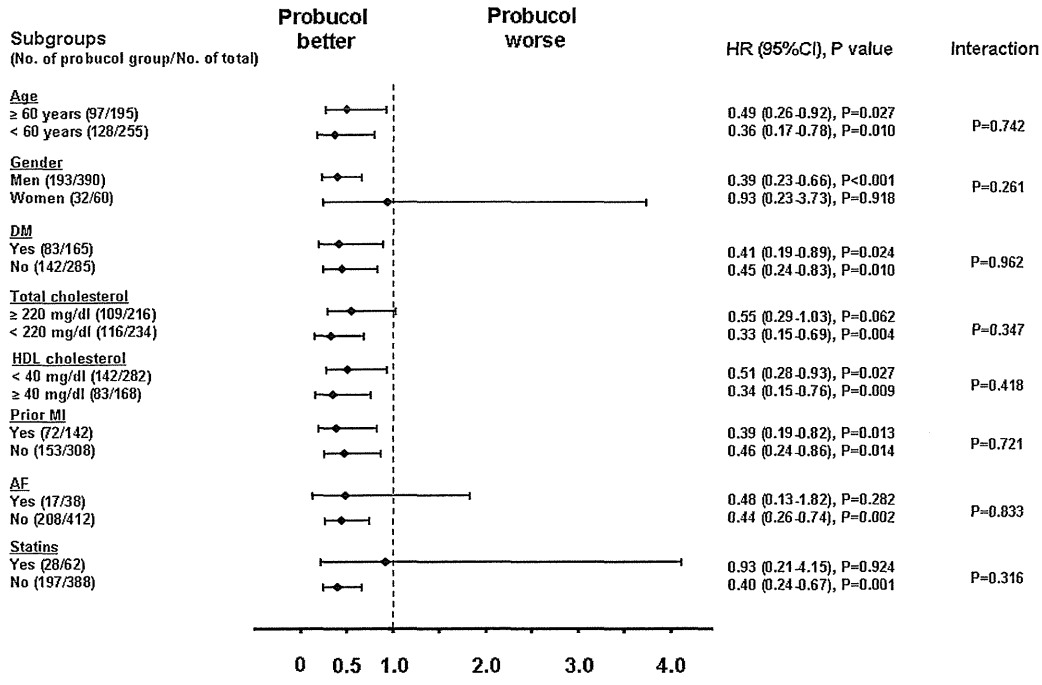

Fig. 2. Association between probucol use and all-cause mortality in subgroups of propensity score matched patients HR, hazard ratio; CI, confidence interval; HDL, high-density lipoprotein; MI, myocardial infarction; AF, atrial fibrillation.

large-scale randomized, double-blind, placebo-controlled trial to assess the effect of succinobucol, the monosuccinic acid ester of probucol, which has greater intracellular antioxidant efficacy *in vitro* than probucol without a QT interval prolongation effect [10,26], on the incidence of cardiovascular events after acute coronary syndrome. The ARISE trial showed that succinobucol significantly reduces the incidence of composites of cardiovascular death, resuscitated cardiac arrest, non-fatal MI, or stroke as pre-specified secondary endpoints. However, this study failed to show a benefit for primary endpoint, a composite of the abovementioned secondary endpoints as well as unstable angina or coronary revascularization. Although this is a large-scale randomized clinical trial using very similar advantageous drug based on QT interval prolongation as compared to probucol, specific beneficial effects of probucol on long-term morbidity and mortality in patients with severe CAD remains unclear. The Probucol Observational Study Illuminating Therapeutic Impact on Vascular Events (POSITIVE) study [21] assessed the efficacy of long-term probucol treatment on reduction of cardiovascular event risk in Japanese patients with heterozygous familial hypercholesterolemia. The POSITIVE study enrolled relatively large number of patients ($N=410$) from 15 institutions and showed that in a subset of patients with prior cardiovascular diseases ($N=88$), probucol therapy was associated with a reduced risk of long-term cardiovascular event incidence. This study indicates that probucol therapy may be beneficial in reducing cardiovascular events in patients with prior cardiovascular diseases. However, number of subjects in the secondary prevention cohort was very small and it is not clear whether patients enrolled in their study had undergone coronary revascularization. In the present study, we showed that probucol therapy at the time of complete coronary revascularization is associated with reduced all-cause mortality for a long-term follow-up period (>10 years). To the best of our knowledge, there have been no studies involving patients with significant CAD treated with coronary revascularization. Furthermore, it was important to assess data only from patients who had achieved complete revascularization because initial CAD events may be prevented or delayed by complete coronary revascularization, even in patients with severe coronary atherosclerosis. This minimizes the bias of treatment procedures for initial CAD events. Therefore, benefits of probucol use in long-term mortality among a secondary prevention cohort of patients with CAD were assessed in this study.

Mechanisms contributing to the association of probucol use and reduced all-cause mortality may include a combination of oxidative stress reduction [1–3] and inflammatory response [4,5] in addition to lowering LDL cholesterol. Anti-oxidant and anti-inflammatory effects may explain why all-cause mortality, not cardiac mortality, was reduced in the probucol group. These two effects may affect not only progression of cardiovascular disease but also development or progression of other diseases such as inflammatory diseases, cancer, and neurodegenerative disease [27]. More importantly, mechanisms promoting cholesterol efflux and enhancing RCT by activation of CETP [14,17] and SR-BI [19] may have contributed to lower all-cause mortality in the probucol group. In our pre-match patients, the probucol group showed a significantly lower baseline HDL level. Although we did not have data regarding changes in HDL cholesterol level before starting probucol, lower HDL cholesterol levels in the probucol group imply that reduced HDL cholesterol levels are caused by probucol through enhancing RCT by activation of CETP and SR-BI. However, this reduction in HDL cholesterol may be due to altered HDL function from increasing preβ1-HDL, which can promote cellular lipid efflux. Therefore, the observed lowered HDL cholesterol levels in the probucol group may not be harmful, but rather may be a reflection of increased cholesterol efflux, which can beneficially affect mortality. Furthermore, based on reductions in new-onset DM cases and glycated hemoglobin levels among patients with succinobucol in the ARISE trial [25], probucol treatment contributes to reduced mortality through the prevention of new-onset DM or through the improvement of its control level [28]. However, we did not study new-onset DM or glycemic control during follow-up in this study.

In the present study, we observed a significant relationship between probucol and all-cause mortality but did not observe a significant relationship between probucol and cardiac

mortality. In addition, PS adjusted and PS matched analyses indicate that patients using probucol generally died due to non-cardiac-related causes. Therefore, we expected to observe a significant relationship between probucol and major sub-specific causes of non-cardiac death. However, we did not observe a significant relationship between probucol and deaths associated with cancer, which were the most frequent sub-specific cause of non-cardiac death. However, the probucol group tended to have reduced cardiac mortality in all models, as well as non-cardiac mortality. This suggests that reduced all-cause mortality in the probucol group is consistent with combined risk reduction in both cardiac and some sub-specific causes of non-cardiac death other than cancer-related deaths, which may include deaths related to heart failure. Since it is difficult to distinguish deaths due to heart failure from other non-cardiac causes of death, we did not include heart failure in the definition of cardiac death, which may have affected the results. Nevertheless, all-cause mortality is an objective endpoint and should be used as a primary endpoint in this type of observational study.

Importantly, no significant subgroup-treatment effect interactions were observed in any subgroup analyses. This suggests that the efficacy of probucol may not be affected by age, gender, presence or absence of DM, total and HDL cholesterol levels, presence or absence of prior MI, AF, and statins use. However, the absence of a significant relationship between probucol use and all-cause mortality in women and patients with AF might suggest an incidence of QT interval prolongation and fatal ventricular arrhythmia. Women and patients with a high risk of drug interaction such as those with AF treated with anti-arrhythmic drugs were at risk to have torsades de pointes among patients treated with drugs that prolong the QT interval such as probucol [29]. Absence of a significant relationship between probucol use and mortality in patients who receive statins should be taken into account, although the probucol group showed no significant overall mortality risk and there were no significant subgroup treatment effect interactions. In addition, negative results can be ignored because of the small number of patients in those subgroups and the high rate of false-negative results in the analysis within individual subgroups. Thus, studies specifically evaluating the effects of probucol on mortality in women, patients with AF, and statin-treated patients as well as large-scale studies are required.

4.1. Study limitations

This was a single center observational study of daily clinical practice. Although propensity analyses are powerful, they are inherently limited by the number and accuracy of variables evaluated. Furthermore, recent evidence suggests that PS is not always an accurate indicator for adjusting confounding factors by indication [30] and that results from propensity analyses pertain to population-averaged effects rather than the effect of receiving or not receiving probucol therapy within an individual. In addition, even after adjusted analysis was performed, other unknown confounders may have affected the outcomes. Further studies are required to determine if there is a benefit in long-term outcomes for patients undergoing probucol treatment.

In addition, the total duration of probucol use and changes in dosage after complete revascularization was not examined and there was crossover between the two groups. However, if crossover bias was present, it would have led to underestimation of the association between probucol administration and survival. This further emphasizes that probucol therapy at the time of complete revascularization is associated with better long-term mortality. Although this may have affected the results of our study, the effect was very small.

