研究成果の刊行に関する一覧表

書籍

著者氏名	論文タイトル名	書籍全体の編集者名	書　籍　名	出版社名	出版地	出版年	ページ
小谷和彦, 河野幹彦	糖尿病に合併する脂質異常症（高脂血症）の治療薬.	河盛隆造, 綿田裕二孝	改訂版　糖尿病治療薬ハンドブック	羊土社 （東京）	日本	2012	166-180

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Ⅳ. 研究成果の刊行物・別冊

Enhanced Circulating Soluble LR11 in Patients With Diabetic Retinopathy

MAO TAKAHASHI, HIDEAKI BUJO, TOMOAKI SHIBA, MEIZI JIANG, TAKATOSHI MAENO, AND KOHJI SHIRAI

• PURPOSE: To investigate the relationship of circulating levels of soluble form of LR11 (sLR11; also called SorLA or SORL1), with the progression of proliferative diabetic retinopathy (PDR) in patients with type 2 diabetes mellitus.

• DESIGN: Cross-sectional study.

• METHODS: Fifty-four patients with type 2 diabetes mellitus were divided into 2 sex- and age-matched groups: one with PDR (n = 29) and the other with nonproliferative diabetic retinopathy (n = 25). The serum sLR11 levels were measured with an immunodetection system followed by chemifluorescence quantification.

• RESULTS: The serum sLR11 levels were higher in the PDR group than in the nonproliferative diabetic retinopathy group (5.8 ± 1.2 U vs 3.7 ± 1.3 U; P < .01). A multivariate regression analysis showed that circulating sLR11 is a factor contributing to the prediction of PDR independent of other classical risk factors, and an area under the receiver operating characteristic curve analysis revealed that the sensitivity and the specificity were equivalent to or more than those of other factors. Among the classical risk factors for PDR, glycosylated hemoglobin levels showed the highest correlation coefficient (P < .01) for the sLR11 concentrations.

• CONCLUSIONS: Serum sLR11 concentration may reflect the progression of PDR in patients with type 2 diabetes mellitus. sLR11, released from immature vascular cells and indicating the development of atherosclerosis, is expected to be a novel candidate biomarker indicating diabetic retinopathy in patients with type 2 diabetes mellitus. (Am J Ophthalmol 2012;154: 187–192. © 2012 by Elsevier Inc. All rights reserved.)

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From the Cardiovascular Center, Sakura Hospital Medical Center, Toho University, Chiba, Japan (M.T.); the Department of Ophthalmology, Sakura Hospital Medical Center, Toho University, Chiba, Japan (T.S., T.M.); the Department of Internal Medicine, Sakura Hospital Medical Center, Toho University, Chiba, Japan (K.S.); and the Department of Genome Research and Clinical Application, Graduate School of Medicine, Chiba University, Chiba, Japan (H.B., M.J.)

Inquiries to Mao Takahashi, Department of Cardiovascular Center, Sakura Hospital, Medical Center, Toho University, 564-1 Shimoshizu, Sakura-shi, Chiba Prefecture 285-8741, Japan; e-mail: takahashi-04@sakura.med.toho-u.ac.jp

SUSTAINED HYPERGLYCEMIA, EVEN IN THE ABSENCE of other risk factors, can increase the risk of microvascular complications.[1] Given the substantial quality-of-life burden that diabetic retinopathy can confer, the ability to detect early retinal vascular abnormalities sensitively in patients with diabetes mellitus is desirable. The detection of such markers of pathologic cell function in combination with treatment of hyperglycemia is needed.

LR11 (also called SorLA or SORL1), an low-density lipoprotein (LDL)-receptor family member, has been identified as a molecule expressed in intimal smooth muscle cells in the development of atherosclerosis and endothelial cells under the condition of dyslipidemia.[2,3] The released soluble form of LR11 (sLR11) promotes pathologic infiltration of macrophages into the damaged vessels.[2] We have shown that the circulating sLR11 levels were increased in patients with coronary artery disease[4] and dyslipidemic subjects with carotid atherosclerosis.[5] A multivariate analysis in these independent studies in patients with atherosclerosis indicated that the sLR11 levels were correlated distinctly with the glycemic level among the classical risk factors for atherosclerosis.[4,5]

Diabetic retinopathy mainly is caused by diffuse endothelial damage at the microvascular level. However, the interesting observations are that the retinopathy is tightly associated with increased cardiovascular mortality,[6–8] reduced coronary reactivity,[9] and poorer prognosis of coronary revascularization procedures.[10,11] Thus, high glucose levels may change the phenotype of endothelial cells as well as that of arterial smooth muscle cells; the pathologic cell phenotype in microvessels of the retina possibly is detected by the circulating sLR11 released from the damaged cells. In this analysis, we investigated the significance of circulating sLR11 with regard to proliferative diabetic retinopathy (PDR) in patients with type 2 diabetes mellitus. The factors contributing to the elevation of the serum sLR11 also were analyzed.

METHODS

• STUDY POPULATION: The subjects consisted of 56 consecutive Japanese patients with type 2 diabetes mellitus seeking treatment at the Department of Laboratory Vascular Function, Toho University Sakura Medical Center, who had already given blood samples. PDR was defined

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TABLE 1. Comparison of Type 2 Diabetes Mellitus Patient Background Factors between Nonproliferative Diabetic Retinopathy and Proliferative Diabetic Retinopathy

	NPDR Group	PDR Group	*P* Value
No.	25	29	—
Male (%)	68.2	69	.95
Age (y)	66.0 ± 8.6	62.4 ± 9.7	.15
Duration of diabetes (y)	11.4 ± 7.8	11.9 ± 7.8	.71
Body mass index (kg/m^2)	23.8 ± 4.0	25.8 ± 3.7	.06
Hypertension (%)	63.6	58.6	.72
Dyslipidemia (%)	64.0	44.8	.16
eGFR (mL/minute per 1.73m^2)	60.2 ± 15.3	58.2 ± 28.0	.72
HbA1c (%)	6.5 ± 0.8	7.0 ± 1.4	.10
Fasting blood sugar (mg/dL)	124.6 ± 33.1	132.1 ± 38.2	.63
Total cholesterol (mg/dL)	183.8 ± 34.8	202.6 ± 40.7	.12
LDL cholesterol (mg/dL)	111.7 ± 30.6	124.1 ± 33.3	.26
HDL cholesterol (mg/dL)	47.7 ± 16.4	49.4 ± 10.6	.58
Triglyceride (mg/dL)	122.4 ± 43.1	122.7 ± 52.7	.93
Medications			
Insulin therapy (%)	13.6	65.5	< .0001
Administration of statin (%)	45.5	24.1	.11
Administration of ACE-I or ARB (%)	54.5	41.4	.43

ACE-I = angiotensin converting enzyme inhibitor; ARB = angiotensin receptor blocker; eGFR = estimated glomerular filtration rate; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = high-density lipoprotein; NPDR = nonproliferative diabetic retinopathy; PDR = proliferative diabetic retinopathy.

The data are presented as mean ± standard deviation or number of subjects (%). The unpaired *t* test was used for continuous variables, and the chi-square test was used for categorized variables.

according to the international clinical classification of diabetic retinopathy as neovascularization in the retina.[12] Vitreous surgeries had been performed to treat macular edema (n = 7), vitreous hemorrhage (n = 13), traction retinal detachment (n = 5), or neovascular glaucoma (n = 4). None of the nonproliferative diabetic retinopathy (NPDR) cases had retinal neovascularization. Patients with chronic heart disease with an ejection fraction of less than 50% or chronic renal failure with serum creatinine of more than 1.3 mg/dL were excluded from the study analysis.

