Associations Among Serum Lipoprotein(a) Levels, Apolipoprotein(a) Phenotypes, and Myocardial Infarction in Patients With Extremely Low and High Levels of Serum Lipoprotein(a)

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A high serum lipoprotein(a) [Lp(a)] level, which is genetically determined by apolipoprotein(a) [apo(a)] size polymorphism, is an independent risk factor for coronary atherosclerosis. However, the associations among Lp(a) levels, apo(a) phenotypes, and myocardial infarction (MI) have not been studied. Patients with MI (cases, n=101, M/F: 86/15, age: 62±10y) and control subjects (n=92, M/F: 53/39, age: 58±14y) were classified into quintile groups (Groups I to V) according to Lp(a) levels. Apo(a) isoform phenotyping was performed by a sensitive, high-resolution technique using sodium dodecyl sulfate-agarose/gradient polyacrylamide gel electrophoresis (3–6%), which identified 26 different apo(a) phenotypes, including a null type. Groups with higher Lp(a) levels (Groups II, III, and V) had higher percentages of MI patients than that with the lowest Lp(a) levels (Group I) (54%, 56%, or 75% vs. 32%, $p<0.05$). Groups with different Lp(a) levels had different frequency distributions of apo(a) isoform phenotypes: Groups II, III, IV, and V, which had increasing Lp(a) levels, had increasingly higher percentages of smaller isoforms (A1–A4, A5–A9) and decreasingly lower percentages of large isoforms (A10–A20, A21–A25) compared to Group I. An apparent inverse relationship existed between Lp(a) and the apo(a) phenotype. Subjects with the highest Lp(a) levels (Group V) had significantly ($p<0.05$) higher serum levels of total cholesterol, apo B, and Lp(a). Patients with MI and the controls had different distributions of apo(a) phenotypes: ie, more small isoforms and more large size isoforms, respectively (A1–A4/A5–A9/A10–A20/A21–A25: 35.7%/27.7%/20.8%/15.8% and 22.8%/23.9%/29.4%/23.9%, respectively). Lp(a) (parameter estimate±standard error: 0.70±0.20, Wald $\chi^2=12.4$, $p=0.0004$), apo(a) phenotype (−0.43±0.15, Wald $\chi^2=8.17$, $p=0.004$), High-density lipoprotein-cholesterol, apo A-I, and apo B were significantly associated with MI after adjusting for age, gender, and conventional risk factors, as assessed by a univariate logistic regression analysis. The association between Lp(a) and MI was independent of the apo(a) phenotype, but the association between the apo(a) phenotype and MI was not independent of Lp(a), as assessed by a multivariate logistic regression analysis. This association was not influenced by other MI- or Lp(a)-related lipid variables. These results suggest that apo(a) phenotype contributes to, but does not completely explain, the increased Lp(a) levels in MI. A stepwise logistic regression analysis with and without Lp(a) in the model identified Lp(a) and the apo(a) phenotype as significant predictors for MI, respectively. (*Jpn Circ J* 1999; 63: 659–665)

Key Words: Apolipoprotein(a); Lipoprotein(a); Myocardial infarction; Phenotypes

Lipoprotein(a) [Lp(a)] is a heterogeneous lipoprotein¹ that incorporates a low-density lipoprotein (LDL) particle and highly polymorphic apolipoprotein(a) [apo(a)], which is covalently linked to the apolipoprotein B moiety of the LDL by a single disulfide bridge.^{2,3} Apo(a) is highly homologous to plasminogen and affects the human coagulation system.^{4,5} The *apo(a)* gene locus is the major gene that controls plasma Lp(a) concentrations.^{6,7} The alleles at this locus determine the size polymorphism of apo(a), which is the result of a variable number of expressed

