Original Article

Effects of Statin on Small Dense Low-Density Lipoprotein Cholesterol and Remnant-Like Particle Cholesterol in Heterozygous Familial Hypercholesterolemia

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Aim: The effects of statin on small dense low-density lipoprotein cholesterol (sd-LDL-C) and remnant-like particle cholesterol (RLP-C) levels in heterozygous familial hypercholesterolemia (FH) have not been examined. This study aimed to clarify the effects of statin on sd-LDL-C and RLP-C levels in heterozygous FH.

Methods: Seventeen patients with heterozygous FH were randomly assigned to 2 mg/day pitavastatin or 10 mg/day atorvastatin. At baseline and 12 weeks after treatment with statin, we measured sd-LDL-C and RLP-C levels.

Results: Sd-LDL-C levels significantly decreased from 43 ± 24 to 16 ± 10 mg/dL (-63% , $p=0.001$) in the pitavastatin group, and from 44 ± 17 to 19 ± 10 mg/dL (-55% , $p<0.001$) in the atorvastatin group. RLP-C levels decreased from 8.4 ± 2.8 to 6.6 ± 2.7 mg/dL (-16% , $p=0.156$) in the pitavastatin group, and from 9.8 ± 4.7 to 5.9 ± 5.4 mg/dL (-45% , $p=0.044$) in the atorvastatin group. There were no significant differences in percent changes of sd-LDL-C ($p=0.370$) and RLP-C levels ($p=0.097$) between the two groups.

Conclusions: Sd-LDL-C measured by the heparin-magnesium precipitation method and RLP-C levels in heterozygous FH were decreased by 12 weeks of statin therapy. Statin might have additional anti-atherogenic effects by reducing not only LDL-C but also sd-LDL-C and RLP-C.

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Key words; Familial hypercholesterolemia, Small dense low-density lipoprotein cholesterol, Remnant-like particle cholesterol, Statin

Introduction

Familial hypercholesterolemia (FH) is a common autosomal dominant disorder caused by a mutation of the gene for the low-density lipoprotein (LDL) receptor¹⁾. FH is frequently associated with premature coronary artery disease (CAD), and the rate of death from

CAD is several times higher than the general population^{1, 2)}; however, there is a wide variation of coronary risk among FH patients³⁾.

Increasing experimental and clinical evidence suggests that disturbed plasma triglyceride (TG) metabolism plays an important role in the development of premature atherosclerosis^{4, 5)}. Disturbances in TG metabolism are characterized by postprandial accumulation of lipoprotein remnants. A new remnant lipoprotein method based on the immunoseparation principle [remnant-like particle (RLP) cholesterol (RLP-C) assay] offers the possibility of separating lipoprotein remnant particles with the use of an immunoaffinity gel with coupled monoclonal antibodies against apoB-100 and apoA-I^{6, 7)}. Increased levels of RLP-C have

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been associated with the development of CAD⁸⁻¹⁰. FH patients with disturbances in postprandial lipoprotein metabolism also have higher risks for CAD¹¹.

Small dense (sd) LDL has been demonstrated to be a new risk factor for the development of CAD in Westerners as well as in Japanese¹²⁻¹⁴. LDL particle size is usually measured by gradient gel electrophoresis using non-denaturing polyacrylamide¹⁵. Recently, Hirano *et al.* established a simple and rapid method for the measurement of sd-LDL-C concentrations using heparin-magnesium precipitation^{16, 17}. This method is useful for evaluating qualitative and quantitative abnormalities in LDL, and may be applicable to a routine clinical examination¹⁸.

Pitavastatin (Kowa Pharmaceutical Co. Ltd., Tokyo, Japan) is new 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitor (statin) developed in Japan. Total cholesterol (TC) and LDL-C levels in heterozygous FH decreased by 31% and 40% with 2 mg/day pitavastatin, respectively¹⁹. It remains unclear, however, whether statin could reduce sd-LDL-C by mechanisms similar to LDL-C. Sd-LDL-C and RLP-C levels and the response to statin therapy could be relevant for understanding the heterogeneity in coronary risk among FH patients. The goal of this study was to assess the effects of statin therapy on sd-LDL-C and RLP-C levels in heterozygous FH. Furthermore, the comparative effects of two statins (pitavastatin and atorvastatin), both of which appeared to decrease TG levels¹⁹⁻²¹, were evaluated.