• PATIENT DATA ANALYSIS: Blood samples were collected in the morning after an overnight fast. Lipid variables and fasting blood glucose were measured using standard laboratory techniques. The potential risk factors for atherosclerosis were analyzed, including age, sex, body mass index (BMI), smoking, and history of hypertension and dyslipidemia. Hypertension was defined as systolic pressure of more than 140 mm Hg or diastolic pressure of more than 90 mm Hg. Diabetes mellitus was defined as a fasting blood glucose level of more than 126 mg/dL, glycosylated hemoglobin (HbA1c) of more than 5.8%, or both. Dyslipidemia was defined as serum total cholesterol of more than 220 mg/dL and triglycerides of more than 150 mg/dL in the fasting state, or both, and high-density lipoprotein (HDL) cholesterol of less than 40 mg/dL, or a combination thereof. The serum creatinine level was assayed by an enzymatic method. The estimated glomerular flow rate was estimated using a modified traceable Modification of Diet in Renal equation, as proposed by the Working Group of Japan Chronic Kidney Disease Initiative[13]: estimated glomerular flow rate (mL/minute per 1.73 m^2) = $0.741 \times 175 \times \text{age}^{-0.203} \times \text{serum creatinine}^{-1.154}$ (if female × 0.742).

• MEASUREMENT OF SERUM SLR11: For the analysis of sLR11, fasting blood samples were collected and centrifuged immediately at 4000 g for 10 minutes, and the supernatant immediately was frozen in polypropylene tubes and stored at −80 C until use. Fifty microliters of serum was purified using 39-kDa receptor-associated protein-GST affinity beads (Cosmo Bio, Toyo city, Tokyo, Japan). For immunoblotting, equal amounts of protein extracted from pelleted beads were subjected to 10% sodium dodecyl sulfate poly-acrylamide gel electrophoresis (SDS-PAGE) after heating to 95 C for 5 minutes, as described previously[5] under reducing conditions, and were transferred to a nylon membrane. Incubations were carried out with an antibody against LR11 (5-4-30-19-2 at 1:500 dilution),[5] followed by peroxidase-conjugated antimouse immunoglobulin G. The development was performed with the ECL detection reagents (Amersham Pharmacia, Piscataway, New Jersey, USA). The signals were quantified by densi-

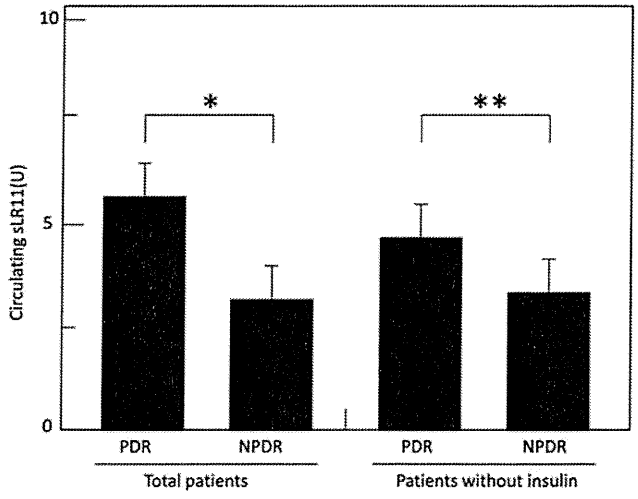

FIGURE 1. Bar graph showing circulating sLR11 levels in the patient groups with proliferative diabetic retinopathy (PDR) or without PDR (NPDR). The sLR11 levels of the total patients and those of the patients without insulin therapy were compared between the PDR and NPDR groups, respectively. Data are expressed as means ± standard deviation. For statistical analysis, the Student t tests was used. *P < .05, **P < .01.

tometric scanning using the NIH image software program (National Institutes of Health, Betheda, Maryland, USA). The sLR11 levels in each serum sample (50 μL) were determined as an averaged value of 3 quantified signal intensities resulting from independent assays using samples with blinded indications and were expressed as a ratio to that of standard serum. The immunologic estimation indicated that the signal of 1 U (in 50 μL serum) corresponded to approximately 50 ng/mL of recombinant sLR11.

• STATISTICAL ANALYSIS: The results are shown as means ± standard deviation or proportion (%) for each index. The statistical analyses were performed using the SPSS Statistical Package for Windows software program version 11.01.1. Comparisons between groups were performed using the Student t test. The data were subjected to a 1-way analysis of variance with the Dunnett multiple comparison of means. A Pearson correlation coefficient analysis was used to assess the associations between measured parameters. Subsequently, multiple linear regression analyses were used to calculate the odds ratio for PDR by controlling for all risk factors. These risk factors were scored as explanatory factors, and the subordinate variable was PDR = 1 and NPDR = 0. The sensitivity and specificity with respect to the presence of PDR were analyzed using a conventional receiver operating characteristic (ROC) curve. P values less than .05 were considered to be statistically significant.

TABLE 2. Results of Multivariate Analysis Investigating Risk Factors for Proliferative Diabetes Retinopathy in Subjects with Type 2 Diabetes Mellitus

	Odds Ratio (95% Confidence Interval)	*P* Values
Age, per 1-y increase	4.12 (0.78 to 0.996)	< .05
Male	0.01 (0.16 to 6.18)	.99
eGFR, per 1-U (mL/minute per 1.73 m^2) increase	0.80 (0.97 - 1.08)	.37
Total cholesterol, per 1-mg/dL increase	1.43 (0.99 to 1.04)	.34
HbA1c, per 1% increase	1.23 (0.24 to 2.04)	.51
sLR11, per 1-U increase	8.50 (1.63 to 12.25)	< .01

eGFR = estimated glomerular filtration rate; HbA1c = glycosylated hemoglobin; sLR11 = soluble form of LR11.

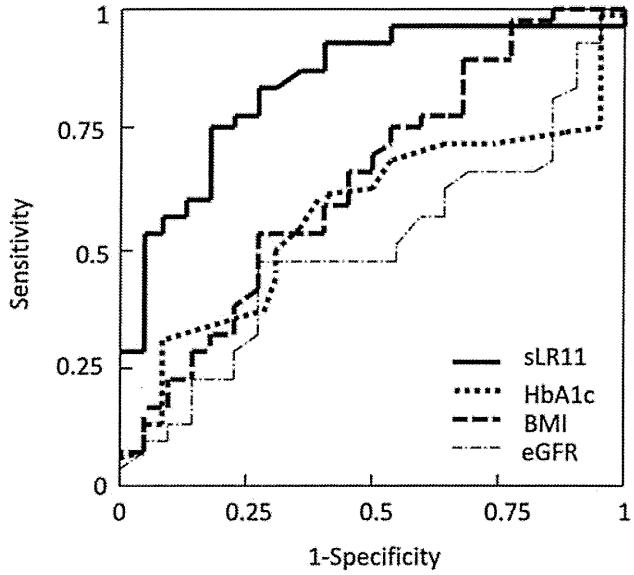

FIGURE 2. Receiver operating characteristic curve for discriminating the probability of type 2 diabetes mellitus patients developing proliferative diabetic retinopathy (PDR) from patients without PDR based on the levels of circulating soluble form of LR11 (sLR11), glycosylated hemoglobin (HbA1c), body mass index (BMI), or estimated glomerular filtration rate (eGFR). The curves show the fraction of true-positive results (sensitivity) and false-positive results (1-specificity) for various cutoff levels of each parameter.

RESULTS

THE PATIENT CHARACTERISTICS ARE SHOWN IN TABLE 1. The age- and gender-matched NPDR and PDR groups comprised 25 and 29 subjects, respectively. There were no statistically significant differences in BMI, duration of diabetes, frequency of hyperlipidemia or dyslipidemia, or estimated glomerular flow rate between the NPDR and PDR subjects. There were also no statistically significant

TABLE 3. Area under the Receiver Operating Characteristic Curve Analysis Investigating Cutoff Values for Proliferative Diabetic Retinopathy

Marker	Cutoff	Sensitivity	Specificity	AUC %
sLR11(U)	4.2	0.78	0.77	85
HbA1c (%)	6.5	0.63	0.60	57
BMI (kg/m^2)	24.5	0.59	0.59	64
eGFR (mL/minute per 1.73 m^2)	120.5	0.5	0.46	50

AUC = area under the receiver operating characteristic curve; BMI = body mass index; eGFR = estimated glomerular filtration rate; HbA1c = glycosylated hemoglobin; sLR11 = soluble form of LR11.