kringle IV repeats^{6–8} and is assessed by apo(a) genotyping.⁷ Utermann et al first reported a size polymorphism of 6 apo(a) isoforms with different molecular weights,⁸ and other researchers have detected additional apo(a) isoforms using different detection methods.^{9,10} We recently detected 26 different apo(a) alleles, including a null allele, at the apo(a) locus by a sensitive high-resolution technique using sodium dodecyl sulfate (SDS)-agarose/gradient polyacrylamide gel electrophoresis (PAGE).¹¹ Approximately 75% of the subjects had a double band of apo(a), and the remaining subjects had a single band. An inverse relationship has been demonstrated between plasma Lp(a) concentrations and the size of apo(a).^{8,11} and a smaller Lp(a) band was well correlated with Lp(a) levels in double-banded phenotypes.^{11–13}

High plasma levels of Lp(a) have been shown to be associated with myocardial infarction (MI),^{14–17} although this association was not confirmed by 2 prospective studies.^{18,19} Apo(a) size polymorphism has been shown to be inversely associated with the risk of coronary heart disease (CHD) in

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patients with end-stage renal disease;²⁰ hypertension (HT)²¹ or diabetes mellitus (DM);¹² those undergoing coronary bypass surgery;²² and hemodialysis patients with or without DM.¹³ However, the relation between apo(a) isoform size polymorphism and MI has rarely been studied,²³ and it would be interesting to determine whether, and how much, apo(a) isoform size polymorphism contributes to the increased serum Lp(a) levels in MI, because an inverse relation does exist between plasma Lp(a) levels and apo(a) size.^{8,11}

In the present study, we studied the associations among serum Lp(a) levels, apo(a) phenotypes, and MI. Because large differences in Lp(a) levels can be used to predict important associations, we initially targeted patients with MI as well as normal controls, and then selected the subjects with extremely low or high serum Lp(a) levels from among these.

Methods

Study Patients

The subjects, consisting of 101 patients with MI and 92 normal controls at Fukuoka University Hospital, were classified into 5 groups according to quintile values of serum Lp(a) levels: Group I, $n=41$, $Lp(a) \leq 10$ mg/dl; Group II, $n=39$, $10 \text{ mg/dl} < Lp(a) \leq 20$ mg/dl; Group III, $n=36$, $20 \text{ mg/dl} < Lp(a) \leq 29$ mg/dl; Group IV, $n=37$, $29 \text{ mg/dl} < Lp(a) < 48$ mg/dl; and Group V, $n=40$, $Lp(a) \geq 48$ mg/dl. Cases were defined as patients with old MI; the diagnosis of MI was made from symptoms, electrocardiogram (ECG) and serum creatine kinase isoenzyme (CK-MB) of more than twice the upper level of normal. Cardiac catheterization analysis, including coronary angiography (CAG) and left ventriculography, were performed in the acute phase, and the definitive diagnosis of MI was made. Controls were defined as those who had normal coronary arteries ($<25\%$ stenosis) as proven by CAG and who had no clinical evidence of CHD on history and exercise ECG. Patients with acute MI (within 3 weeks of the onset), heart failure (Killip Class ≥ 2 after MI), vascular disease (aortitis treated by prednisolone), hepatic dysfunction (viral and non-viral, transaminases more than 3 times the normal value), or uncontrollable DM were excluded from the study. Patients with systolic or diastolic blood pressure greater than 160 mmHg or 95 mmHg, respectively, or who were under anti-hypertensive treatment were considered to have HT. Patients under treatment for DM and/or with symptoms of DM and a fasting glucose concentration ≥ 126 mg/dl were considered to have DM. Otherwise, the results of a 75-g glucose tolerance test were used to diagnose DM. All of the women were in menopause, but none were receiving hormone replacement therapy. This study was approved by the ethics committee of Fukuoka University Hospital and informed consent was obtained from each patient.

