# Soluble LR11 is a Novel Biomarker for Vascular Lesions Late after Kawasaki Disease

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## Abstract

### Objective

Coronary artery lesions (CALs) and a risk for early onset of atherosclerosis are major concerns following Kawasaki disease (KD). Intimal smooth muscle cells (SMCs) have an important role in vascular lesions in KD. It is known that soluble LR11 (sLR11) is a novel biomarker for vascular lesions and LR11 is markedly expressed in intimal SMCs in atherosclerotic lesions. In this study, we hypothesized that sLR11 reflects the presence of vascular lesions late after KD.

### Methods

Twenty-three age-matched controls (group 1) and 59 patients with a history of KD were enrolled; 36 with KD had normal coronary arteries or regressed aneurysms (group 2), and 23 had CALs (group 3).

### Results

Serum sLR11 levels in group 3 (median, interquartile range (IQR): 11.1 ng/mL, 9.3–13.9 ng/mL) were significantly higher than those in groups 1 (8.4 ng/mL, 7.1–10.2 ng/mL, $p < 0.001$) and 2 (9.0 ng/mL, 7.7–10.1 ng/mL, $p < 0.01$). Levels of sLR11 were positively correlated with levels of high-sensitivity C-reactive protein ($r = 0.480$, $p < 0.01$) and lipoprotein (a) ($r = 0.486$, $p < 0.01$).

## Conclusion

These findings suggest that sLR11 reflects the development of vascular lesions in patients with serious CALs.

## Keywords

LR11; smooth muscle cell; Kawasaki disease; biomarker

## Abbreviations

CAL, coronary artery lesion

KD, Kawasaki disease

hs-CRP, high-sensitivity C-reactive protein

SMCs, smooth muscle cells

## 1. Introduction

Kawasaki disease (KD) is an acute, self-limited generalized systemic vasculitis, which develops most frequently around 1 year of age. Over 40 years have passed since KD was first reported in 1967 [1]. The total number of patients with a history of KD is over 200,000 and almost half of the patients have reached adulthood in Japan. Therefore, many patients are now managed by cardiovascular internists. Coronary artery lesions (CALs), including aneurysms, stenosis, and occlusion, are the most serious cause of morbidity and mortality in patients with a history of KD. Coronary aneurysms occur because of vasculitis in 20–25% of untreated patients in the acute phase of KD [2, 3]. Coronary artery aneurysms sometimes progress to stenosis or occlusion because of intimal thickening late after onset and may lead to myocardial infarction or cardiac sudden death. Furthermore, the risk for early onset of atherosclerosis is becoming a major concern in patients with a history of KD. However, whether patients after KD may be at an increased risk for atherosclerosis remains unclear.

We have previously reported that LR11, a member of the low-density lipoprotein (LDL) receptor family, is markedly expressed in intimal smooth muscle cells (SMCs) in atherosclerotic lesions, and overexpression of LR11 enhances SMC migration through activation of the urokinase-type plasminogen activator receptor [4-6]. Recently, we

showed that serum concentrations of the soluble form of LR11 (sLR11) might be a useful biomarker for vascular lesions, including atherosclerosis and coronary disease [7-9]. Intimal SMCs play an important role in development of CALs in KD, as well as atherosclerotic lesions [2, 3]. In this study, we tested the hypothesis that sLR11 reflects late vascular lesions in patients with KD.

## 2. Methods

### 2.1. Patients

We enrolled patients who had had acute KD more than 12 months prior to the study enrollment. All of the patients fulfilled the diagnostic criteria of KD during the acute phase. The patients were seen at outpatient clinics in Niigata University Hospital, and its three affiliated hospitals Saiseikai Niigata Daini Hospital, Tsuruoka Municipal Shonai Hospital, and Saiseikai Sanjo Hospital between April 2009 and December 2012. Fifty-nine KD patients and 23 age-matched controls were enrolled in this study. The KD patients were classified into two subgroups based on coronary sequelae evaluated by echocardiography and/or coronary angiography; group 1 (controls) consisted of 23 patients who had mild arrhythmias or tiny congenital heart diseases without hemodynamic abnormalities. Group 2 (n = 36) included those without CALs from the

onset of KD, those with slight and transient dilatation which subsided within 30 days from the onset of KD, and those with regression of aneurysms. Group 3 (n = 23) included those with CALs, including aneurysms, stenosis, and occlusion.