TABLE 4. Correlation Analysis of Circulating sLR11 with Various Markers in All Subjects with Type 2 Diabetes Mellitus

	Pearson Correlation Coefficient	*P* Value
Age	−0.07	.63
Male	0.15	.29
Body mass index (kg/m^2)	0.21	.14
HbA1c (%)	0.32	< .01
Fasting blood sugar (mg/dL)	0.19	.17
eGFR (mL/minute per 1.73 m^2)	−0.19	.17
Total cholesterol (mg/dL)	0.27	.05
LDL cholesterol (mg/dL)	0.31	< .05
HDL cholesterol (mg/dL)	−0.10	.51
Triglyceride (mg/dL)	0.25	.07

eGFR, estimated glomerular filtration rate; HbA1c = glycosylated hemoglobin; HDL = high-density lipoprotein; LDL = low-density lipoprotein.

differences in HbA1c, fasting blood sugar, or lipid concentrations between the NPDR and PDR subjects. Although there was no significant difference in the use frequency of statin, angiotensin converting enzyme inhibitor (ACE-I), or angiotensin II receptor type 1 blocker (ARB) between the 2 groups, the frequency of patients using insulin was significantly higher in the PDR subjects than that in the NPDR subjects.

The circulating sLR11 levels in the NPDR and PDR groups were 3.7 ± 1.8 U and 5.8 ± 2.7 U, respectively (Figure 1), indicating that the sLR11 levels in the PDR group were higher than those in the NPDR group ($P <$ .01). Note that we previously reported that the mean circulating sLR11 levels in 400 dyslipidemic subjects was 3.0 ± 1.0 U.[5] The sLR11 analysis restricted for the patients not treated with insulin showed that the sLR11 levels again were higher in the subjects with PDR (4.8 ± 1.2 U; n = 10) than in those with NPDR (3.7 ± 1.3; n = 12; $P <$ .05). Thus, circulating sLR11 levels were increased in type 2 diabetes mellitus patients with PDR regardless of medication with insulin therapy.

We analyzed the significance of the sLR11 concentration in comparison with other risk factors for PDR, including age, male gender, estimated glomerular flow rate, and the total cholesterol and HbA1c concentrations, in all subjects (Table 2). The multivariate analysis using all variables for PDR showed that the circulating sLR11 level, as well as younger age, strongly associated with PDR independent of other variables.

The ROC curves of the various factors were examined for discriminating the probability of the type 2 diabetes mellitus patients with PDR from the NPDR patients based on the levels of sLR11, the levels of HbA1c, the BMI, or the estimated glomerular filtration rate (Figure 2). The curves showed the fraction of true-positive results (sensitivity) and false-positive results (1-specificity) for various cutoff levels of each parameter. The cutoff level of sLR11 that gave the maximum sensitivity and specificity for PDR was 4.2 U. At the cutoff level, the sensitivity of sLR11 for PDR was 78%, and the specificity was 77%, equivalent to or more than the other classical risk factors, HbA1c, BMI, or estimated glomerular flow rate (Table 3).

Finally, to clarify the correlation between the sLR11 concentration and various clinical parameters in the studied patients, simple regression analyses were performed for the dependent variable (Table 4). The HbA1c levels and LDL cholesterol levels correlated positively with sLR11 ($r = 0.32$, $P <$ .01, and $r = .31$, $P <$ .05, respectively). No significant correlation was observed between the sLR11 and age, sex, BMI, fasting blood glucose, estimated glomerular flow rate, total cholesterol, HDL cholesterol, or triglyceride.

DISCUSSION

LR11 IS HIGHLY EXPRESSED IN THE ENDOTHELIAL CELLS under the condition of dyslipidemia as well as in the intimal smooth muscle cells migrated from media in the development of atherosclerosis.[2,3] Two recent independent studies for the subjects with dyslipidemia or coronary heart diseases have shown that the concentrations of soluble form, sLR11, were associated with the HbA1c levels in these subjects with different backgrounds.[4,5]

The key cytokines underlying the pathogenesis and development of PDR are similar to those leading to atherosclerosis. The barrier dysfunction of microvessels and retinal ischemia provokes an increase in the ocular levels of inflammatory cytokines and growth factors, including vascular endothelial growth factor, platelet-derived growth factor BB (PDGF-BB), and angiotensin II,[14–16] with increased expression of adhesion molecules,[17] all promoting retinal neovascularization. PDGF-BB and

angiotensin II trigger the increased expression of LR11 on vascular smooth muscle cells.[2,5] The LR11 expression in endothelial cells is induced under conditions of dyslipidemia, possibly through the activations of combination of cytokines and adhesion molecules.[2,3] Thus, considering that endothelial dysfunction is the first sign of microvascular injury at the organ level[18] and that the progression of diabetic microvascular complications is modulated by the severity of hyperglycemia through the gradual damages of the endothelium,[19] a high sLR11 concentration in the serum of diabetic patients with PDR may reflect the pathophysiologic endothelial dysfunction associated with diabetes, although the mechanism responsible for the release of sLR11 in circulation remains unresolved.

In the present study, the sLR11 levels in the PDR group were increased compared with the NPDR group, regardless of medication with insulin therapy (see Figure 1). The multivariate analysis of all variables showed that the circulating sLR11 level, as well as age, strongly associated with PDR, independent of other variables (see Table 2). The ROC analysis indicated that the sensitivity and specificity of sLR11 is the highest at a cutoff level of 4.2 as a marker of PDR (see Figure 2 and Table 3). Finally, the sLR11 concentration was correlated positively with the HbA1c level (see Table 4), which was consistent with previous observations with subjects with different profiles.[4,5]

Various studies on the pathogenesis of and risk factors for the development of PDR have been conducted, and hypertension and renal failure have been identified as important risk factors, along with poor blood glucose control.[20–23] ROC analyses using the present study subjects showed that the area under the ROC of sLR11 was equivalent to or more than those of the so-far established risk markers (see Table 3). Thus, LR11 may be an additional tool for discriminating patients with a high risk of developing diabetic retinopathy from the increasing population of patients with type 2 diabetes mellitus. Considering the lack of enough data for the role of LR11 in the basic mechanism of PDR, to clarify the clinical significance of LR11 in patients with PDR, further pathophysiologic studies to address the question that sLR11 is a marker or a triggering factor are required.

Thus, one limitation of the present investigation is the lack of information about the sLR11 data in the retina and proliferative membrane in patients with PDR. Second, the data may have been influenced by the continuous use of medication. The sLR11 levels of subjects with insulin therapy were not significantly different from those of subjects without insulin therapy (see Figure 1). In addition, most of the patients had received medication against hypertension with ARBs (see Table 1). Considering the fact that statins and ARBs inhibit the sLR11 expression in cultured cells,[2,5] the circulating sLR11 levels may be modified by these treatments. In this context, there was no significant difference in sLR11 levels between the subject groups with or without use of statin, or ACE-I, or ARB in the PDR subjects (data not shown). Finally, our results were obtained using relatively small sample sets. Clearly, further careful validation studies with larger sample sets to evaluate the effects of sLR11 on microvascular outcomes as primary end points will be required.

In summary, this study presented a novel and potentially clinically relevant new correlation of sLR11 with PDR, thus potentially providing a serum test to indicate patients at greater risk of developing PDR.