Coronary Angiography

Coronary arteries were cannulated by the Judkins technique²⁴ with 5F catheters, and recorded on Kodak 35-mm cinefilm at a rate of 25 frames/s. Coronary arteries were divided into 15 segments, according to the classification of the American Heart Association Grading Committee. The presence of stenosis was determined using a computer-assisted CAG analysis system (Mipron 1; Kontron Co) after the direct intracoronary injection of isosorbide dinitrate (ISDN) (2.5 mg/5-ml solution). One minute after the

injection of ISDN through the Judkins catheter, which took 20 s, CAG was performed from several projections during 7 min, as described previously.^{25,26}

Determination of Serum Lipids, Lipoproteins, Apolipoproteins, and Apo(a) Isoform Phenotype

A blood sample was drawn in the morning after an overnight fast. Serum total cholesterol (TC) and triglyceride (TG) concentrations were determined enzymatically. High-density lipoprotein-cholesterol (HDL-C) was determined by the heparin Ca^{2+} precipitation method. HDL subfractions (HDL₂ and HDL₃) were separated by standard sequential preparative ultracentrifugation techniques. Apolipoprotein (apo) A-I, apo A-II, apo B, apo C-II, apo C-III, and apo E were determined by the turbidity immunoassay method.²⁷ Serum Lp(a) was determined by enzyme-linked immunosorbent assay (ELISA) using a Tint Eliza Lp(a) kit (Biopool, Sweden).²⁸ For all measurements in our laboratory, the interassay and intra-assay coefficients of variation were less than 7% and 5%, respectively, and blinded quality-control specimens were included in each assay.

Apo(a) phenotyping was performed by the method of Kikuchi et al,¹¹ as we described previously.^{12,13}

Statistical Analysis

Statistical analysis was performed using the SAS Software Package (Version 6.12, Statistical Analysis System, SAS Institute Inc, Cary, NC, USA). Categorical variables (such as gender) and frequency distribution patterns of the apo(a) phenotype between groups were compared by a chi-square analysis. The distribution of continuous variables was examined by the Shapiro-Wilk test.²⁹ Quintile values of Lp(a) (20th percentile: 10 mg/dl; 40th percentile: 20 mg/dl; 60th percentile: 29 mg/dl; 80th percentile: 48 mg/dl) were used to classify subjects into 5 groups with increasing Lp(a) levels from Group I to Group V. Differences of continuous variables between groups were examined by an analysis of variance (ANOVA), and age and gender were adjusted for by an analysis of covariance.³⁰ The associations among serum Lp(a) levels, apo(a) phenotype, and MI after controlling for age, gender, and potentially confounding variables were evaluated by a multiple logistic function analysis.³¹ For logistic parameter estimates, we showed the standard error. Independent associations of variables with MI were also examined by a stepwise logistic regression analysis.³¹ All *p* values are 2-tailed. The significance level was considered to be 5% unless otherwise indicated.

Results

Table 1 shows the clinical characteristics of the 5 groups of subjects classified according to the quintile values of serum Lp(a) levels, and the frequency distribution of apo(a) isoform phenotypes. Age, gender, body mass index (BMI), and prevalence of HT, DM, and smokers were not significantly different among the 5 groups. However, there were significantly ($p < 0.05$) more MI patients in each of Groups II, III, and/or V (with extremely high Lp(a) levels) than in Group I (with extremely low Lp(a) levels), and the linear trend of the prevalence of MI across these quintile groups of Lp(a) was significant as assessed by the Mantel-Haenszel chi-square test. The frequency distributions of the apo(a) phenotypes in Groups II, III, IV, and/or V were different

Table 1 Clinical Characteristics of Subjects Grouped According to Quintile Values of Serum Levels of Lipoprotein (a) [Lp(a)], and the Frequency Distribution of the Apolipoprotein(a) [Apo(a)] Isoform Phenotype

