Jpn J Electroph 2001; 45: 65

〔Full Paper〕

# Cholesteryl ester transfer protein deficiency is correlated with thyroid disease

**Yan-Ping Feng,* Akira Kondo,** Masato Maekawa* and Takashi Kanno***

## SUMMARY

We investigated whether cholesteryl ester transfer protein (CETP) deficiency is a risk factor for atherosclerosis or other disease. Ninety-seven individuals with increased levels of high density lipoprotein-cholesterol (HDL-C) (≥85 mg/dl) were selected. CETP deficiency was assessed in all 97 subjects by polymerase chain reaction (PCR) and either single stranded conformation polymorphism (SSCP) or restriction fragment length polymorphism (RFLP). Of the 97 subjects, 7 (7%) were heterozygous for the intron 14 splicing defect, which decreases CETP activity, and 9 (9%) were heterozygous for D442G missense mutation, which has no effect on CETP activity. One patient was found to be a compound heterozygote for the intron 14 and D442G mutations. These mutations were found in only 3 atherosclerotic patients; 2 carried the D442G mutation, and 1 had the intron 14 mutation. Although CETP activity was not associated with atherosclerosis, we discovered a novel relation between CETP gene mutations and thyroid gland disorders. Specifically, 5 of 7 subjects with CETP gene intron 14 mutation had thyroid disease. Regarding the D442G mutation, there was no difference between subjects with and without thyroid disease. It is possible that CETP activity affects thyroid function.

Key words: cholesteryl ester transfer protein deficiency, high density lipoprotein cholesterol, mutation, thyroid disease.

## INTRODUCTION

Cholesteryl ester transfer protein (CETP) is a plasma glycoprotein that transfers cholesteryl ester formed by lecithin: cholesterol acyltransferase (LCAT) in high density lipoprotein (HDL) to apolipoprotein B (apoB)-containing lipoproteins and regulates plasma HDL-cholesterol (HDL-C) levels.[1] Lipoprotein profiles of subjects with CETP deficiency are characterized by increased HDL-C levels and decreased low density lipoprotein (LDL) levels with the change of these lipoprotein components.[1,2]

Two common mutations, intron 14 and D442G in the CETP gene, have been reported in Japanese with CETP deficiency.[3] These mutations are found at high frequencies, 0.98% and 7.3%, respectively, in heterozygotes in the Japanese population.[2,4,5] The intron 14 mutation, which is a G(+1)-to-A mutation in the 5′ splice donor site of intron 14, greatly increases HDL-C plasma levels and decreases CETP activity. The D442G

* Yan-Ping Feng, 近藤 明, 前川真人, 菅野剛史; Department of Laboratory Medicine, Hamamatsu University School of Medicine.

** 近藤 明; Diagnostics Research Laboratories, Daiichi Pure Chemicals Co.

Correspondence address: Takashi Kanno, Department of Laboratory Medicine, Hamamatsu University School of Medicine, 3600, Handa-cho, Hamamatsu 431-3192, Japan.

Abbreviations: CETP, cholesteryl ester transfer protein; LCAT, lecithin: cholesterol acyltransferase; HDL, high density lipoprotein; ApoB, apolipoprotein B; HDL-C, high density lipoprotein-cholesterol; LDL, low density lipoprotein; CHD, coronary heart disease; TC, total cholesterol; TG, triglyceride; ApoA-I, apolipoprotein A-I; ApoE, apolipoprotein E; PCR, polymerase chain reaction; SSCP, single stranded conformation polymorphism; RFLP, restriction fragment length polymorphism.

(Received October 16, 2000, Accepted November 11, 2000, Published March 15, 2001)

mutation, which is a missense mutation ($Asp^{442}$ to Gly) in exon 15, has only weak effects on HDL-C plasma levels and CETP activity.

