



Short Communication

Disease-associated marked hyperalphalipoproteinemia



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ARTICLE INFO

Article history:

Received 4 May 2014

Received in revised form 3 June 2014

Accepted 3 June 2014

Available online 30 June 2014

Keywords:

Atherosclerosis

Cardiovascular disease

Cholesteryl ester transfer protein deficiency

High density lipoprotein

Hyperalphalipoproteinemia

Stroke

ABSTRACT

Marked hyperalphalipoproteinemia (HAL) is a heterogeneous syndrome. To clarify the pathophysiological significance of HAL, we compared clinical profiles between marked HAL subjects with and without cholesteryl ester transfer protein (CETP) deficiency. CETP deficiency was associated with cardiovascular diseases and strokes in the HAL population, particularly in female. HAL women without CETP deficiency tended to have higher prevalence with cancer history. HAL may not always be a longevity marker, but be sometimes accompanied with pathological conditions.

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1. Introduction

Hyperalphalipoproteinemia (HAL) had been regarded as a longevity syndrome. Matsuzawa et al. reported that a man with HAL unexpectedly had a corneal opacity which is a clinical sign for

high density lipoprotein (HDL) deficiency [1]. Following studies revealed that genetic deficiency of cholesteryl ester transfer protein (CETP) is a major cause for HAL in Japan [2,3]. CETP is a plasma glycoprotein which facilitates the transfer of cholesteryl ester from HDL to apolipoprotein

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B-containing lipoproteins, then determine the plasma levels of HDL-cholesterol and low-density lipoprotein (LDL)-cholesterol levels [4]. This protein also regulates the lipid composition and particle size of lipoproteins.

CETP deficiency presents marked HAL and relative decrease in LDL-cholesterol level [5]. Such lipid profiles are generally believed protective for cardiovascular diseases (CVDs) and strokes, however, there has been a controversy whether this genetic deficiency is overall anti- or pro-atherogenic [6–8]. In addition, it is noteworthy that some clinical trials with CETP inhibitors recently failed and terminated [9], suggesting that further understanding pathophysiological significance for HAL is obviously required.

Here, we examined the prevalence of CVDs and strokes in HAL subjects with and without CETP deficiency along with their respective lipid profiles in a specific community, Akita Prefecture, Japan, where we reported that genetic CETP deficiency accumulates [10].

2. Subjects and methods

2.1. Subjects

The surveyed population comprised residents aged over 20-years-old in a community in Daisen City, Akita Prefecture, Japan (<http://www.city.daisen.akita.jp/content/docs/english/>), which includes Omagari area where genetic CETP deficiency accumulates [9,10].

After the opt-out in the community journal, we directly sent a request letter to 343 people with marked HAL (HDL-C > 100 mg/dL) based upon the annual health examination for the last three years. Unrelated 181 individuals (53%) agreed to participate in this study. Physical examination, blood test, and interview for medical histories and records of CVDs and strokes were performed. Based upon the analyses of the CETP gene and the protein levels, the subjects with HAL were divided into CETP-deficient and non-CETP-deficient groups.

This study was approved by the ethical committee in Osaka University.

2.2. Medical interview

We performed interviews on smoking, alcohol consumption, and medical histories for CVDs, stroke, diabetes mellitus, hypertension, hyperlipidemia, and cancer.

Diagnoses of hypertension and diabetes mellitus were made according to the criteria of Japanese Society of Hypertension and Japan Diabetes Society. CVDs include non-fatal myocardial infarction, angina pectoris, congestive heart failure, and arteriosclerosis obliterans. Strokes include cerebral infarction and cerebral hemorrhage, but exclude subarachnoid hemorrhage and strokes associated with atrial fibrillation. Cancers included any malignant tumors treated previously and currently.

2.3. CETP gene analyses

We performed direct sequencing of the DNA fragments amplified by polymerase chain reaction to detect two common CETP gene mutations [11,12]: intron 14 splicing defect (c.1321 + 1G>A, rs5742907) and missense mutation in exon 15 (c.1376A>G, rs2303790).

