Original Article

Effects of Statin Therapy on Plasma Proprotein Convertase Subtilisin/kexin Type 9 and Sortilin Levels in Statin-Naive Patients with Coronary Artery Disease

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Aim: Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of serum low-density lipoprotein (LDL) cholesterol levels, and sortilin is linked to lipoprotein metabolism. Although statin therapy increases PCSK9 levels, effects of this therapy on plasma sortilin levels have not been evaluated. The purpose of the present study was to examine the effects of statins on plasma PCSK9 and sortilin levels, and association of statin-induced increase in PCSK9 levels with sortilin.

Methods: Serum lipid levels and plasma PCSK9 and sortilin levels were measured at baseline and 8 months after statin therapy in 90 statin-naive patients with coronary artery disease (CAD). Pitavastatin 4 mg/day was used to treat 44 patients and pravastatin 20 mg/day to treat the remaining 46 patients.

Results: For both statin groups, significant increases in hetero-dimer PCSK9 levels (pitavastatin: 31%, $p < 0.0001$; pravastatin: 34%, $p = 0.03$) and decreases in sortilin levels (pitavastatin: -8%, $p = 0.02$; pravastatin: -16%, $p = 0.002$) were observed. Although a reduction in LDL cholesterol was greater in the pitavastatin group than in the pravastatin group, no significant differences were observed in percentage changes in hetero-dimer PCSK9 and sortilin levels. A significant positive correlation was observed between percentage changes in hetero-dimer PCSK9 levels and those in sortilin levels (pitavastatin: $r = 0.359$, $p = 0.02$; pravastatin: $r = 0.276$, $p = 0.06$).

Conclusions: Use of pitavastatin and pravastatin increased plasma PCSK9 and decreased sortilin levels. Statin-induced increases in PCSK9 were associated with changes in sortilin in statin-naive patients with CAD.

Introduction

Elevated low-density lipoprotein (LDL) cholesterol levels are key factors associated with the occur-

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rence of atherosclerosis and coronary artery disease (CAD), which are the leading causes of morbidity and mortality worldwide. Proprotein convertase subtilisin/kexin type 9 (PCSK9) is a key regulator of serum LDL cholesterol levels[1-3] and is secreted by the liver into the circulation and binds the hepatic LDL receptors, causing their subsequent degradation[4-7].

Recent genome-wide association studies have identified that a genetic variant at a locus on chromosome 1p13.3 is strongly associated with LDL cholesterol levels and the risk of myocardial infarction[8-10]. This locus contains the following four genes: *SORT1*,

Table 1. Baseline characteristics of patients

	All patients ($n=90$)	Pitavastatin ($n=44$)	Pravastatin ($n=46$)	p value
Age (years)	67 ± 10	67 ± 9	67 ± 11	0.92
Men (%)	73 (81%)	38 (86%)	35 (76%)	0.33
Body mass index (kg/m^2)	24.2 ± 3.2	23.9 ± 3.2	24.4 ± 3.3	0.52
Status of coronary artery disease				0.89
Stable angina pectoris (%)	63 (70%)	30 (68%)	33 (72%)	
Unstable angina pectoris (%)	27 (30%)	14 (32%)	13 (28%)	
Hypertension (%)	59 (66%)	27 (61%)	32 (70%)	0.55
Diabetes mellitus (%)	39 (43%)	16 (36%)	23 (50%)	0.27
Smoker (%)	20 (22%)	11 (25%)	9 (20%)	0.66
ACE inhibitors or ARBs (%)	49 (54%)	22 (50%)	27 (59%)	0.54
Calcium channel blockers (%)	47 (52%)	15 (34%)	32 (70%)	0.002
β blockers (%)	9 (10%)	5 (11%)	4 (9%)	0.74

Data are expressed as mean ± SD or as number (percentage).
ACE, angiotensin-converting enzyme; ARB, angiotensin-receptor blocker.