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Increased circulating soluble LR11 in patients with acute coronary syndrome

Manabu Ogita [a], Katsumi Miyauchi [a,*], Tomotaka Dohi [a], Shuta Tsuboi [a], Tadashi Miyazaki [a], Takayuki Yokoyama [a], Ken Yokoyama [a], Kazunori Shimada [a], Takeshi Kurata [a], Meizi Jiang [b], Hideaki Bujo [b], Hiroyuki Daida [a]

[a] Department of Cardiovascular Medicine, Juntendo University Graduate School of Medicine, Tokyo, Japan
[b] Department of Genome Research and Clinical Investigation, Chiba University Graduate School of Medicine, Chiba, Japan

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ABSTRACT

Background: LR11 (also so called SorLA or SORL1) is a novel marker of intimal smooth muscle cell (SMC) proliferation. Vascular SMCs play important roles in the development of atherosclerosis interacting with macrophages in a vulnerable plaque of patients with acute coronary syndrome (ACS).The present study determines whether soluble LR11 (sLR11) is associated with ACS.
Methods: We studied 100 patients with coronary artery disease (CAD) comprising 50 consecutive patients with acute coronary syndrome (ACS; mean age 62.3 ± 13.0 years; male 78.0%) who were successfully treated with percutaneous coronary intervention and 50 age- and sex-matched stable angina pectoris (SAP) patients as control. Concentration of sLR11 was measured by sandwich enzyme-linked immunosorbent assay method.
Results: Circulating sLR11 was significantly increased in patients with ACS compared with SAP (9.88 ± 2.78 vs. 8.18 ± 1.11 ng/ml, $p<0.01$). Multivariate logistic regression analysis indicated that sLR11 was independently associated with ACS (odds ratio (OR), sLR11 quartile increment, 2.18, 95% confidence interval (CI) 1.21–4.19, $p<0.01$). Among various biomarkers of acute coronary syndrome, hsCRP were significantly correlated with LR11 ($r=0.480$, $p<0.01$).
Conclusions: There is a statistical significant association between LR11 and ACS and may be a useful biomarker for the development of acute coronary syndrome.

1. Introduction

Acute coronary syndrome (ACS) remains a major cause of mortality and morbidity despite advances in cardiovascular therapies [1–3] and it is mainly characterized by the rupture of lipid-rich vulnerable atherosclerotic plaque with subsequent thrombus formation [4,5]. Many studies have investigated the pathological mechanisms of ACS and inflammation is recognized as a key step in the pathogenesis of acute thrombotic events [6,7]. A novel diagnostic biomarker derived from ruptured plaque would be useful but remains to be established.

LR11 (also so called SorLA or SORL1) is a member of the LDL receptor family, and is highly expressed in atheromatous plaques of animal experimental model, especially in intimal smooth muscle cell (SMC) but not in medial SMC [8,9]. The overexpression of LR11 protein enhances SMC migration via the activation of the urokinase-type plasminogen activator receptor that regulates inflammatory monocyte adhesion [9,10]. We previously reported that circulating LR11 can be immunologically detected in serum using a novel sandwich enzyme-linked immunosorbent assay (ELISA) and specific monoclonal antibodies against human LR11 [11,12]. Circulating soluble LR11 levels positively correlate with the intima-media thickness in patients with dyslipidemia [11]. In addition, we also demonstrated increased levels of soluble LR11 in patients with stable coronary artery disease [13]. However, circulating LR11 levels in ACS have not been evaluated. The present study therefore evaluated the clinical significance of circulating LR11 in patients with ACS.

2. Materials and methods

2.1. Subjects

The present study is a cross-sectional case–control study. We enrolled 50 patients with ACS who were successfully treated with percutaneous coronary intervention (PCI) at Juntendo University Hospital between November 2008 and December 2009 and 50 age- and sex-matched patients with stable angina pectoris (SAP) as controls (mean age 62.4 ± 12.7 years; male, 78 %). Patients with previous coronary revascularization, malignant disease, inflammatory disease, and hemodialysis were excluded from the study. We defined ACS as acute myocardial

Abbreviations: ACS, acute coronary syndrome; CRP, C-reactive protein; PCI, percutaneous coronary intervention; SAP, stable angina pectoris; SMC, smooth muscle cell; STEMI, ST-segment elevation myocardial infarction; UAP, unstable angina pectoris; uPAR, urokinase-type plasminogen activator.

* Corresponding author at: Department of Cardiovascular Medicine, Juntendo University Graduate School of Medicine, 2-1-1 Hongo, Bunkyo-ku, Tokyo 113-0033, Japan. Tel.: +81 3 3813 3111; fax: +81 3 5689 0627.
E-mail address: ktmmy@med.juntendo.ac.jp (K. Miyauchi).

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infarction (AMI) and unstable angina (UAP). AMI was defined as increased cardiac enzymes (troponin or MB fraction of creatine kinase) with ischemic symptoms and subclassified AMI as ST-segment elevation myocardial infarction (STEMI) and non ST-segment elevation myocardial infarction (NSTEMI) according to the presence or absence, respectively, of at least 0.1 mV ST-segment elevation in at least 2 contiguous leads. Unstable angina (UAP) was diagnosed based on the presence of ischemic symptoms with the ST–T change but without an increase in cardiac enzymes, or myocardial necrosis indicated as an increase in cardiac enzymes regardless of ST-segment change. The ACS group was divided into STEMI and UAP/NSTEMI subgroups. SAP was defined as effort angina with a stable profile of symptoms for at least 3 months before admission. Written informed consent was obtained from all patients to underego PCI using standard techniques. This study adhered to the Declaration of Helsinki and was approved by our institutional internal review board. The choice of stent type and device was left to the discretion of the operators at our cardiology center.

2.2. Blood samples

Arterial blood samples were collected from all patients before coronary angiography in the operating room. The samples were centrifuged at 1000×g for 10 min and serum samples were stored at −80 C. Soluble LR11 (sLR11) was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) method with our specific monoclonal antibodies directed against human LR11 that we previously established [12]. In brief, sLR11 in serum was immunologically identified as 250-kDa protein in serum fluid by SDS–PAGE separation, and was purified using a receptor-associated protein and monoclonal antibodies that bind to intact sLR11 without prior purification. The sLR11 immunoassay used a combination of anti-LR11 monoclonal antibodies (M3 and R14). Assay characteristics concerning sLR11 immunoassay, for example, the inter-, and intra-assay CVs, as well as the working range, and the mean backfit value for the lowest standard giving acceptable precision, and the lower limit of detection has been described in our previous article [12]. Concentrations of serum high-sensitive C-reactive protein (hs-CRP) were measured using a validated immunoassay and an autoanalyzer. Levels of circulating Troponin T (TnT) and CD40 ligand (CD40L) were measured in patients with ACS. Serum cardiac troponin T was measured using a chemiluminescent enzyme immunoassay kit (Determiner CL TnT, Kyowa Medex, Tokyo, Japan). CD40L was quantified using human CD40L ELISA kit (R&D systems, Minneapolis, MN). Other markers were determined by routine laboratory methods.

2.3. Statistical analysis

Results are expressed as means ± SD or as ratios (%) and numbers for categorical data. The distribution of continuous variables was visually assessed from frequency histograms and using the Kolmogorov–Smirnov test. The hs-CRP and blood glucose on admission (BG on Ad) were skewed and thus we used natural log-transformed hs-CRP and BG on Ad. Continuous variables were compared using an unpaired *t*-test or Mann–Whitney U-test. Categorical variables (presented as frequencies) were compared using either chi-square test or Fisher's exact probability test. LR11 values across the three groups were compared using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Associations between measured parameters were evaluated using Spearman's rank correlation coefficient analysis. Predictive variables for ACS adjusted for potential confounding factors were identified by multiple logistic regression analysis. The effects of biomarkers including hs-CRP, blood glucose on admission and sLR11 in this model were evaluated as quartile increments in the concentration of each. The univariate model included the variables of age, gender, diabetes, hypertension, dyslipidemia, current smoking, use of statins, angiotensin converting enzyme and/or angiotensin receptor blockers, hs-CRP, blood glucose on admission (BG on Ad) and sLR11. Statistically significant variables in the multivariable logistic regression analysis selected using stepwise forward selection were subsequently included in a new model. All data were statistically analyzed using JMP8.0 (SAS Institute Inc., Cary, NC) and SPSS v.18.0 (Chicago, IL). A $p<0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of subjects

A comparison of the baseline characteristics between the ACS and SAP groups is shown in Table 1. Diabetes, metabolic syndrome, and hypertension were comparable between them, whereas current smoking and dyslipidemia were more frequent in the ACS and SAP group, respectively. The levels of total cholesterol, LDL-C, hs-CRP, WBC, and blood glucose on admission were significantly higher in the ACS group.