5. Conclusion

In the present study involving consecutive revascularization patients, we showed that the use of probucol is significantly associated with reduced long-term all-cause mortality after complete revascularization. Although probucol should be used with caution in specific subgroups, findings of the present study may contribute to reappraisal of probucol as a therapeutic drug in patients with CAD.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.atherosclerosis.2011.09.051.

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Original Article

Patients with CD36 Deficiency Are Associated with Enhanced Atherosclerotic Cardiovascular Diseases

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Aim: The clustering of dyslipidemia, impaired glucose tolerance and hypertension increases the morbidity and mortality from cardiovascular events. A class B scavenger receptor, CD36, is a receptor for oxidized LDL and a transporter of long-chain fatty acids. Because of the impaired uptake of oxidized LDL in CD36-deficient macrophages and from the results of CD36 knockout mice, CD36 deficiency (CD36-D) was supposed to be associated with reduced risks for coronary artery disease (CAD); however, CD36-D patients are often accompanied by a clustering of coronary risk factors. The current study aimed to investigate the morbidity and severity of cardiovascular diseases in CD36-D patients.
Methods: By screening for CD36 antigen on platelets and monocytes using FACS or the absent myocardial accumulation of ^{123}I-BMIPP by scintigraphy, 40 patients with type I CD36-D were collected, the morbidity of CAD and their features of atherosclerotic cardiovascular diseases were observed. Screening for CD36-D in both CAD patients (n=319) and healthy subjects (n=1,239) were underwent.
Results: The morbidity of CAD was significantly higher in CD36-D patients than in the general population; 50% of patients (20 out of 40) had CAD identified by BMIPP scintigraphy and 37.5% (3 out of 8) by FACS screening, respectively. Three representative CD36-D cases demonstrated severe CAD and atherosclerosis. The frequency of CD36-D was three times higher in CAD patients than in healthy subjects (0.9% vs 0.3%, $p<0.0001$).
Conclusion: The morbidity of CAD is significantly higher in CD36-D patients suffering from severe atherosclerosis, implying that the status of CD36-D might be atherogenic.

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Key words; CD36 deficiency, Long-chain fatty acid transporter, Atherosclerotic cardiovascular disease, Insulin resistance, Metabolic syndrome

Introduction

Patients with metabolic syndrome (MetS) are characterized by a clustering of coronary risk factors, such as dyslipidemia including hypertriglyceridemia and a low level of high density lipoprotein-cholesterol (HDL-C), impaired glucose tolerance and hypertension along with the accumulation of abdominal visceral fat. The morbidity and mortality of atherosclerotic cardiovascular events are significantly high in patients with MetS, and the reduction of abdominal visceral fat by diet and exercise therapy is very important for treatment of the clustering of these coronary risk fac-

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tors and atherosclerotic cardiovascular diseases.

CD36 is an 88-kDa membrane glycoprotein belonging to a class B scavenger receptor[1]. CD36 is expressed in a variety of cells and tissues including platelets, monocyte/macrophages, heart, skeletal muscle, adipose tissue and small intestines[1]. CD36 is a receptor for oxidized low density lipoproteins (LDL)[2] and a transporter of long-chain fatty acids (LCFA)[3]. CD36-deficient patients were first identified from subjects who were refractory to platelet transfusion[4]. Kashiwagi *et al.* identified several genetic mutations of human CD36 deficiency (CD36-D)[5]. We previously investigated the metabolic phenotypes of CD36-D patients[6-7] and reported that they (high fasting serum triglycerides level, low HDL-C level, fasting hyperglycemia, insulin resistance and hypertension) were frequently observed and clustered in patients with CD36-D, similar to those with MetS[8-9]. It was later reported that the accumulation of these metabolic phenotypes is not due to the deposition of abdominal visceral fat, but to insulin resistance or impaired metabolism of lipoproteins and free fatty acids (FFA) in the postprandial state in patients with CD36-D[8-10]. It is well known that these metabolic profiles are independent coronary risk factors in the general population[11-13]; therefore, the status of human CD36-D was supposed to be atherogenic and the morbidity of atherosclerotic cardiovascular diseases might be high in patients with CD36-D.

In contrast, the status of human CD36-D was supposed to be anti-atherogenic since CD36 is a scavenger receptor for oxidized LDL when the foam cell formation of CD36-D macrophages by exposure of oxidized LDL is impaired[14]. Nozaki et al. showed that the uptake of oxidized LDL was reduced by approximately 40% in macrophages from patients with CD36-D compared with normal controls[15]. Janabi *et al.* showed that the responses of oxidized LDL-induced NF-kappa B activation and subsequent cytokine expression were impaired in monocyte-derived macrophages from CD36-D patients[16]. Furthermore, there have been two reports by Febbraio *et al.* and Moore *et al.* concerning the atherogenicity of genetic disruption of CD36 in mice[17-18]. Both reports showed that macrophage foam cell formation when treated with oxidized LDL was impaired when they crossed CD36 null mice with atherogenic apoE-null mice; however, atherosclerotic lesion development in CD36-apoE double knockout mice was different in these two reports. Febbraio *et al.* showed a 76.5% decrease in aortic tree lesion areas when mice were fed a Western diet and a 45% decrease in the aortic sinus lesion area when fed a normal diet in CD36-apoE double knockout mice, respectively, compared with wild-type mice[17]. In contrast, Moore et al. showed that CD36-apoE double knockout (DKO) mice did not show amelioration of the progression of atherosclerotic lesions but foam cell accumulation at aortic sinus rather increased and the severity of atherosclerotic lesions was advanced in DKO mice compared with apoE-KO mice[18]. From these controversial results, the atherogenicity of CD36-D, especially that of human CD36-D patients, remains unclear.

We have so far identified 40 patients with CD36-D by myocardial scintigraphy using an analogue of LCFA, ^{123}I-BMIPP, or by screening with immunofluorescent flow cytometric analysis (FACS). We experienced three typical cases of severe atherosclerotic cardiovascular diseases in patients with CD36-D who were identified in our previous study[9] and evaluated by imaging studies. In the current study, in order to elucidate whether the morbidity and severity of atherosclerotic cardiovascular diseases are high in patients with CD36-D, we evaluated the prevalence of atherosclerotic cardiovascular diseases in 40 patients with CD36-D. Furthermore, to exclude the patient collection bias and to extend the knowledge to the general population, we compared the prevalence of CD36-D between patients with CAD and healthy subjects. We demonstrate that the morbidity of CAD is significantly higher in CD36-D patients suffering from severe atherosclerosis, implying that the status of human CD36-D might be atherogenic.

Subjects and Methods

Diagnosis of CD36 Deficiency

In our previous study, 40 patients without myocardial accumulation of an LCFA analogue, ^{123}I-beta-methyl-p-iodophenyl-pentadecanoic acid (^{123}I-BMIPP), were identified among patients whose heart was evaluated by single photon emission computed tomography (SPECT) for the evaluation of cardiac performance screening at Osaka University Hospital and related hospitals[9]. In order to confirm the diagnosis of CD36-D in these patients, immunofluorescent flow cytometric analysis was performed by using mouse monoclonal antibodies against CD36 (OKM5; Ortho Diagnostic System Inc., Raritan, NJ) at the Department of Blood Transfusion, Osaka University Hospital[7]. Briefly, 20 ml of blood was drawn, anticoagulated with heparin (10 U/ml), layered over 10 ml Ficoll-Paque (GE Healthcare UK Ltd., Buckinghamshire, UK) and centrifuged at 1,000 g for 30 minutes. A 50 μl suspension of platelets ($2\times10^5/\mu$l) or mononuclear cells ($2\times10^4/\mu$l) was incubated with FITC-conjugat-

ed anti-human CD36 monoclonal antibody OKM5 (Ortho Diagnostic Systems) (final concentration: 2.5 μg/ml) or FITC-conjugated mouse IgG (final concentration: 2.5 μg/ml) for 30 minutes at 40℃ and assayed on a FACScan® system (Becton Dickinson Co., Mountain View, CA) as previously reported[7]. Appropriate cell fractions for the analysis of monocytes were selected by a gating method with a two-dimensional display of forward scatter and side scatter of analyzed cells[15]. We diagnosed patients with type I CD36-D whose CD36 antigen was not detected in either monocytes or platelets. Each subject gave written informed consent before participating in the study, and the ethics committee of Osaka University Hospital approved the study design.

Analysis of Clinical Profile and Atherosclerotic Cardiovascular Diseases in Patients with CD36-D

The presence or absence of atherosclerotic cardiovascular diseases was extensively investigated in patients with CD36-D based upon their medical history and symptoms. We assessed the severity of atherosclerotic cardiovascular diseases and risk factors of these patients. Blood pressure was determined in the sitting position, and peripheral venous blood was drawn in the fasting state after overnight fasting and centrifuged for serum separation. Serum levels of total cholesterol (TC), triglycerides (TG), and HDL-cholesterol (HDL-C) as well as fasting plasma glucose levels were measured by enzymatic methods as reported in our previous study[9]. HbA1c was measured by HPLC (Sekisui Medical Co., Tokyo, Japan). All samples were treated in accordance with the Helsinki Declaration. In some patients with CD36-D, coronary angiography was performed for an extensive evaluation of coronary artery atherosclerosis. In order to evaluate atherosclerotic lesions in arteries other than coronary arteries in detail, the thoracic and abdominal aorta and their branches were examined by aortic angiography or magnetic resonance angiography.