CELSR2, *PSRC1*, and *MYBPHL*, and a subsequent study has shown that *SORT1* affects lipid metabolism[11]. *SORT1* encodes sortilin, a type 1 transmembrane protein[12, 13], which acts as a multiligand receptor and is linked to systemic lipoprotein metabolism and PCSK9 secretion[14-17].

Lowering LDL cholesterol levels is currently considered the most effective strategy for preventing CAD. The use of statins results in the elevation of LDL receptor expression that increases the uptake of LDL particles from the circulation[18]. However, the use of statins causes an increase in the expression of PCSK9[19], counteracting the beneficial effects of statins. Thus, PCSK9 is an attractive drug target for hypercholesterolemia, and results from clinical trials are promising[20, 21]. Although statin therapy increases PCSK9 levels, the effects of statins on plasma sortilin levels have not been evaluated. Therefore, in this observational, longitudinal study, we examined the effects of statin therapy on plasma PCSK9 and sortilin levels and the association of statin-induced increase in PCSK9 with sortilin in statin-naive patients with CAD. Furthermore, we evaluated the effects of two types of statins, intensive lipid-lowering therapy with pitavastatin and moderate lipid-lowering therapy with pravastatin, on plasma PCSK9 and sortilin levels.

Methods

Patients and Study Design

Data were obtained from the Treatment With Statin on Atheroma Regression Evaluated by Intravascular Ultrasound With Virtual Histology (TRUTH) study, which was a prospective, open-labeled, randomized, multicenter trial performed at 11 Japanese centers to compare the effects of 8-month treatment with pitavastatin versus pravastatin on coronary atherosclerosis using virtual histology intravascular ultrasound[22]. In brief, 164 patients with angina pectoris who were not receiving lipid-lowering therapy were randomly treated with either pitavastatin (4 mg/day, intensive lipid-lowering group) or pravastatin (20 mg/day, moderate lipid-lowering group).

The patients included in the TRUTH study were considered for the present study if they fulfilled the following criteria: the allocated statins were continued during the study period (8 months) and an adequate plasma volume was available in frozen samples for the required measurements. In total, 90 patients met the inclusion criteria. Pitavastatin was used to treat 44 patients and pravastatin to treat the remaining 46 patients.

The TRUTH study was conducted in accordance with the Declaration of Helsinki and with the approval of the ethical committees of the 11 participating institutions. Each patient enrolled in the present study provided written informed consent.

Laboratory Analysis

Serum lipid levels were measured before treatment (at baseline) and at the 8-month follow-up[22]. Serum total cholesterol, LDL cholesterol, high-density lipoprotein cholesterol, and triglycerides levels were measured using standard enzymatic methods (AU2700; Beckman Coulter, CA, USA) and commercial enzymatic kits (Kyowa Medex, Tokyo, Japan). Plasma

Table 2. Serum lipid levels and plasma PCSK9 and sortilin levels at baseline and at the 8-month follow-up