Materials and Methods

Subjects

Seventeen patients with heterozygous FH (male/female=3/14, mean age=61±11 years) were studied. FH was diagnosed according to the following two criteria: (1) primary hypercholesterolemic patients (TC level above 230 mg/dL in any age group) with tendon xanthomas, or (2) primary hypercholesterolemic patients with and without tendon xanthomas in a first-degree relative of familial hypercholesterolemic patients²². After a washout period of 8 weeks, patients were randomly assigned to 2 mg/day pitavastatin or 10 mg/day atorvastatin. At baseline and 12 weeks after treatment with statin, we measured sd-LDL-C and RLP-C levels. Written informed consent was obtained from all patients.

Laboratory Determination

Blood samples were obtained after an overnight fast. Serum TC and TG levels were measured by standard enzymatic methods. Serum LDL-C and high-den-

sity lipoprotein cholesterol (HDL-C) levels were measured by direct homogeneous assay using detergents (LDL-EX and HDL-EX, Denka Seiken Co. Ltd., Tokyo). Sd-LDL-C level was measured using a commercially available test kit (sd-LDL-C "Seiken", Denka Seiken Co. Ltd.). The details and validation of this method have been described elsewhere^{16, 17}. Serum RLP-C level was measured using an immune-separation technique (Otsuka Pharmaceuticals Co., Ltd., Tokyo)⁷. Serum lipoprotein (a) [Lp(a)] levels were assayed by a commercially available enzyme-linked immunosorbent assay (Daiichi Pure Chemicals Co., Ltd., Tokyo)²³. Large buoyant (lb)-LDL-C was calculated by subtracting sd-LDL-C from LDL-C.

Statistical Analysis

Statistical analyses were performed using Statview 5.0 software (SAS Institute, Cary, NC, USA). All values are expressed as the mean ± SD. Differences between the means were compared with the unpaired *t*-test. The significance of any differences in proportions was tested with Chi-square analysis. One-way analysis of variance (ANOVA) was used to compare mean changes of lipid parameters, and multiple comparisons were made by Bonferroni's test. Significant difference was defined as $p < 0.05$.

Results

The baseline characteristics of the patients are shown in **Table 1**. Three patients were male, and 14 patients were female. The mean age was 61 ± 11 years. Mean TC, LDL-C, sd-LDL-C, and RLP-C levels at baseline were 303 ± 45 mg/dL, 218 ± 47 mg/dL, 44 ± 20 mg/dL, and 9.2 ± 3.9 mg/dL, respectively. There were no significant differences in age, gender, body mass index, TC, LDL-C, HDL-C, TG, sd-LDL-C, and RLP-C levels between the two groups.

Mean LDL-C and apo B levels 12 weeks after treatment with pitavastatin decreased significantly from 201 ± 27 to 111 ± 11 mg/dL (−45%, $p < 0.0001$) and from 160 ± 9 to 101 ± 11 mg/dL (−37%, $p < 0.0001$). Mean LDL-C and apo B levels 12 weeks after treatment with atorvastatin decreased significantly from 234 ± 57 to 142 ± 52 mg/dL (−40%, $p = 0.001$) and from 170 ± 38 to 114 ± 32 mg/dL (−34%, $p < 0.001$). Serum high-sensitivity C-reactive protein (hs-CRP) levels tended to be decreased by statin therapy, but were not statistically significant (**Table 2**).