3.2. Levels of LR11

Levels of circulating soluble LR11 were significantly higher in patients with ACS than that with SAP (9.88 ± 2.78 vs. 8.18 ± 1.11 ng/ml, $p<0.01$). We also compared LR11 levels among 50 ACS patients subclassified as 32 patients represented STEMI and 18 diagnosed as NSTEMI/UAP, respectively. Levels of sLR11 were significantly higher in patients with STEMI compared with the other 2 groups (Fig. 1).

Table 1
Baseline Clinical Characteristics.

	SAP (n = 50)	ACS (n = 50)	P
Age	62.4 ± 12.5	62.3 ± 13.0	NS
Male Gender, n (%)	39 (78.0)	39 (78.0)	NS
Diabetes, n (%)	18 (36.0)	17 (34.0)	NS
Metabolic syndrome	30 (60.0)	23 (46.0)	NS
Hypertension, n (%)	38 (76.0)	33 (66.0)	NS
Dyslipidemia, n (%)	43 (86.0)	34 (68.0)	< 0.05
Current Smoker, n (%)	9 (18.5)	28 (56.0)	< 0.01
Family history, n (%)	15 (30.0)	13 (61.1)	NS
CKD, n (%)	9 (18.0)	11 (22.0)	NS
Angiographic degree of CAD			NS
1-vessel disease, n (%)	26 (52.0)	26 (52.0)	
2-vessel disease, n (%)	11 (22.0)	14 (28.0)	
3-vessel disease, n (%)	13 (26.0)	10 (20.0)	
LVEF, %	62.7 ± 10.3	62.8. ± 9.9	NS
ACE-I/ARB	31 (62.0)	13 (26.0)	<0.01
Statin	37 (74.0)	13 (26.0)	<0.01
BMI, kg/m^2	24.2 ± 2.9	25.2 ± 3.5	NS
Waist, cm	88.2 ± 8.2	90.7 ± 8.2	NS
SBP, mmHg	146.3 ± 24.4	139.3 ± 24.0	NS
DBP, mmHg	77.5 ± 13.2	80.2 ± 17.9	NS
TC, mg/dl	171.1 ± 37.4	204.3 ± 38.5	<0.01
LDL-C, mg/dl	93.1 ± 30.2	131.3 ± 29.5	<0.01
HDL-C, mg/dl	49.3 ± 13.1	45.1 ± 11.1	0.09
TG, mg/dl	131.5 ± 52.8	145.7 ± 105.1	NS
FBG, mg/dl	103.5 ± 21.9	109.9 ± 28.2	NS
HbA1c, %	5.8 ± 1.1	6.2 ± 1.5	NS
BNP, ng/dl	56.3 ± 83.1	105.2 ± 209.4	NS
hs-CRP, mg/dl	0.25 ± 0.68	1.33 ± 2.67	<0.01
eGFR, ml/min/1.73 m^2	70.9 ± 16.3	76.2 ± 26.1	NS
WBC, /μl	5784 ± 1562	9489 ± 3125	<0.01
BG on admission	120.0 ± 38.8	166.3 ± 74.5	<0.01
sLR11, ng/ml	8.18 ± 1.11	9.88 ± 2.78	<0.01

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BG, blood glucose; BNP, brain natriuretic peptide; CAD, coronary artery disease; CKD, chronic kidney disease; DBP, diastolic blood pressure; FBG, fasting blood glucose; LAD, left anterior descending artery; LCX, left circumflex coronary artery; LVEF, left ventricular ejection fraction; RCA, right coronary artery; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides.

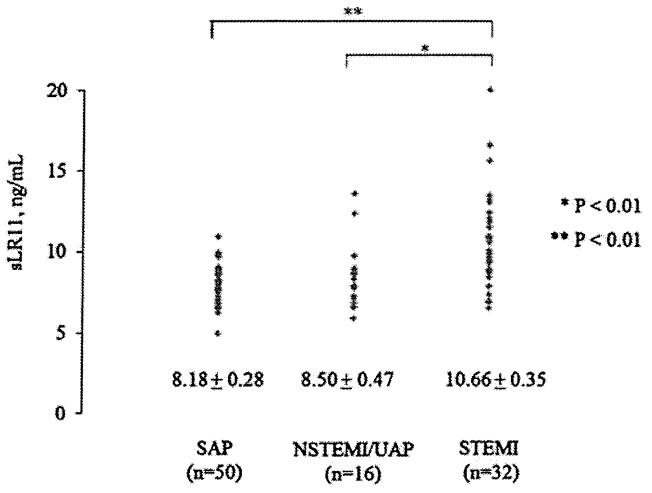

Fig. 1. Comparison of sLR11 among SAP, NSTEMI/UAP and STEMI. SAP, stable angina pectoris; NSTEMI/UAP, non ST-segment elevation myocardial infarction/unstable angina; STEMI, ST-segment elevation myocardial infarction.

3.3. Correlations between LR11 and clinical parameters

As shown in Table 2, serum LR11 levels positively correlated with BMI and LDL-C in all patients ($r=0.217$, $p<0.05$ and $r=0.304$, $p<0.01$, respectively). In ACS patients, LR11 positively correlated with hs-CRP ($r=0.480$, $p<0.01$, Fig.2A), WBC ($r=0.413$, $p<0.05$) and blood glucose on admission ($r=0.437$, $p<0.01$, Fig. 2B). LR11 tended to correlate swith TnT and CD40L but not significant ($r=0.231$, $p=0.11$ and $r=0.230$, $p=0.11$, respectively).

3.4. Multiple logistic regression analysis

We evaluated predictors of ACS for the entire study population using univariate and multivariate logistic regression analysis. The multivariate logistic regression analysis in Table 3 shows that sLR11 is independently associated with ACS after adjusting for confounding factors (odds ratio (OR), sLR11 quartile increment, 2.18, 95% CI 1.21–4.19, $p<0.01$).

4. Discussion

The present findings demonstrated that circulating soluble LR11 levels is significantly higher in patients with ACS than with SAP. We also showed that sLR11 is significantly and positively correlated with hs-CRP. Multivariate analysis indicated that increased sLR11 could be an independent variable for ACS after adjustment.