	All patients (*n*=90)			Pitavastatin (*n*=44)			Pravastatin (*n*=46)		
	Baseline	Follow-up	*p* value	Baseline	Follow-up	*p* value	Baseline	Follow-up	*p* value
Total cholesterol (mg/dL)	203 ± 34	157 ± 27	<0.0001	196 ± 31	143 ± 24	<0.0001	210 ± 36	172 ± 22	<0.0001
% change		− 22 ± 13			− 26 ± 13*			− 17 ± 12	
LDL cholesterol (mg/dL)	130 ± 31	84 ± 25	<0.0001	123 ± 24	71 ± 20	<0.0001	137 ± 36	96 ± 23	<0.0001
% change		− 35 ± 17			− 42 ± 15**			− 28 ± 16	
Triglycerides (mg/dL)	114 (36 to 573)	98 (34 to 396)	0.07	116 (36 to 573)	91 (34 to 292)	0.02	112 (53 to 316)	108 (40 to 396)	0.75
% change		− 17 (−75 to 168)			− 20 (−76 to 121)			− 12 (−75 to 168)	
HDL cholesterol (mg/dL)	47 ± 12	51 ± 13	0.001	48 ± 12	51 ± 14	0.03	47 ± 11	51 ± 13	0.02
% change		11 ± 24			9 ± 21			12 ± 28	
PCSK9									
Total (ng/mL)	125 ± 40	148 ± 42	<0.0001	126 ± 40	154 ± 43	0.0001	124 ± 41	142 ± 41	0.03
% change		31 ± 66			29 ± 40			33 ± 83	
Hetero-dimer (ng/mL)	113 ± 36	135 ± 41	<0.0001	113 ± 35	141 ± 41	<0.0001	112 ± 37	130 ± 40	0.03
% change		33 ± 69			31 ± 40			34 ± 89	
Furin-cleaved (ng/mL)	12 (4 to 44)	12 (6 to 31)	0.58	12 (4 to 44)	12 (6 to 31)	0.7	11 (5 to 23)	12 (6 to 30)	0.3
% change		0 (−63 to 233)			− 6 (−57 to 225)			8 (−63 to 233)	
Sortilin (ng/mL)	8.7 ± 2.3	7.4 ± 2.7	<0.0001	8.2 ± 1.9	7.3 ± 2.0	0.02	9.2 ± 2.6	7.5 ± 3.3	0.002
% change		− 12 ± 27			− 8 ± 28			− 16 ± 26	

Data are expressed as mean ± SD or median (range).
LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.
*p=0.0003; **p=0.0001 compared with pravastatin group.

PCSK9 and sortilin levels at baseline and at the 8-month follow-up were measured at a central laboratory (BML, Kawagoe, Japan) using sandwich enzyme-linked immunosorbent assays (ELISA)[23]. For soluble sortilin, sortilin-specific antibodies were used for detection. In brief, mAb A9E [0.3 µg/mL in phosphate-buffered saline (PBS)] was coated onto a microplate (Nunc Maxisorp, Thermo Scientific) by incubation at 4°C overnight. The wells were then blocked with PBS containing 1% bovine serum albumin for 2 h at room temperature. After the plate had been washed with PBS containing 0.1% Tween20 (PBST), the calibrator (recombinant soluble sortilin) and plasma/serum samples (1:100) diluted with PBST containing 0.3% BSA was added and incubated for 2 h at room temperature. After washing the plate, horseradish peroxidase-labeled mAb E12A (0.03 µg/mL in PBST containing 0.3% BSA) was allowed to react for 1 h at room temperature. After washing, 3, 3′, 5, 5′-tetramethylbenzidine substrate solution (Dako) was added and incubated for 0.5 h. The reaction was then stopped by adding 0.5M sulfuric acid. The absorbance was measured at 450 nm using a microplate reader.

Statistical Analysis

Statistical analysis was performed using StatView, version 5.0 (SAS Institute, Cary, North Carolina). The results are expressed as mean ± SD or median (range).

Differences in continuous variables between the two groups were compared using the unpaired *t*-test when the variables had a normal distribution and the Mann-Whitney *U*-test when they were not normally distributed. Differences in continuous variables within each group were compared using the paired *t*-test when the variables had a normal distribution and the Wilcoxon signed rank-sum test when they were not normally distributed. Categorical variables between the two groups were compared using the chi-square test or the Fisher's exact test. Univariate regression analyses were performed to assess the relation between percentage changes in sortilin and those in various lipid parameters. Statistical significance was set at *p*<0.05.

Results

The baseline characteristics of the subjects are listed in **Table 1**. No differences in the baseline characteristics were found between the two groups, except for the frequency of calcium channel blocker use.