Sd-LDL-C levels 12 weeks after treatment with statin significantly decreased from 43 ± 24 to 16 ± 10 mg/dL (−63%, $p = 0.001$) in the pitavastatin group and from 44 ± 17 to 19 ± 10 mg/dL (−55%, $p < 0.001$)

Table 1. Baseline characteristics

	All patients (<i>n</i> = 17)	Pitavastatin (<i>n</i> = 8)	Atorvastatin (<i>n</i> = 9)	<i>p</i> value
Age (years)	61 ± 11	62 ± 6	60 ± 15	0.758
Gender (male/female)	3/14	1/7	2/7	0.600
Height (cm)	157.3 ± 9.4	159.0 ± 7.6	155.8 ± 11.1	0.500
Body weight (kg)	56.5 ± 9.7	58.0 ± 7.0	55.2 ± 11.9	0.566
BMI (kg/m ²)	22.7 ± 2.5	22.9 ± 1.7	22.6 ± 3.2	0.790
TC (mg/dL)	303 ± 45	286 ± 32	318 ± 51	0.146
LDL-C (mg/dL)	218 ± 47	201 ± 27	234 ± 57	0.167
HDL-C (mg/dL)	60 ± 13	58 ± 15	62 ± 11	0.514
TG (mg/dL)	152 ± 60	165 ± 64	141 ± 58	0.443
RLP-C (mg/dL)	9.2 ± 3.9	8.4 ± 2.8	9.8 ± 4.7	0.477
Lp(a) (mg/dL)	33 ± 27	27 ± 23	37 ± 31	0.462
sd-LDL-C (mg/dL)	44 ± 20	43 ± 24	44 ± 17	0.859
lb-LDL-C (mg/dL)	175 ± 39	159 ± 28	189 ± 43	0.113
Smoking (%)	4 (24%)	2 (25%)	2 (22%)	0.893
History of CAD (%)	4 (24%)	2 (25%)	2 (22%)	0.893
History of CVA (%)	1 (6%)	0 (0%)	1 (11%)	0.331
DM (%)	2 (12%)	0 (0%)	2 (22%)	0.156
Hypertension (%)	4 (24%)	2 (25%)	2 (22%)	0.893

All values are expressed as the mean ± SD or number (%).

BMI, body mass index; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; RLP-C, remnant-like particle cholesterol; Lp(a), lipoprotein (a); sd-LDL-C, small dense low-density lipoprotein cholesterol; lb-LDL-C, large buoyant low-density lipoprotein cholesterol; CAD, coronary artery disease; CVA, cerebrovascular accident; DM, diabetes mellitus.

in the atorvastatin group (**Table 2**). **Fig. 1** shows serial changes in sd-LDL-C levels during statin therapy. There was variability in the baseline sd-LDL-C levels. The highest and lowest levels of sd-LDL-C were 95 mg/dL and 24 mg/dL. Sd-LDL-C levels decreased significantly 4 weeks after treatment with statin and remained depressed to 12 weeks in both groups.

Fig. 2 shows serial changes in RLP-C levels during statin therapy. RLP-C levels decreased significantly 4 weeks after treatment with statin and tended to be depressed to 12 weeks in both groups.

Percent changes in LDL-C, apo B, sd-LDL-C, lb-LDL-C, and RLP-C levels after 12 weeks of statin therapy were -42%, -35%, -59%, -38%, and -31%, respectively (**Fig. 3**). There were no significant differences in the reduction of sd-LDL-C ($p=0.370$) and RLP-C levels between the two groups ($p=0.097$). Percent changes of sd-LDL-C levels were higher than percent changes of lb-LDL-C levels in both groups. In simple linear regression analysis, mean changes of sd-LDL-C were positively correlated with mean changes of TG ($r=0.828$, $p=0.011$) in the pitavastatin group, but not in the atorvastatin group ($r=0.146$, $p=0.708$) (**Table 3**). Mean changes of RLP-C levels were positively correlated with mean changes of TG in both

groups ($r=0.823$, $p=0.012$ in the pitavastatin group and $r=0.751$, $p=0.020$ in the atorvastatin group) (**Table 4**).

Discussion

This study is the first report to examine the effects of statin on sd-LDL-C and RLP-C levels in heterozygous FH. These results suggested that sd-LDL-C and RLP-C levels in heterozygous FH were decreased by statin therapy.

