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Human scavenger receptor class B type I is expressed with cell-specific fashion in both initial and terminal site of reverse cholesterol transport

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Abstract

The reverse cholesterol transport (RCT) is one of the major protective systems against atherosclerosis, in which high-density lipoprotein (HDL) removes cholesterol from lipid-laden cells and delivers it to the liver. Scavenger receptor class B type I (SR-BI) is a HDL receptor in the liver and adrenal glands and is involved in the selective uptake of cholesteryl ester from HDL, which has been extensively, analyzed using rodent models. However, the expression and regulation of the human homologue of this receptor are not known yet. We previously reported that this receptor is expressed in in vitro differentiated macrophages and its expression is up-regulated by the addition of modified lipoproteins into the medium [Hirano K, Yamashita S, Nakagawa Y, et al. Expression of human scavenger receptor class B type I in cultured human monocyte-derived macrophages and atherosclerotic lesions. Circ Res 1999;85:108–16]. In order to further investigate the physiological significance of this receptor in humans, we have performed extensive immunohistochemical analyses with specimens of the liver and adrenal glands as well as arteries with different stages of atherosclerotic lesions. In human liver and adrenal glands, a positive SR-BI immunoreactivity was detected in both hepatic and adrenal parenchymal cells as well as Kupffer cells. These parenchymal cells had a strong signal on the cell surface, whereas Kupffer cells showed a heterogeneous and punctate pattern. In human aorta and coronary arteries, SR-BI was highly expressed in atherosclerotic plaques, but not in non-atherosclerotic lesions. Double immunostaining revealed that SR-BI was expressed in a subpopulation of macrophages, of which staining pattern was similar to that observed in Kupffer cells. These data clearly demonstrated that SR-BI was expressed with cell-specific fashions in both the initial and terminal step of RCT in humans. Thus, SR-BI might be physiologically relevant and have distinct tissue-specific functions.

Keywords: Human scavenger receptor class B type I; Reverse cholesterol transport; Macrophages; Atherosclerosis; Liver parenchymal cells; Kupffer cells; Adrenal gland

1. Introduction

Numerous epidemiological studies showed that the incidence of coronary heart disease is inversely correlated with plasma levels of HDL-cholesterol [1–4]. The reverse cholesterol transport (RCT) is a concept for the protective system against atherosclerosis, in which HDL particles remove cholesterol from lipid-laden cells in the arterial wall and deliver it as a shuttle to the liver, a terminal of RCT [5–7]. We have investigated the mechanism for RCT in a series of clinical and biochemical studies with a genetic cholesteryl ester transfer protein (CETP) deficiency as a model [8–10]. These studies have shown that a marked elevation of HDL-cholesterol due to the accumulation of large and cholesterol-rich HDL particles is a sign of impaired RCT and rather atherogenic, despite markedly high levels of plasma HDL-cholesterol.

Abbreviations: AEC, 3amino-9-ethylcarbazole; BSA, bovine serum albumin; HDL, high density lipoprotein; LDL, low density lipoprotein; LSAB, labeled streptavidin biotin; PBS, phosphate-buffered saline; RCT, reverse cholesterol transport; SR-BI, scavenger receptor class B type I

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Scavenger receptor class B type I (SR-BI) is the first identified HDL receptor by Krieger et al. [11,12]. Expression and function of this receptor have been extensively analyzed in rodent models, showing that this molecule is expressed mainly in the terminal of RCT, liver and steroidogenic tissues, to mediate selective uptake of HDL-derived lipids. The elimination of this receptor caused a marked elevation of HDL-cholesterol and apolipoptotein AI-containing lipoproteins and an accelerated atherosclerosis in the apolipoprotein E knockout mice, indicating that a marked accumulation of large HDL particles is a sign of impairment of RCT in rodents as well [13]. Considering that the genetic cause for the majority of patients with hyperalphalipoproteinemia is unknown yet, it is obviously important to know the physiological relevance of this type of HDL receptor in humans. However, information even about the expression and regulation of human homologue of this receptor is still very limited.

We previously reported the results of immunohistochemical analyses on SR families such as SR class A and CD36 [14,15]. These results suggested that the distribution of these receptors was extremely diverse, and that these receptors might have their own function. We have reported that SR-BI is expressed in in vitro differentiated macrophages and up-regulated by the addition of modified low-density lipoproteins (LDL) [16]. To further investigate the physiological significance of the human homologue of SR-BI, we have performed extensive immunohistochemical analyses, showing that this receptor is expressed with cell-specific patterns probably in both the initial and terminal site of RCT.

2. Materials and methods

Seventeen human aorta and 19 coronary arteries were obtained from 31 autopsied cases. Autopsy was performed within 5 h after death with the informed consent from the bereaved families. Table 1 depicts clinical features in each autopsied case and atherosclerotic stages of tissues examined. Seven tissues from the liver and two tissues from adrenal gland were obtained from operation with an informed consent and autopsied cases, respectively. These tissues were embedded in Tissue-Tek® O.C.T. compound (Sakura Finetechnical Co., Ltd., Tokyo, Japan), frozen in liquid nitrogen and stored at −80 °C until use for immunohistochemical staining. Serial cryostat sections cut at 6 μm-thick were air-dried for 30 min, and fixed for 10 min in cold acetone.

Table 1
Clinical features of 31 autopsied cases and atherosclerotic stages of tissues examined

Case	Age	Gender	Clinical features	Tissues examined	
				Aorta	Coronary
1	64	M	Non-Hodgkin's lymphoma	Fatty streaks	DIT
2	56	M	Liver cirrhosis, rupture of varix	Fatty streaks	DIT
3	60	M	Acute myocardial infarction	Plaque	Plaque
4	54	M	Esophageal cancer	Plaque	Plaque
5	52	F	Colon cancer	Plaque	Plaque
6	47	M	Glioblastoma	Normal	
7	48	M	Dilated cardiomyopathy	Normal	
8	25	M	Myelodysplastic syndrome	Normal	
9	76	F	Cerebral infarction, Old myocardial infarction	Normal	
10	59	F	Thyroid cancer	Fatty streaks	
11	78	F	Sick sinus syndrome	Fatty streaks	
12	72	M	Hepatocellular carcinoma	Plaque	
13	63	M	Prostate cancer	Plaque	
14	68	M	Angina pectoris, Hypertension	Plaque	
15	68	M	Aortic abdominal aneurysm, CABG	Plaque	
16	66	M	Chronic lymphoblastic leukemia	Plaque	
17	68	M	Angina pectoris, Sudden death	Plaque	
18	45	M	Lung cancer		DIT
19	72	F	Cerebral hemorrhage		DIT
20	55	M	Hepatocellular carcinoma		DIT
21	72	M	Pancreatic cancer		DIT
22	72	F	Ovarian cancer		DIT
23	66	F	Aplastic anemia		Plaque
24	53	M	Hepatocellular carcinoma		Plaque
25	82	M	Acute myocardial infarction		Plaque
26	74	M	Spinal cord injury, angina pectoris		Plaque
27	67	M	Cerebral hemorrhage		Plaque
28	46	M	Liver cirrhosis		Plaque
29	78	F	Pneumonia		Plaque
30	41	F	Progressive systemic sclerosis, Sudden death		Plaque
31	59	M	Myocardial infarction, diabetes mellitus		Plaque

Normal: macroscopically normal aorta; DIT: diffuse intimal thickening; plaque: atherosclerotic plaque.

3. SR-BI Antibodies

Polyclonal antibodies raised against the peptides corresponding to the carboxyl-terminus of human SR-BI (amino acids 490–509; SR-BI-490) and against those to the extracellular domain (amino acids 75–93; SR-BI-75) were prepared in rabbits, as described before [15]. These antibodies were confirmed to be fully utilized for immunoblotting and immunohistochemical assays. In the current study, we used SR-BI-75 for immunohistochemical analysis.

4. Immunohistochemical detection of SR-BI in human liver

To detect SR-BI in human liver, a confocal immunofluorescent microscopy was utilized. Non-specific reaction was blocked with Block Ace (Snow Brand Milk Products Co., Ltd., Sapporo, Japan) containing 10% normal goat serum, and sections were incubated for 1 h with rabbit anti-SR-BI polyclonal antibodies diluted 1:500 in phosphate-buffered saline (PBS) containing 1% bovine serum albumin (BSA), followed by a 30-minute incubation with Alexa® 488 goat anti-rabbit IgG (H+L) (Molecular Probes Inc., Oregon) diluted 1:500 in PBS containing 1% BSA. Then sections were incubated with CD68 (EBM11, mouse IgG_1 fraction, DAKO Co.) diluted 1:200 in PBS containing 1% BSA as a marker for Kupffer cells. These sections were further incubated with Alexa® 594 goat anti-mouse IgG (H+L), $F(ab')_2$ fragment (Molecular Probes Inc., Oregon) diluted 1:500 in PBS containing 1% BSA. Images were obtained by a confocal laser microscopy using a Zeiss LSM510 (Carl Zeiss Co., Ltd., Germany).