Prevalence of CAD in Patients with CD36-D Identified by Absence of Cardiac Uptake of ^{123}I-BMIPP

In order to assess whether patients with CD36-D had a higher mortality and severity of CAD, we evaluated the morbidity of CAD in these patients by checking medical records. The diagnosis of CAD was established when a patient had coronary artery stenosis (≥ 75%) assessed by coronary angiography. The CAD patients were divided into 3 groups by their clinical course and results of coronary angiography: 1) acute or old myocardial infarction, 2) unstable angina, and 3) stable angina.

Prevalence of CAD in Patients with CD36-D Identified by Screening of CD36-D by FACS Analysis in the General Population

For the screening study of CD36-D by FACS analysis, normal healthy volunteers were recruited for over ten years in our laboratory and we found 8 patients with type I CD36-D. We traced their medical records, especially the result of coronary angiography, in order to confirm whether they were accompanied by CAD.

Prevalence of CD36-D in Patients with CAD and Healthy Subjects

In order to evaluate whether the frequency of CD36-D in patients with CAD is higher than in normal healthy subjects, we performed screening examinations in patients with CAD and healthy subjects. Patients with coronary artery stenoses (≥ 75%) were diagnosed with CAD by coronary angiography (n=319). Normal healthy volunteers were recruited using the following criteria: no ST-T abnormalities in ECG, no chest symptoms on effort and no significant coronary artery stenosis (≥ 75%) if they received coronary angiography (n=1,239). Their cell surface CD36 antigen on monocytes and platelets was analyzed by FACS analysis and type I CD36-D was diagnosed by an absence of CD36 antigen in both cells. Statistical significance was assessed by Pearson's chi-square test using JMP 8 software (SAS Institute Japan, Tokyo, Japan).

Results

Case Presentations

Out of 40 patients with CD36-D, we experienced three representative cases of severe atherosclerotic cardiovascular diseases. The metabolic parameters of these patients are shown in **Table 1**, and compared with those of patients with CD36-D in our previous study[9]. As found in that study, these three cases were accompanied by hypertriglyceridemia and low HDL-C, and hypertension (Case 2 received anti-hypertensive drugs), while one case showed high fasting plasma glucose.

Case 1 is a 74-year-old man. At the age of 62, he suffered acute myocardial infarction. Coronary angiography demonstrated severe and diffuse stenoses in 3 major coronary arteries (**Fig. 1A** and **1B**) and he underwent percutaneous coronary revascularization. At the age of 66, angiographic restenosis was detected in the right coronary artery (RCA) and left anterior descending artery (LAD), which were later revascularized successfully. At the same time, we found total oc-

Table 1. Metabolic Profiles of Three Cases of CD36-D Associated with Severe Atherosclerotic Cardiovascular Diseases

	Case 1	Case 2	Case 3	CD36-D ($n=40$)*	Healthy subjects ($n=84$)*
Age (year)	74	73	73	62±14	60±14
Sex (m/f)	male	male	female	(25, 15)	(63, 21)
BMI (kg/m^2)	21.6	24	21.4	23.5±3.6	23.5±2.0
TC (mg/dl)	193	166	220	201±39	205±32
TG (mg/dl)	192	152	156	178±89	126±62
HDL-C (mg/dl)	29	34	34	46±15	61±11
FPG (mg/dl)	100	87	210	110±22	95±18
sBP (mmHg)	152	128	154	135±18	115±15
dBP (mmHg)	94	86	53	80±10	77±18

*CD36-D ($n=40$) and healthy, age, sex, and BMI-matched controls ($n=84$) were quoted from our previous study (Reference 9).
Abbreviations: BMI, body mass index; TC, total cholesterol; TG, triglycerides; FPG, fasting plasma glucose; sBP, systolic blood pressure; dBP, diastolic blood pressure.

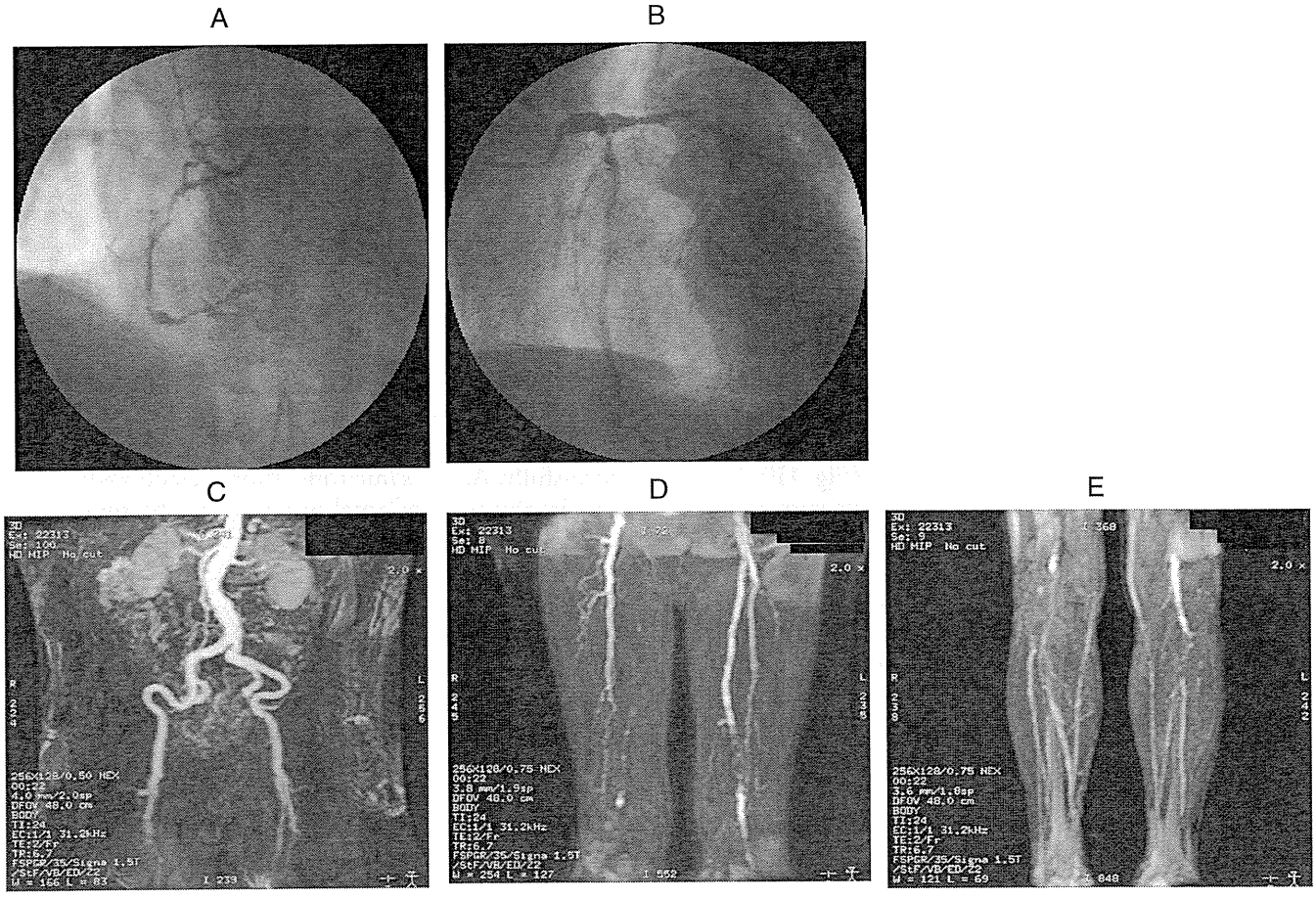

Fig. 1. Case 1, a 74-year-old male patient with CD36-D

At the age of 62, he suffered from acute myocardial infarction, and emergent cardiac catheterization revealed severe and diffuse stenosis in the triple coronary arteries (1-A, right coronary artery (RCA); 1-B, left coronary artery (LCA), respectively). Magnetic resonance angiography revealed total occlusion of bilateral femoral arteries (1-C and 1-D), total occlusion of left anterior tibial artery and severe stenosis of right anterior tibial artery (1-E).