Some researchers have reported that CETP deficiency is associated with coronary heart disease (CHD) and is a significant, independent risk factor for CHD.[6,7] However, a recent study showed that increased HDL levels due to CETP deficiency or other causes are associated with reduced risk of CHD,[8] and the significance in relation to atherosclerosis is still unclear. Genetic analysis may be useful for evaluating the predictive values of risk factors for specific diseases. Thus, we investigated the clinical role of CETP in subjects with mutations in the CETP gene and high levels of HDL-C. Unexpectedly, we found a new relation between CETP activity deficiency and thyroid function.

## MATERIALS AND METHODS

### Subjects

Ninety-seven individuals with increased serum HDL-C levels ($\geq$85 mg/dl) (high HDL-C group) were selected randomly from patients treated at Hamamatsu University School of Medicine Hospital. Subjects comprised 36 men and 61 women with a mean age of 52.8±15.3 years. Ninety-seven age- and sex-matched control individuals (36 men, 61 women; mean age, 52.6±15.8) (control group) with serum levels of 45 mg/dl$\leq$HDL-C$<$85 mg/dl were chosen.

### Subjects with thyroid disease

We checked all subjects whether they have thyroid disease or not. Thyroid disease included hypothyroidism, hyperthyroidism and euthyroid goiter. Patients were confirmed as having thyroid disease, by levels of free T4, free T3 and thyroid stimulating hormone (TSH).

### Lipid and apolipoprotein determinations

Total cholesterol (TC), triglyceride (TG), and HDL-C levels in sera were measured by enzymatic methods using commercial kits (Kyowa Medex Co. Ltd., Tokyo, Japan). Sera apolipoprotein A-I (apoA-I), apoB, and apoE were determined by turbidimetric immunoassay (Daiichi Pure Chemicals, Co. Ltd., Tokyo, Japan) with a Hitachi 7250 analyzer. LDL-C assay (Daiichi) was performed with the same analyzer according to the manufacturer's specifications.

### DNA analysis

Genomic DNA was extracted from EDTA-treated venous blood with a slightly modified version of the procedure reported by Kunkel et al.[9] Intron 14 and D442G mutations on the CETP gene were amplified separately by polymerase chain reaction (PCR) as previously described.[10,11] The amplified products were analyzed by PCR-single stranded conformation polymorphism (PCR-SSCP) and the Phast system (Amersham Pharmacia Biotek, Tokyo, Japan). Digestion was performed overnight at 37°C with NdeI (New England Biolabs, Beverly, MA) for the intron 14 splicing defect or MspI (New England Biolabs) for the D442G mutation. The cleaved products were analyzed by electrophoresis on a gel containing 2.5% NuSieve low melting point agarose (FMC Co., Rockland) and 1% standard agarose (Iwai Co., Tokyo, Japan).

### Statistical analysis

Frequencies of thyroid disease and CETP gene mutations in the high HDL-C level and control groups were compared using Fisher's exact probability test. Sex distributions of subjects with and without thyroid disease in the high HDL-C level group were analyzed similarly. The statistical significance of differences in the lipid parameters between patients with uncommon, intron 14 and D442G mutations were evaluated by one-way analysis of variance (ANOVA). Comparison of lipid profiles between subjects with and without thyroid disease were analyzed with Student's $t$-test. $p<0.05$ was considered statistically significant.

## RESULTS AND DISCUSSION

### Relation between CETP gene mutations and specific diseases

Using PCR-based methods, we screened for CETP deficiency caused by two common mutations in the two groups. Of 97 subjects with high serum HDL-C levels, 7 (7%; 2 men and 5 women) were heterozygous for the intron 14 splicing defect, and 9 (9%; 2 men and 7 women) were heterozygous for the D442G mutation. One patient carried both the intron 14 and D442G mutations. Atherosclerotic disease was identified in 3 of the subjects with these mutations; 2 patients had the D442G mutation and 1 patient had the intron 14 mutation. Although CETP activity was not associated with atherosclerosis, we discovered a novel relation between CETP gene mutations and the thyroid gland disorders.