2.4. CETP protein mass

CETP protein mass was measured by the commercial available ELISA kit according to the manufacturer's protocol [13,14].

2.5. Criteria for CETP deficiency

Criteria of CETP deficiency was one of the following: 1) either of the common genetic mutations with c.1321 + 1G>A or c.1376A>G. We previously reported that these two CETP gene mutations contributed to approximately 90% of the genetic CETP deficiency in Japan (13, 14); 2) CETP mass was below 2.0 µg/mL.

We decided to use this cut-off value because the mean CETP mass level of the heterozygote for the missense mutation in exon 15 was $1.65 \pm 0.31 \mu\text{g/mL}$, as reported by Goto et al. [14].

2.6. Lipoproteins analyses

Serum lipoproteins were analyzed by analytical HPLC service system (LipoSEARCH®) at Skylight Biotech Inc. (Akita, Japan), as previously described [15].

2.7. Statistical methods

Data are presented as means (SD). All pair-wise comparisons between CETP- and non-CETP deficient groups were performed with the two-sided Student's *t*-test, and differences in percent values between these two groups were examined by Fisher's exact test. *p* Values < 0.05 were considered significant.

3. Results

Among the 181 participants with marked HAL, the numbers of CETP-deficient and non-CETP-deficient subjects were 71 and 110, respectively. There were no statistical significance of age and listed coronary risk factors, including hypertension, diabetes mellitus, and cigarette smoking (Table 1).

Among 71 CETP-deficient subjects, 2 were revealed to be homozygous. Prevalence of CVDs history was significantly higher in CETP-deficient group than in non-CETP-deficient group ($p = 0.016$). Particularly in female subgroups, the prevalence of CVDs and strokes was significantly higher in CETP-deficient female ($p = 0.02$ for CVD, $p = 0.028$ for ischemic stroke) (Table 1). Furthermore, the prevalence of cancer history tended to be higher in non-CETP-deficient females than in CETP-deficient ones, although not significant statistically (Table 1). Among HAL women without CETP deficiency, the histories for gastric and uterine/breast cancers seem to be higher.

The particle sizes of HDL and LDL were not different significantly between CETP-deficient and non-CETP-deficient groups. HDL-TG/HDL-cholesterol ratio was significantly decreased in CETP-deficient group than non-CETP-deficient group ($p = 0.002$), whereas LDL-TG/LDL-cholesterol ratio was significantly increased in CETP-deficient group ($p = 0.01$) (Table 1), which is compatible with our previous reports [16,17].

4. Discussion

In the previous cross-sectional study in Omagari area, Japan, where CETP deficiency accumulates, we found that there was a U-shaped relationship between plasma HDL-cholesterol and ischemic electrocardiographic changes for the first time [10]. Zhong et al. reported that heterozygous CETP deficiency may be associated with CVDs in Japanese-American population in Hawaii [18], consistent with results of our previous study. Further, recent reports have drawn U-shaped relationship between plasma HDL-C levels and prevalence of CVDs in the other subjects and population [19,20]. The results of this study, together with those of previous studies, provide evidence that HAL is not always promising for the preventions of CVDs and strokes.

We and others reported that CETP deficiency results in qualitative and quantitative abnormalities in both HDL and LDL [16,17], as shown in Table 1. Triglyceride-rich LDL had lower affinity for LDL receptor [17] and may be susceptible for oxidation in plasma. There seems to be controversial whether large and cholesterol-rich HDL from CETP deficiency had reduced or improved ability for cholesterol efflux from lipid-laden macrophages, depending on their experimental settings [16,21,22].

Unexpectedly, we noticed that the cancer history tended to be more frequent in HAL without CETP deficiency than with CETP deficiency (Table 1). It is known that the Akita Prefecture has one of the highest cancer mortalities among all prefectures in Japan last couple of decades. Further study would be of significance to know the association between HAL and cancer for public health as well as medical science.