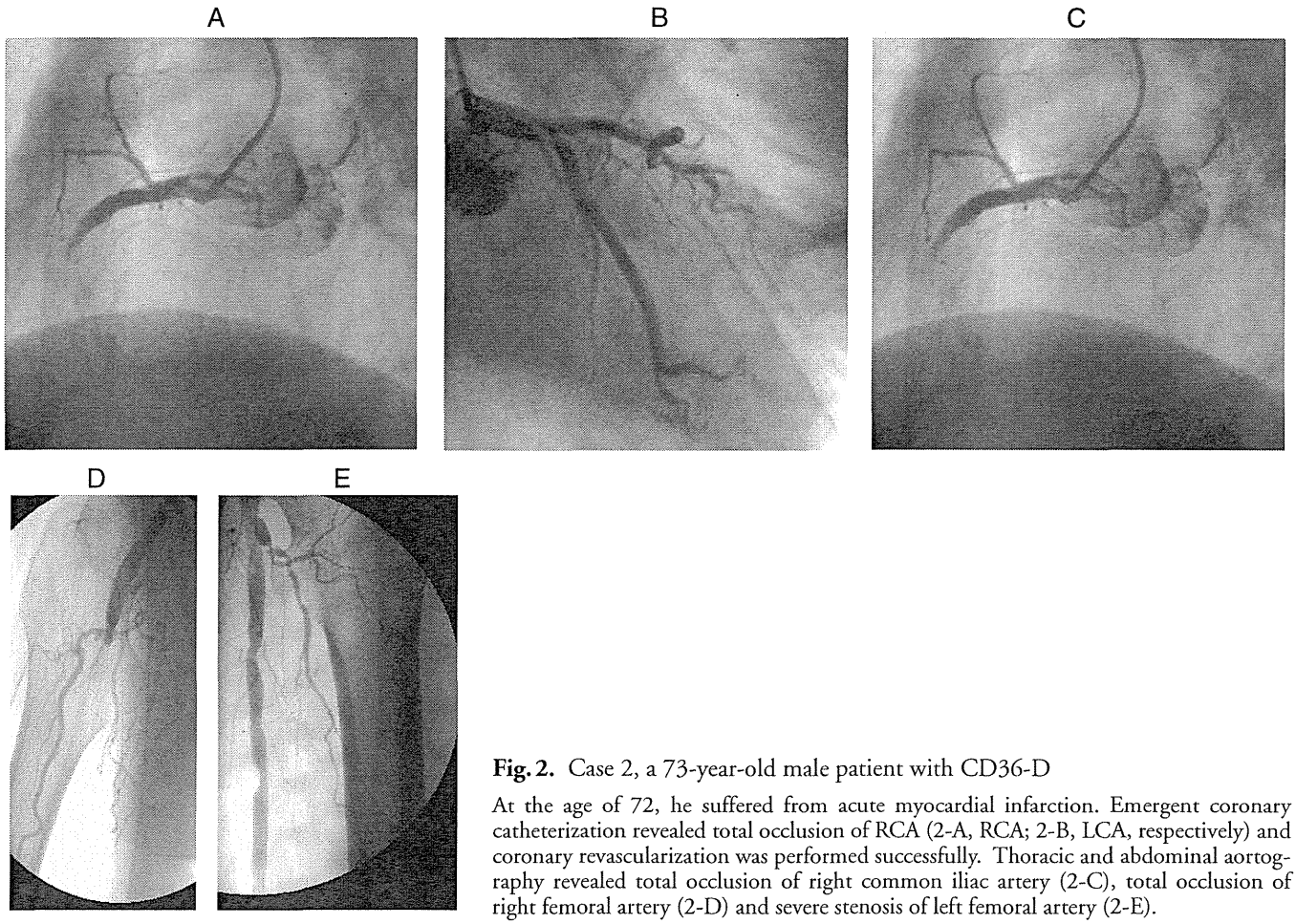

Fig. 2. Case 2, a 73-year-old male patient with CD36-D

At the age of 72, he suffered from acute myocardial infarction. Emergent coronary catheterization revealed total occlusion of RCA (2-A, RCA; 2-B, LCA, respectively) and coronary revascularization was performed successfully. Thoracic and abdominal aortography revealed total occlusion of right common iliac artery (2-C), total occlusion of right femoral artery (2-D) and severe stenosis of left femoral artery (2-E).

clusion of bilateral femoral arteries (**Fig. 1C** and **1D**), complete obstruction of the left anterior tibial artery and severe stenosis of the right anterior tibial artery by magnetic resonance angiography (MRA) (**Fig. 1E**). Up to the age of 73, the serum level of brain natriuretic peptide (BNP) gradually increased and left ventricular ejection fraction assessed by echocardiography gradually decreased, although repeated revascularization was undergone successfully. At the age of 74, ^{123}I-BMIPP scintigraphy revealed no myocardial uptake of BMIPP, an analogue of LCFA, and he was diagnosed with type I CD36-D by FACS analysis. Regarding his risk factors for cardiovascular diseases, he had a history of smoking and impaired glucose tolerance was observed by an oral glucose tolerance test (data not shown) in addition to the metabolic disorders shown in **Table 1**.

Case 2 is a 73 year-old man. He had a history of excessive alcohol consumption, but he had never smoked. For over 10 years he regularly attended Osaka University Hospital and received medical treatments for hypertension and intermittent claudication. At the age of 72, he suffered acute myocardial infarction. On emergent coronary angiography, total occlusion of RCA was identified (**Fig. 2A** and **2B**), and thereafter coronary revascularization was performed successfully. At the same time, thoracic and abdominal aortography revealed total occlusion of the right common iliac artery (**Fig. 2C**), complete obstruction of the right femoral artery (**Fig. 2D**) and severe stenosis of the left femoral artery (**Fig. 2E**). Thus, we decided to start anticoagulant therapy. Left ventricular ejection fraction in echocardiography did not improve although coronary revascularization was successful; therefore, we tested whether LCFA metabolism was impaired by scintigraphy using ^{123}I-BMIPP, an analogue of LCFA, and found marked reduction of myocardial uptake of ^{123}I-BMIPP. He was finally diagnosed with type I CD36-D by FACS analysis. This was accompanied by moderate hypertension and dyslipidemia, including hypertriglyceridemia and low HDL-C, as shown in **Table 1**.

Case 3 is a 73 year-old woman. She had no history of smoking or regular alcohol intake. At the age of 64, she began to feel chest discomfort and muscle

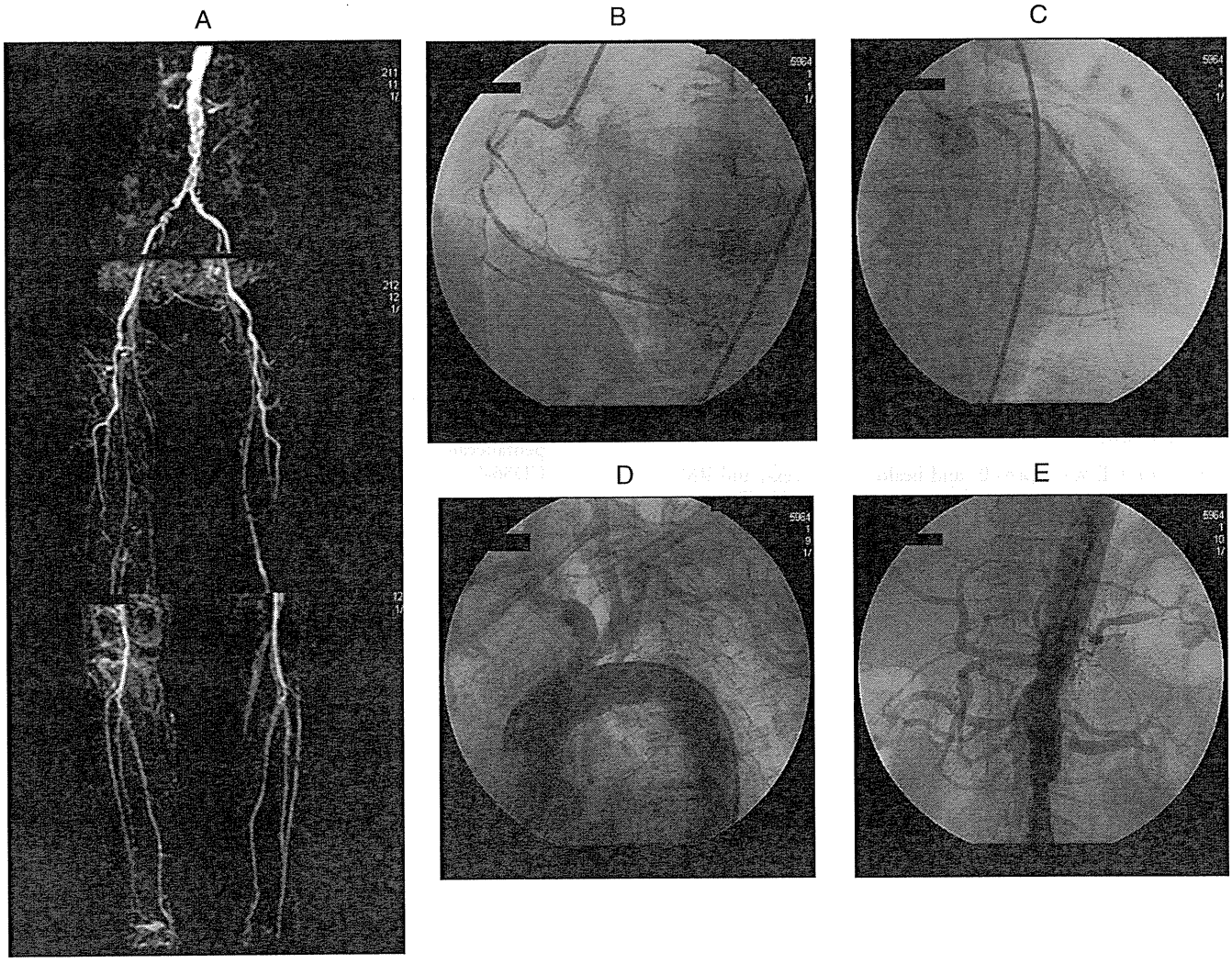

Fig. 3. Case 3, a 73-year-old female patient with CD36-D

At the age of 64, she felt chest discomfort and muscle fatigue of the bilateral legs after climbing stairs. Magnetic resonance angiography (MRA) of lower limbs revealed total occlusion of bilateral femoral arteries and severe stenosis of right common iliac artery and bilateral popliteal arteries (3-A). The following year she was hospitalized for refractory unstable angina, and diagnostic cardiac catheterization revealed severe stenosis of triple coronary arteries (3-B and 3-C). Thoracic and abdominal aortography revealed severe stenosis of trunks brachiocephalicus, right common carotid artery (3-D), abdominal aorta and left renal artery (3-E).