Of 7 subjects with the intron 14 mutation, 5 (71%) had thyroid disease, whereas 1 (11%) of 9 subjects with the D442G mutation had thyroid disease (Table 1). Of

Table 1. Frequency of intron 14 and D442G mutation in the CETP gene of subjects with and without thyroid disease in the high HDL-C group.

| CETP gene mutation status | Thyroid disease[a] | | Frequency (%) |
|---|---|---|---|
| | (−) | (+) | |
| | $n$ | $n$ | |
| No mutation[b] | 67 | 13 | 16.3 |
| Intron 14 heterozygous | 2 | 5 | 71.4[§] |
| D442G heterozygous | 8 | 1 | 11.1 |
| Intron 14/D442G[c] | 0 | 1 | 100 |
| Total | 77 | 20 | 20.6 |

a, consisting of hyperthyroidism, hypothyroidism, and euthyroid goiter, (−) negative (+) positive; $n$, number of patients (all had high HDL-cholesterol); b, without 2 common mutations; c, carried both the intron 14 and D442G mutations; §, $p<0.01$ (Fisher's exact probability test).

subjects with the intron 14 mutation, 4 (57%) had hypothyroidism and 1 (14%) had euthyroid goiter. Only 1 patient with thyroid disease (hypothyroidism) was found among subjects with the D442G mutation. Comparison of frequencies of thyroid disease and CETP gene mutations in the group of subjects with high HDL-C levels revealed a significant relationship ($p<0.01$) between the intron 14 mutation and thyroid disease. No relationship between the D442G mutation and thyroid disease was found. Furthermore, we could not do a statistical analysis for the control group because of only one hyperthyroid subject showed CETP gene intron 14 in this group.

**Lipid parameters in subjects with and without CETP mutations**

Serum lipid parameters in subjects with and without CETP mutations in the high HDL-C group are listed in Table 2. HDL-C levels were significantly higher in subjects with intron 14 mutation than in subjects with the D442G mutation or without a CETP gene mutation ($p<0.01$, for both). There was no significant difference between subjects with the D442G mutation and those without common CETP mutations. Our data support a previous report[3] in which HDL-C levels were significantly higher in patients with intron 14 mutation than in other subjects. Concentrations of apoE were higher ($p<0.05$) in our D442G mutation subjects than in our no common mutation subjects. This is also consistent with previous findings.[11] Other lipid parameters did not differ statistically between subjects among these three sub groups. Lipid profiles in subjects with and without thyroid disease in the high HDL-C group are shown in Table 3. There were no remarkable differences in lipid profiles between the groups including sex distributions.

Table 2. Serum lipid parameters in the subjects with and without mutations in the high HDL-C group.

| | | No mutation | Intron 14 | D442G |
|---|---|---|---|---|
| *n* (M/F) | | 80(31/49) | 7(1/6) | 9(2/7) |
| Age | (years) | 53±15 | 59±10 | 47±22 |
| HDL-C | (mg/dl) | 98±11 | 120±41[a] | 96±13[b] |
| TC | (mg/dl) | 226±42 | 243±59 | 218±31 |
| TG | (mg/dl) | 99±53 | 121±92 | 73±31 |
| apoA-I | (mg/dl) | 193±26 | 206±40 | 186±18 |
| apoB | (mg/dl) | 90±30 | 87±26 | 79±13 |
| LDL-C | (mg/dl) | 110±36 | 98±33 | 96±19 |
| apoE | (mg/dl) | 4.5±1.2 | 5.4±2.4 | 6.0±2.7[c] |

Data are the mean ±SD; M/F, male/female; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglyceride; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; LDL-C, low density lipoprotein-cholesterol; apoE: apolipoprotein E. a, $p<0.01$ compared with no mutation; b, $p<0.01$ compared with intron 14 mutation; c, $p<0.05$ compared with no mutation.