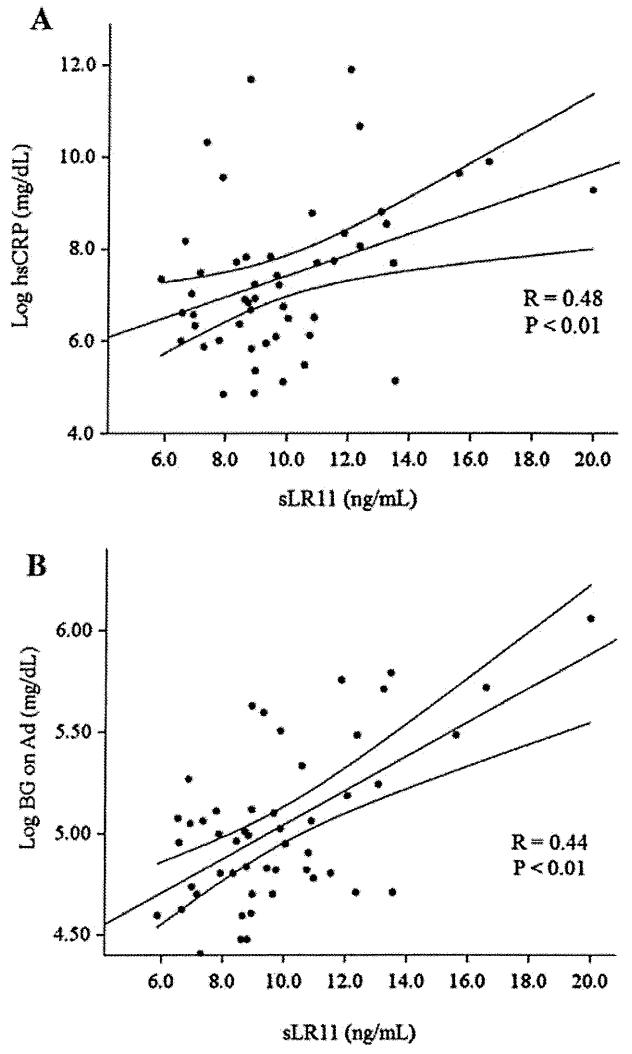

Fig. 2. Correlation between levels of sLR11 and various biomarkers in ACS. Fig. 2A, correlation between levels of sLR11 and hsCRP in ACS; Fig. 2B, correlation between levels of sLR11 and blood glucose on admission in ACS.

LR11, is a potential novel biomarker of vascular smooth muscle cells, that are abundantly expressed in the intimal SMC at the border between intima and media in plaque areas of apo E knockout mice [9]. Circulating LR11 positively correlates with intimal-media thickness in patients with dyslipidemia [11] and levels of soluble LR11 are higher in patients with coronary artery disease [13]. Our previous studies

Table 2
Correlations between LR11 and other parameters.

	r	P
Age	0.017	NS
BMI, kg/m^2	0.217	<0.05
SBP, mmHg	−0.029	NS
DBP, mmHg	0.130	NS
LDL-C, mg/dl	0.304	<0.01
HDL-C, mg/dl	−0.050	NS
TG, mg/dl	0.130	NS
BG, mg/dl	0.111	NS
eGFR, mL/min/1.73 m^2	−0.067	NS
HbA1c, %	0.020	NS
BNP, ng/mL	0.119	NS

BG on Ad, blood glucose on admission; DBP, diastolic blood pressure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglyceride.

Table 3
Univariate and multivariate logistic regression analysis model for prediction of ACS.

	Univariate			Multivaritate		
	OR	95%CI	P	OR	95%CI	P
Age, y	1.00	0.97–1.03	NS	1.04	0.99–1.10	NS
Gender, male	1.00	0.38–2.60	NS	Not selected		
Diabetes, yes	0.92	0.40–2.09	NS	0.24	0.04–0.91	<0.05
Hypertension, yes	0.61	0.25–1.46	NS	Not selected		
Dyslipidemia, yes	0.34	0.12–0.90	0.03	Not selected		
Current smoking, yes	5.80	2.40–15.1	<0.01	11.9	2.59–74.0	<0.01
Statin use, yes	0.13	0.05–0.30	<0.01	0.38	0.10–1.33	NS
ACEI/ARB use, yes	0.22	0.09–0.51	<0.01	0.36	0.09–1.31	NS
High-sLR11, QI	1.92	1.32–2.88	<0.01	2.18	1.21–4.19	<0.01
High-hsCRP, QI	2.48	1.65–3.90	<0.01	2.06	1.16–3.91	<0.05
High-BG on Ad, QI	2.18	1.47–3.32	<0.01	2.83	1.52–5.83	<0.01

ACE-I, angiotensin-converting enzyme inhibitors; ARB, angiotensin receptor blockers; BG on Ad, blood glucose on admission; QI, quartile increment.

implied that circulating LR11 reflects the pathological status of vascular smooth muscle cells in atherosclerotic lesions. Vascular smooth muscle cells play important roles in the development of atherosclerosis. Medial SMC migrate into the subendothelial space, proliferate, and produce extracellular matrix to form atheromatous plaques in response to inflammatory cytokines. The precursor of ruptured culprit lesions in patients with ACS is a thin cap fibroatheroma, characterized by a necrotic core and an overlying thin cap that lacks an extracellular collagen matrix [7,14]. The present study found significantly higher levels of soluble LR11 in patients with ACS. The pathological roles of LR11 in vulnerable plaque remain uncertain, but several possible explanations can be considered. Macrophages and vascular smooth muscle cells promote the local release of matrix metalloproteinases (MMP) that degrade supportive collagen, resulting in fibrous cap breakdown and enhanced plaque vulnerability [15–17]. The phenotypic modulation of smooth muscle cells influences the production of matrix-degenerating enzymes, monocyte recruitment and the expression of pro-inflammatory cytokines [18] and LR11 is expressed in synthetic intimal smooth muscle cells [19] that produce higher levels of matrix-degrading proteases resulting in thinning of the fibrous cap.

LR11 also significantly and positively correlated with hs-CRP, which plays an important role as an inflammatory mediator. Inflammation drives the formation and progression of atherosclerotic plaque and CRP is predominantly produced in the liver during the acute phase, but it is also expressed in smooth muscle cells within atherosclerotic lesions [20], where it is implicated in various aspects of atherogenesis and plaque vulnerability. We reported that soluble LR11 also regulates adhesion, migration and lipid accumulation in macrophages through urokinase-type plasminogen activator receptors [21]. We also demonstrated that sLR11 enhances scavenger receptor expression that contributes to foam cell formation in atherogenesis in vivo. These results imply that LR11 is a potential regulator of vulnerable plaque formation.

Soluble LR11 enhances the expression of urokinase-type plasminogen activator (uPAR) in macrophages and its expression is increased in the circulating monocytes of patients with AMI [22]. Urokinase-type plasminogen activator implicated in a broad spectrum of pathophysiological processes, including inflammation, fibrinolysis, proteolysis, atherogenesis and plaque destabilization, all of which contribute to the pathogenesis of myocardial infarction [23,24]. Cozen AE, et al. reported that macrophage-targeted overexpression of uPAR causes accelerated atherosclerosis, coronary artery occlusion, and premature death in apo E knockout mice [25]. Another study demonstrated that high levels of uPAR expression in vulnerable carotid plaque with high macrophage density and a ruptured fibrous cap [26]. These findings suggest that levels of soluble LR11 in ACS that are elevated via uPAR activation are associated with these pathophysiological conditions.

The present study has several limitations. Firstly, this is a single center study with a small patient cohort and thus unknown confounding factors might have affected the results regardless of the adjusted analysis. Further studies with a larger sample size are needed to confirm the results and to validate the clinical implication of LR11 as a diagnostic biomarker of ACS. Secondly, the limitations inherent in any cross-sectional study that a single sample at a specific time point might not reflect the natural course of a disease must be considered. The relationship between LR11 and other biomarkers at serial time points after patients with ACS are admitted to the hospital require further validation. Thirdly, several experimental studies have found that statin and angiotensin II type1 receptor blockers inhibit LR11 expression in SMCs [11,19]. More patients with the SAP group received statins and ARBs than the ACS group in the present study. This is because stable angina pectoris patients had been medically treated on the basis of current guideline, in contrast, most of patients with ACS admitted to our hospital for the first time without any medical background. These drugs might have attenuated circulating LR11 levels. In conclusion, there is a statistical significant association between LR11 and ACS. We believe that LR11 might serve as new biomarker reflecting different aspects of atherosclerosis, however the mechanism of how sLR11 plays a role in the pathophysiological conditions of ACS requires elucidation.