fatigue of the bilateral legs after climbing stairs. MRA of the lower limbs revealed total occlusion of the bilateral femoral arteries and severe stenosis of the right common iliac artery and bilateral popliteal arteries (**Fig. 3A**); therefore, anticoagulant drugs and vasodilators were administered. The following year she was hospitalized because of refractory unstable angina. Emergent diagnostic cardiac catheterization and thoracic and abdominal aortography were performed, which revealed severe stenoses of triple coronary arteries (**Fig. 3B** and **3C**), the brachiocephalic trunk, right common carotid artery (**Fig. 3D**), abdominal aorta and left renal artery (**Fig. 3E**). At the same time, the patient was diagnosed with type II diabetes, hypertension, hypertriglyceridemia and low HDL-C, and drug treatments were started for these diseases. After stent implantation in the left renal artery, coronary artery bypass graft surgery was performed and a saphenous vein graft was connected to the RCA and left circumflex coronary artery, and the left internal thoracic artery to LAD. At the age of 73, she began to complain of exertional dyspnea and the serum level of BNP gradually increased even though these grafts were patent and native coronary arteries remained intact, as assessed by coronary angiography. ^{123}I-BMIPP scintigraphy revealed no myocardial uptake of BMIPP and she

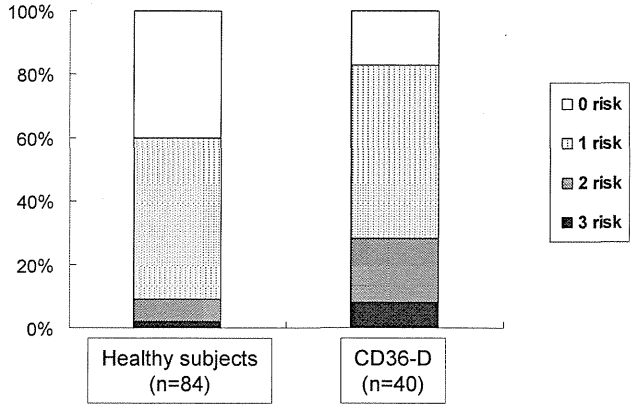

Fig. 4. Number of risk factors for CAD in patients with CD36-D

Patients with CD36-D (n=40) and healthy, age-, sex-, and BMI-matched controls (n=84) were from our previous study (Reference 9). Diabetes mellitus, hypertension and dyslipidemia were counted as risk factors for CAD. Patients with CD36-D had more risk factors for CAD than healthy subjects (patients with CD36-D vs healthy subjects: 1.20±0.80 vs 0.76±0.72 risk factors, P=0.005), and were associated with multiple risk factors for CAD.

was diagnosed with type I CD36-D by FACS analysis.

Number of Risk Factors for CAD in Patients with CD36-D and Healthy Control Subjects

We compared the number of risk factors for CAD in patients with CD36-D (n=40) and healthy, age, sex, and BMI-matched control subjects (n=84) from our former study[9]. These risk factors included diabetes mellitus, hypertension and dyslipidemia. As shown in **Fig. 4**, patients with CD36-D had more risk factors for CAD than healthy subjects (1.20±0.80 vs. 0.76±0.72 risks, p=0.005), and were often associated with multiple risk factors for CAD.

Frequency of CAD in Patients with CD36-D

The frequency of CAD was examined among 40 patients with CD36-D who were identified by an absence of cardiac uptake of ^{123}I-BMIPP. As shown in **Table 2**, the frequency of CAD in CD36-D patients was significantly high (50%, 20 of 40 CD36-D patients). Furthermore, in 20 CD36-D cases of CAD, coronary stenoses with high severity, acute or old myocardial infarction and unstable angina pectoris were observed in 65% (13 of 20 patients with CD36-D). These data suggest that CD36-D patients are accompanied by enhanced atherosclerotic cardiovascular diseases. By a screening study of FACS analysis, we found 8 patients with type I CD36 deficiency. Three patients (37.5%) out of 8 had coronary artery stenoses by coronary angiography.

Table 2. Frequency of Coronary Artery Disease in Patients with CD36 Deficiency

Patients with CD36-D (n=40)	
BMIPP	
CAD negative	50% (20/40)
CAD positive	50% (20/40)
acute MI	22.5% (9/40)
unstable angina	10% (4/40)
stable angina	17.5% (7/40)
Screening Study by FACS	
CAD negative	62.5% (5/8)
CAD positive	37.5% (3/8)

Abbreviations: BMIPP, ^{123}I-beta-methyl-p-iodophenyl-pentadecanoic acid; CAD, coronary artery disease; CD36-D, CD36 deficiency; MI, myocardial infarction.

Table 3. Frequency of CD36-D in Patients with Coronary Artery Disease

	Patients with CAD (n=319)	Healthy subjects (n=1239)
Frequency of CD36-D	0.94%* (3/319)	0.32% (4/1239)

*p<0.0001, assessed by Pearson's chi-square test

Prevalence of CD36-D in Patients with CAD and Healthy Subjects

In order to investigate whether CD36-D may increase the prevalence of CAD, we also compared the morbidity of CD36-D between healthy subjects (n=1,239) and patients with CAD diagnosed by coronary angiography (n=319). As shown in **Table 3**, the frequency of CD36-D in patients with CAD was approximately 3-fold higher than in healthy subjects [CAD patients vs healthy subjects, 0.94 % (3/319) vs 0.32 % (4/1239)]. The statistical significance was assessed by Pearson's chi-square test, and the frequency of CD36-D was significantly higher in patients with CAD (p<0.0001). These data suggest that patients with CD36-D are susceptible to CAD.

Discussion

In patients with CD36-D, compared with healthy CD36-positive controls, metabolic phenotypes such as high TG levels, low HDL-C levels, high fasting glucose and hypertension were observed more frequent-

ly[7, 9]. Furthermore, we also found that patients with CD36-D are accompanied by insulin resistance[10], postprandial hyperlipidemia, and high levels of remnant lipoprotein cholesterol and FFA[8-9]. These coronary risk factors were clustered in each CD36-D patient, which may appear to be partly similar to the profiles of patients with MetS[8-9]. Another report showed that Pro90Ser CD36 mutation was associated with elevated FFA levels[19]. These profiles have been shown to be independent coronary risk factors by many clinical investigations[11-13]; however, the morbidity of atherosclerotic cardiovascular diseases in patients with CD36-D has not been clarified beside the reports of Ma *et al.*[20] and Yasunaga *et al.*[21]. Ma *et al*[20] showed that a common haplotype at the CD36 locus was associated with high FFA levels and increased cardiovascular risk in Caucasians. Yasunaga *et al.*[21] reported a 45-year-old male CD36-D patient with acute coronary syndrome without major cardiovascular risk factors. Emergency coronary angiography demonstrated 90% stenosis at segment 7 of LAD. We compared the number of risk factors for CAD in patients with CD36-D and healthy subjects (**Fig. 4**). Patients with CD36-D had more risk factors for CAD than healthy subjects and were associated with multiple risk factors for CAD. We also suggested that the clustering of coronary risk factors might increase the morbidity of cardiovascular disease in patients with CD36-D compared with healthy subjects.

In the current study, we investigated for the first time whether the morbidity of atherosclerotic cardiovascular diseases in CD36-D patients is higher. The clinical observations of three representative CD36-D patients were demonstrated in detail for those whose atherosclerotic lesions of not only coronary arteries but also the aorta and its branches could be assessed. As demonstrated in **Table 1**, dyslipidemia, hypertension and hyperglycemia were clustered in these three cases. Aortography and MRA revealed severe and multiple stenoses and occlusion of the aorta, its branches and arteries of lower limbs. We also found that atherosclerotic lesions were relatively long (up to 8-10 cm) and their collateral circulation was developed sufficiently. It was suggested that multiple and sequential stenoses along with long distance occlusion were not due to acute thrombotic occlusion but to chronic progression of atherosclerotic plaques. These three patients were rather older than the average CD36-D patients and were associated with multiple risk factors; therefore, we could not exclude the possibility that aging and the simple clustering of risk factors might have enhanced the atherogenicity in these three cases; however, a similar tendency of the clustering of CAD risk factors and the association of atherosclerotic cardiovascular diseases were also observed in younger patients with CD36-D.