5. Immunohistochemical detection of SR-BI in human adrenal glands, aorta and coronary arteries

Labeled streptavidin biotin (LSAB)-peroxidase method was applied to detect SR-BI in human adrenal glands, aorta and coronary arteries (DAKO LSAB-peroxidase Kit, DAKO Co., CA). Endogenous peroxidase activity was quenched by incubating the sections for 30 min with methanol containing 0.3% hydrogen peroxide. Non-specific reaction was blocked by a 5-min incubation with 10% normal goat serum at room temperature. These sections were incubated with rabbit anti-SR-BI polyclonal antibodies diluted the same as detecting SR-BI in liver, followed by sequential 10-min incubations with goat biotinylated anti-rabbit immunoglobulins and peroxidase-labeled streptavidin. Antibody binding was visualized with 3-amino-9-ethylcarbazole (AEC). These sections were counterstained with hematoxylin. As a negative control, preimmune serum was used.

6. Double immunohistostaining for SR-BI and macrophages

Double immunohistostaining was performed to identify the type of cells expressing SR-BI in human arteries. After immunostaining for SR-BI using LSAB-peroxidase method as described above, sections were further incubated for 5 min with 10% normal goat serum. For detecting macrophages, these sections were incubated for 1 h with HAM56 (mouse IgM fraction, DAKO Co.) diluted 1:200 in PBS containing 1% BSA. After a 10-min incubation with goat biotinylated anti-mouse immunoglobulins, sections were incubated with FITC avidin D (Vector Laboratories, Inc., CA) diluted 1:200 in PBS containing 1% BSA, and were observed using a light and a fluorescence microscopes (PROVIS AX80T and AX80TR, OLYMPUS, Tokyo, Japan).

7. Results

We first examined immunohistochemically the expression of SR-BI in human liver as the terminal of RCT. As shown in Fig. 1a, there were two staining patterns of SR-BI antibody in human liver, one was a cell surface staining and the other was an intracellular one. Double immunostaining with SR-BI (Fig. 1a) and CD68 as a marker for Kupffer cells (Fig. 1b) revealed that the intracellular staining was detected only in Kupffer cells (Fig. 1c). A higher power view showed that the immunoreactivity to SR-BI was mainly localized on the cell surface of hepatic parenchymal cells (Fig. 1d). In contrast, in Kupffer cells (Fig. 1e), distinctly different from parenchymal cells, the immunostaining of SR-BI showed an heterogeneous and punctate appearance (Fig. 1f). There was no significant staining of the liver tissues when the SR-BI antibody was replaced by preimmune serum (data not shown).

Human adrenal gland was investigated as another terminal site of the RCT. Fig. 2a shows that cells in the adrenal cortex expressed SR-BI, while no immunoreactivities to SR-BI were detected in the adrenal medulla. The locations of the cortical zones (zona glomerulosa, zona fasciculata, and zona reticularis) are indicated. Immunoreactivity to SR-BI was detected in zona fasciculata and in zona reticularis, but was seen only faintly within the zona glomerulosa. At a higher magnification of zona fasciculata, immunoreactivity to SR-BI was most prominent on the surface of parenchymal cells (Fig. 2b). No immunoreactivity was detected when incubated with preimmune serum (Fig. 2c).

We next evaluated immunohistochemically the expression of SR-BI in human aorta as a possible site of the initial step of RCT. As shown in Table 1, we examined four samples of macroscopically normal aorta, four of fatty streaks, and nine of atherosclerotic plaques of human aorta. Representative figures of each atherosclerotic stage are shown in Fig. 3. In sections of normal aorta, the immunoreactivity to SR-BI was not detected in any cells of either the intima or the media (Fig. 3a). In the region of fatty streaks, some intimal cells had a positive

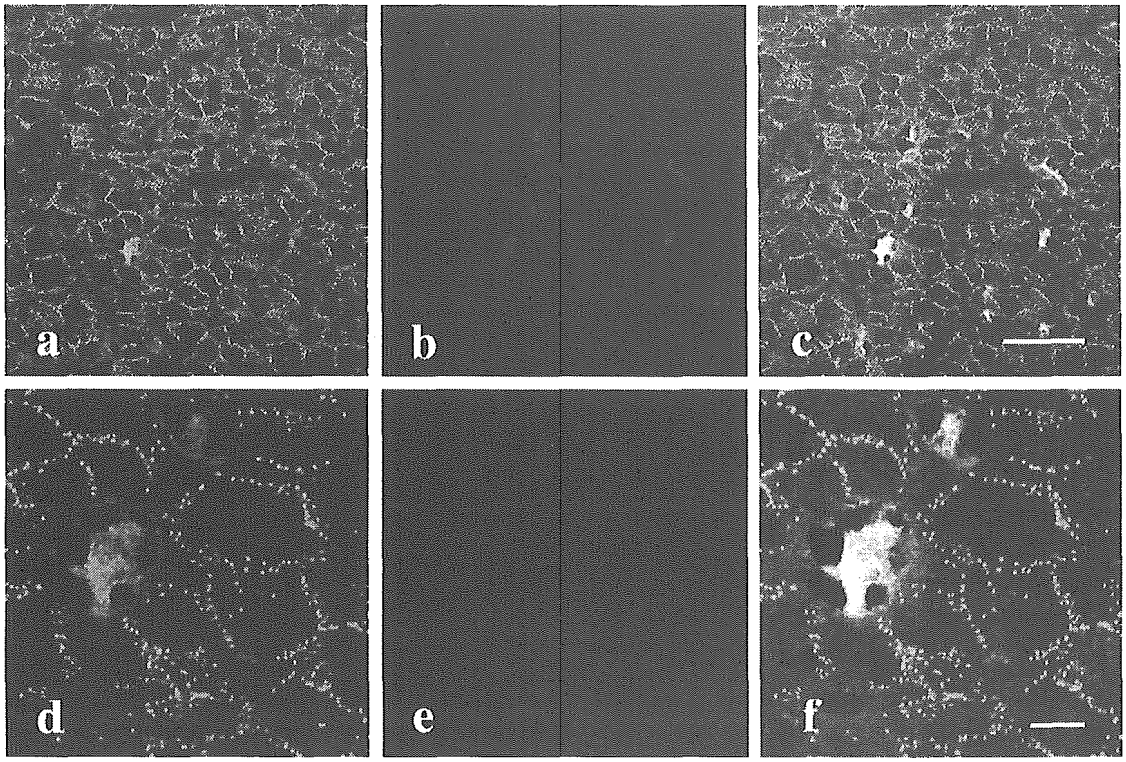

Fig. 1. Immunohistochemical detection of SR-BI in human liver. Low power views of human liver stained with SR-BI antibodies (a, green) and with CD68 (b, red) as a marker for Kupffer cells revealed that the immunoreactivity to SR-BI was localized both in hepatic parenchymal cells and in Kupffer cells (c). A higher power view clearly showed that immunostaining for SR-BI in the hepatic parenchymal cells was distributed on the cell surface (d). Different from hepatic parenchymal cells, Kupffer cells stained with red color (e) had an immunoreactive mass of SR-BI with a heterogeneous, punctate appearance (f). (a, b and c, Bar = 50 μm; d, e and f, Bar = 10 μm).

immunoreactivity to SR-BI (Fig. 3b). With the development of atherosclerosis, more SR-BI-positive cells were observed in the atherosclerotic plaques (Fig. 3c). In all sections of macroscopically normal aorta, the immunoreactivity to SR-BI was undetectable, while in all sections of fatty streaks and atherosclerotic plaques of human aorta, some population of intimal cells expressed SR-BI protein. There was no positive staining with preimmune serum (Fig. 3d). This character-

