and 5′-ACT CTC AGA GGA AGG GAA AG-3′ (antisense). The products were digested with *Pae*I and *BseL*I for detection of the V73M variant and the G119S mutation, respectively. For the L334F variant, exon 7 was amplified using a pair of primers: 5′-TAA ATT TAA AAT CAC TGC TT-3′ (sense) and 5′ CAC CTA GGG GGC TAC ACC TC-3′ (antisense); and the products were digested with *Mse*I. After digestion, the reaction mixture was electrophoresed on 2% agarose gels; and DNA fragments were visualized under UV light. The DNA from 50 unrelated healthy subjects was also investigated for the presence of novel mutations.

2.6. Bioinformatic studies

Both the PolyPhen (http://genetics.bwh.harvard.edu/pph/) and Protein Analysis Through Evolutionary Relationships (PANTHER; www.pantherdb.org) programs were used to determine dysfunction of the a novel missense mutation (G119S). The PANTHER program uses multiple alignments of protein sequences to estimate the probability of different amino acids occurring at different positions. Whereas high-probability amino acids are more likely to result in a functional protein, low-probability amino acids are likely to result in a protein with impaired function. A substitution position-specific evolutionary conservation (subPSEC) score can be calculated from an equation to determine if a given coding variant will cause a deleterious effect on the protein function. Smaller (more negative) subPSEC scores indicate a higher likelihood of a coding variant being deleterious.

$P_{\text{deleterious}}$, the probability of a coding variant being deleterious, can be calculated using a subPSEC score. A subPSEC score less than −3, which corresponds to a $P_{\text{deleterious}}$ of .5, has previously been identified as a cutoff point for functional significance. A greater $P_{\text{deleterious}}$ value tends to indicate more severe impairment [23,24].

Table 1
Clinical characteristics of subjects in the HALP group and control subjects

Baseline characteristics	Control (n = 38)	HALP (n = 38)	*P* value
Mean age (year)	56 ± 2	57 ± 2	.93
Male-female (n)	1:37	2:36	.56
Years after menopause	10 ± 1.4	9 ± 1.8	.68
SBP/DBP (mm Hg)	125 ± 4/71 ± 2	123 ± 3/73 ± 2	.81/.37
Body weight (kg)	57 ± 2	52 ± 2	.05
Body mass index (kg/m^2)	23 ± 1	22 ± 1	.09
Waist-hip ratio	0.82 ± 0.01	0.80 ± 0.01	.12
Presence of corneal arcus (%)	6/38 (16%)	11/38 (29%)	.27
Total cholesterol (mg/dL)	237 ± 6	258 ± 7	.03
HDL-C (mg/dL)	65 ± 3	119 ± 2	<.001
Triglyceride (mg/dL)	136 ± 11	72 ± 6	<.001
LDL-C (mg/dL)	146 ± 6	126 ± 7	.04
CETP activity (pmol/[μL h])	44 ± 3	34 ± 4	.04
HL activity (nmol FFA/[mL min])	227 ± 16	150 ± 17	.002

Values are means ± SEM. SBP indicates systolic blood pressure; DBP, diastolic blood pressure.