The present study has the following limitations: 1) we focused on subjects with marked HAL who voluntarily participated. Therefore, residents with some clinical problems might be more motivated to participate compared with those without any clinical problems, which might raise a possibility that the

Table 1

Clinical profiles in subjects with marked hyperalphalipoproteinemia with and without CETP deficiency.

	CETP deficiency	Non-CETP deficiency	P
Total number	71	110	
Age (y)	67 ± 12	64 ± 13	0.263
CETP mass (mg/mL)	1.7 ± 0.5	2.8 ± 0.5	0.0009
Coronary risk factors			
Hypertension	22 (31%)	36 (33%)	0.878
LDL-cholesterol (mg/dL)	98 ± 24	103 ± 29	0.284
Diabetes mellitus	4 (6%)	10 (9%)	0.572
Smoking habit	18 (26%)	29 (26%)	1.00
Triglycerides:cholesterol ratio in HDL	0.15 ± 0.03	0.21 ± 0.03	0.002
Triglycerides:cholesterol ratio in LDL	0.28 ± 0.04	0.22 ± 0.04	0.01
Cardiovascular disease	10 (14%)	3 (3%)	0.016
Stroke	5 (7%)	4 (4%)	0.487
Ischemic	5 (7%)	3 (3%)	0.271
Hemorrhagic	0 (0%)	1 (1%)	1.00
Cancers	8 (11%)	19 (17%)	0.399
Gastric cancer	5 (7%)	10 (9%)	0.786
Male (n)	28	44	
Cardiovascular disease	3 (11%)	2 (5%)	0.386
Stroke	1 (4%)	4 (9%)	0.645
Ischemic	1 (4%)	3 (7%)	1.00
Hemorrhagic	0 (0%)	1 (2%)	1.00
Cancers	5 (18%)	6 (14%)	0.747
Gastric cancer	4 (14%)	5 (14%)	0.734
Others	1 (4%)	1 (4%)	1.00
Female (n)	43	66	
Cardiovascular disease	7 (16%)	1 (2%)	0.02
Stroke	4 (9%)	0 (0%)	0.028
Ischemic	4 (9%)	0 (0%)	0.028
Hemorrhagic	0 (0%)	0 (0%)	1.00
Cancers	3 (7%)	13 (20%)	0.165
Gastric cancer	1 (2%)	5 (8%)	0.404
Uterine, breast cancers	2 (5%)	7 (11%)	0.48
Others	0 (0%)	1 (2%)	1.00

Data are presented as mean ± SD (p value assessed by use of Student's *t*-test) and percentages by Fisher's exact test.

Diagnoses of hypertension and diabetes mellitus were made according to the criteria of the Japanese Society of Hypertension and the Japan Diabetes Society.

Cardiovascular diseases include non-fatal myocardial infarction, angina pectoris, congestive heart failure, and arteriosclerosis obliterans.

Stroke includes cerebral infarction and cerebral hemorrhage, and excludes subarachnoid hemorrhage and strokes associated with atrial fibrillation. Cancers include any malignant tumors treated previously and currently.

disease prevalence might be overestimated in both CETP- and non-CETP deficient HAL groups. It would be of importance to compare the disease prevalence in subjects with marked HAL with that in normolipidemic subjects in the same community; 2) we did not know the molecular basis for HAL without CETP deficiency, although molecules such as hepatic triglyceride lipase [7,22] were reported as responsible for some types of HAL.

In conclusion, marked HAL is not always beneficial for the prevention of CVDs and strokes. Rather, marked HAL may be occasionally associated with the developments of these life-threatening diseases, depending on their sexes and genetic backgrounds.

Acknowledgment

The authors would like to thank Dr. Yoshinobu Ikeda, Dr. Yoshiya Toyoshima, and Professor Mitsuyo Okazaki for their helpful comments and discussion. This study was partially supported by research grant

from rare and intractable disease (H21-Nanchi-Ippan-165) from the Ministry of Health, Labor, and Welfare, Japan.

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