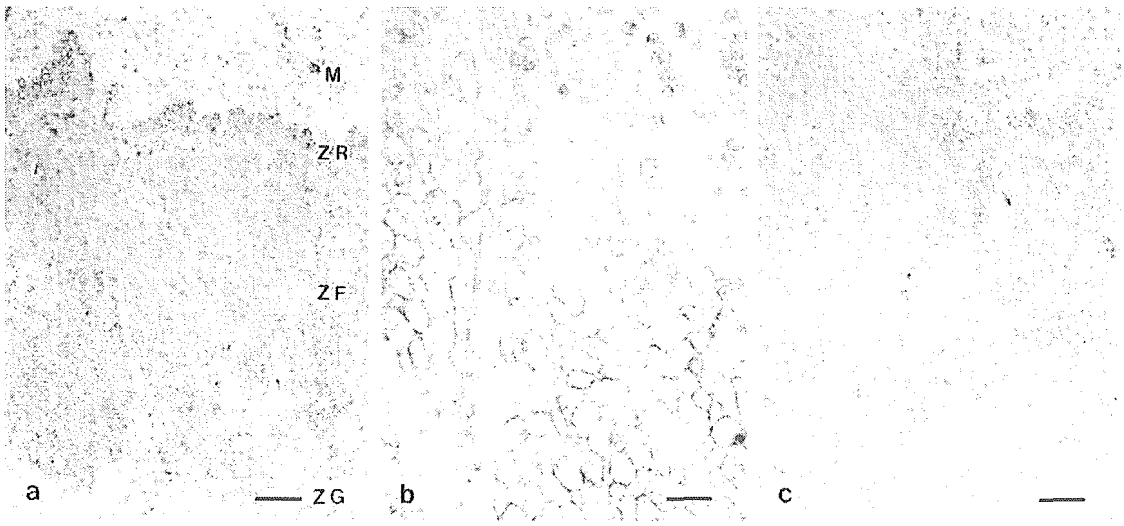

Fig. 2. Localization of SR-BI in human adrenal gland. Cells composing adrenal cortex expressed SR-BI, while no immunoreactivities to SR-BI were detected in adrenal medulla (M). Immunostaining to SR-BI was detected in zona fasciculata (ZF) and in zona reticularis (ZR), but was seen only faintly within the zona glomerulosa (ZG) (a). At a higher magnification of zona fasciculata, immunostaining to SR-BI was most prominent on the surface of parenchymal cells (b). No immunoreactive mass was detected when incubated with preimmune serum (c). M, medulla; ZR, zona reticularis; ZF, zona fasciculata; ZG, zona glomerulosa. (a and c, Bar = 220 μm; in b, Bar = 55 μm).

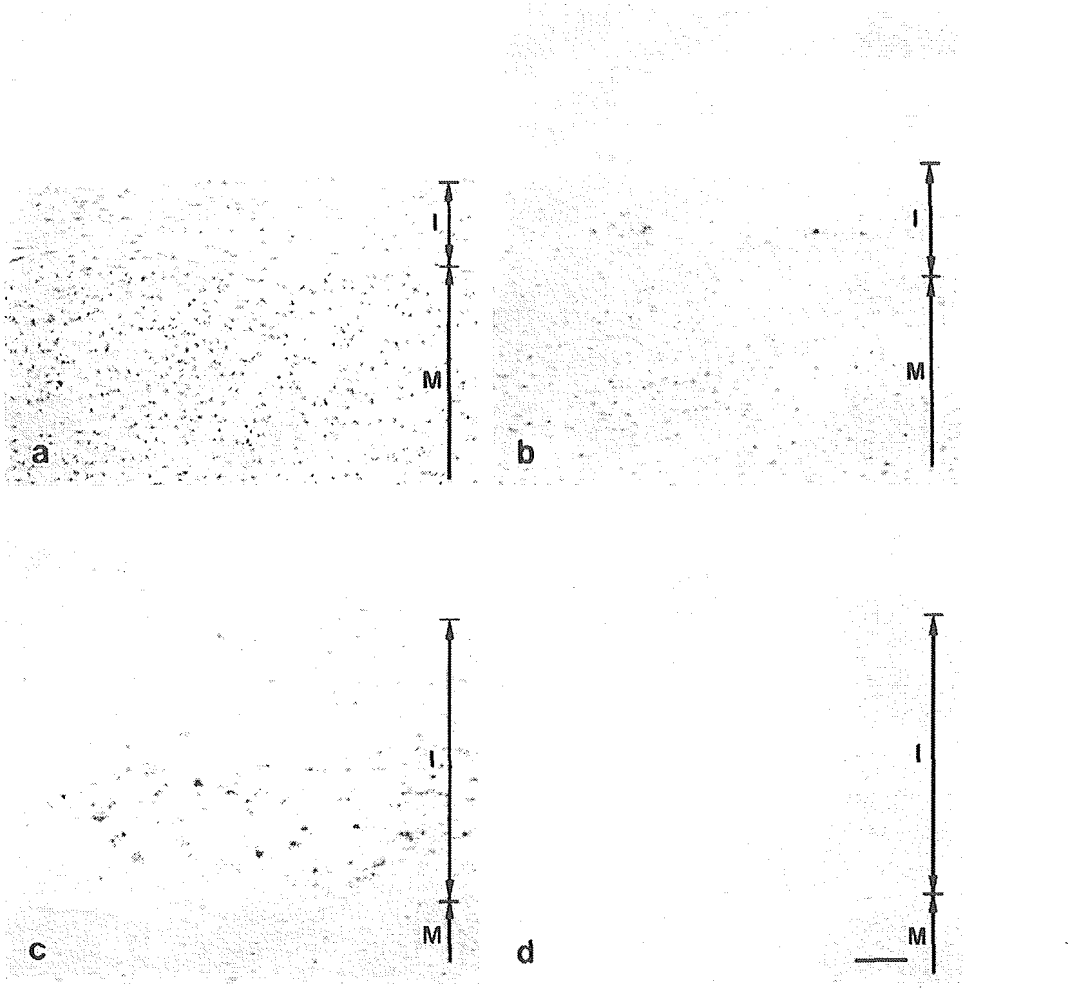

Fig. 3. Expression of SR-BI in the process of atherosclerosis in human aorta. Representative figures of localization for immunoreactive mass of SR-BI are shown. In macroscopically normal aorta from a 47-year-old male (case 6), no SR-BI-positive cells were found neither in the media nor in the intima (a). In the region of fatty streaks from a 59-year-old female (case 10), some intimal cells had immunoreactivity to SR-BI (b). With the progress of atherosclerosis, immunoreactive mass of SR-BI was more abundantly expressed in the intimal cells (c, atherosclerotic plaques from a 63-year-old male (case 13)). No positive staining was detected for preimmune serum (d). I, intima; M, media. (Bar = 220 μm).

istic staining pattern was also observed in human coronary arteries as shown in Fig. 4. In the region of diffuse intimal thickening, no immunoreactive mass of SR-BI was detected (Fig. 4a and b), while a number of cells expressed SR-BI protein in atherosclerotic plaques (Fig. 4c and d). In all seven sections with diffuse intimal thickenings, immunoreactivity to SR-BI was at an undetectable level and in 10 out of 12 cases with atherosclerotic plaques of human coronary arteries, many intimal cells showed a positive immunostaining to SR-BI antibodies.

A double immunohistostaining was performed to identify the cell types expressing SR-BI protein. Fig. 5 shows high-power views of fatty streaks (Fig. 5a and b) and atherosclerotic plaques (Fig. 5c and d) of human aorta. Double immunostaining for SR-BI and macrophages revealed that the cells expressing SR-BI were macrophages. The staining pattern of SR-BI in the macrophages in the atherosclerotic lesions was similar to that in hepatic Kupffer cells: it appeared as a heterogeneous and punctate pattern. It is noteworthy that not all macrophages in the intima expressed SR-BI protein. Smaller sized macrophages (arrowheads in Fig. 5b and d) showed little immunoreactivity to SR-BI. To the contrary, lipid-laden foam cells (arrows in Fig. 5b and d) expressed a more abundant immunoreactivity to SR-BI than smaller macrophages.

8. Discussion

We have for the first time demonstrated that human SR-BI is abundantly expressed under physiological conditions in multiple tissues and that the staining patterns are apparently different between hepatic and adrenal parenchymal cells and cells with phagocytotic activity such as macrophages of aorta and coronary arteries and Kupffer cells. Immunohistochemically, such diverse staining patterns against one antibody are very rare. Prosaposin, known as a large precursor protein that is proteolytically cleaved to form four