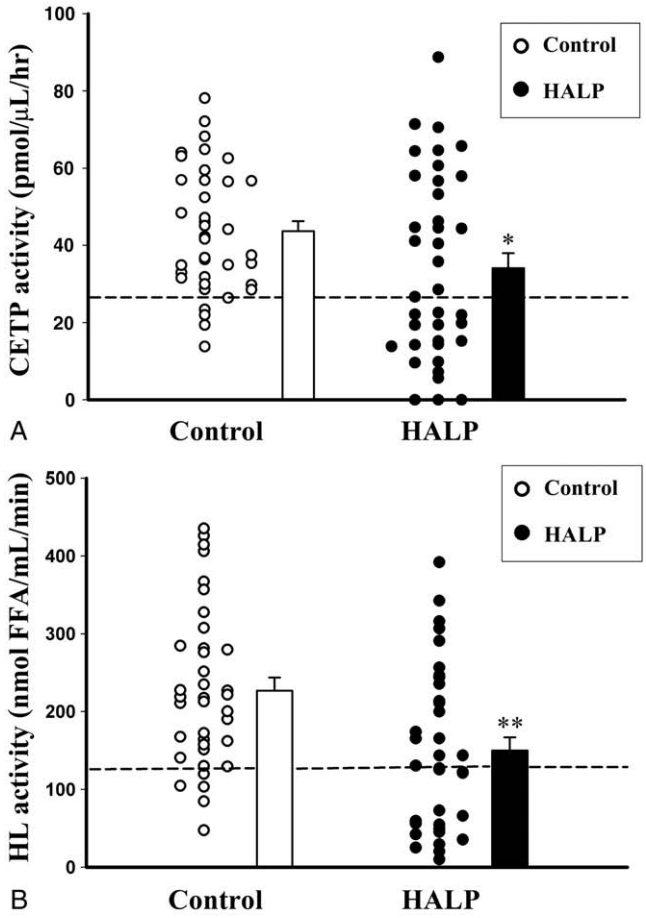

Fig. 1. A, CETP activity in the control group and the HALP group. Individual data (circles), mean values (vertical bars), and SEM (error bars) are shown. The dashed line indicates a cutoff point of plasma CETP activity less than 28 pmol/(μL h). Unpaired Student *t* test was used for comparison between the 2 groups (*P = .04). B, HL activity in the control group and the HALP group. Individual data (circles), mean values (vertical bars), and SEM (error bars) are shown. The dashed line indicates a cutoff point of plasma HL activity less than 127 nmol FFA/(mL min). Unpaired Student *t* test was used for comparison between the 2 groups (**P = .002).

2.7. Statistical analysis

Statistical analysis was performed using SPSS (version 12, Chicago, IL). Normality of distribution of data was tested by Kolmogorov-Smirnov test. The results are presented as mean ± SEM. Statistical significance was evaluated by use of the Student *t* test for comparison of unpaired data, the χ^2 test for frequency, and the Pearson correlation for the relationship between the 2 groups. P value less than .05 was considered statistically significant.

3. Results

3.1. Clinical characteristics

Because of a high level of HDL-C as a cutoff point (≥ 2.59 mmol/L), only 38 subjects with HALP were included in the study. Comparison of clinical characteristics between the HALP group and the control group is shown in Table 1. One case in the HALP group had a history of cerebrovascular accident, and 1 case in the control group had a history of transient ischemic attack. Body weight was significantly lower than those in the control group, whereas body mass index and waist-hip ratio were not significantly different. Presence of corneal arcus was not significantly different between the 2 groups.

Plasma concentrations of total cholesterol and HDL-C in the HALP group were significantly higher than those in the control group (Table 1). In contrast, plasma triglyceride and LDL-C concentrations in the HALP group were significantly lower than those in the control group (Table 1).

3.2. CETP activity

We next determined plasma CETP activity in both groups. As shown in Table 1 and Fig. 1A, we found that the mean CETP activity in the HALP group was significantly lower than that in the control group (34 ± 4 vs 44 ± 3 pmol/[μL h], $P = .04$). In our study, a cutoff point for plasma CETP activity less than 28 pmol/(μL h), which corresponded to 1 SD (SD = 16) less than the mean of the CETP activity of the control group, was chosen to indicate a low CETP activity. We found 19 subjects who had CETP activity lower than this cutoff point (50%) (Table 2). However, these subjects were not significantly different from the whole HALP group in terms of lipoprotein levels or anthropometric parameters (data not shown).

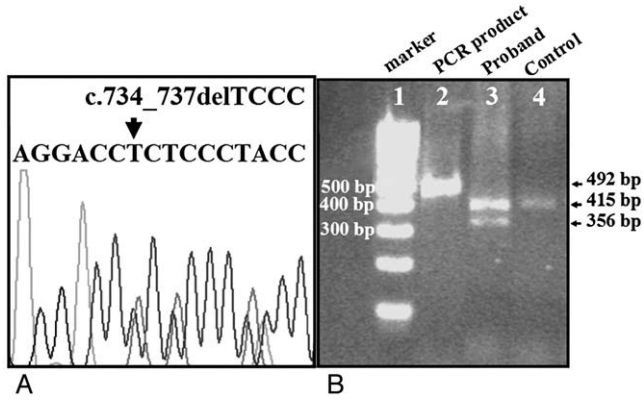

Fig. 2. A, Genomic DNA sequence of exon 9 of the *CETP* gene showing a novel mutation, c.734_737delTCCC, in 1 subject in the HALP group. B, The PCR-RFLP of the *CETP* mutation. Cleavage of PCR-amplified 492–base pair (bp) product of exon 9 (lane 2) with *NLa*IV normally results in 415-, 51-, and 26-bp fragments (lane 4). The presence of the mutant allele is disclosed by the appearance of a 356-bp fragment (lane 3). Lane 1 shows molecular size marker.

