Impact of LR11 as Residual Risk on Long-Term Clinical Outcomes in Patients with Coronary Artery Disease Treated with Statins after First Percutaneous Coronary Intervention

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Summary

Cardiovascular events still occur despite statin-based lipid-lowering therapy in patients with coronary artery disease (CAD). LR11, a member of the low-density lipoprotein receptor family, is a novel marker for the proliferation of intimal smooth muscle cells, which are critical to atherosclerotic plaque formation. We evaluated the impact of LR11 on long-term clinical outcomes in CAD patients treated with statins after percutaneous coronary intervention (PCI).

This study included 223 consecutive CAD patients (age, 64.5 ± 9.6 years; male, 81.2%) treated with statin after first PCI between March 2003 and December 2004 at our institution. Patients were stratified to two groups according to LR11 levels (median). Composite cardiovascular disease (CVD) endpoints that included cardiovascular death, non-fatal acute coronary syndrome and non-fatal stroke were compared between groups.

The rate of CVD endpoints was significantly higher in the high LR11 group (log-rank, $P = 0.0029$) during the median follow-up period of 2844 days. Multivariate Cox regression analysis showed that a higher LR11 level was significantly associated with adverse clinical outcomes (adjusted hazard ratio for composite CVD endpoints, 2.47; 95% confidence interval, 1.29-4.92; $P = 0.006$).

Elevated levels of LR11 were significantly associated with long-term clinical outcomes among CAD patients treated with statins after first PCI.

Key words: Ischemic heart disease, Long-term outcomes, Biomarker

Coronary artery disease (CAD) is the leading cause of mortality and morbidity worldwide. Statins significantly reduce the frequency of cardiovascular events in patients with CAD and represent the most widely prescribed pharmacotherapy for individuals with CAD as secondary prevention. However, residual cardiovascular risk persists despite lipid-lowering therapy with statins. Factors associated with this residual cardiovascular risk remain to be elucidated.

LR11 (also called SorLA or SORL1) belongs to the low-density lipoprotein (LDL) receptor family and is highly expressed in atheromatous plaques from animal experimental models, particularly in the intimal smooth muscle cells (SMCs) at the border between the arterial intima and media. Overproduction of LR11 promotes the enhanced migration of SMCs via the activation of urokinase-type plasminogen activator receptor (uPAR). The action of LR11 via the upregulation of uPAR enhances the scavenger receptor expression that contributes to foam cell formation during atherogenesis.

We developed an enzyme-linked immunosorbent assay (ELISA) system to detect circulating levels of sLR11 using specific antibodies against LR11. Accumulated clinical findings have recently shown that circulating levels of the soluble form of LR11 (sLR11) levels are associated with atherosclerotic processes, carotid intima-media thickening (IMT), and the atherosclerotic plaque burden of CAD, are increased in patients with acute coronary syndrome (ACS) as compared to those with stable angina. We also reported that circulating sLR11 are associated with restenosis following coronary angioplasty and long-term outcomes in patients with CAD. However, the association between LR11 and residual cardiovascular risk in the setting of secondary prevention using statins after PCI remains uncertain. We therefore evaluated the impact of LR11 on long-term clinical outcomes in CAD patients treated with statins after first PCI.
Methods

Study subjects: The present study was a single-center observational study of consecutive 223 CAD patients treated with statins after a first PCI at our hospital between March 2003 and December 2004. Demographic data, coronary risk factors and medication were collected from the institutional database. Patients were stratified into two groups according to a median LR11 of 10.84 mg/dL (Figure 1). Written informed consent was obtained from all patients. The present study was carried out in accordance with the Declaration of Helsinki and with approval from the institutional review board.

Blood samples: Blood samples were obtained in a fasted state before performance of coronary angiography in the operating room and were centrifuged at 1000 × g for 10 minutes. Serum samples were stored at −80°C. Levels of sLR11 were measured using sandwich ELISA with the specific monoclonal antibodies directed against human LR11, as we previously established. Other biochemical parameters were determined by standard laboratory methods at our institution.