	Group I (n=41)	Group II (n=39)	Group III (n=36)	Group IV (n=37)	Group V (n=40)
Age (years)	59±13	59±15	60±11	63±13	60±11
Gender (male/female)	29/12	31/8	28/8	23/14	28/12
Body mass index (kg/m ²)	22.4±2.8	23.5±3.2	23.0±2.6	23.4±2.7	23.6±3.8
Hypertension (%)	34	33	53	46	25
Diabetes mellitus (%)	20	38	25	27	18
Smokers (%)	46	51	58	35	60
Myocardial infarction (%)	32	54*	56*	46	75*†
Apo(a) isoform phenotype					
I (A1–A4)	0	5*	6*	12*	34*†
II (A5–A9)	6	12	8	21	3
III (A10–A20)	8	17	17	3	3
IV (A21–A25)	27	5	5	1	0

Group I, subjects with Lp(a) levels ≤ 10 mg/dl (20th percentile); Group II, subjects with Lp(a) levels > 10 mg/dl and ≤ 20 mg/dl (40th percentile); Group III, subjects with Lp(a) levels > 20 mg/dl and ≤ 29 mg/dl (60th percentile); Group IV, subjects with Lp(a) levels > 29 mg/dl and < 48 mg/dl (80th percentile); Group V, subjects with Lp(a) levels ≥ 48 mg/dl.

*p<0.05 vs Group I, by chi-square analysis. †p<0.05, linear trend as assessed by the Mantel-Haenszel chi-square test.

Fig 1. Plot of serum lipoprotein(a) [Lp(a)] levels versus the apolipoprotein(a) [apo(a)] isoform phenotype in subjects grouped according to quintile values of Lp(a) [Group I (quintile I), (patients with MI) and (controls); Group II (quintile II), (patients with MI) and (controls); Group III (quintile III), (patients with MI) and (controls); Group IV (quintile IV), (patients with MI) and (controls); Group V (quintile V), (patients with MI) and (controls)].

($p<0.05$) from those in Group I; Groups II, III, IV and V had more small isoforms (low molecular weight, A1–A4 and A5–A9) and fewer large isoforms (high molecular weight, A10–A20 and A21–A25) compared with Group I.

Fig 1 shows a plot of serum Lp(a) levels vs the apo(a) isoform phenotype in all of the subjects. An inverse relationship between serum Lp(a) levels and apo(a) size polymorphism was apparent: Group I subjects, who had the lowest Lp(a) levels, were associated with high molecular weight apo(a) phenotypes (Fig 1, left); Group V subjects, who had the highest Lp(a) levels, were associated with low molecular weight apo(a) phenotypes (Fig 1, right). Lp(a) levels were strongly and inversely related to apo(a) isoform phenotypes in all of the subjects combined ($r=-0.73$; regression coefficient $\pm$ standard error: -0.159 ± 0.011 , $p<0.001$): the cases ($r=-0.75$, regression coefficient $\pm$ SE: -0.143 ± 0.013 , $p<0.001$), and the controls ($r=-0.68$, regression coefficient $\pm$ SE: -0.170 ± 0.020 , $p<0.001$). As also shown in Fig 1, over the whole range of apo(a) phenotypes, cases had higher levels of Lp(a) than controls.

Table 2 compares the serum levels of lipids, lipoproteins,

apolipoproteins, and Lp(a) in subjects grouped according to their Lp(a) levels. Group V subjects had higher ($p<0.05$) serum levels of apo B and TC than Group I subjects, as assessed by the multiple comparison method of Dunnett. Lp(a) levels in Groups II, III, IV, and V were significantly ($p<0.05$) higher than those in Group I, as expected. The linear trends of apo B, TC, and Lp(a) levels across the quintile groups of Lp(a) were significant, as assessed by ANOVA.