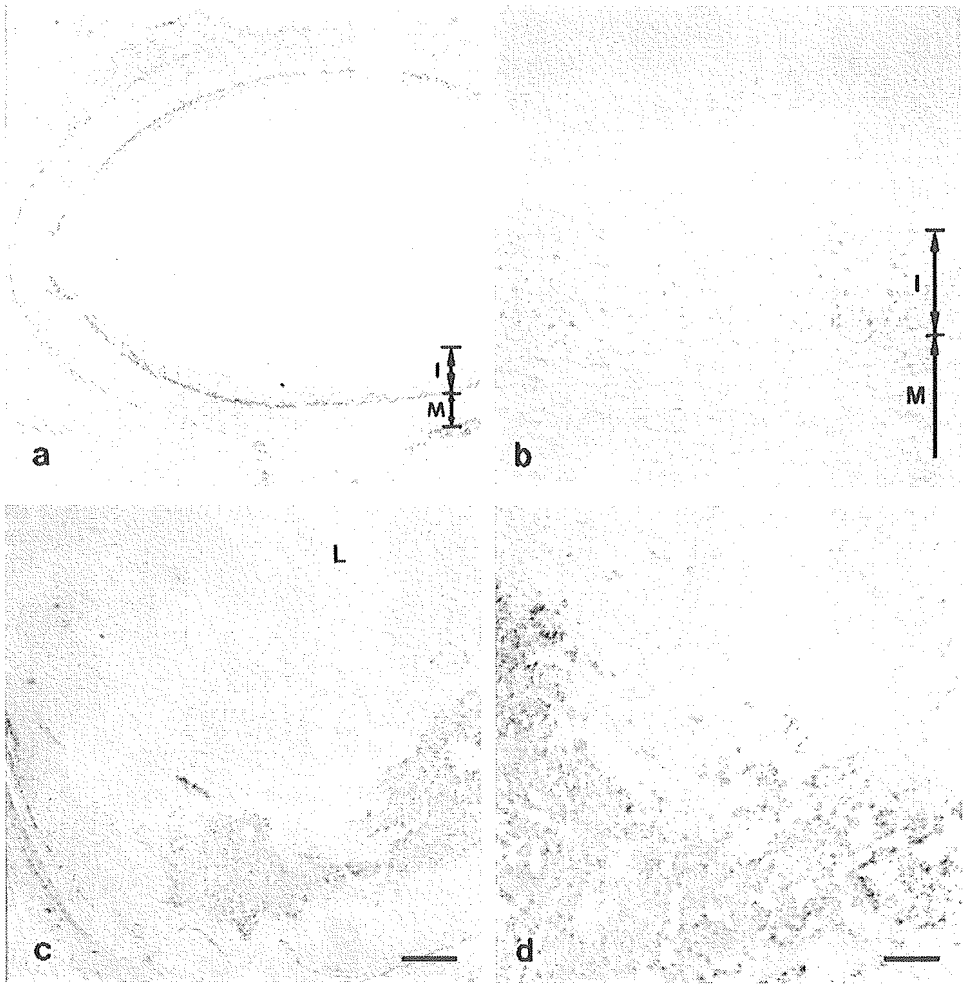

Fig. 4. Localization of SR-BI in human coronary arteries. In the region of diffuse intimal thickenings in the coronary artery from a 45-year-old male (case 18), no positive staining for SR-BI was observed (a, b). In the advanced atherosclerotic plaques of the coronary artery from a 46-year-old male (case 28), numerous cells had an immunoreactivity to SR-BI (c). High power view of atherosclerotic plaques showed SR-BI-positive cells were presumably macrophages with lipids (d). I, intima; M, media; L, lumen. (a and c, Bar = 550 μm; b and d, Bar = 110 μm).

distinct sphingolipid activator proteins, had two staining patterns in different kinds of cells [17,18]. In epithelial cells, immunoreactivities to prosaposin showed a granular reaction in the cytoplasm. In Sertoli cells, those showed a homogeneous reaction. These different staining patterns appeared to be correlated with its functional difference. Granular reaction in the cytoplasm was a characteristic feature of lysosomes where prosaposin gets processed to saposin. Those cells, which expressed prosaposin immunoreactive mass homogeneously in the cytoplasm secreted it without processing to saposin, and it is anticipated to have several biological activities. In this way, different staining patterns suggested distinct cell-specific functions. In the current study, we showed that hepatic and adrenal parenchymal cells expressed SR-BI on the cell surface, whereas the macrophages of aorta and coronary arteries and Kupffer cells had heterogeneous and punctate patterns of SR-BI expression. Although it should await for further detailed analyses such as electron microscopy, our finding indicates that human SR-BI might have a cell- and tissue-specific function.

Function of murine SR-BI expressed in the liver and adrenal glands has been extensively analyzed by the studies with nutritional and hormonal modification as well as genetically engineered mice technology. The hepatic and adrenal SR-BI appeared to be cholesterol-sensitive, in that cholesterol feeding decreased the expression of SR-BI in rat liver and estrogen treatment increased that in adrenal gland [19–24]. These data have been supported by the finding that promoter of SR-BI possesses a sterol-responsive element [25]. However, immunohistochemical analyses on this receptor have not been fully performed. The available information about hepatic expression of SR-BI was that SR-BI was expressed in the bile canalicular membrane of murine liver overexpressing SR-BI, which was introduced by adeno virus-mediated gene transfer [26]. However, this was not a physiological condition. Kocher et al. reported the hepatic expression of mouse SR-BI with immunohistochemical method [27]. Mardones and Stangl both showed that endogenous SR-BI is primarily located in the sinusoidal domain of hepatocytes in the rodent liver [28,29]. They both used the rabbit polyclonal anti-

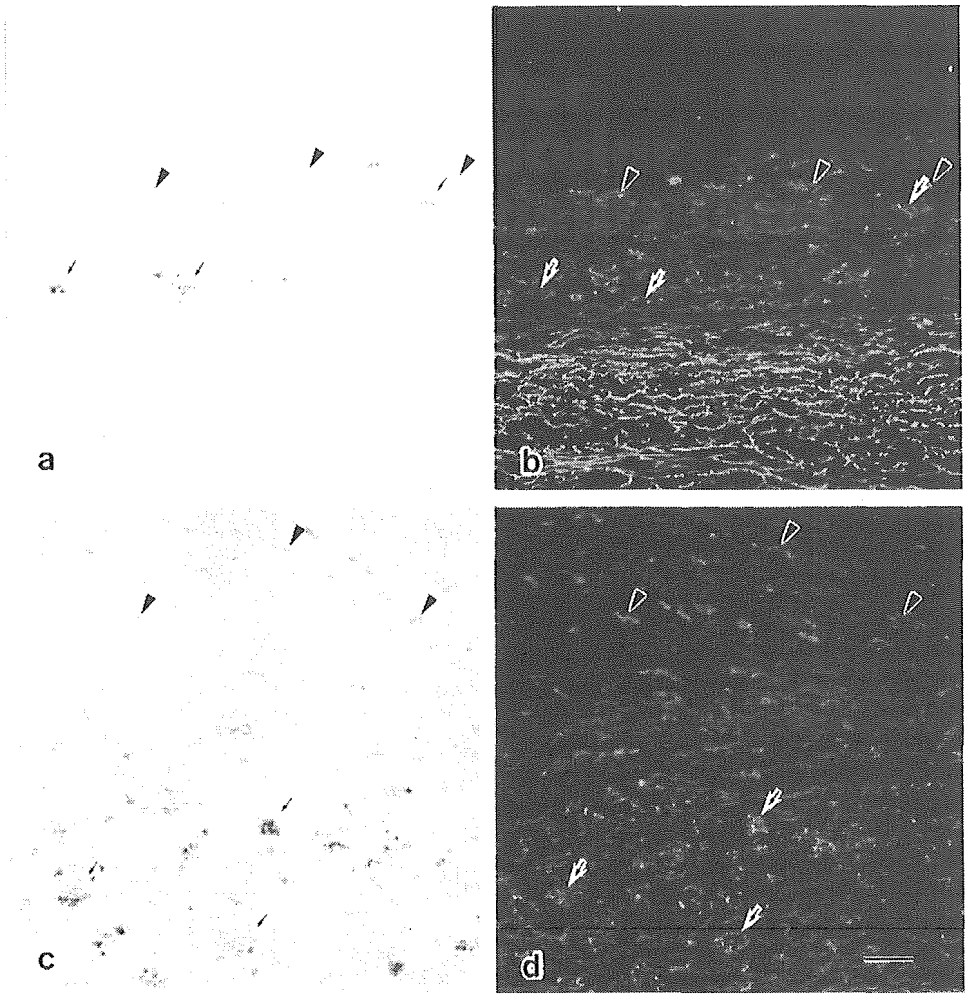

Fig. 5. Identification of the type of cells expressing SR-BI in fatty streaks and in atherosclerotic plaques of human aorta by double immunostaining. Although no positive immunostaining for SR-BI was detected (arrowheads in a), there existed macrophages (arrowheads in c, indicating the same cells as arrowheads in a) in the fatty streak lesions. To the contrary, cells expressed an immunoreactivity to SR-BI (arrows in a) were macrophages (arrows in c, indicating the same cells as arrows in a). In the more advanced atherosclerotic plaques, punctate, heterogeneous immunostaining for SR-BI (arrows in b) was detected in macrophages (arrows in d, indicating the same cells as arrows in b). Smaller macrophages (arrowheads in d) had little immunoreactive mass of SR-BI (arrowheads in b, indicating the same cells as arrowheads in d). Immunoreactivity to SR-BI was localized only in macrophages and no other cell types expressed SR-BI. I, intima; M, media. (Bar = 110 μm).

peptide antibody directed against the carboxy-terminal 14 amino acids of mSR-BI. We used rabbit polyclonal antibody raised against the peptides corresponding to the extracellular domain (amino acids 75–93) of human SR-BI. Using our antibody, SR-BI was located not only in the sinusoidal domain of human hepatocytes but also in the intercellular one. It is hard to distinguish apical surface and intercellular domain of hepatocytes with single staining, however, we could conclude at least that the immunoreactivity to SR-BI was mainly localized on the cell surface of hepatic parenchymal cells. The present data clearly showed that human SR-BI in a physiological condition appeared to be localized mainly on the cell surface of hepatic and adrenal parenchymal cells, suggesting that SR-BI might be physiologically relevant in these cells in humans as well as in rodents.