Outcome measurements: The primary endpoint was adverse cardiovascular events defined as a composite of cardiovascular death, non-fatal ACS, and non-fatal stroke. Clinical follow-up included reviews of medical charts, telephone contact and questionnaires sent to patients or their families. Mortality data were collected from the medical records of patients who died or were treated at the present institution, and the details and cause of death were requested from other hospitals to which patients had been admitted. We defined cardiovascular death as death from CAD, cardiogenic shock, stroke or sudden death. ACS was defined as acute myocardial infarction (AMI) and unstable angina pectoris (UAP) requiring urgent coronary revascularization. AMI was defined as ischemic symptoms, a ≥2-fold increase in creatine kinase, or a positive result for troponin T. UAP was diagnosed in the presence of ischemic symptoms regardless of ST-T change. Stroke was diagnosed based on the presence of a neurologic deficit with rapid onset that persisted for ≥24 hours.

Statistical analysis: Continuous variables are presented as the mean ± standard deviation and were compared using the unpaired t test or the Mann-Whitney U-Test. Categorical variables were compared using the chi-square test. Event-free survival rates for the primary endpoint were compared using Kaplan-Meier curves and log-rank tests overall and in groups with and without statins. Variables showing P < 0.10 in univariate analysis (diabetes mellitus, diastolic blood pressure, high-density lipoprotein cholesterol, multivessel disease, left ventricular ejection fraction) were included in the multivariate analysis, in addition to age and sex. Hazard ratios (HRs) and 95% confidence intervals (CIs) were calculated. All data were analyzed using JMP for Windows version 12 (SAS Institute, Cary, NC, USA).

Results

We analyzed data from 223 CAD patients treated with statins after a first PCI at our institution during the study period. The median follow-up period was 2844 days (interquartile range, 2422-3021 days). Table I shows the baseline clinical characteristics and laboratory findings. Patients with high sLR11 levels showed significantly higher proportions of old age, female sex, DM and renal impairment. The rate of the cumulative incidence of CVD endpoints was significantly higher in the high LR11 group (27.0% versus 12.5%, log-rank P = 0.0029; Figure 2). Kaplan-Meier curves of each CVD endpoint are shown in Figure 3(A: CVD death; B: non-fatal ACS; C: non-fatal stroke). The incidence of each endpoint was not significant but was slightly higher in the high LR11 group (10.8% versus 4.5%, log-rank P = 0.0554; 11.6% versus 5.4%, log-rank P = 0.585; 9.0% versus 4.5%, log-rank P = 0.12, respectively). Table II shows Cox proportional hazard analyses for composite CVD endpoints. Table III shows the Cox proportional hazard model for each CVD endpoint. Multivariate logistic regression analysis showed that sLR11 was independently associated with CVD events after adjusting for confounding factors.
Figure 2. Kaplan-Meier curve of composite cardiovascular endpoints

ACE-I indicates angiotensin converting enzyme inhibitors; ACS, acute coronary syndrome; ARBs, angiotensin receptor blockers; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL-C, high-density lipoprotein cholesterol; LAD prox, proximal lesion of left anterior descending coronary artery; LDL-C, low-density lipoprotein cholesterol; LMT, left main trunk; NSTEMI, non ST-segment elevation myocardial infarction; SBP, systolic blood pressure; STEMI, ST-segment elevation myocardial infarction; TG, triglycerides; and UAP, unstable angina.
Figure 3. Kaplan-Meier curves of each cardiovascular endpoint. A: Cardiovascular death; B: Non-fatal acute coronary syndrome; and C: Non-fatal stroke.