Table 3 shows the clinical characteristics of patients with MI (cases) and the normal controls, and the frequency distribution of apo(a) isoform phenotypes. The cases were older and there were significantly ($p<0.05$) more male subjects (85% vs 74%) and smokers (61% vs 38%) than the controls. BMI and prevalence of HT and DM were not different between the cases and controls. The cases had relatively more subjects in Groups II, III, and V compared with the controls, as assessed by the chi-square test. Increasing Lp(a) levels were associated with an increased prevalence of MI, as assessed by the Mantel-Haenszel chi-squared analysis. The apo(a) phenotypes were also distributed

Table 2 Serum Levels of Lipids, Lipoproteins, and Apolipoproteins in Patients Grouped According to Quintile Values of Serum Levels of Lipoprotein(a) [Lp(a)]

	Group I (Quintile I)	Group II (Quintiles II)	Group III (Quintiles III)	Group IV (Quintiles IV)	Group V (Quintiles V)
HDL-C	41.9±14.9	39.9±12.5	43.7±17.5	38.3±12.3	40.0±10.2
HDL ₂ -C	27.1±11.0	26.8±11.2	28.6±14.8	27.7±11.7	31.7±9.0
HDL ₃ -C	15.5±4.6	16.2±3.5	17.8±3.4	15.5±3.3	16.7±2.8
Apo A-I	112±28	105±22	113±31	105±23	104±21
Apo A-II	28.2±7.2	27.3±5.6	28.8±7.3	26.5±6.4	26.2±4.8
Apo B	96.0±30.8	95.9±22.6	108.1±24.3	106.3±31.2	112.5±31.0*†
Apo C-II	3.3±2.4	3.5±1.5	3.7±1.4	3.5±1.6	3.2±1.4
Apo C-III	9.9±8.8	8.7±3.4	9.9±3.5	9.2±4.3	7.8±2.1
Apo E	5.3±3.4	4.9±1.1	5.2±1.4	5.0±1.8	5.0±1.7
TC	182±40	183±32	204±34	198±46	211±47*†
TG	145±162	122±55	124±55	139±73	106±44
Log _e [Lp(a)]	1.5±0.7	2.7±0.2*	3.2±0.1*	3.6±0.1*	4.4±0.4*†

HDL-C, high density lipoprotein cholesterol; Apo, apolipoprotein; TC, total cholesterol; TG, triglyceride.

Data are presented as means±standard error, and the units of measurement for lipids and lipoproteins are mg/dl.

**p*<0.05 vs Group I, as assessed by the multiple comparison method of Dunnett; †*p*<0.05, linear trend as assessed by an analysis of variance.

Table 3 Clinical Characteristics of Patients With Myocardial Infarction (Cases) and With Normal Coronary Arteries (Controls), and the Frequency Distribution of Serum Lipoprotein(a) [Lp(a)] Levels and the Apolipoprotein(a) [Apo(a)] Isoform Phenotype

	Controls (n=92)	Patients with MI (n=101)
Age (years)	58±14	62±10*
Gender (male/female)	53/39	86/15*
Body mass index (kg/m ²)	23.0±3.3	23.4±2.9
Hypertension (%)	35	41
Diabetes mellitus (%)	20	31
Smokers (%)	38	61*
Quintiles of Lp(a)		
I	28 (30.4%)	13 (12.9%)*
II	18 (19.6%)	21 (20.8%)*
III	16 (17.4%)	20 (19.8%)*
IV	20 (21.7%)	17 (16.8%)*
V	10 (10.9%)	30 (29.7%)*
Apo(a) isoform phenotype		
I (A1–A4)	21 (22.8%)	36 (35.7%)*
II (A5–A9)	22 (23.9%)	28 (27.7%)*
III (A10–A20)	27 (29.4%)	21 (20.8%)*
IV (A21–A25)	22 (23.9%)	16 (15.8%)*

**p*<0.05, by chi-squared analysis; †*p*<0.01, linear trend as assessed by the Mantel-Haenszel chi-square.

differently (*p*<0.01) in the cases and controls: the cases had lower percentages of larger isoforms (A5–A9, A10–A20, and A21–A25) relative to small isoforms (A1–A4) than the controls. A decrease in the size of apo(a) isoforms was associated with an increase in the prevalence of MI as assessed by the Mantel-Haenszel chi-square analysis.