Sd-LDL-C levels have been positively correlated with both TG and LDL-C levels and inversely correlated with HDL-C levels¹⁸; however, the most powerful determinant of LDL size may be TG levels²⁴. It has been demonstrated that cholesteryl ester transfer protein (CETP) is also an important determinant of LDL particle size^{25, 26}. In addition, lipoprotein lipase (LPL) activity and hepatic lipase activity have been shown to contribute to the formation of sd-LDL particles and could well represent significant determinants of LDL-peak particle diameter (LDL-PPD) in FH²⁷. In the previous report, LDL-PPD in FH was significantly smaller than in controls. FH subjects had 11% higher CETP mass and there was an inverse rela-

Table 2. Effects of statins on lipid and apolipoprotein levels

		Baseline	4 weeks	8 weeks	12 weeks	<i>p</i> value
TC (mg/dL)	Pitava	286 ± 32	201 ± 23 ****	206 ± 22 ****	196 ± 16 ****	< 0.0001
	Atorva	318 ± 51	236 ± 42 ***	229 ± 43 ***	230 ± 41 ***	< 0.001
LDL-C (mg/dL)	Pitava	201 ± 27	118 ± 18 ****	120 ± 16 ****	111 ± 11 ****	< 0.0001
	Atorva	234 ± 57	151 ± 53 **	143 ± 52 **	142 ± 52 **	0.002
HDL-C (mg/dL)	Pitava	58 ± 15	59 ± 13	62 ± 16	54 ± 11	0.720
	Atorva	62 ± 11	63 ± 12	63 ± 10	61 ± 10	0.983
TG (mg/dL)	Pitava	165 ± 64	135 ± 48	144 ± 56	171 ± 56	0.556
	Atorva	141 ± 58	112 ± 47	109 ± 55	117 ± 92	0.715
RLP-C (mg/dL)	Pitava	8.4 ± 2.8	5.1 ± 1.6 *	5.4 ± 2.9 *	6.6 ± 2.7	0.060
	Atorva	9.8 ± 4.7	5.3 ± 1.8 *	5.3 ± 2.8 *	5.9 ± 5.4 *	0.060
Lp(a) (mg/dL)	Pitava	27 ± 23	27 ± 23	26 ± 23	25 ± 20	0.996
	Atorva	37 ± 31	37 ± 30	37 ± 30	35 ± 28	0.998
sd-LDL-C (mg/dL)	Pitava	43 ± 24	17 ± 9 **	18 ± 9 **	16 ± 10 **	0.003
	Atorva	44 ± 17	22 ± 13 ****	17 ± 10 ****	19 ± 10 ****	< 0.001
lb-LDL-C (mg/dL)	Pitava	159 ± 28	101 ± 14 ****	102 ± 16 ****	95 ± 12 ****	< 0.0001
	Atorva	189 ± 43	129 ± 43 **	126 ± 44 **	123 ± 47 **	0.009
Apo AI (mg/dL)	Pitava	128 ± 21	140 ± 20	141 ± 24	142 ± 22	0.568
	Atorva	135 ± 26	139 ± 23	142 ± 23	150 ± 27	0.599
Apo B (mg/dL)	Pitava	160 ± 9	102 ± 13 ****	105 ± 7 ****	101 ± 11 ****	< 0.0001
	Atorva	170 ± 38	119 ± 31 **	112 ± 30 ***	114 ± 32 ***	0.002
Apo E (mg/dL)	Pitava	5.6 ± 1.1	4.3 ± 1.0 *	4.5 ± 0.9 *	4.5 ± 0.9 *	0.056
	Atorva	5.4 ± 1.2	4.1 ± 0.6 **	3.9 ± 0.7 ***	4.0 ± 0.6 ***	< 0.001
hs-CRP (mg/L)	Pitava	0.19 ± 0.15	0.15 ± 0.18	0.08 ± 0.05	0.14 ± 0.14	0.533
	Atorva	0.06 ± 0.07	0.05 ± 0.06	0.08 ± 0.17	0.04 ± 0.04	0.847

All values are expressed as the mean ± SD.

Pitava, pitavastatin; Atorva, atorvastatin; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; RLP-C, remnant-like particle cholesterol; Lp(a), lipoprotein (a); sd-LDL-C, small dense low-density lipoprotein cholesterol; lb-LDL-C, large buoyant low-density lipoprotein cholesterol; Apo, apolipoprotein; hs-CRP, high-sensitivity C-reactive protein.