Table II. Univariate and Multivariate Cox Hazard Model Predicting Composite CVD Endpoints

|                      | Univariate |              | Multivariate |              |
|----------------------|------------|--------------|--------------|--------------|
|                      | HR         | 95% CI       | P            | HR           | 95% CI       | P            |
| LR11 high/low        | 2.53       | 1.38-4.93    | 0.003        | 2.21         | 1.13-4.49    | 0.020        |
| Age                  | 1.02       | 0.99-1.05    | 0.27         | 1.004        | 0.97-1.04    | 0.78         |
| Male sex             | 0.95       | 0.47-2.20    | 0.90         | 1.05         | 0.39-2.07    | 0.90         |
| Diabetes mellitus    | 1.99       | 1.10-3.73    | 0.023        | 1.60         | 0.86-3.06    | 0.14         |
| Hypertension         | 1.14       | 0.62-2.18    | 0.69         |              |              |              |
| Current smoking      | 0.92       | 0.44-1.76    | 0.81         |              |              |              |
| Renal impairment     | 1.46       | 0.78-2.65    | 0.23         |              |              |              |
| BMI                  | 0.93       | 0.83-1.04    | 0.21         |              |              |              |
| SBP                  | 0.97       | 0.98-1.01    | 0.67         |              |              |              |
| DBP                  | 0.98       | 0.95-1.00    | 0.09         | 0.98         | 0.95-1.01    | 0.14         |
| LDL                  | 1.00       | 0.99-1.01    | 0.89         |              |              |              |
| HDL                  | 0.97       | 0.95-1.004   | 0.029        | 0.98         | 0.95-1.001   | 0.06         |
| Triglycerides        | 0.998      | 0.99-1.002   | 0.31         |              |              |              |
| ACE-I+ARB            | 0.98       | 0.54-1.78    | 0.96         |              |              |              |
| β-Blocker            | 1.35       | 0.74-2.47    | 0.33         |              |              |              |
| Ca-channel blocker   | 1.13       | 0.60-2.052   | 0.33         |              |              |              |
| Multivessel disease  | 1.78       | 0.98-3.32    | 0.059        | 1.63         | 0.88-3.10    | 0.12         |
| Type B2/C lesion     | 1.75       | 0.63-7.23    | 0.31         |              |              |              |
| LVEF                 | 0.97       | 0.95-0.996   | 0.023        | 0.97         | 0.94-0.99    | 0.014        |
| LAD proximal or LMT lesion | 1.12   | 0.48-2.28    | 0.78         |              |              |              |
| AMI at presentation  | 0.94       | 0.32-2.17    | 0.89         |              |              |              |

HR indicates hazard ratio; 95% CI, 95% confidence interval; AMI, acute myocardial infarction; ACE inhibitors, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; high-density lipoprotein cholesterol; LAD, left anterior descending coronary artery; LDL-C, low-density lipoprotein cholesterol; LMT, left main trunk; LVEF, left ventricular ejection fraction; and SBP, systolic blood pressure.
Clinical events in patients with CAD, especially for residual risk after statin therapy.

A considerable amount of data has suggested the utility of new therapeutic non-statin lipid-modifying therapies such as ezetimibe, and proprotein convertase subtilisin/kexin type 9 (PCSK9) inhibitors. The Improved Reduction of Outcomes: Vytorin Efficacy International Trial (IMPROVE-IT) demonstrated that addition of ezetimibe to statin therapy reduced the primary outcome by 1.8% over 7 years.20 In particular, alirocumab, as a fully human monoclonal antibody to PCSK9 synthesized and secreted by hepatocytes, binds to LDL-receptors, thereby targeting it for lysosomal degradation. In the Further Cardiovascular Outcomes Research with PCSK9 Inhibition in Subjects with Elevated Risk (FOURIER) study, evolocumab reduced CVD events to a frequency of 1.5% over 2.2 years among subjects with suboptimal low-density lipoprotein cholesterol (LDL-C) levels receiving maximally tolerated statin therapy.20 ODYSSEY OUTCOMES (Evaluation of Cardiovascular Outcomes After an Acute Coronary Syndrome During Treatment with Alirocumab) demonstrated that alirocumab significantly reduced recurrent CVD events in patients with recent ACS.25 Although these novel non-statin lipid-lowering therapies reduce the risk of CVD, the magnitude of risk reduction has become smaller as high-dose statin therapy has become standard care.25 Subgroup analyses in these trials demonstrated greater risk reduction in higher-risk patients. LR11 might serve as a biomarker for residual risk stratification in CAD patients treated with statin therapy and could provide some indications for additional lipid-modifying agents.