Chinetti showed the expression of SR-BI in atherosclerotic plaques of human carotid artery [30]. We investigated various stages of atherosclerotic lesions in human both aorta and coronary arteries as shown in Table 1. It appeared that more SR-BI-positive cells were observed with the progress of atherosclerosis. We also performed a double immunostaining to identify the cell types expressing SR-BI protein, and clarified that SR-BI is expressed in some but not all subpopulations of macrophages in the lesions and that foamy macrophages tend to have a higher expression of SR-BI than smaller macrophages, indicating that SR-BI is not just a marker of macrophages. Although the function of SR-BI expressed in macrophages remains to be investigated, the response to cholesterol loading appears to be opposite to that in the liver (Hirano et al., unpublished observation). Furthermore, although numerous numbers of SRs, including SR class A, CD36 and LOX-1, have been cloned and identified, it should be noted that SR-BI is very unique in that it has a strong ability to mediate cholesterol efflux from the cells expressing

this molecule, which has raised a possibility that this receptor may mediate cholesterol efflux in vivo [31–33]. It is of great importance to know whether or not this type of HDL receptor contributes to cholesterol efflux from macrophages in the arterial wall.

On the other hand, it was also reported that SR-BI was expressed in the Sertoli cells of rats and that this molecule bound phosphatidylserine [34]. Many cell biological studies showed that this receptor might have multiple ligands in vivo as other SRs class B do [35–39]. Considering the present results that the staining pattern of SR-BI appears cell-specific, it will be the issue for the future to elucidate tissue- and cell-specific function of this receptor especially in extrahepatic tissues such as blood vessels.

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The New Worldwide Definition of Metabolic Syndrome Is Not a Better Diagnostic Predictor of Cardiovascular Disease in Japanese Diabetic Patients Than the Existing Definitions

Additional analysis from the Japan Diabetes Complications Study

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We previously reported (1) the limited clinical significance for Japanese diabetic patients of the widely used World Health Organization (WHO) (2) and National Cholesterol Education Program (NCEP) (3) definitions of metabolic syndrome and suggested that an international definition of metabolic syndrome that was applicable regardless of ethnicity was necessary (1).

Recently, the International Diabetes Federation published a long-awaited new worldwide definition of metabolic syndrome (4) that is intended to be applicable to various ethnic groups. The new definition is similar to the NCEP definition (3) but has several important differences. Notably, most components of the new definition now include subjects who are receiving specific treatments for the abnormalities that comprise metabolic syndrome. Also, central obesity (defined by waist circumference with ethnic modification in its thresholds) has become a mandatory component in the new definition. In this report, we evaluated the predictive power of the new international definition for cardiovascular disease (CVD), as compared with that of previous definitions, in Japanese diabetic patients.

RESEARCH DESIGN AND METHODS — The Japan Diabetes Complications Study (JDCS) has been described in detail elsewhere (1,5). The same dataset was used for evaluation so that the new definition of metabolic syndrome could be directly compared with the WHO and NCEP definitions (1–4). A total of 1,424 Japanese patients (771 men and 653 women, age 58.4 ± 7.4 years [means ± SD]) with previously diagnosed type 2 diabetes but without known CVD were followed for 8 years for coronary heart disease (CHD) and stroke events. Fatal and nonfatal CHD and stroke were defined as previously reported (1). The new International Diabetes Federation definition (4) was used with a recommended ethnic modification for Japanese subjects in relation to waist circumference (men ≥85 cm, women ≥90 cm). Since all of the subjects had diabetes, metabolic syndrome diagnosis was made in patients who met criteria for central obesity plus one or more of the following: increased triglycerides, increased blood pressure, or reduced HDL cholesterol (see Table 1 for detailed thresholds). Incidence rates in the two groups (with and without metabolic syndrome) were estimated under the Poisson assumption using person-year methods. Cox regression analysis was used to calculate the age-adjusted hazard ratio (HR) and 95% CI of metabolic syndrome risk factors with CHD, stroke, or both. The SAS software package (version 8.0; SAS Institute, Cary, NC) was used for all analyses. $P < 0.05$ was considered statistically significant.

RESULTS — At baseline, the prevalence of metabolic syndrome, using the new definition (Table 1), was notably lower, especially in female patients, than the prevalence under the WHO (2) and NCEP (3) definitions, which was ~50% on average (1). Diabetes duration in patients with (9.9 ± 6.9 years) or without (10.7 ± 7.3 years) metabolic syndrome did not differ significantly ($P = 0.07$). The proportion of patients that met the central obesity criterion (an essential component of the new definition) was 36.7% for men and 9.7% for women, such that 87% of men and 95% of women with central obesity had metabolic syndrome.

The incidence (per 1,000 patient-years) of CHD (13.5 [with metabolic syn-

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Abbreviations: CHD, coronary heart disease; CVD, cardiovascular disease; JDCS, Japan Diabetes Complications Study; NCEP, National Cholesterol Education Program; WHO, World Health Organization.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

Table 1—*Patient prevalence at baseline, age-adjusted HRs with 95% CIs, and incidence of CHD, stroke, or both in 1,424 Japanese patients with type 2 diabetes (771 men and 653 women) according to individual cardiovascular risk factors comprising the metabolic syndrome as defined by the International Diabetes Federation (b, c, and d include specific treatment for each abnormality)*

	Prevalence at baseline (%)		HR for CHD		HR for stroke		HR for CHD and/or stroke	
	Men	Women	Men	Women	Men	Women	Men	Women
a) Waist circumference ≥85 cm (men), ≥90 cm (women)	36.7	9.7	1.68 (0.92–3.08)	1.13 (0.26–4.86)	0.91 (0.44–1.86)	1.11 (0.31–4.05)	1.32 (0.83–2.10)	1.13 (0.43–2.97)
b) Triglycerides ≥150 mg/dl	26.5	23.4	2.93 (1.55–5.53)	2.03 (0.81–5.04)	1.10 (0.51–2.36)	0.59 (0.20–1.78)	1.96 (1.21–3.19)	1.13 (0.56–2.26)
c) HDL cholesterol <40 mg/dl (men), <50 mg/dl (women)	19.3	36.3	1.82 (0.94–3.54)	1.48 (0.63–3.49)	0.99 (0.41–2.40)	1.34 (0.61–2.94)	1.53 (0.90–2.61)	1.34 (0.74–2.40)
d) SBP ≥130 mmHg or DBP ≥85 mmHg	64.1	68.8	1.04 (0.53–2.01)	1.05 (0.39–2.84)	2.08 (0.90–4.82)	1.63 (0.60–4.37)	1.29 (0.77–2.17)	1.29 (0.64–2.59)
a plus one or more of *b*, *c*, or *d*	32.0	9.2	1.72 (0.94–3.15)	1.15 (0.27–4.90)	1.14 (0.56–2.34)	1.13 (0.31–4.11)	1.47 (0.91–2.35)	1.14 (0.44–3.01)

DBP, diastolic blood pressure; SBP, systolic blood pressure.

drome] vs. 8.1 [without metabolic syndrome] in men; 5.8 vs. 5.5 in women) or stroke (8.1 vs. 7.5 in men; 8.8 vs. 7.0 in women) did not differ significantly between subjects with or without metabolic syndrome. Age-adjusted HRs were calculated to determine whether the new metabolic syndrome definition or its components could predict cardiovascular events (Table 1). Patients diagnosed as having metabolic syndrome, even when subgrouped by therapeutic contents (oral hypoglycemic agents or insulin use), did not show significantly raised HRs for CHD, stroke, or both compared with subjects without metabolic syndrome. However, male patients with raised triglyceride levels and/or having specific treatment for this condition had a significantly increased risk of CHD (HR 2.93, $P < 0.001$) and combined CHD and stroke (1.96, $P = 0.006$), regardless of whether they had metabolic syndrome (Table 1).

CONCLUSIONS— Our previous analysis (1) showed that HRs for CVD in patients with WHO-defined metabolic syndrome were significantly elevated compared with HRs in subjects without metabolic syndrome (although the HR for CHD in male patients was not elevated). Diagnosis of metabolic syndrome by the NCEP definition was less predictive but still associated with a significantly elevated HR for CHD in male patients. However, metabolic syndrome diagnosis by the new definition was not predictive for CVD in either male or female patients in the same prospective setting. Therefore, the new definition did not improve the prediction of adverse cardiovascular events, and its clinical usefulness in Japanese diabetic patients is rather less than that of the existing definitions or of hypertriglyceridemia alone in male patients.