3.3. Genetic analyses of the CETP gene

All 16 exons and all exon-intron junctions of the *CETP* gene in 19 subjects who had low CETP activity were sequenced, and we found a known D442G mutation and a novel heterozygous deletion mutation, c.734_737delTCCC, in 6 and 1 subjects, respectively (Table 2 and Fig. 2). An intron 14 splicing defect (Ivs14+1G>A) commonly found in

Table 2
CETP mutations, CETP activity, and HDL-C concentrations in subjects in the HALP group who had low CETP activity

Subject no.	CETP activity (pmol/[μL h])	HDL-C (mg/dL)	D442G known mutation	c.734_737delTCCC novel mutation
1	14	101	Not detected	Not detected
2	7	143	Not detected	Heterozygous
3	14	117	Not detected	Not detected
4	14	151	Heterozygous	Not detected
5	27	111	Heterozygous	Not detected
6	19	110	Heterozygous	Not detected
7	19	108	Not detected	Not detected
8	20	102	Not detected	Not detected
9	0	121	Heterozygous	Not detected
10	0	103	Not detected	Not detected
11	15	113	Not detected	Not detected
12	22	120	Heterozygous	Not detected
13	6	114	Not detected	Not detected
14	10	136	Not detected	Not detected
15	0	110	Heterozygous	Not detected
16	15	131	Not detected	Not detected
17	23	112	Not detected	Not detected
18	10	110	Not detected	Not detected
19	22	137	Not detected	Not detected

Table 3
LIPC variants, HL activity, and HDL-C concentrations in subjects in the HALP group who had low HL activity

Subject no.	HL activity (nmol FFA/[mL min])	HDL-C (mg/dL)	G119S novel mutation	V73M known variant	L334F known variant
1	59	101	Not detected	Not detected	Not detected
2	30	114	Not detected	Heterozygous	Not detected
3	20	111	Not detected	Not detected	Not detected
4	42	108	Not detected	Heterozygous	Not detected
5	73	102	Not detected	Not detected	Not detected
6	36	110	Not detected	Not detected	Not detected
7	121	120	Not detected	Heterozygous	Not detected
8	126	100	Not detected	Heterozygous	Not detected
9	56	116	Not detected	Heterozygous	Heterozygous
10	122	131	Not detected	Not detected	Not detected
11	66	107	Not detected	Not detected	Not detected
12	45	107	Not detected	Heterozygous	Heterozygous
13	55	112	Not detected	Heterozygous	Not detected
14	50	102	NA	NA	NA
15	10	132	Not detected	Heterozygous	Not detected
16	25	137	Heterozygous	Not detected	Not detected

NA indicates DNA not available.

Table 4
CETP and *LIPC* variants in the entire group

Variants	Controls (38)	HALP (35)[a]
D442G Known mutation (*CETP* gene)	0	9 All were heterozygous.
c.734_737delTCCC Novel mutation (*CETP* gene)	0	1
G119S Novel mutation (*LIPC* gene)	0	1
V73M Known variant (*LIPC* gene)	17 Heterozygous =14 Homozygous = 3	16 Heterozygous =10 Homozygous = 6
L334F Known variant (*LIPC* gene)	4 All were heterozygous.	4 All were heterozygous.

[a] DNA was not available in 3 subjects.

the Japanese was not found in all HALP subjects. We further analyzed the D442G mutation in the entire group (Table 4). Using PCR-RFLP, the D442G mutation was found in 9 subjects in the HALP group (all of them were heterozygous mutations); but none was found in the control group. Plasma CETP activity was significantly lower in those who had the D442G mutation than in those without the mutation in subjects with HALP (20 ± 5 vs 36 ± 5 pmol/[μL h], respectively; P = .03). However, there were no significant differences in terms of lipoprotein levels or anthropometric parameters between those HALP subjects who had the D442G mutation and those who did not (data not shown).