Serum lipid levels and plasma PCSK9 and sortilin levels at baseline and at the follow-up are shown in **Table 2**. Serum LDL cholesterol levels decreased significantly in both statin groups, with a significantly greater reduction in the pitavastatin group (− 42% vs. − 28%, *p*=0.0001). Furthermore, high-density lipoprotein cholesterol levels increased significantly in
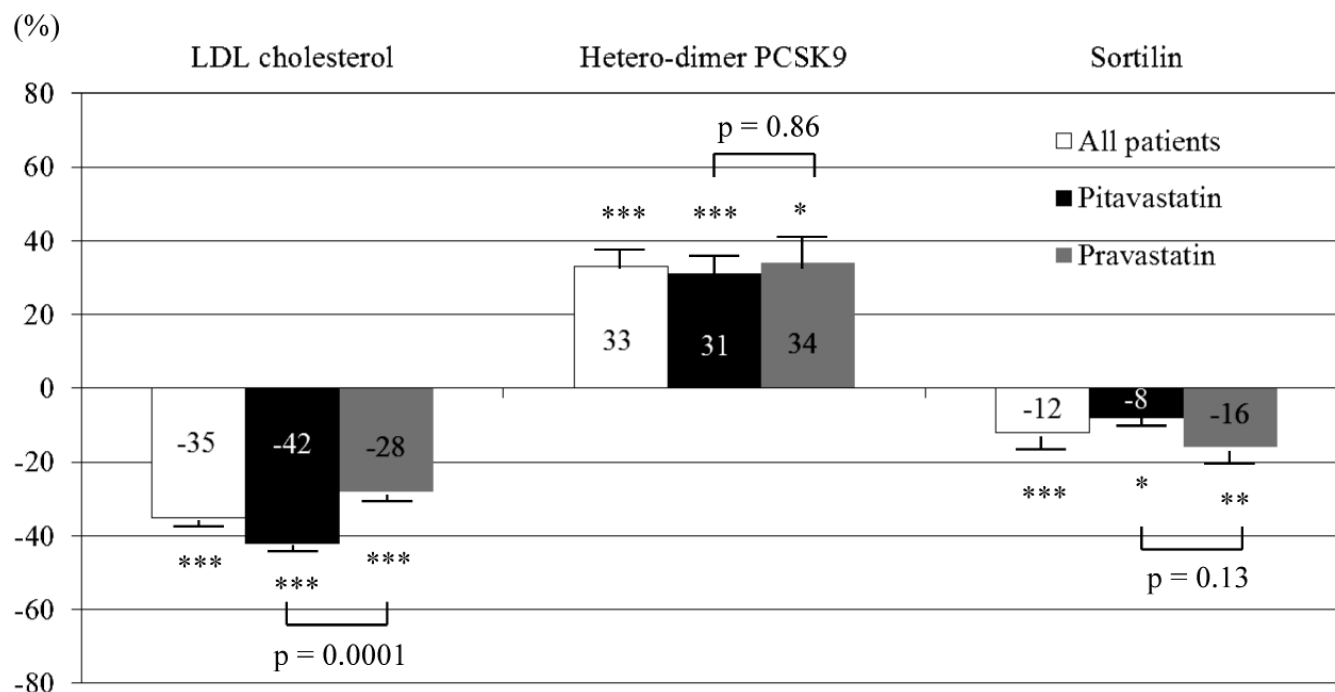
Fig. 1. Percentage changes in LDL cholesterol, hetero-dimer PCSK9, and sortilin levels at the 8-month follow-up.

Although the reduction in LDL cholesterol levels was greater in the pitavastatin group than in the pravastatin group, no significant differences in percentage changes in hetero-dimer PCSK9 and sortilin were observed. Data are expressed as mean ± SEM. *$p < 0.05$, **$p < 0.01$, ***$p < 0.0001$ compared with baseline.

both statin groups. Significant increases in total and hetero-dimer PCSK9 levels were observed in both statin groups (pitavastatin: 29%, $p = 0.0001$, and 31%, $p < 0.0001$; pravastatin: 33%, $p = 0.03$, and 34%, $p = 0.03$, respectively). Plasma furin-cleaved PCSK9 levels were not significantly changed from baseline in either group. Significant decreases in sortilin levels from baseline were observed in both statin groups (pitavastatin: −8%, $p = 0.02$; pravastatin: −16%, $p = 0.002$). Although a reduction in LDL cholesterol level was greater in the pitavastatin group than in the pravastatin group, percentage changes in hetero-dimer PCSK9 and sortilin levels were not different (**Fig. 1**).