We also investigated the morbidity and severity of atherosclerotic cardiovascular diseases in 40 patients with CD36-D who were identified by BMIPP scintigraphy and a screening study by FACS analysis. As shown in **Table 2**, we found extremely high morbidity of CAD (50%, 20 of 40 patients with CD36-D). Among 20 CD36-D patients with CAD, 13 (65%) were accompanied by unstable angina or acute myocardial infarction due to the stenosis and occlusion of coronary arteries; therefore, these data suggest that the morbidity and severity of CAD were significantly higher in patients with CD36-D than CD36-positive control subjects. Furthermore, many patients with both CD36-D and CAD suffered from other atherosclerotic cardiovascular diseases involving the stenosis and occlusion of arteries in the upper and lower limbs.

Since ^{123}I-BMIPP scintigraphy was performed in order to evaluate the myocardial damage of FFA metabolism in subjects with possible ischemic heart disease, the possibility could not be rejected that the 40 patients in the current study were extracted from a population with high morbidity of CAD. Watanabe *et al.*[22] also reported patients with type I and type II CD36-D, many of whom were accompanied by CAD or cardiomyopathy, although these patients were found by ^{123}I-BMIPP scintigraphy. Therefore, in the current study, we also examined the morbidity of CAD from a screening study. The morbidity of CAD was 50% in CD36-D patients identified by ^{123}I-BMIPP scintigraphy, while 37.5% (3 CAD of 8 CD36-D patients) in the population in the screening study. Although these data further imply that the morbidity of CAD in patients with CD36-D is definitely high, the possibility of patient selection bias cannot be excluded.

To explore further the contribution of CD36-D to the development of CAD in the general population, we compared by FACS analysis the frequency of CD36-D between patients with CAD diagnosed by coronary angiography (n=322) and non-CAD subjects (n=1,239). As shown in **Table 3**, the frequency of CD36-D was significantly three times higher in patients with CAD than in non-CAD subjects; therefore, the risk for the development of CAD is significantly higher in CD36-D patients, although the uptake of oxidized LDL *in vitro* is reduced in monocyte-derived macrophages.

Since foam cell formation by the uptake of oxidized LDL was shown to be reduced in monocyte-derived macrophages of CD36-D patients, it may be necessary to explore novel mechanisms for the en-

hanced atherogenicity in a CD36-deficient condition. We will discuss these mechanisms in more detail as follows (**Fig. 5**):

1) Increased Lipoprotein Remnants and Postprandial Hyperlipidemia

In the postprandial state of CD36-D patients, we demonstrated that not only hypertriglyceridemia but also increased levels of apoB-48, chylomicron remnants, and small dense LDL were observed[9]. In our previous papers, we demonstrated that CD36-null mice showed higher TG concentrations in plasma and intestinal lymph than wild-type mice even in a high fat loading state, suggesting that CD36-null mice may have intestinal overproduction of chylomicrons and may be a good mouse model of postprandial hyperlipidemia[23-24]. Furthermore, patients with CD36-D were also associated with insulin resistance, as we reported[10]. These profiles associated with impaired lipid and glucose metabolism proved to be independent coronary risk factors in the general CD36-positive population[11-13]. Moreover, these profiles were linked; the increase in chylomicron remnants in the postprandial state was shown to be associated with insulin resistance[25]; the production of small dense LDL was shown to be associated with the impaired postprandial clearance of TG-rich lipoproteins including remnants[26-27]; the accumulation of TG-rich lipoproteins caused an increase in FFA levels; high levels of FFA may suppress lipoprotein lipase (LPL) activity and the clearance of TG-rich lipoproteins, resulting in increased remnants[28]. Therefore, these lipoprotein phenotypes clustered in patients with CD36-D might have a synergistic influence and enhance the cardiovascular risk. Furthermore, as nicely reviewed by Fujioka *et al.*[29], increased remnant lipoproteins (mainly chylomicron remnants) contribute to form atherosclerotic lesions through a variety of mechanisms. It was demonstrated that chylomicron remnants invade directly into the subendothelial spaces of arteries and are taken up by macrophages via several receptors, such as LDL receptor-related protein (LRP) or apoB-48 receptor, resulting in macrophage foam cell formation[30-33]. We reported that increased serum chylomicron remnants are directly associated with enhanced carotid atherosclerosis in subjects with apparently normal TG levels[34]. Chylomicron remnants induce the secretion of monocyte chemoattractant protein 1 (MCP-1), which stimulates the migration of monocytes through arterial endothelial layers[35] and the production of plasminogen activator inhibitor-I (PAI-I), which regulates thrombus formation on endothelial cells[33]. Thus, abundant chylomicron remnants in the blood of CD36-D patients might enhance the foam cell formation of CD36-deficient macrophages, leading to the development of atherosclerotic cardiovascular diseases.

2) Reduced Serum HDL-C Levels

In CD36-D patients, we demonstrated a reduction of serum HDL-C[9], although there is a report showing an increase of serum HDL-C[36]. More recently, Love-Gregory *et al.*[37] reported a homozygote of SNP32 who was CD36-D accompanied by hypertriglyceridemia and reduction of serum HDL-C, although heterozygotes showed an opposite profile. The reduced serum HDL-C in our CD36-D patients could be one of the causes of enhanced atherogenicity.

3) Increased Free Fatty Acids Levels Caused by Deficiency of LCFA Transporter

CD36 is distributed in the heart, skeletal muscles and adipose tissues where it functions as one of the transporters of LCFA[38]. CD36 may be a major transporter of LCFA in the heart, since the uptake of ^{123}I-BMIPP, an analogue of LCFA, in cardiac scintigraphy is markedly deficient in CD36-D patients, which causes increased serum FFA and the 2-fold-enhanced influx of LCFA into the liver[39]. Increased FFA flux into the liver may cause overproduction of VLDL and hypertriglyceridemia as well as insulin resistance.

4) Insulin Resistance and Impaired Glucose Metabolism

CD36-D was shown to be accompanied by insulin resistance[10, 40-43]; however, this is controversial[36, 44]. CD36 knockout mice developed marked glucose intolerance, hyperinsulinemia and decreased muscle glucose uptake on a fructose-rich diet, but not on a high-starch, low-fat diet[42]. Goudriaan *et al.*[43] demonstrated that CD36-D increases insulin sensitivity in muscle, but induces insulin resistance in the liver. Insulin resistance may lead to the down-regulation of lipoprotein lipase and finally to hypertriglyceridemia.

5) Hypertension

The average systolic and diastolic blood pressure in our CD36-D patients was significantly high compared with CD36-positive subjects, similar to the reported case of MetS and vasospastic angina[45]. The mechanism for increased blood pressure is unknown; however, it may accelerate the development of atherosclerosis.

6) Increased PAI-I Levels

Low plasma fibrinolytic activity in association with increased PAI-I levels has been demonstrated to

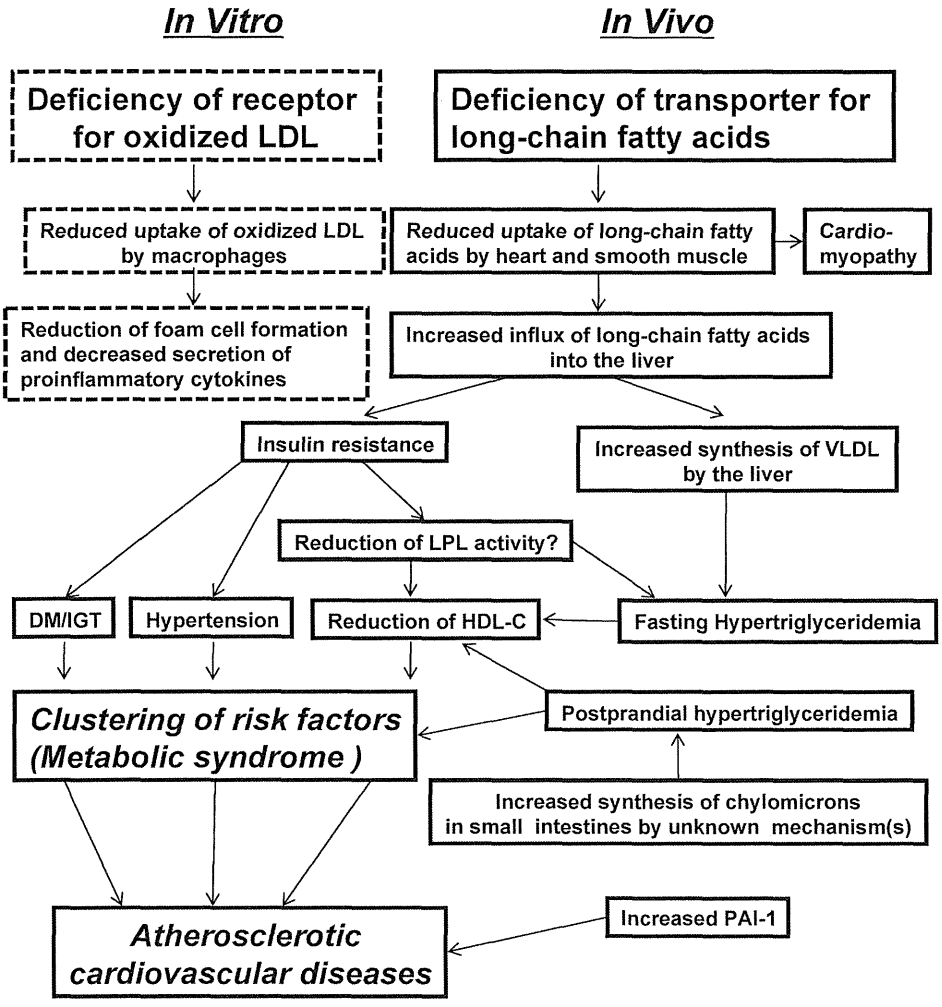

Fig. 5. Possible molecular mechanisms for enhanced atherosclerosis in human CD36-D