Table 3. Lipid profiles in subjects with and without thyroid disease in the high HDL-C group.

| | | HDL-C≥85 | |
|---|---|---|---|
| | | (−) | (+) |
| *n* (M/F) | | 20(6/14) | 77(30/47) |
| Age | (years) | 52±16 | 56±12 |
| HDL-C | (mg/dl) | 99±17 | 99±9 |
| TC | (mg/dl) | 228±45 | 221±29 |
| TG | (mg/dl) | 95±51 | 110±68 |
| apoA-I | (mg/dl) | 192±27 | 196±25 |
| apoB | (mg/dl) | 90±28 | 84±18 |
| LDL-C | (mg/dl) | 109±36 | 102±27 |
| apoE | (mg/dl) | 4.7±1.7 | 4.4±1.1 |

(−) subjects without thyroid disease; (+) subjects with thyroid diseases. Data are the mean ±SD; M/F, male/female; HDL-C, HDL cholesterol; TC, total cholesterol; TG, triglyceride; apoA-I, apolipoprotein A-I; apoB, apolipoprotein B; LDL-C, low density lipoprotein-cholesterol; apoE: apolipoprotein E.

**Comparison of the prevalence of thyroid disease between the two groups**

Of the 97 subjects with high HDL-C level, 20 had thyroid disease. Of these 20, 12 (12%) were hypothyroid, 3 (3%) were hyperthyroid, and 5 (5%) had euthyroid goiter. On the other hand, of the 97 control subjects, 18 had thyroid disease. Of these 18, 3 (3%) were hypothyroid, 7 (7%) were hyperthyroid, and 8 (8%) had euthyroid goiter. Therefore, there was no difference on the prevalence of thyroid disease between the patient and control groups (Table 4).

CETP deficiency results in markedly increased HDL-C levels.[1] However, the clinical significance of CETP deficiency in atherosclerosis, including a risk factor for CHD, remains unclear. Two common gene mutations have been reported in Japanese persons with CETP deficiency.[3] A G(+1)-to-A mutation in the 5′ splice donor site of intron 14 of the CETP gene elevates HDL-C plasma levels and decreases CETP activity levels markedly, and the D442G missense mutation ($Asp^{442}$ to Gly) in exon 15 weakly affects HDL-C plasma level and CETP activity. To determine whether CETP activity is related to atherosclerosis or other diseases, we scanned for these two common CETP gene mutations in subjects with elevated levels of HDL-C (≥85 mg/dl) in sera. Although CETP activity was not associated with atherosclerosis, we discovered a novel

Table 4. Comparison of the prevalence of thyroid disease between groups.

| | Normal (*n*) | Thyroid disease (*n*) |
|---|---|---|
| HDL-C≥85 | 77 | 20 |
| 45≤HDL-C<85 | 79 | 18 |

HDL-C, high density lipoprotein-cholesterol; Normal, without any thyroid diseases; *n*=number of subjects with and without thyroid disease.

relation between CETP gene mutation and disorders of the thyroid gland. Specifically, out of the 7 subjects with the intron 14 mutation, 5 had thyroid disease, and out of the 9 subjects with the D442G mutation, 1 had such disease. A significant relationship ($p<0.01$) was found between the intron 14 mutation and thyroid disease, but no relationship was found between the D442G mutation and thyroid disease. These findings suggest that thyroid disease may exist in certain individuals with decreased CETP activity.

Various effects of thyroid dysfunction on HDL-C levels have been reported.[12,13] In our present study, high HDL-C levels in sera were used as a screen for CETP deficiency. Of 97 subjects with elevated HDL-C, 20 had thyroid disease. We observed the 18 cases with thyroid disease in our control group. As a result of comparison between both groups, it indicates that patients with thyroid disease exist in the same proportion, regardless of HDL-C concentration in sera. Thus, in this study, we confirmed that thyroid disease concomitant with decreased CETP activity occurs primarily in persons with high serum HDL-C. Because of only 1 hyperthyroid subject in the control group showing the intron 14 mutation, it was not confirmed whether there is a relationship between thyroid disease and CETP deficiency in the control group.