Correlations between baseline sortilin levels and lipid parameters are shown in **Table 3**. Baseline sortilin levels did not correlate with baseline PCSK9 or LDL cholesterol levels or percentage changes in these levels.

We assessed correlations between percentage changes in sortilin and lipid parameters (**Table 4**) and found weak but significant positive correlations between percentage changes in hetero-dimer PCSK9 and those in sortilin ($r = 0.272$, $p = 0.009$) (**Fig. 2**). These correlations were observed in both statin groups (pitavastatin: $r = 0.359$, $p = 0.02$; pravastatin: $r = 0.276$,

$p = 0.06$) (**Table 4**).

Discussion

The major findings of the present study are as follows. First, plasma hetero-dimer PCSK9 levels increased significantly 8 months after statin therapy in both the pitavastatin and pravastatin groups. Second, plasma sortilin levels were significantly reduced in both statin groups. Although the reduction of LDL cholesterol was greater in the pitavastatin group than in the pravastatin group, the differences in the increase in hetero-dimer PCSK9 levels and the decrease in sortilin levels were not significant between these two statin groups. Finally, percentage changes of hetero-dimer PCSK9 levels were positively correlated with those of sortilin levels.

PCSK9, a serine protease produced by the liver, is a newly identified protein that plays a key role in cholesterol homeostasis[24]. PCSK9 degrades hepatic LDL receptors and subsequently increases LDL cholesterol levels[4-7]. In addition, plasma PCSK9 is also involved in the inflammatory process[24, 25]. Thus, PCSK9 may have a broader physiological role in the vascular system. The use of statins is associated with

Table 3. Correlations between baseline sortilin levels and lipid parameters

	All patients		Pitavastatin		Pravastatin	
	r	*p* value	r	*p* value	r	*p* value
Baseline level						
Total cholesterol	0.118	0.27	0.229	0.13	− 0.004	0.98
LDL cholesterol	0.104	0.33	0.133	0.39	0.026	0.87
Triglycerides	− 0.032	0.76	0.220	0.15	− 0.267	0.07
HDL cholesterol	− 0.023	0.83	− 0.106	0.5	0.048	0.75
Hetero-dimer PCSK9	0.085	0.43	0.084	0.59	0.094	0.54
Percentage change						
Total cholesterol	0.008	0.94	− 0.035	0.82	− 0.113	0.45
LDL cholesterol	0.039	0.71	0.124	0.42	− 0.166	0.27
Triglycerides	0.119	0.26	− 0.047	0.76	0.159	0.29
HDL cholesterol	0.052	0.63	− 0.026	0.87	0.074	0.63
Hetero-dimer PCSK9	0.020	0.85	− 0.286	0.06	0.110	0.47

LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

Table 4. Correlations between percentage changes in sortilin and lipid parameters

	All patients		Pitavastatin		Pravastatin	
	r	*p* value	r	*p* value	r	*p* value
Total cholesterol (% change)	0.100	0.35	0.166	0.28	0.182	0.22
LDL cholesterol (% change)	− 0.105	0.32	− 0.008	0.96	− 0.085	0.57
Triglycerides (% change)	0.141	0.19	0.020	0.9	0.308	0.04
HDL cholesterol (% change)	0.108	0.31	0.061	0.69	0.169	0.26
Hetero-dimer PCSK9 (% change)	0.272	0.009	0.359	0.02	0.276	0.06