KD sequelae were defined as CALs persisting beyond 30 days after onset. In all patients, echocardiograms were performed to evaluate left ventricular function, valvular insufficiency, and proximal coronary artery dimensions. Coronary artery aneurysms during the acute phase were defined as follows on the basis of the diagnostic criteria recommended by the Ministry of Health and Welfare in Japan [10]: the internal diameter of the coronary artery was 3 mm or greater in children aged < 5 years of age, the internal diameter of the coronary artery was 4 mm or greater in children ≥ 5 years of age, the internal diameter of a segment was 1.5 times or greater the adjacent segment, and the lumen of the coronary artery was clearly irregular. CALs in the long term included aneurysms, stenosis and occlusion. This study was approved by the Institution Ethics Review Board at Niigata University Graduate School of Medical and Dental Sciences, and written informed consent was obtained from all participants.

### 2.2. Laboratory measurements

Serum samples were obtained at an outpatient clinic or during catheter angiography. We measured sLR11 levels and the following markers associated with atherosclerotic

risk: serum total cholesterol (Tchol), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), apolipoprotein (Apo) A1, Apo A2, Apo B, lipoprotein (a), and high-sensitivity C-reactive protein (hs-CRP) levels. Serum levels of Tchol, HDL-C, LDL-C, Apo A1, Apo A2, and Apo B were measured in the local laboratory. Blood samples were centrifuged after collection, and the supernatant was frozen in polypropylene tubes and stored at −80°C until use. Soluble LR11 was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) [11]. Serum levels of lipoproteins were measured using the latex agglutination method (Sekisui Medical, Tokyo, Japan). Serum levels of hs-CRP were measured using nephelometry (Siemens Healthcare Diagnostics, Tokyo, Japan).

### 2.3. Statistical analysis

Data in tables are shown as mean±standard error of the mean (SEM). To compare the differences in clinical characteristics between two or three groups, Student's t test or one-way analysis of variance (ANOVA) was performed. Differences in proportions were tested with $\chi^2$ analysis. Because sLR11 and CRP levels had a skewed distribution, data are shown as the median and interquartile range (IQR). The Kruskal–Wallis test, followed by the Steel–Dwass test for multiple comparisons were used to assess the significance of differences among three groups. Correlations were analyzed with

Pearson correlation coefficient analysis in KD patients. Differences were considered significant when the p value was $< 0.05$. All data analyses were performed with JMP 9.0 for Windows software (SAS institute Inc., Cary, NC, USA).

## 3. Results

### 3.1. Patients

Of the 59 KD patients, 52 had received various doses of intravenous immunoglobulin during the acute phase of the illness. In addition, 32 of 36 patients in group 2, and 20 of 23 patients in group 3 had been treated with immunoglobulin therapy. The detailed clinical characteristics of the study population are shown in Table 1. There were no significant differences in age, gender, height, body weight, and body mass index among the three groups. The mean age at the onset of KD and the mean time from the onset were similar between the two KD groups.

Three (8%) patients in group 2 took regular antiplatelet doses of aspirin. All of the patients in group 3 received antiplatelet agents, 21 (91%) patients received antiplatelet dose of aspirin, one (4%) received dipyridamole, and 14 (61%) received ticlopidine. Nine (39%) patients in group 3 received warfarin.

Blood pressure, Tchol, HDL-C, and LDL-C levels were all within normal limits in the

two KD groups. There was a high tendency of diastolic blood pressure in group 3 (p=0.063). Apo A1, A2, and B levels were also all within normal limits and were not significantly different between the two KD groups. Lipoprotein (a) levels tended to be higher in group 3 (10.9 ± 2.2 mg/dL) than in group 1 (4.5 ± 2.3 mg/dL) and group 2 (4.9 ± 1.6 mg/dL) ($p = 0.073$).