Because the cases and controls differed with regard to age and gender, Lp(a) levels and serum levels of lipids, lipoproteins, and apolipoproteins were compared between the cases and controls after adjusting for age and gender by an analysis of covariance.³⁰ Table 4 shows the age- and gender-adjusted least-square means of lipid variables in the cases and controls. Cases had significantly (*p*<0.05) lower levels of HDL-C and apo A-I and higher levels of apo B, apo C-II, and Lp(a) than the controls after considering for age and gender.

The significance of the associations among serum levels of Lp(a), apo(a) phenotype, MI-related lipid variables, and MI were examined by a logistic regression analysis after

Table 4 Age- and Gender-Adjusted Least-Square Means of Serum Levels of Lipids, Lipoproteins, Apolipoproteins, and Lipoprotein(a) in Patients With Myocardial Infarction (Cases) and With Normal Coronary Arteries (Controls)

	Controls (n=92)	Patients with MI (n=101)
HDL-C	43.2±1.4	38.4±1.4*
HDL ₂ -C	29.9±1.4	26.9±1.4
HDL ₃ -C	16.0±0.5	16.6±0.4
Apo A-I	113±3	104±3*
Apo A-II	28.0±0.7	26.8±0.6
Apo B	97.5±3.1	109.0±2.9*
Apo C-II	3.1±0.2	3.7±0.2*
Apo C-III	8.3±0.5	9.7±0.5
Apo E	4.9±0.2	5.2±0.2
TC	191±5	199±4
TG	115±10	138±9
Log _e [Lp(a)]	2.7±0.1	3.4±0.1*

HDL-C, high density lipoprotein cholesterol; Apo, apolipoprotein; TC, total cholesterol; TG, triglyceride.

Values are age- and gender-adjusted least-square means±standard error.

**p*<0.05 vs the controls, as assessed by an analysis of covariance.

controlling for age, gender, and confounding risk factors of MI (Table 5). Table 5 shows the results of a univariate logistic regression analysis. Serum Lp(a) levels and the apo(a) isoform phenotype were significantly associated with MI (positively and inversely, respectively), after controlling for age and gender (parameter estimate±standard error, 0.69±0.20, Wald ²=12.5, *p*=0.0001; and −0.39±0.15, Wald ²=7.24, *p*=0.007; respectively, and additionally controlling for HT, DM, and current smoking status (0.70±0.20, Wald ²=12.4, *p*=0.0004; and −0.43±0.15, Wald ²=8.17, *p*=0.004; respectively), suggesting that Lp(a) and the apo(a) isoform phenotype are independent indicators of MI. As also shown in Table 5, HDL-C and apo A-I levels were inversely and apo B levels were positively related to MI, independent of age and gender.

Table 5 also shows the results of a multivariate logistic regression analysis. When the apo(a) isoform phenotype was included in the model, the association between serum Lp(a) levels and MI remained significant after controlling for age and gender (0.61±0.25, Wald ²=5.92, *p*=0.015), and additionally controlling for HT, DM, and current

Table 5 Logistic Regression Analysis of the Association of Serum Lipoprotein(a) [Lp(a)] Levels, the Apolipoprotein(a) [Apo(a)] Phenotype, and Serum Lipoprotein and Apolipoprotein Levels With Myocardial Infarction After Adjusting for Age, Gender, and Other Factors

Independent variables	Adjusted for effects					
	Age and gender			All*		
	Parameter estimates ± standard error	Wald	² p value	Parameter estimates ± standard error	Wald	² p value
<i>Univariate model</i> [including either Lp(a) or apo(a) phenotype]						
Lp(a)	0.69±0.20	12.5	0.0001	0.70±0.20	12.4	0.0004
Apo(a) phenotype	-0.39±0.15	7.24	0.007	-0.43±0.15	8.17	0.004
HDL-C	-0.65±0.32	4.13	0.042	-0.61±0.33	3.52	0.061
Apo A-I	-0.91±0.32	8.39	0.004	-0.93±0.32	8.41	0.004
Apo B	0.65±0.32	4.05	0.044	0.56±0.33	2.81	0.094
<i>Multivariate analysis</i> [including both Lp(a) and apo(a) phenotype]						
Lp(a)	0.61±0.25	5.92	0.015	0.58±0.26	5.14	0.023
Apo(a) phenotype	-0.10±0.19	0.28	0.60	-0.15±0.20	0.56	0.46

², chi-square.