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Monosodium glutamate is not associated with overweight in Vietnamese adults

Vu Thi Thu Hien[1,2], Nguyen Thi Lam[1], Nguyen Cong Khan[3], Andrea Wakita[2] and Shigeru Yamamoto[2,*†]

[1]National Institute of Nutrition, Hanoi, Vietnam: [2]Department of International Nutrition, Graduate School of Humanities and Sciences, Ochanomizu University, Tokyo, Japan: [3]Vietnam Food Administration Organization, Hanoi, Vietnam

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Abstract

Objective: To determine the prevalence of and associated factors for overweight, especially to determine the relationship between the intake of monosodium glutamate (MSG) as a seasoning and overweight in Vietnam.

Design: A cross-sectional survey was conducted of Vietnamese adults aged ≥20 years in 2008. Dietary intake was assessed by the 24 h recall method for 3 d. MSG intake was evaluated by the weighing method on three consecutive days. Physical activity was assessed based on the Global Physical Activity Questionnaire recommended by the WHO. Overweight was defined as BMI ≥ 23·0 kg/m^2. Other characteristics such as age and lifelong occupation were determined by a structured questionnaire.

Setting: Some rural and urban areas of Hanoi, Thua Thien Hue Province and Ho Chi Minh City, Vietnam.

Subjects: A total of 1528 adults living in surveyed areas were randomly selected by the multistage cluster sampling method.

Results: The prevalence of overweight was 27·9%, and 81·0% of participants were MSG users. Average MSG intake was 2·2 (SD 1·8) g/d. Multiple logistic regression analysis revealed that factors associated with overweight were age, region of residence, lifelong occupation, physical activity and intakes of energy, carbohydrates, saturated fat and animal protein. There was no significant association between MSG intake and overweight.

Conclusions: The study demonstrated that overweight was not associated with MSG intake in Vietnamese adults. Further longitudinal studies should be done in different populations to determine the relationship between MSG and overweight.

Keywords
Overweight
Monosodium glutamate

Even though chemical, biochemical and toxicological evaluations made by the Joint Expert Commission on Food Additives of the Codex Alimentarius Commission have shown that there is no need for establishing an Acceptable Daily Intake value for monosodium glutamate (MSG)[1,2], there is a need to conduct research on the health consequences of MSG since it has become one of the world's most widely used food additives. One of the issues of concern is weight gain related to MSG consumption. This has been a controversial problem not only in animal but also in human studies. In animal reports, it has been shown that weight gain was a result of destruction in several brain regions by high doses of MSG by injection, without food and during the neonatal period[3,4]. However, these animal studies involved doses that people are incapable of consuming. Moreover, other animal experiments have shown that there was no brain damage[5] or any positive association between MSG and obesity[6,7]. In human studies, there have been controversial reports on the association between MSG consumption and obesity in different areas of China since 2008. The MSG intakes in these studies were found to range from a mean of 0·33 to 3·8 g/d[8–10]. At present, overweight and obesity are increasing around the world, even in developing countries[11–13]. In Vietnam, along with a growing economy, dietary patterns and lifestyles have changed profoundly. Consumption of meat and cholesterol-rich food has increased compared with previously, which would suggest that overweight and obesity have been increasing among the Vietnamese population[14–16].

† Correspondence address: International Nutrition, Department of Food and Nutritional Sciences, Graduate School of Human Life Sciences, Jumonji University, 2-1-28 Sugasawa, Niiza-City, Saitama 352-8510, Japan.

*Corresponding author: Email syamamototokushima@hotmail.com

In addition, the Vietnamese people have long been familiar with the L-glutamate taste from traditional fermented seasonings, such as fish sauce and soya sauce. MSG has been also used as a food seasoning to enhance the taste of foods and meals, by shaking MSG on to foods during preparation. Since there have been controversial reports concerning the association between MSG intake and overweight/obesity[8–10], there is an urgent need to investigate this relationship in the Vietnamese population.

For these reasons, a cross-sectional survey was done to determine the prevalence of and associated factors with overweight, especially to determine the relationship between intake of MSG as a seasoning and overweight in Vietnamese adults.

Materials and methods

Setting and study participants

The study was conducted according to the guidelines laid down in the Declaration of Helsinki and it was approved by the Research and Ethical Committee of the Vietnamese National Institute of Nutrition. The survey was carried out in Hanoi, Thua Thien Hue Province and Ho Chi Minh City, which are located in the north, centre and south of Vietnam, respectively.

A multistage sampling method was used to select participants. In the first step, in each selected province/city, one commune in a rural area and one ward in an urban area were chosen randomly from the list of all communes/wards. Second, lists of all households in the selected communes/wards were established and family codes were created. From this list, a first family was selected by randomly picking a family code. Households in which all family members usually took their meals at home and had at least one member aged ≥20 years were selected for the study. All family members aged ≥20 years were invited to participate in the study. Individuals were excluded if they had any of the following factors: malformation; chronic or acute disease (such as cancer or acute infection); pregnant and lactating women; or on a special diet for weight loss, weight gain, vegetarianism, salt reduction, diabetes mellitus or other reasons.

After choosing the first family, we approached other families by using the 'random walking' method to obtain 255 adults aged ≥20 years (about eighty households) for each commune/ward. By this sampling procedure, a total of 1530 adults in six communes/wards were selected for participation in the survey. Written informed consent was obtained from all participants before conducting the survey.

Assessment of variables

Data were collected by trained researchers and doctors at the participant's home. In interviews and examinations, the doctors employed a specially designed questionnaire which included questions related to demographic variables (age, sex, region of residence and ethnic background), education, occupation, physical activities and lifestyle factors. Medical history of the participant was also requested.

Lifelong occupation was defined as the occupation that the participant engaged in most frequently in her/his life. It was classified as heavy worker (farmers, manual workers), office worker (office clerks and other sedentary jobs) or domestic work (housewife).

Education level was categorized into three groups by years of schooling: low level (≤5 years), medium level (6–8 years) and high level (≥9 years).

Body height and weight were measured while the participant was standing on a stable plane, wearing light clothing and no shoes. BMI (kg/m^2) was calculated as the ratio of weight (in kilograms) to the square of height (in metres). Waist circumference was measured at the minimum circumference between the umbilicus and iliac crest, and hip circumference was measured at the widest circumference around the buttocks. Overweight was defined as BMI ≥ 23·0 kg/m^2 based on WHO recommendations for Asian populations[17].

Physical activity was assessed according to the Global Physical Activity Questionnaire recommended by WHO[18]. Activity levels were categorized as low, moderate and high based on MET (metabolic equivalent of task) values.

Dietary intake was assessed by the 24 h recall method for three consecutive days at the participant's home, while MSG intake was evaluated by the weighing method using a precise scale on the same days. The participant was asked to eat meals prepared at home during the three survey days. On those days, a researcher came to the survey household before and after each meal. Before each meal, the researcher weighed bottles of pure MSG and any seasonings (including sauces or powder) which included MSG. After the meal, the researcher returned to the household to weigh the seasoning bottles again, and to ask the participant to recall the type and amount of any food consumed during the meal. To improve the accuracy of food descriptions, a full-size photograph album of common foods and household measures (such as bowls, cups and spoons) was used during interviews to define appropriate amounts. The participant was also asked to recall the amounts of any snacks and drinks consumed between meals.