### 3.2. Levels of sLR11

Serum sLR11 levels in group 3 (median, IQR; 11.1 ng/mL, 9.3–13.9 ng/mL) were significantly higher than those in group 1 (8.4 ng/mL, 7.1–10.2 ng/mL, $p < 0.001$), group 2 (9.0 ng/mL, 7.7–10.1 ng/mL , $p < 0.01$) (Figure 1).

### 3.3 Levels of hs-CRP

Levels of hs-CRP in group 3 (median, IQR; 0.22 mg/L, 0.14–0.28 mg/L) were significantly higher than those in group 1 (0.08 mg/L, 0.05–0.14 mg/L, $p = 0.03$), group 2 (0.09 mg/L, 0.05–0.12 mg/L, $p < 0.01$).

### 3.4 Correlation between sLR11 and other markers

Serum sLR11 levels were positively correlated with hs-CRP levels ($r = 0.480$, $p < 0.01$, Figure 2A) and lipoprotein (a) ($r = 0.486$, $p < 0.01$, Figure 2B).

## 4. Discussion

The current study demonstrated that sLR11, a novel biomarker for vascular lesions, is elevated in KD patients with CALs. Levels of an inflammatory marker hs-CRP are also elevated in KD patients with CALs, and levels of sLR11 and hs-CRP and lipoprotein (a) were positively correlated. These results suggest that sLR11 reflects remaining vascular lesions associated with smooth muscle cell dysfunction and chronic inflammation and atherosclerosis in the long-term follow-up of KD patients.

We have previously reported that LR11, a member of the LDL receptor family, is markedly expressed in intimal SMCs in atherosclerotic lesions, but not medial SMCs [4]. Intimal SMCs play an important role in the development of atherosclerosis. Medial SMCs migrate into the intima under the influence of various stimuli from infiltrating cells, including endothelial cells and macrophages. In this process, SMCs change from a contractile to a synthetic phenotype, lose vascular contractility, and are involved in the development of atherosclerosis. In LR11 knockout mice, cuff-induced intimal thickening of femoral arteries is decreased [8]. These studies suggest that LR11 plays an important role in medial-to-intimal migration of SMCs in injured arteries and the development of atherosclerotic plaques.

We recently demonstrated that serum sLR11 levels are positively correlated with intima-media thickness in patients with dyslipidemia [8], are significantly elevated in

patients with coronary stenosis compared with those without stenosis [7], and are significantly increased in those with acute coronary syndrome compared with those with stable angina pectoris [9].

Similar to atherosclerotic lesions, intimal SMCs are involved in vascular lesions of KD from the acute to the late phase. In the acute phase of KD, medial SMCs migrate into the intima in response to various cytokines, resulting in intimal thickening. Persistent CALs and the potential risk for early onset of atherosclerosis are problems that occur late after KD. Regression of coronary aneurysms often occurs within 1–2 years after illness onset in patients with small- or moderate-sized aneurysms [3, 12]. Pathological and intravascular ultrasound studies of regressed coronary aneurysms have demonstrated that this regression mainly results from marked intimal thickening, but not complete normalization [13, 14]. Coronary artery stenosis is caused by intimal thickening, which usually occurs in the inflow and outflow of aneurysms [2, 3, 15]. Furthermore, whether intimal SMCs that remain late after the onset of KD causes vascular lesions or early onset of atherosclerosis in systemic arteries is another concern [16].

The current study showed that serum sLR11 levels were positively correlated with hs-CRP levels. This finding is consistent with our previous report [9] demonstrating that

serum hs-CRP is the most strongly correlated with serum sLR11 among various biomarkers. In KD patients with CALs, endothelial dysfunction has been demonstrated in several studies [17, 18]. The relation between sLR11 and hs-CRP and lipoprotein (a) levels in the current study suggests that chronic inflammation and endothelial and smooth muscle cell dysfunction may be closely related and involved in the development of atherosclerosis late after KD.

Furthermore, LR11 is a potential novel therapeutic target. Recent studies have demonstrated that statins, which are hydroxymethylglutaryl-coenzyme A reductase inhibitors, prevent cardiovascular events with or without dyslipidemia [19, 20], and reduce coronary plaque volume in patients with acute coronary syndrome [21]. We have shown that pitavastatin, a member of the statin family, inhibits the migratory activity of cultured intimal SMCs, and reduces aortic plaques from hypercholesterolemic rabbits via suppression of LR11 expression [22, 23]. Statin therapy also improves vascular lesions late after KD [24, 25]. These findings indicate that a portion of the effect of statin therapy in KD patients with CALs may arise via suppression of LR11 expression. Further studies of LR11 as a therapeutic target and application to KD patients are expected in the future.