It has been reported that thyroid hormone may be involved in regulation of CETP activity and that CETP activity may play a role in alterations of HDL metabolism.[14] In contrast, our results indicate that decreased CETP activity due to the mutation in intron 14 inversely effected on incidence of thyroid disease. In conclusion, it is possible not only that CETP activity is regulated by thyroid hormone, but also CETP activity affects thyroid function.

## REFERENCES


1) Tall AR. Plasma cholesteryl ester transfer protein. J Lipid Res 1993；34：1255-74.
2) Koizumi J, Inazu A, Yagi K, Koizumi I, Uno Y, Kajinami K. Serum lipoprotein lipid concentration and composition in homozygous and heterozygous patients with cholesteryl ester transfer protein deficiency. Atherosclerosis 1991；90：189-96.
3) Akita H, Chiba H, Tsuchihashi K, Tsuji K, Kumagai M, Matsuno K, Kobayashi K. Cholesteryl ester transfer protein gene：Two common mutations and their effect on plasma high-density lipoprotein cholesterol content. J Clin Endocrinol Metab 1994；79：1615-8.
4) Inazu A, Jiang X, Haraki T, Yagi K, Kamon N, Koizumi J, et al. Genetic cholesteryl ester transfer protein deficiency caused by two prevalent mutations as a major determinant of increased levels of high density lipoprotein cholesterol. J Clin Invest 1994；94：1872-82.
5) Hirano K, Yamashita S, Funahashi T, Sakai N, Menju M, Ishigami M, et al. Frequency of intron 14 splicing defect of cholesteryl ester transfer protein gene in the Japanese general population-relation between the mutation and hyperalphalipoproteinemia. Atherosclerosis 1993；100：85-90.
6) Zhong S, Sharp DS, Grove JS, Bruce C, Yano K, Curb. JD, Tall AR. Increased coronary heart disease in Japanese-American men with mutation in the cholesteryl ester transfer protein gene despite increased HDL levels. J Clin Invest 1996；97：2917-23.
7) Hirano K, Yamashita S, Nakajima N, Arai T, Maruyama T, Yoshida Y, et al. Genetic cholesteryl ester transfer protein deficiency is extremely frequent in the Omagari area of Japan. Marked hyperalphalipoproteinemia caused by CETP gene mutation is not associated with longevity. Arterioscler Thromb Vasc Biol 1997；17：1053-9.
8) Moriyama Y, Okamura T, Inazu A, Doi M, Iso H, Mouri Y, et al. A low prevalence of coronary heart disease among subjects with increased high-density lipoprotein cholesterol levels, including those with plasma cholesteryl ester transfer protein deficiency. Prev Med 1998；27：659-67.
9) Kunkel LM, Smith KD, Boyer SH. Analysis of human Y chromosome-specific reiterated DNA in chromosome variants. Proc Natl Acad Sci USA 1977；74：1245-9.
10) Arai T, Tsukada T, Nakayama T. Simple and rapid detection of cholesteryl ester transfer protein deficiency by using nonradioisotopic single-strand conformation polymorphism analysis. Clin Chem 1994；40：2227-9.
11) Sakai N, Yamashita S, Hirano K, Menju M, Arai T, Kobayashi K, et al. Frequency of exon 15 missense mutation (442 D：G) in cholesteryl ester transfer protein gene in hyperalphalipoproteinemic Japanese subjects. Atherosclerosis 1995；114：139-45.
12) Agdeppa D, Macaron C, Mallik T, Schnuda ND. Plasma high density lipoprotein cholesterol in thyroid disease. J Clin Endocrinol Metab 1979；49：726-9.
13) Raziel A, Rosenzweig B, Botvinic V, Beigel I, Landau B, Blum I. The influence of thyroid function on serum lipid profile. Atherosclerosis 1982；41：321-6.
14) Dullaart RPF, Hoogenberg K, Groener JEM, Dikkeschei LD, Erkelens DW, Doorenbos H. The activity of cholesteryl ester transfer protein is decreased in hypothyroidism：A possible contribution to alterations in high-density lipoproteins. Eur J Clin Invest 1990；20：581-7.