One-way analysis of variance (ANOVA) was performed for each variable.

Multiple comparisons were made by Bonferroni's test.

p* < 0.05, *p* < 0.01, ****p* < 0.001, *****p* < 0.0001 vs. baseline.

Fig. 1. Serial changes in small dense low-density lipoprotein cholesterol levels during statin therapy. (A): Pitavastatin group, (B): Atorvastatin group.

Fig. 2. Serial changes in remnant-like particle cholesterol levels during statin therapy.

(A): Pitavastatin group, (B): Atorvastatin group.

Fig. 3. Percent changes in LDL-C, apo B, sd-LDL-C, lb-LDL-C, and RLP-C levels after 12 weeks of statin therapy.

■: All patients, ■: Pitavastatin group, □: Atorvastatin group.

LDL-C, low-density lipoprotein cholesterol; Apo, apolipoprotein; sd-LDL-C, small dense low-density lipoprotein cholesterol; lb-LDL-C, large buoyant low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol.

tionship between LDL-PPD and CETP mass. TG levels and CETP mass concentrations, as well as gender were independent predictors of LDL-PPD and represented nearly 27% of its variance in FH²⁸). These findings indicate that a large proportion of the variability in LDL-PPD remained unexplained by these parameters. Hogue *et al.* reported that the genotype of the LDL receptor was associated with LDL particle size heterogeneity in FH. They showed that the LDL

receptor status accounted for 5.7% of the variance in the LDL-PPD²⁹). In the present study, the highest and lowest levels of sd-LDL-C at baseline were 95 mg/dL and 24 mg/dL, revealing the variability of sd-LDL-C levels in FH. We previously reported that sd-LDL-C determined by this method was a useful marker of metabolic syndrome (MetS) in patients with CAD³⁰). In this report, mean levels of sd-LDL-C with MetS were 26.4 ± 2.6 mg/dL. Tokuno *et al.* reported that

Table 3. Simple linear regression analysis between mean change of small dense low-density lipoprotein cholesterol levels and various parameters

Variables	Pitavastatin		Atorvastatin	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
ΔTC (mg/dL)	0.125	0.768	0.333	0.381
ΔLDL-C (mg/dL)	0.446	0.268	0.263	0.494
ΔHDL-C (mg/dL)	0.037	0.931	0.065	0.868
ΔTG (mg/dL)	0.828	0.011	0.146	0.708
ΔApo AI (mg/dL)	0.115	0.787	0.127	0.746
ΔApo B (mg/dL)	0.365	0.374	0.165	0.671
ΔApo E (mg/dL)	0.382	0.351	0.425	0.255
ΔIb-LDL-C (mg/dL)	0.300	0.471	0.627	0.071
ΔRLP-C (mg/dL)	0.804	0.016	0.123	0.752

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo, apolipoprotein; Ib-LDL-C, large buoyant low-density lipoprotein cholesterol; RLP-C, remnant-like particle cholesterol.

sd-LDL-C levels determined by this method were 37 ± 20 mg/dL in hypercholesterolemic subjects with type 2 diabetes and 45 ± 21 mg/dL in hypertriglyceridemic subjects with type 2 diabetes³¹. Compared with these data, mean levels of sd-LDL-C (44 ± 20 mg/dL) in heterozygous FH were equal to or more than in subjects with MetS or type 2 diabetes.

As several statins have been known to decrease sd-LDL levels³² or increase LDL particle size³³⁻³⁵, our data also indicate the effect of statin on lowering sd-LDL-C levels in heterozygous FH. Percent changes of sd-LDL-C levels were 63% in the pitavastatin group and 55% in the atorvastatin group. Thus, the effect of statin on lowering sd-LDL-C levels was similar in both 2 mg/day of pitavastatin and 10 mg/day of atorvastatin.

In the present study, RLP-C levels in FH were decreased by statin therapy. Furthermore, mean changes of RLP-C levels were positively correlated with mean changes of TG in both groups. This observation was reported previously^{36, 37}; however, not all statins could reduce RLP-C levels. Stein *et al.* reported that RLP-C levels were reduced by simvastatin 20 mg and by atorvastatin 10 mg but not by pravastatin 40 mg in patients with combined dyslipidemia³⁸. A recent study indicated that the LDL receptor may regulate the rate of entrance of very low-density lipoprotein (VLDL) into the circulation³⁹, indicating that reduced RLP-C levels might be mainly due to decreasing hepatic VLDL synthesis in both statins.