The novel deletion mutation, c.734_737delTCCC, occurs in exon 9 and is predicted to result in a premature stop codon 30 amino acids downstream, causing deletion of the C terminus of the protein, which is part of lipid binding site. The proband was a 73-year–old single woman. Besides hypertension, she had no symptoms or signs of cardiovascular diseases. Her HDL-C concentration was 143 mg/dL, and her CETP activity was 7 pmol/(μL h). This novel mutation was found only in this subject and was not found in the rest of the HALP group, the control group, or 50 healthy subjects.

A further study of the proband's family revealed that her only surviving immediate family member was her 65-year–old brother, who also harbored this mutation. He was a chronic smoker for 44 years with hypertension and had a cerebrovascular accident at age 57 years; and his HDL cholesterol concentration was 55 mg/dL while on simvastatin 10 mg/d. Unfortunately, his CETP activity was not available; and he recently died from lung cancer.

3.4. HL activity

HL activity was evaluated next in our subjects. We found that postheparin plasma HL activity in the HALP group was significantly lower than that in the control group (150 ± 17 vs 227 ± 17 nmol free fatty acid [FFA]/[mL min] P = .002, Fig. 1B). A cutoff point for plasma HL activity less than 127 nmol FFA/(mL min), which corresponded to 1 SD (SD = 100) less than the mean of the HL activity in the control group, was chosen to indicate a low HL activity. We found 16 subjects who had HL activity lower than this cutoff point (42%). However, these subjects were not significantly different from the whole HALP group in terms of lipoprotein levels or anthropometric parameters (data not shown).

3.5. Genetic analyses of the LIPC gene

All 9 exons and all exon-intron junctions of the *LIPC* gene in 16 subjects who had low HL activity were sequenced; and we found 2 known genetic variants, V73M and L334F, in 8 and 2 subjects, respectively (Table 3). In addition, a novel heterozygous missense mutation, G119S, was found in 1 subject (Table 3 and Fig. 3).

Using PCR-RFLP, the V73M variant was found in 16 subjects in the HALP group and 17 subjects in the control group (Table 4). In the entire group, we found no significant differences in HL activity, lipoprotein levels, or anthropometric parameters among subjects with normal, heterozygous, or homozygous genotypes (data not shown), suggesting that it was not a cause of HALP. The L334F variant was found in 4 subjects in both the HALP group and the control group (all were heterozygous) (Table 4). Similarly, we did not find a difference in HL activity, lipoprotein levels, or anthropometric parameters between subjects with or without the L334F variant, suggesting that it was not a cause of HALP (data not shown).

The G119S proband was a 72-year–old woman. Except for hypertension, she had no evidence of cardiovascular diseases. Her HDL-C was 137 mg/dL, and her HL activity was 25 nmol FFA/(mL min). A further study on her family

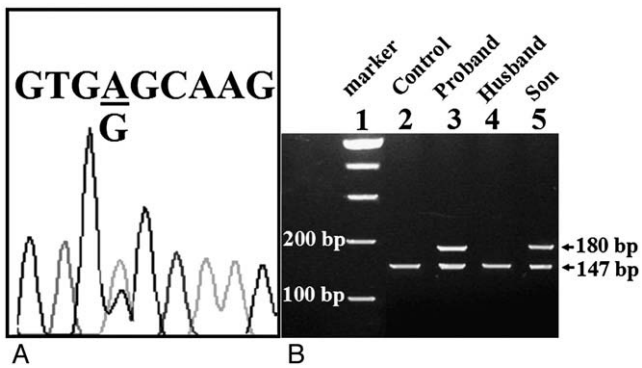

Fig. 3. A, Genomic DNA sequence of exon 3 of the *LIPC* gene showing a novel missense mutation, G119S, in 1 subject in the HALP group. B, The PCR-RFLP of the *LIPC* mutation. Cleavage of PCR-amplified 317-bp product of exon 3 with *BseL*I normally results in 147-, 70-, 67-, and 33-bp fragments (lanes 2 and 4). The presence of the mutant allele is disclosed by appearance of an extra 180-bp fragment (lanes 3 and 5). Lane 1 shows molecular size marker.