In vitro, CD36 deficiency causes the reduced uptake of oxidized LDL by macrophages, leading to decreased foam cell formation and secretion of proinflammatory cytokines; however, *in vivo*, CD36 deficiency results in reduced uptake of long-chain fatty acids (LCFA) by the heart and skeletal muscles, causing impaired metabolism of LCFA by the liver and circulation. These abnormalities as a whole may eventually lead to atherosclerotic cardiovascular diseases.

be linked with an increased risk of atherosclerotic cardiovascular diseases in obesity, insulin resistance and diabetes mellitus[46]. The increase of PAI-I was partly attributed to the accumulation of abdominal visceral fat[47]. Yanai *et al.*[48] reported elevated PAI-I levels in patients with CD36-D, although the mechanism was speculated to be linked to abnormal fatty acid metabolism.

Taken together, as illustrated in **Fig. 5**, despite the anti-atherosclerotic aspects of monocyte-derived macrophages from CD36-D patients due to the reduced uptake of oxidized LDL and decreased secretion of proinflammatory cytokines *in vitro*, the pro-atherogenic profiles *in vivo* may exceed the anti-atherosclerotic properties, thus enhancing the development of atherosclerosis. These pro-atherogenic profiles of CD36-D patients include: 1) increased lipoprotein remnants and postprandial hyperlipidemia, 2) reduced serum HDL-C levels, 3) increased FFA levels because of deficiency of LFCA transporter, 4) insulin resistance and impaired glucose metabolism, 5) hypertension, and 6) increased levels of PAI-I. These risk parameters may cluster and interact, finally leading to the marked enhancement of atherosclerosis; therefore, early screening and detection of CD36-D patients and assessment of atherosclerotic cardiovascular diseases are essential, especially in a population such as the Japanese in which their frequency is extremely high. Fur-

ther investigations into the molecular and vascular biological mechanisms of the progression of atherosclerosis in patients with CD36-D may be necessary in future studies.

Conclusions

Patients with CD36-D are associated with severe and enhanced atherosclerotic diseases. The morbidity of CAD is significantly higher in patients with CD36-D than in healthy subjects, and the frequency of CD36-D is significantly higher in patients with CAD than in healthy subjects. The clustering of atherogenic metabolic profiles such as dyslipidemia, including the accumulation of FFA and remnants, hypertension and insulin resistance, may enhance atherogenicity in patients with CD36-D.

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Conflicts of Interest

S. Yamashita has received consultancy fees from Otsuka Pharmaceutical Company and Skylight Biotech Co. The other co-authors have nothing to disclose.

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Special Report

Diagnosis and Management of Type I and Type V Hyperlipoproteinemia

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Both type I and type V hyperlipoproteinemia are characterized by severe hypertriglyceridemia due to an increase in chylomicrons. Type I hyperlipoproteinemia is caused by a decisive abnormality of the lipoprotein lipase (LPL)- apolipoprotein C-II system, whereas the cause of type V hyperlipoproteinemia is more complicated and more closely related to acquired environmental factors. Since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and V hyperlipoproteinemia are relatively rare, few guidelines for their diagnosis and treatment have been established; however, type I and V hyperlipoproteinemia are clinically important as underlying disorders of acute pancreatitis, and appropriate management is necessary to prevent or treat such complications. Against such a background, here we propose guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

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Key words; Chylomicronemia, Gene mutation, Hyperlipidemia, Lipase, Triglyceridemia

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Background

According to Fredrickson's classification of hyperlipoproteinemia (WHO classification), type I and V hyperlipoproteinemia (hyperlipidemia) are characterized by an increase in chylomicrons alone and an

increase in very low-density lipoprotein (VLDL) in addition to chylomicrons, respectively[1]. Type I hyperlipoproteinemia is a clinical condition showing the severest hypertriglyceridemia and is classically represented by two rare genetic disorders, i.e., familial lipoprotein lipase (LPL) deficiency (MIM 238600) and familial apolipoprotein C-II deficiency (MIM 207750)[2]. Even rarer conditions such as familial inhibitor of lipoprotein lipase (MIM 118830) and the presence of autoantibodies also cause type I hyperlipoproteinemia[3, 4]. More recently, patients with mutations in two additional genes have also been reported to manifest primary type I hyperlipoproteinemia, i.e., genes for glycosylphosphatidylinositol-anchored high density lipoprotein-binding protein 1 (GPIHBP1) (MIM 612757) and for lipase maturation factor 1 (LMF1) (MIM 611761)[5, 6]. Since LPL is an insulin-dependent enzyme, diabetic lipemia observed in insulin-deficient conditions such as type 1 diabetes is well-known as secondary type I hyperlipoproteinemia. Therefore, type I hyperlipoproteinemia is caused by a decisive abnormality of either LPL, which is a rate-limiting enzyme involved in the hydrolysis of triglyceride (TG)-rich lipoproteins such as chylomicrons and VLDL, or apolipoprotein C-II, a cofactor necessary for the expression of LPL activity.

The cause of type V hyperlipoproteinemia is more complicated, and more miscellaneous clinical conditions are considered to belong to this category. It rarely shows familial occurrence, but its inheritance pattern is variable; therefore, type V hyperlipoproteinemia is usually considered to be triggered by acquired environmental factors in individuals with some congenital susceptibility to altered TG metabolism (genetic factors). While the involved environmental factors vary, involvement of heavy drinking, type 2 diabetes, hormonal therapy using steroids and estrogen, and drugs such as diuretics and β-blockers are frequently observed[7].

Many guidelines concerning the diagnosis and treatment of hypercholesterolemia have been formulated[8], and outstanding results of clinical intervention using lipid-lowering drugs, particularly statins, have been reported by large-scale clinical studies. On the other hand, since the relationship of hypertriglyceridemia with atherosclerosis is not as clear as that of hypercholesterolemia, and since type I and type V hyperlipoproteinemia, in particular, are relatively rare, few guidelines for their diagnosis and treatment have been established either in Japan or abroad; however, diagnostic criteria for primary hyperchylomicronemia were issued in the 1988 report by the Study Group on Primary Hyperlipidemia of the Ministry of Health and Welfare (Group leader: Seiichiro Tarui)[9]. Type I and V hyperlipoproteinemia are important as underlying disorders of acute pancreatitis, which is often lethal, and appropriate management, including restriction of fat intake, is necessary to prevent or treat such complications. Against such a background, the Study Group on Primary Hyperlipidemia of the Ministry of Health, Labour and Welfare (Group leader: Nobuhiro Yamada) proposes guidelines primarily concerning the diagnosis and management of type I and V hyperlipoproteinemia in Japanese.

Characteristics of Hyperchylomicronemia

The half-life of chylomicrons is about 5 minutes, and no chylomicron is observed in the plasma of normotriglyceridemic to moderately hypertriglyceridemic individuals after 12-hour fasting. Chylomicrons are considered to appear in fasting plasma in those with a serum TG level of about 1,000-2,000 mg/dl or above, and physical symptoms usually occur above this level ($\geq$ 2,000 mg/dl); therefore, there is a strict viewpoint defining hyperchylomicronemia as a serum TG level of 2,000 mg/dl or above accompanied by characteristic complaints or findings. However, caution is necessary, because there are patients showing no clinical symptom even at a serum TG level of 20,000-30,000 mg/dl, even though they are rare. From a clinical standpoint, it must be explained to the patient that there is risk of pancreatitis when the TG level is 1,000 mg/dl or higher even on casual sampling. This may also apply to neonates whose blood sampling after a long period of fasting is usually difficult. It must also be remembered in clinical laboratory testing that a marked increase in the serum TG level often affects the measurement system, causing apparently low serum amylase, hemoglobin, and electrolyte levels (e.g., sodium appears to be reduced by about 2-4 mEq/l with every 1,000 mg/dl increase in the TG). In particular, acute pancreatitis secondary to hypertriglyceridemia must not be misdiagnosed due to apparently low serum amylase.