The indispensability of central obesity to the new definition was a major cause of the decrease in the prevalence of metabolic syndrome observed using the new definition. The fact that most patients with central obesity were classified as having metabolic syndrome revealed that metabolic syndrome diagnosis by the new definition was highly dependent on waist circumference when applied to Japanese diabetic subjects. It also denoted that most patients with central obesity had at least one other cardiovascular risk factor, suggesting a close relationship between central obesity and other cardiovascular risk factors. However, this combination was not necessarily associated with an increased risk of CVD in our patients. This latter observation led us to further evaluate the significance of waist circumference in our patients by modifying the threshold within the 65- and 105-cm range and recalculating the HRs. Interestingly, we could not find any thresholds associated with significantly elevated HRs for cardiovascular events in either male or female subjects (data not shown). Therefore, the new definition's lower prediction power for CVD seemed to be derived from the indispensability of the waist circumference component.

To date, prospective trials examining the significance of metabolic syndrome as a predictor of CVD in diabetic patients (1,6–9) have been inadequate (10,11). Many important issues remain to be resolved. *1*) Is the new definition of metabolic syndrome a good predictor of CVD in diabetic patients of differing ethnicities (12)? 2) Are there any other combinations of components (or different thresholds) that are better predictors of CVD in Asian diabetic patients (13–15)? 3) Is the concept of metabolic syndrome truly applicable or relevant to diabetic patients in general? Investigations of these issues would aid the screening of diabetic patients at especially high risk of CVD, as well as inform and direct ethnic group–specific management of diabetes (16–19).

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Effect of the Cholesteryl Ester Transfer Protein Genotypes on Plasma Lipid and Lipoprotein Levels in Vietnamese Children

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ABSTRACT

Cholesteryl ester transfer protein (CETP) is understood to play a regulatory role in HDL cholesterol (HDLC) metabolism. In this study, the effect of CETP genotypes on plasma lipid and lipoprotein levels in 348 Vietnamese girls (aged 7–9) with different nutritional conditions was analyzed. The two mutations, intron 14 G(+1)-to-A (I14A) and Asp 442 to Gly within exon 15 (D442G), and the *Taq*IB polymorphism in the CETP gene were identified by an Invader assay. The D442G mutation was present with a frequency of 0.034, while the I14A mutation was absent. HDLC levels were significantly higher in carriers of the D442G mutation than in noncarriers, regardless of the nutritional status. Low-density lipoprotein (LDL) cholesterol and triglyceride levels were not significantly lower in carriers of D442G mutation. The frequency of the TaqIB2 allele was 0.34, which was lower than that observed in other Asian populations. TaqIB2B2 carriers also had significantly higher HDLC levels, but this association was weaker than that of the D442G mutation. Overall, genetic variations at the CETP gene locus may account for a significant proportion of HDLC variation in Vietnamese children. (*Pediatr Res* 58: 1249–1253, 2005)

Abbreviations

CETP, cholesteryl ester transfer protein
HDLC, high-density lipoprotein cholesterol
TG, triglyceride

HDLC levels in plasma may be altered by a variety of environmental factors including alcohol consumption, a low fat diet, obesity, smoking, and exercise (1). In the general population, about 50% of plasma HDLC variability derives from genetic factors (2). CETP is a plasma glycoprotein that transfers cholesterol ester from HDLC to triglyceride (TG)-rich lipoproteins and regulates plasma HDLC levels (3,4). Two CETP gene mutations, an intron 14 G(+1)-to-A mutation (I14A) and a missense mutation, Asp442 to Gly within exon 15 (D442G), first described in Japanese population, were found to be associated with a CETP deficiency and increased HDLC levels (5,6). In addition, several common restriction fragment length polymorphisms (RFLPs) have also been reported in the CETP gene locus (7–9). The most studied RFLP to date has been *Taq*IB, which has been shown to be a silent base change affecting the 277th nucleotide in the first intron of the gene. The B2 allele (in which the *Taq*IB restriction site is absent) at this polymorphic site has been associated with increased HDLC levels and decreased CETP activities and levels in normolipemic subjects, thus resembling a mild form of CETP deficiency (10–12). The risk of coronary artery disease is inversely related to plasma HDLC levels. Therefore, identification of the underlying genetic basis of plasma HDLC levels is key to the understanding of atherosclerosis-related diseases, which are among the 10 leading mortality causes in Vietnam (13). The aim of this study was to determine the importance of genetic variants in the CETP gene to predict the HDLC levels for the Vietnamese children under different nutritional statuses.

METHODS

Study subjects. The study subjects included 348 schoolgirls, aged 7 to 9 y old, who were randomly selected from two schools in the center of Hochiminh city (an urban area) and three schools in the suburban areas in Hochiminh city (a rural area). All the subjects were Kinh, which is the major ethnic group in Vietnam. The characteristics of the children have been described in detail elsewhere (14). The research protocol was approved by the Research and Ethical Review Board of the Ho Chi Minh Child Nutrition Center. The

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informed consent to participate in the study was given by the parents of the subjects.

Anthropometric and dietary intake measurement. Anthropometric measurements including the weight, height, and left mid-arm circumference were examined. The body mass index was calculated from the baseline measurements of body weight and height (kg/m^2). The body fat was measured using a bioelectric impedance method on a body fat analyzer (TBF-511, Tanita Co., Ltd., Tokyo, Japan).

The parents were interviewed regarding the dietary intake of their children for three consecutive days and nutritionists carried out these interviews. The dietary intake was calculated according to Vietnamese food composition table (15)

Laboratory analyses. Fasting blood samples were obtained in EDTA-coated Vacuttainer tubes during clinical examinations for children. The samples were stored at −80°C until analysis. The total cholesterol (TC), LDL cholesterol (LDLC), HDLC, and TG levels were determined by enzymatic methods (Determiner L, Kyowa Medex, Tokyo, Japan).

Genomic DNA was isolated from 2 mL of whole blood from the subjects using a commercially available kit (QIA Amp DNA Mini Kit; QIAGEN, Valencia, CA). The Invader assay was used to determine any mutations of CETP I14A, D442G, and *Taq*IB polymorphism, as previously described (16).

Statistical analyses. The χ^2 test was used to examine any differences in the distribution of the CETP genotypic frequencies among rural and urban groups. The Hardy-Weinberg equilibrium of the CETP polymorphism was also assessed by the χ^2 test. Continuous variables were expressed as the mean ± SD, and significant differences of the plasma lipids between the CETP genotypes were evaluated by analysis of variance (ANOVA) and the *post hoc* test (Scheffé test). Statistical procedures were performed using the StatView statistical program 5.0 (SAS Institute Inc., Cary, NC). A p value of <0.05 was considered to be statistically significant.

RESULTS

Frequencies for the I14A and D442G mutations and the TaqIB polymorphism in CETP gene. As shown in Table 1, none of subjects carried the I14A mutation. In contrast, the D442G mutation was detected in 17 people (8.8%) from the rural subgroup and in seven people (4.5%) from the urban subgroup. The difference in the frequency between two groups was insignificant. Only the D442G heterozygous mutant was found in these Vietnamese children. On analyzing the *Taq*IB polymorphism, the frequencies for the B1B1, B1B2, and B2B2 variants were 43.5%, 44.6%, and 11.9% in rural group and 44.5%, 45.8%, and 9.7% in urban group, respectively. The distribution of D442G mutation and *Taq*IB polymorphism were in Hardy-Weinberg equilibrium.

Nutritional statuses and the CETP genotypes. The urban group had a higher quantity of energy and fat intake and better anthropometric parameters than the rural group (Tables 2 and 3). There was no difference in the profiles regarding the dietary intake and anthropometric factors among CETP D442G or *Taq*IB genotypes in the rural group. However, the quantity of fat intake in the D442G mutant carriers was significantly higher than that of the wild-type carriers ($p < 0.05$, Table 2), and the quantity of fat intake was also significantly higher in the B2B2 carriers than that in the B1B1 carriers ($p < 0.05$, Table 3).

Associations between CETP gene D442G mutation and the plasma lipid and lipoprotein levels. The D442G mutant carriers had significantly higher HDLC levels than nonmutant carriers, and the HDLC levels in the rural group were 1.14 ± 0.3 and 0.96 ± 0.2 mmol/L ($p < 0.005$) for mutant and nonmutant carriers, respectively, while in the urban group they were 1.50 ± 0.1 and 1.25 ± 0.3 mmol/L ($p < 0.05$) for mutant and nonmutant carriers, respectively (Table 4).