In conclusion, our results are consistent with the hypothesis that LR11 is associated with vascular lesions in patients late after KD. Soluble LR11 is a marker that might be related to smooth muscle cell and endothelial dysfunction, and chronic inflammation. Therefore, sLR11 could serve as a useful marker reflecting the risk for early onset of atherosclerosis in the long-term follow-up of KD patients.

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## Figure Legends

**Figure 1.** The levels of soluble LR11 (sLR11) in the three groups. Serum sLR11 levels were higher in Group 3 (median, interquartile range (IQR); 11.1 ng/mL, 9.3–13.9 ng/mL) than in Groups 1 (8.4 ng/mL, 7.1–10.2 ng/mL, $p < 0.001$) and Group 2 (9.0 ng/mL, 7.7–10.1 ng/mL, $p < 0.01$).

Group 1, controls; Group 2, Kawasaki disease (KD) patients with normal coronary arteries and regressed aneurysms; Group 3, KD patients with coronary artery lesions. Box plots show median, first and third quartiles, the bars show minimum and maximum values.

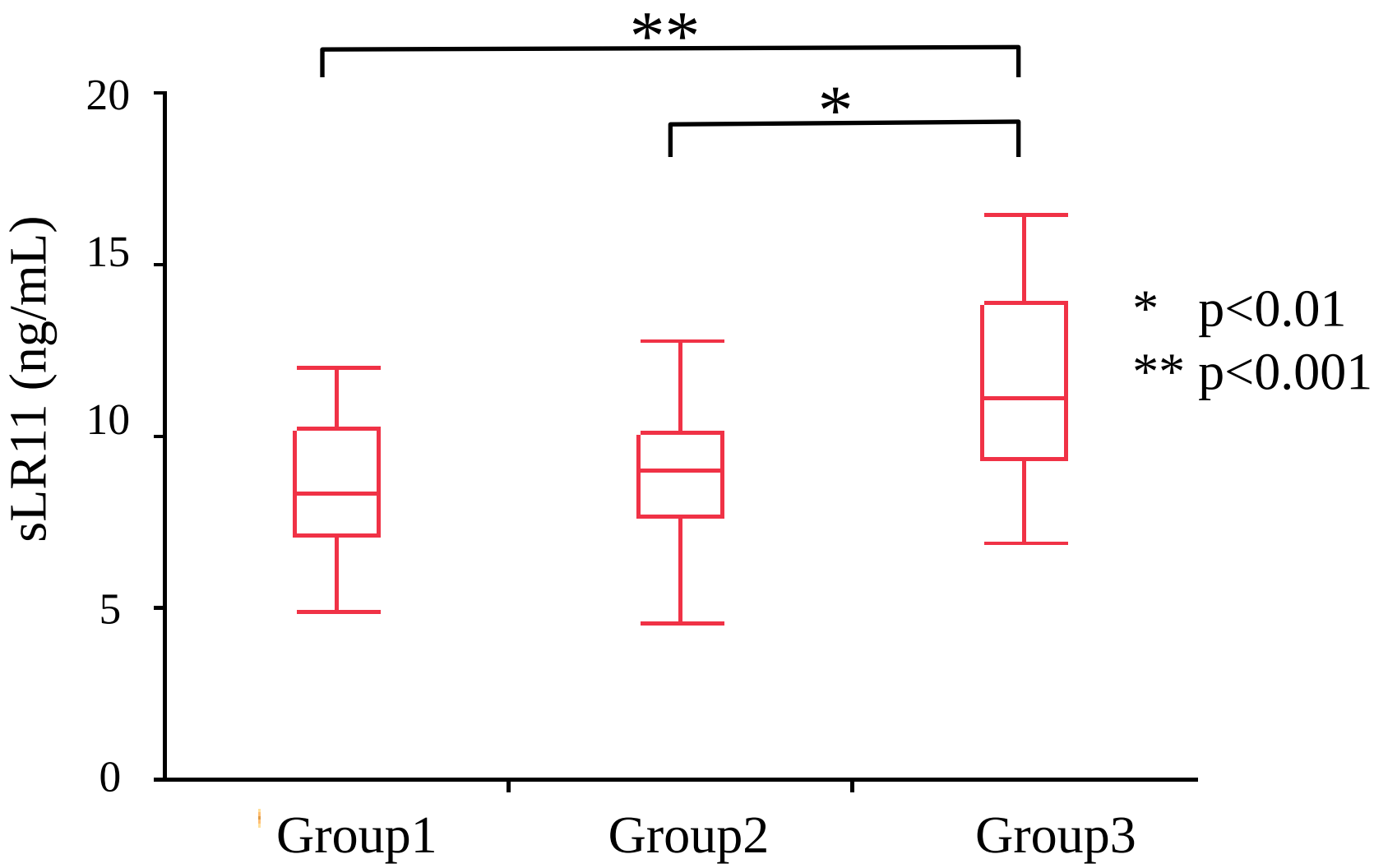
**Figure 2A.** Correlation between high-sensitivity C-reactive protein (hs-CRP) and soluble LR11 (sLR11) levels.