On the other hand, mean changes of sd-LDL-C levels were positively correlated with mean changes of TG in the pitavastatin group, but not in the atorva-

Table 4. Simple linear regression analysis between mean change of remnant-like particle cholesterol levels and various parameters

Variables	Pitavastatin		Atorvastatin	
	<i>r</i>	<i>p</i> value	<i>r</i>	<i>p</i> value
ΔTC (mg/dL)	0.035	0.934	0.430	0.248
ΔLDL-C (mg/dL)	0.294	0.480	0.410	0.273
ΔHDL-C (mg/dL)	0.151	0.721	0.101	0.797
ΔTG (mg/dL)	0.823	0.012	0.751	0.020
ΔApo AI (mg/dL)	0.030	0.945	0.161	0.679
ΔApo B (mg/dL)	0.293	0.481	0.460	0.213
ΔApo E (mg/dL)	0.431	0.287	0.419	0.262
ΔIb-LDL-C (mg/dL)	0.310	0.455	0.228	0.556
Δsd-LDL-C (mg/dL)	0.804	0.016	0.123	0.752

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; TG, triglyceride; Apo, apolipoprotein; Ib-LDL-C, large buoyant low-density lipoprotein cholesterol; sd-LDL-C, small dense low-density lipoprotein cholesterol.

statin group. It is possible that the mechanisms of decreased sd-LDL-C levels might be different in both statins. Furthermore, percent changes of sd-LDL-C levels were higher than percent changes of Ib-LDL-C levels in both statins. Sd-LDL particles have weaker affinity to LDL receptors than Ib-LDL particles⁴⁰; however, a recent study indicated that the induction of LDL receptors by statin stimulated the uptake of all LDL particles, irrespective of their size³¹. According to this report, a low dose of pitavastatin (1 mg) decreased sd-LDL-C levels by 26% and Ib-LDL-C levels by 22%. We speculate that the effect of statin on lowering sd-LDL-C was not only by upregulating LDL receptors but also by decreasing hepatic VLDL synthesis and increasing LPL activities. In previous studies, statin reduced CETP levels⁴¹ and increased LPL activity²¹. These factors may partially contribute to the reduction of sd-LDL-C levels. Lariviere *et al.* reported that 41.6% of the variation in the LDL-PPD response to atorvastatin was attributable to the initial LDL-PPD, apo E polymorphism, nature of the LDL receptor gene mutation, and change in TG levels³³. Thus, genetic and multiple factors associated with lipoprotein metabolism may be important determinants of statin-induced changes of LDL heterogeneity.

Sd-LDL display enhanced susceptibility to copper transition metal-induced oxidation when compared with normal-sized LDL particles^{42, 43}, and it is recognized that oxidized LDLs are highly atherogenic⁴⁴. Thus, sd-LDL particles have been associated with the development of CAD¹²⁻¹⁴. Furthermore, increased levels of RLP-C have been associated with the develop-

ment of CAD⁸⁻¹⁰). Sd-LDL-C and RLP-C might play an important role in the acceleration of atherosclerosis in heterozygous FH. Thus, pitavastatin and atorvastatin have anti-atherogenic effects by reducing not only LDL-C levels but also sd-LDL-C and RLP-C levels.

In conclusion, sd-LDL-C measured by the heparin-magnesium precipitation method and RLP-C levels in heterozygous FH were decreased by 12 weeks of statin therapy. Statin might have additional anti-atherogenic effects by reducing not only LDL-C but also sd-LDL-C and RLP-C.

Study Limitations

There are some limitations regarding the interpretation of these results and drawing conclusions. This study included only 17 patients with heterozygous FH. The sample size allowed for only limited analysis of sd-LDL-C and RLP-C levels and response to statin therapy. Furthermore, we did not perform genetic analysis of the LDL receptor, nor were CETP concentrations and LPL activities measured.

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