Associations between the CETP gene TaqIB polymorphism and plasma lipid and lipoprotein levels. An elevated plasma HDLC level in B2 allele carriers was observed dose-dependently in both groups (Table 5). B1B1 carriers had lower HDLC levels (0.93 ± 0.2 mmol/L) than the B1B2 carriers (1.00 ± 0.2 mmol/L) and B2B2 carriers (1.07 ± 0.3 mmol/L) in the rural group ($p < 0.05$). Likewise, TaqIB2 carriers tended to have elevated HDLC levels in the urban group (1.33 ± 0.3 in B2B2 carriers, 1.27 ± 0.3 in B1B2 carriers and 1.23 ± 0.3 mmol/L in B1B1 carriers) [p = not significant (NS)].

The interaction of the D442G mutation and TaqIB polymorphism regarding the plasma HDLC levels. The genetic effects of D442G and *Taq*IB on the HDLC levels in both the urban and rural groups are shown in Table 6. The D442G mutant carriers had higher HDLC levels than the nonmutant carriers in all types of *Taq*IB polymorphism (p = NS). In wild-type D442G carriers, the HDLC levels tended to increase in the *Taq*IB B2 carriers in both groups (0.93 ± 0.2, 0.98 ± 0.2, and 1.04 ± 0.3 mmol/L in B1B1, B1B2, and B2B2 carriers in the rural group, respectively, and 1.22 ± 0.3, 1.26 ± 0.3 and 1.32 ± 0.3 mmol/L in B1B1, B1B2 and B2B2 carriers in the urban group, respectively). In the D442G mutant carriers, this trend was observed in the rural group (1.12 ± 0.2 and 1.25 ± 0.4 mmol/L in B1B2 carriers and B2B2 carriers in rural group, respectively), but it was not clear in the urban group (1.38 and 1.73 in B1B1 carriers, 1.45 ± 0.1 in B1B2 carriers, and 1.57 mmol/L in B2B2 carriers).

Table 1. *Frequencies of CETP TaqIB polymorphism and D442G mutation in Vietnamese children*

		CETP D442G		CETP *Taq*IB		
	CETP I14A	Mutant	Wild type	B1B1	B1B2	B2B2
Frequency % (no.)						
Total (n = 348)	0	6.9 (24)	93.1 (324)	44.0 (153)	45.1 (157)	10.9 (38)
Rural (n = 193)	0	8.8 (17)	91.2 (176)	43.5 (84)	44.6 (86)	11.9 (23)
Urban (n = 155)	0	4.5 (7)	95.5 (148)	44.5 (69)	45.8 (71)	9.7 (15)
Allele frequency I14A		D442G mutant		B1 allele		B2 allele
Total	0	0.034		0.66		0.34
Rural	0	0.044		0.66		0.34
Urban	0	0.023		0.67		0.33

Table 2. *Nutritional status of Vietnamese children according to CETP D442G genotypes*

	Mutant	Wild type
Rural (n = 193)	17	176
Energy intake (kcal)	1390 ± 307	1255 ± 310
Fat intake E (%)	16.1 ± 5.2	15.5 ± 5.7
Carbohydrate intake E (%)	69.1 ± 6.1	70.1 ± 6.5
Protein intake E (%)	14.8 ± 2.0	14.4 ± 2.1
Body mass index (kg/m^2)	13.9 ± 1.5	14.0 ± 1.2
Body fat (%)	10.2 ± 3.4	10.7 ± 2.9
Left mid-arm circumference (cm)	16.0 ± 1.3	16.0 ± 1.3
Urban (n = 155)	7	148
Energy intake (kcal)	1991 ± 384	1772 ± 346
Fat intake E (%)	25.8 ± 5.1*	22.2 ± 4.2
Carbohydrate intake E (%)	56.2 ± 3.9	59.6 ± 5.2
Protein intake E (%)	15.8 ± 2.3	15.9 ± 2.2
Body mass index (kg/m^2)	16.4 ± 1.8	15.8 ± 2.4
Body fat (%)	14.3 ± 4.7	15.2 ± 6.0
Left mid-arm circumference (cm)	18.3 ± 2.6	18.0 ± 2.3

Values are the mean ± SD. E (%) is the percentage of total energy intake.
* $p < 0.05$ when compared to wild type.

Table 3. *Nutritional status of Vietnamese children according to CETP TaqIB genotypes*

	B1B1	B1B2	B2B2
Rural (n = 193)	84	86	23
Energy intake (kcal)	1315 ± 331	1234 ± 287	1214 ± 316
Fat intake E (%)	15.2 ± 5.3	16.1 ± 6.2	15.0 ± 4.5
Carbohydrate intake E (%)	70.3 ± 6.2	69.4 ± 6.9	71.0 ± 5.5
Protein intake E (%)	14.5 ± 2.1	14.5 ± 2.1	14.0 ± 2.1
Body mass index (kg/m^2)	14.1 ± 1.2	13.9 ± 1.3	13.6 ± 0.9
Body fat (%)	10.8 ± 2.6	10.7 ± 3.4	10.1 ± 2.4
Left mid-arm circumference (cm)	16.0 ± 1.2	16.0 ± 1.4	15.6 ± 1.3
Urban (n = 155)	69	71	15
Energy intake (kcal)	1772 ± 356	1768 ± 359	1893 ± 265
Fat intake E (%)	22.1 ± 3.9	22.3 ± 4.6	24.5 ± 4.5*
Carbohydrate intake E (%)	60.2 ± 4.4	59.2 ± 5.8	56.8 ± 4.2*
Protein intake E (%)	16.0 ± 2.5	15.7 ± 2.2	16.0 ± 1.4
Body mass index (kg/m^2)	15.7 ± 2.4	15.9 ± 2.5	16.2 ± 1.8
Body fat (%)	15.1 ± 6.0	15.0 ± 6.2	16.1 ± 4.2
Left mid-arm circumference (cm)	18.0 ± 2.3	17.9 ± 2.6	18.2 ± 1.3

Values are the mean ± SD. E (%) is the percentage of total energy intake.
* $p < 0.05$ when compared to B1B1 carriers.

Table 4. *Plasma levels of lipids and lipoproteins of Vietnamese children according to CETP D442G genotypes*

	D442G		
	Mutant	Wild type	p
Total (n = 348)	24	324	
TC (mmol/L)	3.89 ± 0.7	4.05 ± 0.8	NS
LDLC (mmol/L)	2.03 ± 0.6	2.34 ± 0.6	<0.05
HDLC (mmol/L)	1.25 ± 0.3	1.09 ± 0.3	<0.05
TG (mmol/L)	1.09 ± 0.4	1.22 ± 0.7	NS
Rural (n = 193)	17	176	
TC (mmol/L)	3.70 ± 0.5	3.69 ± 0.6	NS
LDLC (mmol/L)	1.94 ± 0.5	2.11 ± 0.5	NS
HDLC (mmol/L)	1.14 ± 0.3	0.96 ± 0.2	<0.005
TG (mmol/L)	1.17 ± 0.4	1.19 ± 0.5	NS
Urban (n = 155)	7	148	
TC (mmol/L)	4.36 ± 0.7	4.48 ± 0.7	NS
LDLC (mmol/L)	2.26 ± 0.7	2.61 ± 0.6	NS
HDLC (mmol/L)	1.50 ± 0.1	1.25 ± 0.3	<0.05
TG (mmol/L)	0.90 ± 0.3	1.26 ± 0.8	NS

Values are the mean ± SD.

Table 5. *Plasma levels of lipids and lipoproteins of Vietnamese girls according to CETP TaqIB polymorphism*

	CETP *Taq*IB		
	B1B1	B1B2	B2B2
Total (n = 348)	153	157	38
TC (mmol/L)	3.96 ± 0.7	4.09 ± 0.9	4.17 ± 0.7
LDLC (mmol/L)	2.27 ± 0.7	2.36 ± 0.6	2.36 ± 0.4
HDLC (mmol/L)	1.06 ± 0.3	1.12 ± 0.3	1.18 ± 0.3*
TG (mmol/L)	1.28 ± 0.7	1.16 ± 0.6	1.17 ± 0.4
Rural (n = 193)	84	86	23
TC (mmol/L)	3.65 ± 0.6	3.69 ± 0.7	3.84 ± 0.7
LDLC (mmol/L)	2.08 ± 0.4	2.10 ± 0.6	2.14 ± 0.5
HDLC (mmol/L)	0.93 ± 0.2	1.00 ± 0.2	1.07 ± 0.3*
TG (mmol/L)	1.23 ± 0.5	1.15 ± 0.6	1.22 ± 0.5
Urban (n = 155)	69	71	15
TC (mmol/L)	4.34 ± 0.7	4.57 ± 0.8	4.68 ± 0.5
LDLC (mmol/L)	2.48 ± 0.6	2.67 ± 0.7	2.71 ± 0.5
HDLC (mmol/L)	1.23 ± 0.3	1.27 ± 0.3	1.33 ± 0.3
TG (mmol/L)	1.34 ± 0.9	1.18 ± 0.7	1.09 ± 0.4

Values are the mean ± SD.
* $p < 0.05$ when compared to B1B1 carriers.