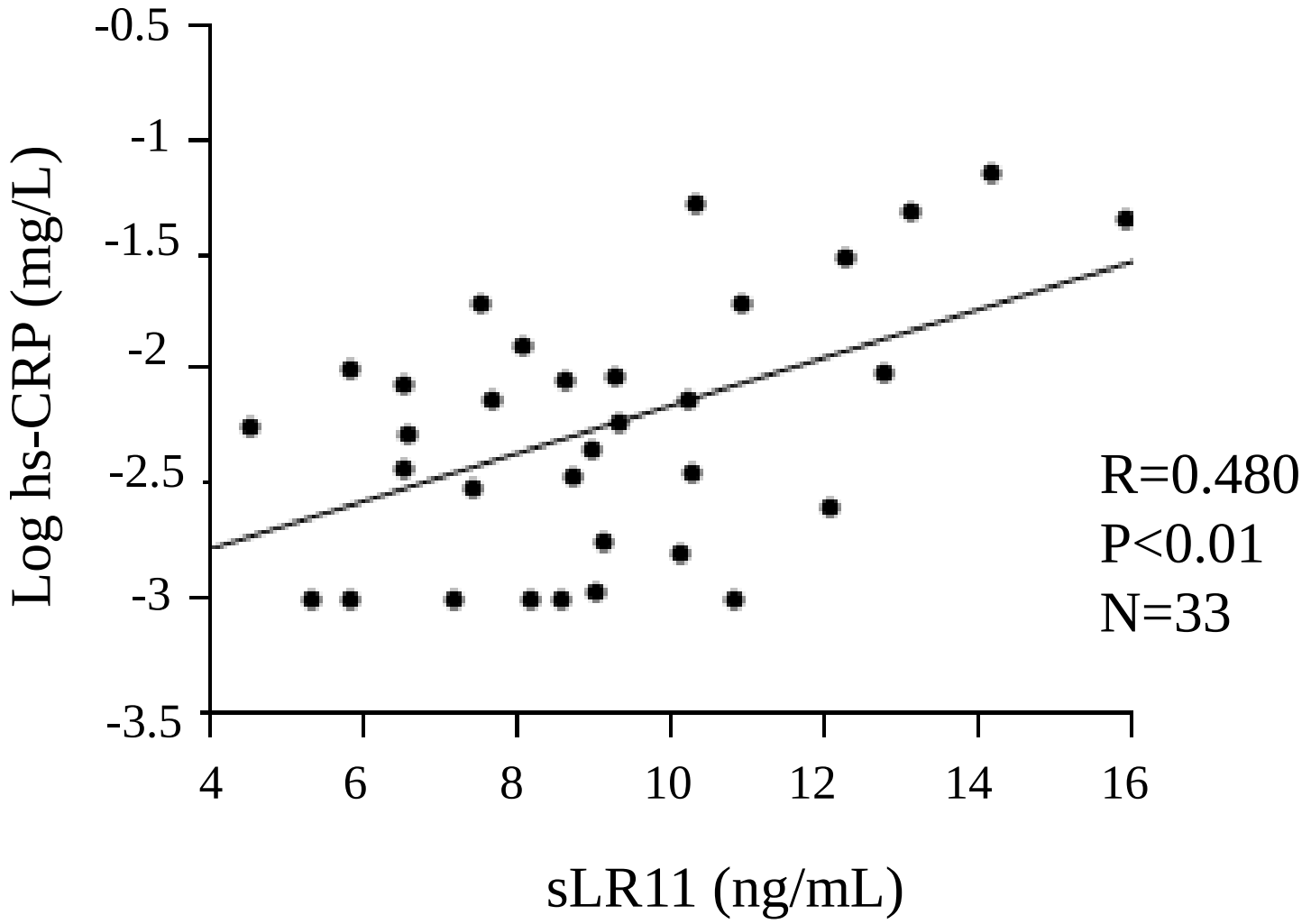
**Figure 2B.** Correlation between lipoprotein (a) and soluble LR11 (sLR11) levels.