LDL, low-density lipoprotein; HDL, high-density lipoprotein; PCSK9, proprotein convertase subtilisin/kexin type 9.

an increase in the expression of PCSK9[19], counteracting the beneficial effects of statins. The mechanism underlying PCSK9 degradation of LDL receptors is extremely complex. Recently, PCSK9 was found to bind to LDL receptors, subsequently targeting them for intracellular destruction within the hepatocyte[26-28]. The effect of 4 mg of pitavastatin on the reduction of LDL cholesterol levels was significantly greater than that of 20 mg of pravastatin. However, no significant difference in percentage changes in PCSK9 levels was observed between these two statin groups. The amount of plasma PCSK9 may not reflect the total amount of statin-induced increases in hepatic PCSK9 secretion because with high levels of PCSK9, greater levels are bound to hepatic LDL receptors, removing them from circulation. In addition, increases in circulating PCSK9 levels caused by statin therapy differ over the short- and long-term[29]. This explains why a significant difference was not observed in the increase of PCSK9 levels from baseline between the two statin groups.

The main functions of sortilin are to transport ligands between the trans-Golgi network and the early endosomes and to bind and internalize various ligands across the cell membrane by receptor-mediated endocytosis[13, 16]. Although the exact mechanism underlying the effects of sortilin on lipid metabolism has not been fully examined, sortilin binds to LDL on the cell surface[30] and increases the amount of LDL internalized[18, 31]. In addition, sortilin has been shown to bind apolipoprotein A-V and lipoprotein lipase[32, 33]. The effect of sortilin on very low-density lipoprotein (VLDL) synthesis, as determined by overexpression and knockdown studies, is conflicting[11, 34, 35]. Musunuru *et al.*[11] reported that sortilin reduced VLDL synthesis and thereby reduced LDL cholesterol levels, whereas Kjolby *et al.*[34] reported that sortilin increased VLDL synthesis and thereby increased LDL cholesterol levels. Strong *et al.*[35] reported that increased hepatic sortilin expression reduced hepatic apolipoprotein B secretion and increased LDL catabolism,

$y = -15.711 + 0.108x$
$r = 0.272$
$p = 0.009$
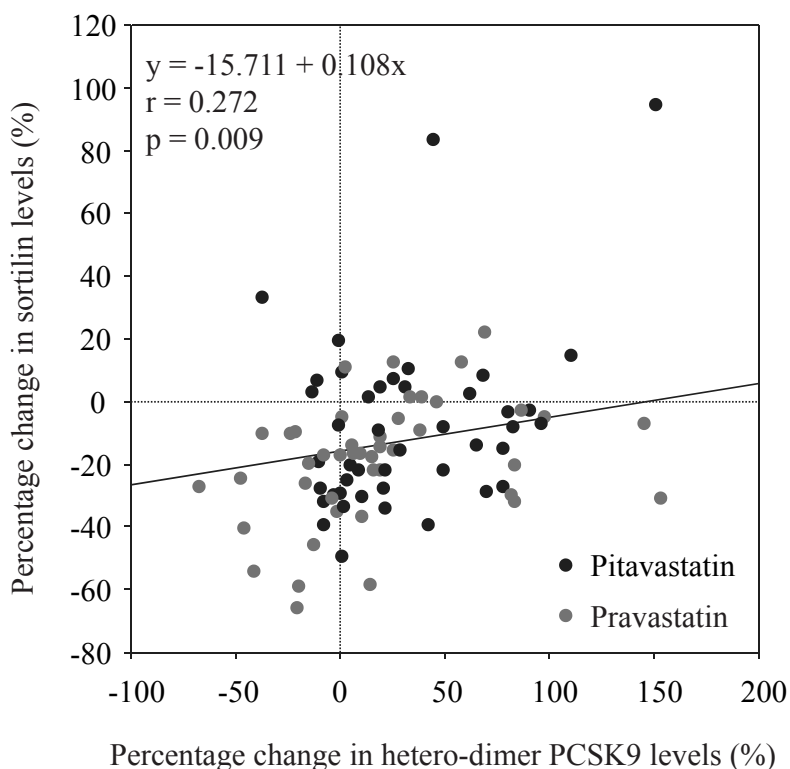
Fig. 2. Correlations between percentage changes in hetero-dimer PCSK9 and those in sortilin.