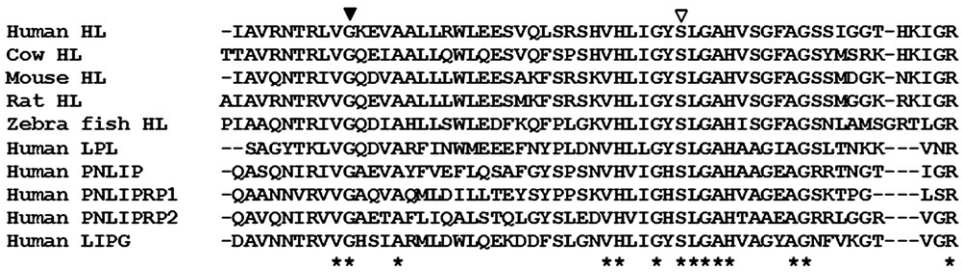

Fig. 4. Amino acid sequence alignment of HL in human, cow, mouse, rat, zebrafish, human lipoprotein lipase, human pancreatic lipase, human pancreatic lipase–related protein 1, human pancreatic lipase–related protein 2, and human endothelial lipase. The position of Gly119 is indicated by filled triangle, and the position of Ser 146 is indicated by open triangle. Asterisks indicate conserved amino acid residues. LPL indicates lipoprotein lipase; PNLIP, pancreatic lipase; PNLIPRP1, pancreatic lipase–related protein 1; PNLIPRP2, pancreatic lipase–related protein 2; LIPG, endothelial lipase.

showed that her 54-year–old son also had a similar heterozygous mutation. His HDL-C concentration was 84 mg/dL. This mutation was not found in the rest of the HALP group, the control group, or 50 unrelated healthy subjects.

3.6. Molecular pathology of the new LIPC mutation (G119S)

We proposed that amino acid substitution at position 119 of HL is functionally relevant because of the following reasons: First, a glycine residue at position 119 of HL is a strictly conserved amino acid across different species and among several related proteins in the lipase superfamily (Fig. 4). Second, the glycine residue at position 119 lies in a conserved helix 3 and is adjacent to Ser 146, which is part of the classic Ser-Asp-His catalytic triad. Third, the PolyPhen program predicted that this mutation is probably damaging; and the PANTHER program similarly predicted this mutation to be deleterious (subPSEC score of −4.43 and $P_{\text{deleterious}}$ of .81). Lastly, this mutation was absent in 38 controls and 50 unrelated subjects.

4. Discussion

In Japan, HALP is common, which is primarily due to CETP deficiency. Cholesteryl ester transfer protein is a key enzyme involved in the transfer of cholesterol ester from HDL to apolipoprotein B–containing lipoproteins. Lack of CETP results in accumulation of cholesterol-rich HDL and elevation of plasma HDL-C levels. Outside Japan, however, information on the cause of HALP is relatively scarce. Besides CETP deficiency, decreased HL activity has been associated with an increase in HDL-C levels [14-19]. Hepatic lipase is an enzyme that hydrolyzes triglyceride and phospholipids in HDL; therefore, HL activity is another important determinant of plasma HDL-C levels. Hepatic lipase deficiency has been identified in several families, which display variable phenotypes; however, the most consistent finding is an elevation of HDL-C levels [15,19].

In our study of Thai subjects with HALP, we found that both CETP and HL activities were significantly lower than those in the control group. Based on enzyme activities, low CETP activity and low HL activity were found in 50% and 42% in our subjects, respectively. We next determined whether low enzyme activities were due to genetic mutation. Our result identified both known and novel mutations of the *CETP* and *LIPC* genes. However, not all subjects with low enzyme activity were found to have a mutation, which is consistent with a study in Japan showing that 34% of subjects with HALP and low CETP activity had no identifiable mutation in the *CETP* gene [8].

In our study, we found the known D442G mutation in the *CETP* gene as previously observed in the Japanese and the Chinese subjects with HALP. Interestingly, an intron 14 splicing defect (Ivs14+1G>A) commonly found in the Japanese was not identified in any of the HALP subjects. In this study, we identified the first deletion mutation in the *CETP* gene. The observed mutation was not present in 38 controls and 50 unrelated subjects, indicating that this mutation is not a common polymorphism. This deletion mutation is predicted to result in a truncated protein lacking exons 10 to 16, which also contains a neutral lipid binding site. It is expected that the c.734_737delTCCC mutant would be inactive and affect lipid binding efficiency, causing severe HALP. Although this mutation was also found in the brother of the proband, his HDL-C concentration was only slightly high and his CETP activity was unavailable. We think that other factors (ie, smoking, hypertension, and metabolic syndrome) could have contributed to his HDL-C level that was lower than expected from the mutation in the *CETP* gene. It is of note that a study in Japanese subjects with mutations in the *CETP* gene causing CETP deficiency has also shown that not all of the subjects displayed HALP [8]. In fact, 5.7% and 7.2% of subjects with an Ivs14+1G>A mutation and a D442G mutation, respectively, had HDL cholesterol concentrations lower than 40 mg/dL [8].