DISCUSSION

This study proved that a significant proportion of the variation in plasma HDLC levels was associated with the CETP genotypes in Vietnamese children. The effects of the CETP gene, including a D442G mutation and the *Taq*IB polymorphism on plasma HDLC levels were examined in girls ranging from 7 to 9 y of age with different nutritional statuses while minimizing confounding factors such as puberty, medicines, and smoking.

Plasma HDLC levels in the children with D442G heterozygote were significantly elevated by 20% in comparison to wild-type children in the rural and urban area. This result was compatible with the findings of other studies carried out using adults in Chinese (17), Taiwanese (18), Japanese (19), and Korean (20) populations. D442G mutant carriers were thus shown to have a lower CETP activity and mass resulting in higher HDLC levels due to a slower rate of apoA-I catabolism (21–23). In contrast, one study on Japanese children (average age of 10) showed that plasma levels of HDLC, apoA-I, and apoA-II did not increase in the D442G heterozygous carriers in comparison to wild-type carriers (24). The authors of this study concluded that the D442G mutation, by itself, might not affect HDLC metabolism in children (24). The discrepancy between this result and our result might be explained by their small sample size (only 32 boys and 33 girls of wild type and 10 boys and 11 girls of heterozygotes). The magnitude of the elevated plasma HDLC levels caused by the D442G mutation in the Vietnamese children with a low fat intake was similar to those of other populations with a higher fat intake (25.2%) in the Japanese (25). Therefore, the effect of this mutation on plasma HDLC levels was not influenced by the low fat intake.

An effect of the TaqIB2 allele on plasma HDLC levels was also observed in Vietnamese children, regardless of the nutritional status, although the elevated effect of the TaqIB2 allele

Table 6. *The interaction between CETP TaqIB and D442G genotypes and HDLC levels in Vietnamese girls*

	B1B1	B1B2	B2B2	Total
Rural (*n*)				
D442G Wild type	84	73	19	176
D442G Mutant	0	13	4	17
Total	84	86	23	193
Plasma HDLC levels (mmol/L)				
D442G Wild type	0.93 ± 0.2	0.98 ± 0.2	1.04 ± 0.3	
D442G Mutant		1.12 ± 0.2	1.25 ± 0.4	
Urban (*n*)				
D442G Wild type	67	67	14	148
D442G Mutant	2	4	1	7
Total	69	71	15	155
Plasma HDLC levels (mmol/L)				
D442G Wild type	1.22 ± 0.3	1.26 ± 0.3	1.32 ± 0.3	
D442G Mutant	1.38 and 1.73	1.45 ± 0.1	1.57	

Values are the mean ± SD.
The plasma HDLC levels in each genotype were not significantly different.

on HDLC levels was found to be weaker than that in the D442G mutation. In both groups of children in rural and urban settings, plasma HDLC levels in TaqIB2 allele carriers were higher than those in TaqIB1 allele carriers. In comparison to the B1 homozygote, plasma HDLC levels of the B2 homozygote increased by 15% and 8% in rural and urban children, respectively. In the present study, no data on the CETP protein level or activity were available; however, previous reports showed that the B2 allele as well as D442G mutant were both associated with a decrease in the CETP protein level or activity (19,24,26). The site of the D442G mutation is close to the active site of CETP, and the cellular expression of mutant cDNA leads to a reduction in the CETP secretion and the specific activity (6). On the other hand, the mechanism by which the *Taq*IB polymorphism may affect the CETP protein level or activity is not well understood. It is plausible to explain that this polymorphism is in linkage disequilibrium with some unknown functional mutation in the CETP gene. In either genotype, the CETP protein level or activity was decreased, resulting in higher HDLC levels.

Other studies showed that the association between CETP genotypes and plasma HDLC levels was decreased due to environmental factors including smoking (27), obesity, and high TG levels (18) and was enhanced by alcohol consumption (27,28). Therefore, the interaction between CETP genotypes and environmental factors on plasma HDLC levels might occur in individuals with high TG levels and/or obese people who are vulnerable to developing metabolic syndrome. However, such interaction might not be found in those with a low fat intake. Obese children have been reported to have increased serum CETP levels and lower plasma HDLC levels (29,30). Only 2.3% of the children (eight children) in this study were overweight (classified as overweight if the weight-for-height *Z* score was >2). These overweight children showed lower plasma HDLC levels than their counterparts (1.16 *versus* 1.26 mmol/L), but the change was not significantly different.

The frequency of the CETP D442G mutation in the Vietnamese was comparable to that in other Asian populations including Chinese (5%) (17), Taiwanese (6.7%) (18), Japanese (7.28%) (19), and Korean (5.9%) (20). This mutation was rarely found in Europeans (27), so it seems that this mutation is particular to Asian populations. The CETP *Taq*IB B2 allele frequency of the Vietnamese was lower than those of the Taiwanese (42.3%) (18), Japanese (49.9%) (26), and nearly the same as those of Koreans (36%) (20). In whites, the frequency of the B2 allele was 44% (31), and therefore it was also higher than that in the Vietnamese. The CETP I14A mutation found neither in Vietnamese children, Taiwanese (18), nor Koreans (20), whereas it is 0.55% in the Japanese (19) and 1% in the Chinese (17). However, the frequency of the I14A mutation also varies in different regions in Japan, and this mutation was found to be 20-fold more frequent in Omagari than in other areas (32).

In conclusion, our findings suggest that the roles of CETP D442G mutation and *Taq*IB polymorphism might explain a significant proportion of the variability in plasma HDLC levels in Vietnamese children. Furthermore, this is the first report to indicate the effects of a D442G mutation and the *Taq*IB polymorphism on the plasma HDLC levels in subjects with a low fat intake. The effect of the D442G and *Taq*IB genotype on the plasma HDLC levels was not related to a low fat diet. Additional studies therefore need to be conducted to demonstrate whether these CETP genotypes play an important role in the risk of coronary artery disease among the Vietnamese population.

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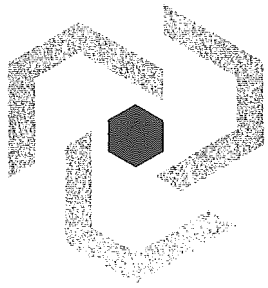

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Soluble TNF Receptors and Albuminuria in Non-obese Japanese Type 2 Diabetic Patients

Abstract

The aim of this study was to investigate the relationships between albuminuria and tumor necrosis factor (TNF)-α or soluble TNF receptors (sTNF-R1, sTNF-R2) in eighty-eight non-obese Japanese type 2 diabetic patients stratified into two groups according to albuminuria status-microalbuminuria or normoalbuminuria. Patients with microalbuminuria were older and had significantly higher concentrations of sTNF-R1 and sTNF-R2 than those with normoalbuminuria. There was, however, no significant difference in sex, diabetes duration, smoking, BMI, systolic and diastolic blood pressure, HbA_{1c}, serum creatinine, and lipid profile between the two groups. Although serum TNF-α was positively correlated to serum sTNF-R1 and sTNF-R2, serum TNF-α level did not differ with respect to albuminuria. Univariate regression analysis showed that urinary albumin concentration was positively correlated to age ($r = 0.380$, $p < 0.001$), serum creatinine ($r = 0.214$, $p < 0.05$) and concentrations of sTNF-R1 ($r = 0.364$, $p < 0.001$) and sTNF-R2 ($r = 0.342$, $p < 0.005$). Other variables, including TNF-α, were not associated with albuminuria. Multiple regression analyses showed that urinary albumin concentration was independently predicted by the level of sTNF-R1 ($F = 32.1$), which explained 26.3% of the variability of urinary albumin concentration. From these results, it can be concluded that serum soluble TNF receptor is an important independent factor associated with albuminuria in non-obese Japanese type 2 diabetic patients.

Key words
sTNF receptors · TNF-alpha · Albuminuria · Diabetes

Introduction

The major clinical consequence of type 2 diabetes is mortality and morbidity from atherosclerotic vascular disease, especially coronary heart disease (CHD). The risk of CHD appears to be similar in patients with type 2 diabetes and impaired glucose tolerance [1,2]. Thus, factors other than the level of glycemia seem to accelerate the development of CHD in type 2 diabetes. This idea is supported by the observation that duration of diabetes and level of glycemia are not risk factors for atherosclerosis including CHD in type 2 diabetic patients [3,4]. Atherosclerosis can be evaluated by urinary albumin excretion rate. Increased urinary albumin excretion rate is not only used as an index of diabetic nephropathy but also as an independent risk factor for atherosclerosis, including cardiac disease, in type 2 diabetic patients. An association between microalbuminuria and cardiac disease in type 2 diabetic patients has been demonstrated [5].

A number of studies published have shown that age, smoking, blood pressure, blood glucose, and abnormalities in lipoprotein

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