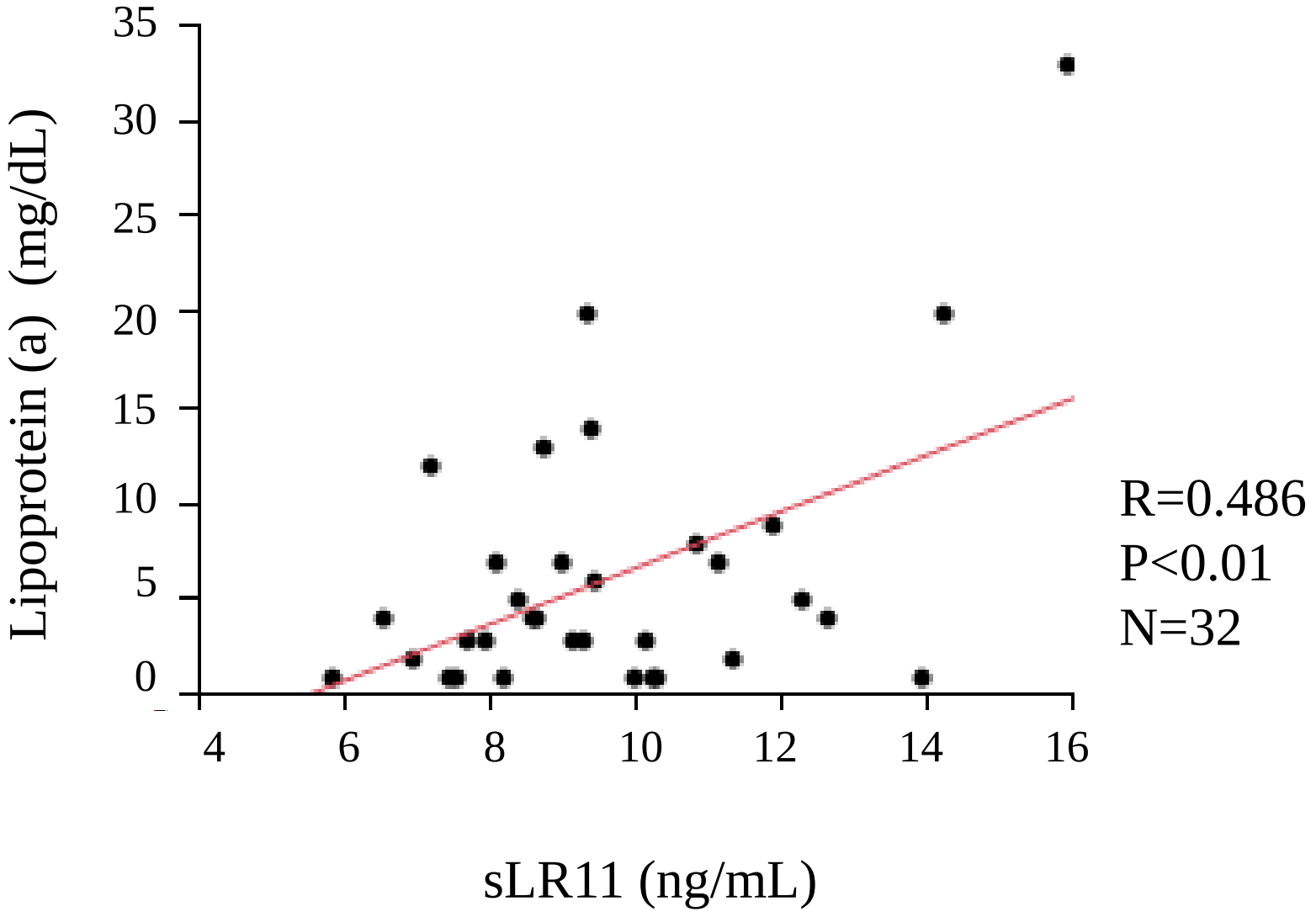
**Clinical Characteristics of Subgroups After Kawasaki Disease and Control Subjects**