We identified 2 previously reported genetic variants of the *LIPC* gene, V73M and L334F, in both groups. The association between the V73M variant and types of dyslipidemia is conflicting. One study reported that the V73M variant was present at a higher frequency in subjects with combined hypertriglyceridemia and HALP [18], whereas others showed no association with various types of dyslipidemia [25]. In our present study, the V73M and

L334F variants were found at a relatively similar frequency in both control and HALP groups, suggesting that these variants are not associated with HALP.

A novel heterozygous missense mutation of the *LIPC* gene, G119S, was identified in 1 subject with low HL activity in the HALP group. Several lines of evidence support that this novel mutation is functionally relevant. First, the glycine residue at position 119 of HL is highly conserved across several animal species and among different proteins in the lipase superfamily, such as lipoprotein lipase, endothelial lipase, pancreatic lipase, and pancreatic lipase–related proteins 1 and 2. Second, Gly119 lies in a conserved helix 3 and is adjacent to Ser 146, which is part of the classic Ser-Asp-His catalytic triad found in several lipase enzymes [26,27]. Third, both the PolyPhen and PANTHER predicted this mutation to be dysfunctional. Lastly, this mutation was absent in 38 controls and 50 unrelated subjects. Although the above evidence suggests that this mutation is functionally relevant, further experiments to define the definitive functional change of this novel mutation are ongoing in our laboratory.

In summary, low CETP or HL activity is common in Thai subjects with HALP. We identified both known and novel mutations in the *CETP* and *LIPC* genes, suggesting that these genes are responsible for some of the severe HALP in the Thai population. This is the first report of deletion mutation, c.734_737delTCCC, in the coding region of *CETP* gene.

Acknowledgments

This work was supported by grants from Ratchadapisek-sompotch Fund (2004 and 2006 to WK), Faculty of Medicine, Chulalongkorn University, and the Hormone Research Fund, Anandamahidol Foundation under the Royal Patronage of His Majesty the King of Thailand. We thank Dr Kenneth R Feingold for critical review of the manuscript, Dr Thiti Snabboon and Dr Vorasuk Shotelersuk for advice, and Ms Natnicha Houngngam and Ms Suchanya Poolsuk for technical assistance.