Type I Hyperlipoproteinemia

A) Familial Lipoprotein Lipase (LPL) Deficiency

a) Concept and Definition

LPL is an enzyme that hydrolyzes TG of lipoprotein particles in blood, and its abnormal activity underlies type I hyperlipoproteinemia in many cases and type V hyperlipoproteinemia in some. Familial LPL deficiency is a rare monogenic disorder that exhibits the severest hyperchylomicronemia. It was first docu-

mented in 1932 in a boy born to a family with a history of consanguineous marriage[10], and the underlying abnormality was demonstrated to be a congenital defect of LPL activity, the rate-limiting enzyme of chylomicron hydrolysis, by Havel *et al.* in 1960[11]. Following the classification of familial hypercholesterolemia, it has been proposed to classify this disease as a class I defect causing complete loss of LPL protein, a class II defect characterized by the production of catalytically inactive protein, and a class III defect characterized by the production of inactive protein lacking affinity to heparan sulfate[12].

b) Etiology

The disease is caused by an abnormality of the human LPL gene, and the patients are homozygotes (including so-called compound heterozygotes) who have inherited LPL gene abnormalities from both parents in an autosomal recessive pattern with penetrance of 100%. The human LPL gene is located on the short arm of chromosome 8 (8p22), is about 35 kb in length, contains 10 exons, and codes for an enzyme protein consisting of 448 amino acids[13-15].

c) Clinical Symptoms

This disease is a relatively rare autosomal recessive disorder, and more than 30 families with this condition have been reported in Japan. The frequency of the occurrence of homozygous patients is estimated to be 1 in every 500,000 to 1 million people. Many patients have a family history of consanguineous marriage, and since patients exhibit chylous serum due to hyperchylomicronemia from early childhood and abdominal pain due to pancreatitis after the intake of fat, the disease is frequently diagnosed during the suckling period or early childhood. In females, the detection of hyperchylomicronemia during pregnancy may lead to the diagnosis. Attacks of abdominal pain due to acute pancreatitis following hyperchylomicronemia are often mistaken for acute abdomen, and the patient may undergo unnecessary laparotomy. While some patients acquire a dietary habit to avoid the intake of fat and suffer growth impairment, some show no marked attack of abdominal pain until adulthood, with consequent overlooking of the disease. It is the primary disease to be differentially diagnosed in a patient with persistent abdominal pain accompanied by hypertriglyceridemia[2].

Hyperchylomicronemia itself is also a major clinical finding, and the serum TG level reaches about 1,500 to even 20,000 mg/dl or more. The presence of chylomicrons can be confirmed by a simple method, i.e., the appearance of a top white cream layer in serum after standing at 4℃ for 24 hours or mild centrifugation. In typical cases, the lower layer is clear and transparent, reflecting an increase in chylomicrons alone. The possibility of LPL deficiency is high if the serum TG level is 1,500 mg/dl or higher, and the serum total cholesterol level is about 1/10 the serum TG level or lower. All other clinical findings are due to the marked increase in chylomicrons. First, eruptive xanthomas, which appear when the serum TG level increases to 2,000 mg/dl or above, are noted in about half of the patients, particularly on the extensor sides of the limbs, buttocks, and shoulders. They appear in association with changes in the serum TG level and disappear gradually over several weeks to a few months. When the serum TG level increases above 4,000 mg/dl, lipemia retinalis, in which the retinal vessels appear whitish pink due to chylous serum on funduscopy, appears, but vision is not impaired. Among other findings, hepatosplenomegaly due to the infiltration of macrophage foam cells that have phagocytozed lipids in the extravascular space, is observed, with hepatomegaly being frequent, but these changes are reversible and are rapidly improved (within 1 week) with correction of the serum lipid levels; however, the most serious complication is acute pancreatitis, and it must be managed carefully as it may be a prognostic determinant. From a clinical viewpoint, the possibility of acute pancreatitis must be explained to the patient if the TG level is 1,000 mg/dl or higher even on casual sampling. Dyspnea and neurological symptoms such as dementia, depression, and memory disorders have been reported as complications of this disorder.

As mentioned above, a major prognostic determinant of homozygous familial LPL deficiency is acute pancreatitis, which is often lethal. LPL deficiency has long been considered not to be closely related to atherosclerosis in humans, because no marked atherosclerotic lesion was noted at the autopsy of several homozygous patients with LPL deficiency who died due to acute pancreatitis. However, detailed research has reported that heterozygotes, which are considered to occur in 1 in every 500 individuals, usually show no marked abnormality in the lipid level but are likely to exhibit hypertriglyceridemia when they develop diabetes or are exposed to burdens such as severe obesity, excessive drinking, and pregnancy[16, 17]. There have also been reports of the frequent occurrence in heterozygotes of familial combined hyperlipidemia (FCHL)[12] and monogenic familial hypertriglyceridemia[16], which are common hyperlipidemia related to atherosclerosis; however, it remains controversial whether homozygotes with LPL gene abnormality are likely to develop atherosclerosis. A Canadian group

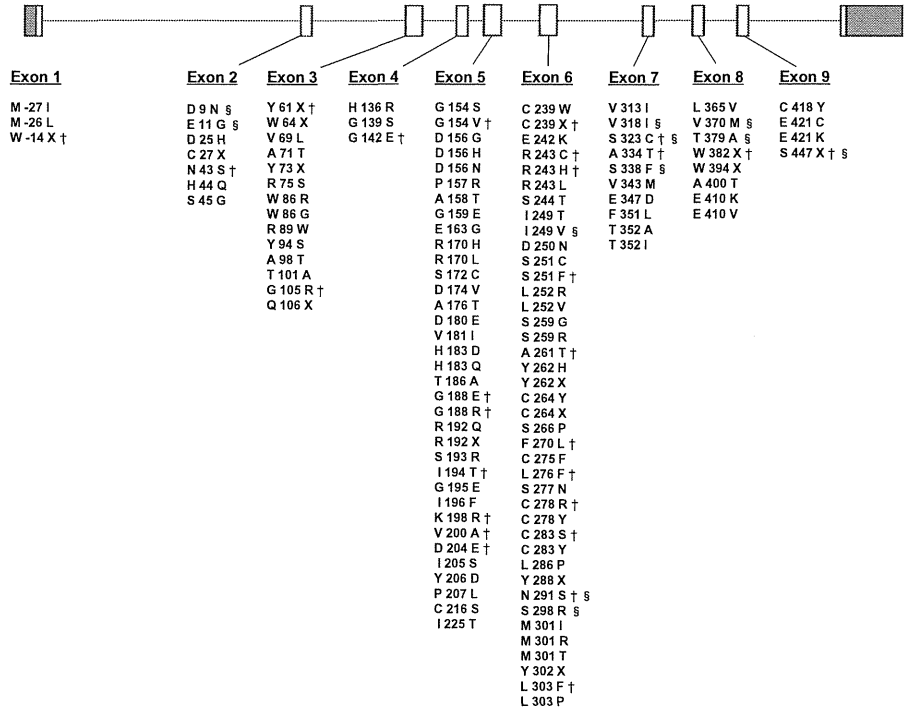

Fig. 1. Missense and nonsense mutations in the human lipoprotein lipase (LPL) gene

Each number indicates the position of affected amino acids, with +1 corresponding to the first amino acid of the mature human LPL protein.
†Mutations identified in Japanese patients with familial LPL deficiency.
§Mutations or polymorphisms not necessarily underlie LPL deficiency.

that followed-up 4 patients with LPL deficiency over 14-30 years reported that coronary angiography established atherosclerotic lesions in all patients before the age of 55 years[18], but studies on homozygotes in Japan[19, 20] both reported no advanced atherosclerotic lesion in those Japanese patients.

d) Diagnosis

Since LPL is anchored by binding with heparan sulfate on the surface of capillary endothelial cells, it appears markedly in the circulation by intravenous injection of heparin; therefore, the diagnosis is usually made by measuring plasma LPL activity and/or protein level 10 minutes after intravenous injection of heparin (10-50 U/kg). LPL protein is also present in plasma before heparin injection, but is markedly reduced or undetectable in patients with LPL null mutation (class I defect). LPL accounts for about 1/3 of the total lipase activity in plasma after heparin injection, and most of the remaining lipase activity is due to hepatic triglyceride lipase (HTGL), so diagnosis of this disorder is impossible by simple measurement of the total lipase activity. Anti-LPL and anti-HTGL antibodies are necessary for the differential measurement of LPL activities, but there is also a method to inactivate LPL using protamine sulfate or 1 M NaCl. Although this technique requires a stable synthetic substrate as well as skill and experience, measurement kits for research use are presently being marketed. Also, if either macrophages derived from peripheral blood monocytes or adipose tissue can be used as samples, differentiation from HTGL becomes unnecessary. If changes in the LPL protein level are involved, the immunological protein assay is effective and there have been a few reports on the use of ELISA in Japan[21-23], which has been adopted as a general clinical laboratory test[21]. If the LPL activity is markedly reduced, and if the concentration of apolipoprotein C-II, a critical cofactor of LPL, is normal or elevated, the diagnosis of this condition would be considered definite. Naturally, close inquiry into the familial history is often very helpful. While very rare cases with an LPL inhibitor or autoantibody are known, they can be eventually excluded by examining whether the patient's serum inhibits LPL activity in the serum of a normal control.

A diagnosis based on the LPL gene level is also widely practiced. To date, at least 163 gene mutations[2, 24, 25], including 35 in Japan alone[26], have been