| | Group 1 (n=23) | Group 2 (n=36) | Group 3 (n=23) | P value |
|---|---|---|---|---|
| Age at study (years) | 10.2±1.2 | 9.3±0.9 | 10.9±1.2 | 0.527 |
| Male/Female (%) | 14/10 (58) | 24/12 (67) | 18/5 (78) | 0.333 |
| Age at KD (years) | - | 2.7±0.3 | 2.4±0.4 | 0.498 |
| Interval (months) | - | 78.5±11.8 | 102.5±14.8 | 0.207 |
| Heght (cm) | 141.5±12.8 | 134.5±8.5 | 133.0±6.8 | 0.838 |
| BW (kg) | 36.6±8.1 | 34.0±5.1 | 34.4±4.3 | 0.995 |
| BMI ($kg/m^2$) | 17.6±1.2 | 18.1±0.8 | 17.7±0.6 | 0.925 |
| Medication | 0 (0) | 3 (8) | 23 (100) | <0.0001 |
| CAG | 0 (0) | 12 (33) | 23 (100) | <0.0001 |
| SBP (mmHg) | 103.7±6.5 | 100.4±3.5 | 103.0±2.3 | 0.812 |
| DBP (mmHg) | 59.3±3.9 | 56.6±2.1 | 62.7±1.4 | 0.063 |
| Tchol (mg/dL) | 165.8±8.8 | 155.7±4.2 | 155.1±4.7 | 0.534 |
| HDL-C (mg/dL) | 60.5±4.6 | 57.2±2.2 | 54.2±2.4 | 0.426 |
| LDL-C (mg/dL) | 89.2±8.4 | 82.7±3.7 | 86.0±4.0 | 0.705 |
| Tchol/HDL-C | 2.7±0.2 | 2.8±0.1 | 2.9±0.1 | 0.714 |
| Apo A1 (mg/dL) | - | 142.4±6.5 | 128.9±7.3 | 0.176 |
| Apo A2 (mg/dL) | - | 30.9±1.5 | 27.9±1.7 | 0.213 |
| Apo B (mg/dL) | - | 72.9±4.8 | 68.6±4.8 | 0.550 |
| Apo B/Apo A1 | - | 0.53±0.04 | 0.54±0.04 | 0.867 |
| lipoprotein(a) (mg/dL) | 4.5±2.3 | 4.9±1.6 | 10.9±2.2 | 0.073 |

KD indicates Kawasaki disease; BW, body weight; BMI, body mass index; CAG, coronary angiography; SBP, systolic blood pressure; DBP, diastolic blood pressure; Tchol, total cholesterol; Apo, apolipoprotein. Values are mean±SEM (%).


**
*
20
15
10
5
0
sLR11 (ng/mL)
Group1
Group2
Group3
* p<0.01
** p<0.001


-0.5
-1
-1.5
-2
-2.5
-3
-3.5
Log hs-CRP (mg/L)
4
6
8
10
12
14
16
sLR11 (ng/mL)
R=0.480
P<0.01
N=33


Lipoprotein (a) (mg/dL)
35
30
25
20
15
10
5
0
4
6
8
10
12
14
16
sLR11 (ng/mL)
R=0.486
P<0.01
N=32