References

[1] Barter PJ, Rye KA. Relationship between the concentration and antiatherogenic activity of high-density lipoproteins. Curr Opin Lipidol 2006;17:399-403.
[2] Linsel-Nitschke P, Tall AR. HDL as a target in the treatment of atherosclerotic cardiovascular disease. Nat Rev Drug Discov 2005;4: 193-205.
[3] von Eckardstein A, Nofer JR, Assmann G. High density lipoproteins and arteriosclerosis. Role of cholesterol efflux and reverse cholesterol transport. Arterioscler Thromb Vasc Biol 2001;21:13-27.
[4] Fielding CJ, Fielding PE. Molecular physiology of reverse cholesterol transport. J Lipid Res 1995;36:211-28.
[5] von Eckardstein A, Assmann G. High density lipoproteins and reverse cholesterol transport: lessons from mutations. Atherosclerosis 1998;137(Suppl):S7-S11.
[6] Miller M, Zhan M. Genetic determinants of low high-density lipoprotein cholesterol. Curr Opin Cardiol 2004;19:380-4.
[7] Yamashita S, Maruyama T, Hirano K, et al. Molecular mechanisms, lipoprotein abnormalities and atherogenicity of hyperalphalipoproteinemia. Atherosclerosis 2000;152:271-85.
[8] Maruyama T, Sakai N, Ishigami M, et al. Prevalence and phenotypic spectrum of cholesteryl ester transfer protein gene mutations in Japanese hyperalphalipoproteinemia. Atherosclerosis 2003;166: 177-85.
[9] Hill SA, Nazir DJ, Jayaratne P, et al. Mutations in cholesteryl ester transfer protein and hepatic lipase in a North American population. Clin Biochem 1997;30:413-8.
[10] Miettinen HE, Gylling H, Tenhunen J, et al. Molecular genetic study of Finns with hypoalphalipoproteinemia and hyperalphalipoproteinemia: a novel Gly230 Arg mutation (LCAT[Fin]) of lecithin:cholesterol acyltransferase (LCAT) accounts for 5% of cases with very low serum HDL cholesterol levels. Arterioscler Thromb Vasc Biol 1998;18:591-8.
[11] Teh EM, Dolphin PJ, Breckenridge WC, et al. Human plasma CETP deficiency: identification of a novel mutation in exon 9 of the CETP gene in a Caucasian subject from North America. J Lipid Res 1998;39: 442-56.
[12] Rhyne J, Ryan MJ, White C, et al. The two novel CETP mutations Gln87X and Gln165X in a compound heterozygous state are associated with marked hyperalphalipoproteinemia and absence of significant coronary artery disease. J Mol Med 2006;84:647-50.
[13] van der Steeg WA, Hovingh GK, Klerkx AH, et al. Cholesteryl ester transfer protein and hyperalphalipoproteinemia in Caucasians. J Lipid Res 2007;48:674-82.
[14] Kuusi T, Ehnholm C, Viikari J, et al. Postheparin plasma lipoprotein and hepatic lipase are determinants of hypo- and hyperalphalipoproteinemia. J Lipid Res 1989;30:1117-26.
[15] Hegele RA, Little JA, Vezina C, et al. Hepatic lipase deficiency. Clinical, biochemical, and molecular genetic characteristics. Arterioscler Thromb 1993;13:720-8.
[16] Hirano K, Yamashita S, Kuga Y, et al. Atherosclerotic disease in marked hyperalphalipoproteinemia. Combined reduction of cholesteryl ester transfer protein and hepatic triglyceride lipase. Arterioscler Thromb Vasc Biol 1995;15:1849-56.
[17] Sich D, Saidi Y, Giral P, et al. Hyperalphalipoproteinemia: characterization of a cardioprotective profile associating increased high-density lipoprotein 2 levels and decreased hepatic lipase activity. Metabolism 1998;47:965-73.
[18] Gehrisch S, Kostka H, Tiebel M, et al. Mutations of the human hepatic lipase gene in patients with combined hypertriglyceridemia/hyperalphalipoproteinemia and in patients with familial combined hyperlipidemia. J Mol Med 1999;77:728-34.
[19] Tilly-Kiesi M, Schaefer EJ, Knudsen P, et al. Lipoprotein metabolism in subjects with hepatic lipase deficiency. Metabolism 2004;53:520-5.
[20] Taenwong S, Sitprija S, Santiyanont R. Reference range of serum lipids and lipoproteins according to age and sex. Chula Med J 1988;32:55-66.
[21] Krauss RM, Levy RI, Fredrickson DS. Selective measurement of two lipase activities in postheparin plasma from normal subjects and patients with hyperlipoproteinemia. J Clin Invest 1974;54:1107-24.
[22] Ogino S, Gulley ML, den Dunnen JT, et al. Standard mutation nomenclature in molecular diagnostics: practical and educational challenges. J Mol Diagn 2007;9:1-6.
[23] Thomas PD, Campbell MJ, Kejariwal A, et al. PANTHER: a library of protein families and subfamilies indexed by function. Genome Res 2003;13:2129-41.
[24] Brunham LR, Singaraja RR, Pape TD, et al. Accurate prediction of the functional significance of single nucleotide polymorphisms and mutations in the ABCA1 gene. PLoS Genetics 2005;1:0739-47.
[25] Hegele RA, Tu L, Connelly PW. Human hepatic lipase mutations and polymorphisms. Hum Mutat 1992;1:320-4.
[26] Derewenda ZS, Cambillau C. Effects of gene mutations in lipoprotein and hepatic lipases as interpreted by a molecular model of the pancreatic triglyceride lipase. J Biol Chem 1991;266:23112-9.
[27] Perret B, Mabile L, Martinez L, et al. Hepatic lipase: structure/function relationship, synthesis, and regulation. J Lipid Res 2002;43:1163-9.