The amount of seasonings consumed by all family members during a meal was determined by the difference between the weight of the seasoning bottles before and after the meal. The MSG content in seasonings was calculated from food labels combined with analysis. Therefore, the MSG intake of all family members was equal to the amount of seasonings multiplied by the MSG content of each seasoning. Individual MSG intake at each meal was calculated according to the MSG intake of all family members multiplied by the proportion of actual food intake of the given individual. The participant's daily

Table 1 Characteristics of the study participants: adults aged ≥20 years (*n* 1528) from rural and urban areas of Hanoi, Thua Thien Hue Province and Ho Chi Minh City, Vietnam, 2008

Variable	Urban (*n* 721)		Rural (*n* 807)	
	Mean	SD	Mean	SD
Age (years)	45·7	15·6	45·5	15·6
BMI (kg/m^2)	21·6	3·2	21·0	3·1
WHR	0·87	0·07	0·86*	0·07
Physical activity level (%)				
Low	49·9		43·0	
Moderate	18·6		16·2	
High	31·5		40·8	
Lowest education level (%)	13·4		27·0**	
Lifelong occupation heavy worker (%)	13·7		21·6**	
Energy intake (kJ/d)	8226	2134	7941*	1937
Energy intake (kcal/d)	1966	510	1898*	463
Plant protein intake (g/d)	41·5	16·3	41·4	15·0
Animal protein intake (g/d)	34·2	18·8	28·2**	18·7
MSG intake (g/d)	2·3	1·8	2·1	1·8

WHR, waist-to-hip ratio; MSG, monosodium glutamate.
Data are presented as mean and standard deviation or percentage. Values were compared between rural and urban areas by the independent *t* test or the χ^2 test.
Values were significantly different from those in urban areas: *$P<0·05$, **$P<0·01$ (two-sided).

MSG intake was calculated by totalling the amount of his/her individual MSG intake at all meals for 3 d, then dividing by three.

Statistical analysis

Data are presented as percentages or means and standard deviations. Continuous variables were $\log_{10}$ transformed if not normally distributed. Student's *t* test (two-tailed) was applied to examine differences in age, BMI, waist-to-hip ratio, energy intake and MSG intake between participants according to region of residence (rural *v.* urban). The χ^2 test was used to examine differences in prevalence of physical activity level, education level and lifelong occupation of heavy worker between participants in rural and urban areas. Multiple logistic regression analysis was used to test several models for the associations between overweight and other variables. Data are presented as odds ratios and 95% confidence intervals. Associations were considered statistically significant at $P<0·05$.

All statistical procedures were performed with the statistical software package SPSS for Windows version 10·0 (SPSS Inc.).

Results

Characteristics of participants

Two persons refused to complete all the procedures for the study. Thus, data for 1528 adults, including 706 males and 822 females, were analysed for the present report. The mean age of participants was 45·6 (SD 15·6) years. As shown in Table 1, there were no significant differences in age and BMI between urban and rural areas. Waist-to-hip ratio and energy intake in rural areas were significantly lower than in urban areas ($P<0·05$). Prevalence of participants with the lowest education level was higher in rural areas than in urban areas ($P<0·01$), as was the prevalence of participants with a lifelong occupation of heavy worker ($P<0·05$).

Table 2 Prevalence of overweight/obesity (%) by sex and region of residence: adults aged ≥20 years (*n* 1528) from rural and urban areas of Hanoi, Thua Thien Hue Province and Ho Chi Minh City, Vietnam, 2008

	Males	Females	Total
Urban	31·1	32·4	31·8
Rural	22·1	26·4	24·4*
Total	26·2	29·4	27·9

Data are presented as percentage. Values were compared between males and females, rural and urban areas by the χ^2 test.
Value was significantly different from that in urban areas: *$P<0·05$.

All participants took their meals at home during the survey period, of whom 81·0% were MSG users. Average MSG seasoning intake was 2·2 (SD 1·8) g/d. Therefore, glutamate (GLU) from MSG was estimated as 1·9 (SD 1·5) g/d. Average animal and plant protein intakes were 31·0 (SD 19·0) g/d and 41·4 (SD 15·2) g/d, respectively. Thus, GLU from food was estimated to be equal to 14·7 (SD 6·7) g/d. There was no significant difference between rural and urban areas with regard to MSG intake as seasoning. There were significant differences in energy intake ($P<0·05$) and animal protein intake ($P<0·01$) between rural and urban areas (Table 1).

Prevalence of overweight

Table 2 shows that there was no significant difference in overweight prevalence between males and females. The prevalence was 26·2% and 29·4% in males and females, respectively. The prevalence in rural areas (24·4%) was significantly lower than that in urban areas (31·8%, $P<0·05$).

Table 3 Odds ratios and 95% confidence intervals of predictors for overweight: adults aged ≥20 years (*n* 1528) from rural and urban areas of Hanoi, Thua Thien Hue Province and Ho Chi Minh City, Vietnam, 2008

	OR	95% CI	*P* value
Region of residence			
Urban	1·00	Ref.	–
Rural	0·77	0·60, 0·98	<0·05
Sex			
Female	1·00	Ref.	–
Male	0·87	0·68, 1·11	NS
Age (per 1 quartile increment)	1·26	1·12, 1·43	0·001
Lifelong occupation			
Office worker	1·00	Ref.	–
Heavy worker	0·45	0·29, 0·68	<0·001
Housewife	1·14	0·83, 1·57	NS
Physical activity level			
High	1·00	Ref.	–
Moderate	1·20	0·91, 1·58	NS
Low	1·50	1·08, 2·09	<0·05
Energy intake (per 1 tertile decrement)	0·64	0·47, 0·85	<0·01
Saturated fat intake (per 1 quartile increment)	1·53	1·33, 1·75	<0·001
Carbohydrate intake (per 1 tertile increment)	1·65	1·28, 2·12	<0·001
Animal protein intake (per 1 quartile increment)	1·20	1·06, 1·35	<0·001
MSG intake			
Non-user	1·00	Ref.	–
1st quartile	1·15	0·80, 1·65	NS
2nd quartile	0·87	0·56, 1·35	NS
3rd quartile	0·69	0·47, 1·00	NS
4th quartile	0·88	0·63, 1·22	NS

MSG, monosodium glutamate; Ref. referent cetagory.
OR and 95% CI from multiple logistic regression analysis, model was adjusted for education level and smoking.

Associated factors for overweight

Table 3 shows results of the multiple logistic regression model applied to analyse associations between overweight and related variables. It reveals that age, region of residence, lifelong occupation, physical activity level and intakes of energy, carbohydrates, saturated fat and animal protein acted as significant predictors for overweight. There was no significant association between overweight and the intake of MSG as food seasoning. The model was adjusted for education level and smoking. Increased age was positively related to the risk of overweight ($P<0·001$). Participants living in rural areas ($P<0·05$) and those whose lifelong occupation was heavy work ($P<0·001$) had a significantly reduced risk of overweight. Participants who did physical activity at low levels had an overweight prevalence 1·5 times higher than those who did so at high levels ($P<0·05$).

Reduced energy intake was associated with a significantly reduced risk of overweight ($P<0·01$). Increased intakes of carbohydrates, saturated fat and animal protein were separately related to a higher risk of overweight ($P<0·001$ for all).

Discussion

The present report demonstrates that the prevalence of overweight was 27·9% in a large sample of adults living in three different regions of Vietnam. This is lower than the prevalence in Western countries and China, which ranges from 48 to 73%[19–21], but similar to that in other Asian countries, which ranges from 17 to 26%[22–25]. Concerning overweight in urban areas, the prevalence in the three regions in the current study was 31·1% and 32·4% in males and females, respectively. These prevalences are similar to those in 2004 in Ho Chi Minh City, the largest city of Vietnam. This shows that overweight is a noteworthy problem in Vietnam, especially in urban areas.

Factors contributing to overweight and obesity have been given great attention and studied extensively[21,26–28]. Similar to previous studies, our data also found that increasing age and less physical activity are predictors of overweight[21,26,27]. Participants who had a lifelong occupation as heavy workers had less risk of overweight than those who did not. In addition, our study confirmed the previous findings that increased intakes of energy, carbohydrates, saturated fat and animal protein were separately associated with increased risk of overweight[28,29]. We observed that the prevalence of overweight in urban areas was significantly higher than in rural areas. This can be explained by the fact that most of the participants in rural areas were farmers who had lifelong occupation as heavy workers and had energy intakes lower than those in urban areas, so they had less risk of overweight than participants in urban areas.

The present survey is the first one done in Vietnam concerning the intake of MSG as seasoning, and its major strengths are that we selected a large number of participants representative of three ecological regions of the country and that we chose an appropriate method for