Several limitations of the present study require consideration. The present study had the limitations inherent to an observational study design such as selection bias and unmeasured confounders which might have affected outcomes regardless of adjustments in the statistical analyses. In addition, relatively few events occurred during the present study, which resulted in the absence of statistically significant differences in outcome measures. Thus, an observational study of a larger cohort is necessary. Furthermore, the choice or dose of statins depended on each physician and we had no information about patient compliance with prescribed pharmacotherapies during follow-up.

Conclusions

Elevated levels of LR11 might serve as a residual risk factor that is significantly associated with long-term clinical outcomes among CAD patients receiving statin therapy after first PCI.

### Table III. Univariate and Multivariate Cox Regression Analysis for Each CVD Endpoint.

|                         | Univariate |          |          |        | Multivariate |          |          |
|-------------------------|------------|----------|----------|--------|--------------|----------|----------|
|                         | HR         | 95% CI   | P        |        | HR           | 95% CI   | P        |
| Overall, LR11 high/low  | 2.53       | 1.38-4.93| 0.003    | 2.47   | 1.29-4.92    | 0.006    |          |
| Cardiovascular death, LR11 high/low | 4.50       | 1.13-29.8| 0.032    | 2.28   | 0.73-8.11    | 0.16     |          |
| Non-fatal ACS, LR11 high/low | 2.47       | 0.97-7.03| 0.06     | 2.51   | 0.90-7.75    | 0.08     |          |
| Stroke, LR11 high/low    | 2.27       | 0.81-7.30| 0.12     | 1.88   | 0.62-6.42    | 0.27     |          |

ACS indicates acute coronary syndrome; CVD, cardiovascular disease; HR, hazard ratio; and 95% CI, 95% confidential interval.

HR, 2.21; 95%CI, 1.13-4.49; P = 0.020).

### Discussion

The major findings of the present study were as follows. First, we showed a significant association between circulating sLR11 levels and long-term outcomes among patients treated using statins. Second, multivariate analysis indicated that higher sLR11 could offer an independent predictor of CAD in patients treated using statins after adjustment.

Atherosclerosis is a progressive vascular injury characterized by lipid accumulation, necrosis and fibrosis and is the principal cause of CAD worldwide.14,15 The migration of vascular SMCs from the media to the intima is a key step in atherosclerotic plaque formation.16,17 LR11 is a relatively newly identified member of the LDL-receptor family that has been shown to regulate the migration of vascular SMCs.5,6 LR11 is released from the intimal SMC membrane by proteolytic shedding, and sLR11 then exerts biological activity on SMC migration by forming complexes with the urokinase-type plasminogen activator receptor (uPAR). In addition, sLR11 enhances macrophage infiltration into injured arteries through the activation of uPAR-mediated pathways.18 LR11 might thus play a central role as a regulator of vulnerable plaque formation through the activation of uPAR. The close association between sLR11 and adverse cardiovascular outcomes in patients with CAD indicates that LR11 derived from intimal SMCs could offer a biomarker for atherosclerotic plaque formation.

Statins significantly reduce the risk of cardiovascular events in patients with CAD and are the most widely prescribed pharmacotherapy for secondary prevention of CAD.20 However, residual risk still exists.24 Previous studies for biomarkers offer a usefulness in clinical practice to improve patient care.20 Insights from the pathophysiology of atherosclerosis have led to the discovery of vascular cell-specific molecules as potential biomarkers.21 Recent clinical studies have evaluated these molecules as potential biomarkers for establishing risk stratification and predicting adverse clinical outcomes in CAD patients.22 In the present study, we found a significant relationship between sLR11 and adverse events among CAD patients treated with statins. In addition, we found that a closer association between sLR11 and each atherosclerotic CVD endpoint (CVD death, non-fatal ACS, non-fatal stroke), although they were not statistically significant. This indicates that LR11 derived from intimal SMCs might serve as a potential biomarker for predicting future atherogenic...
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Disclosure

Conflicts of interest: The authors declare that there is no conflict of interest.

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