*Adjusted for age, gender, hypertension, diabetes mellitus, and smoking status.

Table 6 Stepwise Logistic Regression Analysis of the Independent Association of Serum Lipoprotein(a) [Lp(a)] Levels, Apolipoprotein(a) [Apo(a)] Phenotype, and Other Factors With Myocardial Infarction

Variable	Myocardial infarction incidence			
	Parameter estimate	Standard error	Wald chi-square	p value
Lp(a)*	0.66±0.20		10.92	0.001
Gender	1.47±0.39		14.35	0.000
Age	0.38±0.19		4.07	0.044
Apo A-I	2.77±0.93		8.91	0.003
Apo(a) phenotype†	-0.42±0.16		7.49	0.006
Gender	1.52±0.39		14.94	0.0001
Age	0.47±0.19		5.75	0.017
Apo A-I	-0.98±0.33		8.72	0.003

*Model included age, gender, Lp(a), apo(a) phenotype, hypertension, diabetes, and smoking status as independent variables.

†Model variables were age, gender, apo(a) phenotype, hypertension, diabetes mellitus, and smoking status.

smoking status (0.58±0.26, Wald ²=5.14, p=0.023), whereas that between the apo(a) phenotype and MI was not significant after adjusting for Lp(a) levels (-0.10±0.19, Wald ²=0.28, p=0.60, and -0.15±0.20, Wald ²=0.56, p=0.46, respectively). Because serum levels of apo B and TC were related to Lp(a) levels, as shown in Table 2, and serum levels of HDL-C, apo A-I, and apo C-II were related to MI as shown in Table 4, we added each of these variables (HDL-C, apo A-I, apo B, apo C-II, and TC) to the multivariate model, respectively, to see whether they might influence the association between Lp(a) and MI. The results were similar to those shown in the lower panel of Table 5 (data not shown). These results indicate that the association between serum Lp(a) levels and MI was independent of the apo(a) phenotype, but the association between the apo(a) phenotype and MI was not independent of serum Lp(a) levels, suggesting that the apo(a) isoform phenotype contributes to, but does not completely explain, the high Lp(a) levels in patients with MI.

A stepwise logistic regression analysis was carried out to identify significant predictive variables, with and without serum Lp(a) levels in the model (Table 6). As shown in Table 6, when age, gender, Lp(a), apo(a) phenotype, HDL-C, apo A-I, apo B, apo C-II, TC, HT, DM, and current smoking status were included as independent variables, Lp(a), age, gender, and apo A-I were significant variables

entered into the model, confirming the finding of the multivariate logistic regression analysis (Table 5) that Lp(a) was a significant predictor for MI, independent of the apo(a) phenotype and conventional confounding risk factors. As shown in Table 6, when Lp(a) was excluded from the model, the apo(a) phenotype, age, gender, and apo A-I entered the model as significant variables, confirming the findings of the univariate logistic regression analysis (Table 5) that the apo(a) phenotype was a significant indicator for MI, independent of conventional confounding risk factors.

Discussion

The plasma Lp(a) level is regulated by the *apo(a)* gene⁷ and has a wide range: from less than 1 mg/dl to more than 100 mg/dl. In the present study, we examined the associations among serum Lp(a) level, apo(a) isoform phenotype, and MI in subjects with extremely low and high Lp(a) levels, because such subjects may provide a better understanding of the effects of the interaction of serum Lp(a) levels and the apo(a) phenotype on the occurrence of MI. We excluded subjects with acute MI²³ and uncontrolled DM¹² which are known to affect Lp(a) levels. Our findings indicate that both Lp(a) and the apo(a) isoform phenotype are significantly associated with MI, independent of confounding risk factors, and that the apo(a) phenotype

contributes to, but does not completely explain, the increased Lp(a) in patients with MI. These results were not affected by serum levels of HDL-C, apo A-I, apo B, apo C-II, or TC, which are related to Lp(a) and/or the risk of MI (Tables 2, 4), as assessed by considering each of these variables in a multivariate logistic regression model (data not shown).