Weak but significant positive correlations were observed between percentage changes in hetero-dimer PCSK9 and those in sortilin.

providing dual mechanisms underlying the reduction of plasma LDL cholesterol levels. Thus, the effect of sortilin on LDL cholesterol levels is controversial. In the present study, baseline sortilin levels did not correlate with baseline PCSK9 or LDL cholesterol levels or percentage changes in these levels, indicating that sortilin could not predict the LDL cholesterol-lowering effects of statins.

It is still unclear how the hepatic expression and plasma sortilin levels are regulated. Sortilin is constitutively released from the cell surface following shedding by metalloproteinases[36] and can therefore be detected in human plasma. Once released from the cell surface, sortilin does not influence PCSK9 activity[37]. Recently, two proteins were suggested to enhance PCSK9-mediated degradation of the LDL receptor: one is sortilin, which binds to PCSK9 in the trans-Golgi network and possibly facilitates its secretion[37], and the other is amyloid precursor-like protein 2 (APLP2), which facilitates trafficking of the PCSK9-LDL receptor complex to endosomes/lysosomes[38]. However, Butkinaree *et al.*[39] reported that PCSK9 enhanced LDL receptor degradation independent of sortilin or APLP2 *ex vivo*

and in mice. Interestingly, when co-expressed with PCSK9, both sortilin and APLP2 were targeted for lysosomal degradation, and sortilin enhanced the stability of APLP2. Considering these findings, the effect of sortilin on the functions of APLP2 needs to be studied in specific tissues, especially the brain, small intestine, and colon, where the expression of both transcripts is quite high. We could not explain the precise mechanism of why plasma sortilin levels decreased and how circulating sortilin levels were regulated because this is a clinical study. However, consistent with the previous report that circulating PCSK9 and sortilin are positively correlated[37], we found significant positive correlations between percentage changes in PCSK9 and those in sortilin.

Although the homozygote for the major allele at chromosome 1p13.3 locus is associated with >90% reduced expression of sortilin in the human liver and with increased levels of LDL cholesterol[11], the heterozygote with missense mutations in the *SORT1* gene had no apparent effect on serum cholesterol levels[30], suggesting a marginal effect of the *SORT1* gene on LDL cholesterol levels. Further studies on the func-

tion of sortilin in LDL metabolism will better elucidate the role of genetic variants at the *SORT1* gene in the regulation of lipoprotein metabolism and modulation of CAD risk.

The present study has several limitations. First, it was a post hoc analysis of the TRUTH trial and all subjects had CAD. Second, plasma PCSK9 and sortilin levels were measured using frozen samples at only two timepoints. Third, we did not evaluate the amount of LDL receptor, sortilin, or PCSK9 expression in hepatocytes. Finally, the small number of patients included in the study made the statistical power insufficient.

Despite these limitations, to the best of our knowledge, this is the first study that evaluated the effects of statins, particularly two different types of statins, on plasma PCSK9 and sortilin levels at the same time. Thus, both pitavastatin and pravastatin increased plasma PCSK9 levels and decreased sortilin levels. Moreover, changes in PCSK9 levels were positively correlated with those in sortilin levels, but LDL cholesterol levels were not. A prospective, randomized study with a greater number of patients would be required to confirm our conclusions.

Conclusions

Both pitavastatin and pravastatin increased plasma PCSK9 levels and decreased sortilin levels. Statin-induced increases in PCSK9 levels were associated with changes in sortilin levels in statin-naive patients with CAD.

Disclosures

None.

Conflicts of Interest

None.

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