Our finding that Lp(a) is an independent predictor for MI agrees with the results of most other recent studies,^{14–17} except for 2 prospective studies.^{18,19} Our finding that subjects with extremely high Lp(a) levels had small apo(a) isoforms and those with extremely low Lp(a) levels had large apo(a) isoforms confirms the findings of both our previous studies^{11,12,32} and other studies^{8,9,33} that an inverse relationship exists between apo(a) isoform size and plasma Lp(a) levels. Furthermore, this inverse relationship has been reported to be due to a difference in the rate of production, but not in the rate of catabolism, of Lp(a).³⁴ However, some MI patients had extremely low Lp(a) levels, and some controls had extremely high Lp(a) (Fig 1). The apo(a) that circulates in plasma is synthesized in the liver and secreted from hepatocyte where it joins LDL to form Lp(a). The fate of Lp(a) that circulates in plasma is largely unknown; therefore, an *in vivo* kinetic study of Lp(a) or apo(a) will be needed to answer these questions.

One possible mechanism for the increased serum Lp(a) levels in MI may involve its thrombogenic and fibrinolysis role. Due to its structural homology with plasminogen,^{4,35} Lp(a) may compete with it for binding to endothelial and mononuclear cells as well as platelets, thereby reducing the conversion of plasminogen to plasmin and inhibiting fibrinolysis.^{36–38} The finding that Lp(a) also binds to glycoprotein IIb further supports the important role of Lp(a) in fibrinolysis.³⁹ Therefore, apo(a) size polymorphism may significantly contribute to the thrombogenic potential of Lp(a). A recent study with human apo(a) transgenic mice demonstrated an *in vivo* effect of apo(a) on fibrinolysis and suppression of plasminogen activator-mediated thrombolysis, which may contribute to the pathology of atherosclerosis.⁴⁰

Our finding that the apo(a) isoform phenotype is an independent predictor for MI confirms the results of Sandholzer et al in coronary artery disease.^{33,41,42} However, the influence of the apo(a) isoform phenotype on the risk of MI is not independent of its effects on plasma Lp(a) concentrations (Tables 4, 5), as has been reported in patients with CHD.^{41,42} It has been reported that the *apo(a)* gene may account for more than 90% of the inter-individual difference within a pedigree,¹⁰ a similar tendency to that reported by us.¹² However, the fact that increased Lp(a) levels in patients with MI can not be completely explained by differences in apo(a) isoform size suggest that other factors, such as the abnormalities of thrombosis or fibrinolysis due to increased Lp(a) or the vascular smooth muscle cell proliferation due to the inhibition of transforming growth factor- β by Lp(a),⁴³ may be involved. Because only the interaction of Lp(a), apo(a) phenotype, and history of MI were assessed in the current study, such an analysis in acute coronary syndrome, including unstable angina pectoris, should be done in a future study.

We selected angiographically defined normal subjects as controls. However, a selection bias is known to exist: angiographically defined normal subjects generally have more risk factors for coronary disease than patients with clinical symptoms but who have not been selected for angiography, because a person with both chest pain and a

known risk factor, such as smoking, may be more likely to be referred for angiography than a person with just clinical symptoms. The lack of significance for the fairly substantial difference in the prevalence of DM and HT between cases and controls in the present study may be due to this bias. Thus, limitations regarding the controls may have limited the power of this study.

Conclusions

MI is associated with increased serum Lp(a) levels independent of the size of the circulating apo(a) isoforms.

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