Intraindividual Variations in Lipoprotein (a) Levels and Factors Related to These Changes

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Lp(a) levels are genetically determined and remain stable without major changes throughout lives. However, when an individual's Lp(a) levels are observed over a one-year period, they show spontaneous variation. The rate of intraindividual variation in Lp(a) was observed in 16 patients with hypertension, hyperlipidemia and/or glucose intolerance in a chronic stable state who regularly visited the hospital clinic once a month, at least 10 times during the year, and in whom a total of 42 blood and clinical chemistry tests including serum lipids, Lp(a) and apoproteins were performed. The rate of annual intraindividual variation of Lp(a) averaged out as 16.6%. The rate was 18.8% for isoform S4 (n = 10), 18.6% for S3 (n = 3), and although small in number of subjects, other isoforms showed minor variation rates. There was a significant negative correlation between the rate of variation (y%) and Lp(a) level (x mg/dl) (r = -0.605, p < 0.05, y = -0.461x + 29.8). Therefore, when Lp(a) was high, the rate of variation (SD%) was low. This was consistent with the finding that the rates of variation were low for isoforms S4, S3, and S, whose molecular weights were low, accompanied by high Lp(a) levels. On the other hand, when the relationship between Lp(a) level and the amount of variation (SD mg/dl) was examined, there was no correlation between the two, since the amounts of variation were almost constant at a level of 3.8 mg/dl, regardless of Lp(a) level. The annual variation of Lp(a) level was found to be related to three groups of factors based on comparison of the variations among WHO phenotypes of hyperlipidemias, univariate correlation analysis with the clinical parameters tested, and multivariate analysis: the first group of factors was related to structure and metabolism of very low-density lipoprotein such as triglycerides, phospholipids, apo C-II, C-III, E, A-II and uric acid; the second group was related to thrombosis centering on platelets; and the third group involved those in acute phase reactions represented by 1 hr and 2 hr erythrocyte sedimentation rates.

Key words: Lipoprotein (a) [Lp(a)], LDL-triglycerides, Atherosclerosis, Thrombosis, Acute phase reaction

Lp(a) [Lipoprotein (a)] is a serum lipoprotein consisting of two structural components: low-density lipoprotein (LDL)-like particles and apolipoprotein(a) attached through an S-S bond to the apo B moiety of the LDL-like particle (1-3). Therefore, Lp(a) can induce accumulation of cholesterol in the intima of arterial tissue, as does LDL, and is involved in the occurrence of atherosclerosis (4, 5). At the same time, the kringle structure forming an apoprotein(a) molecule by a maximum of 48 repetitions has a 75-85% similarity in cDNA structure to that of the kringle-4 of plasminogen (1). Thus, Lp(a) can also promote thrombosis by inhibiting plasmin formation as a result of competitive binding through structural similarity at plasminogen binding sites on the membrane surface of platelets and endothelial cells (6).

Lp(a) is considered to act in both atherogenesis and thrombogenesis to increase the incidence of atherothrombotic vascular disease (7). Therefore, Lp(a) is being investigated from many perspectives. Population studies have revealed that there are differences in Lp(a) levels among races (8), but no gender differences (9), and no correlation...
between Lp(a) and other serum lipids such as total cholesterol or high-density lipoprotein (HDL)-cholesterol (9). There were no changes in Lp(a) levels due to age (9), although the number of elderly people aged 83 years or over was reported small in a population (10), and Lp(a) levels were high in the survivors of myocardial infarction and cerebral infarction (11-14).

Further information has accumulated concerning individuals’ Lp(a) levels: individual Lp(a) concentrations are genetically determined and seldom undergo major changes throughout life (15-18). High Lp(a) levels are inherited as a dominant trait in families (19), and Lp(a) levels are shown to remain constant when observed closely over time. These levels do vary spontaneously as do other clinical parameters; e.g. individual’s level of serum total cholesterol varies by an average of 8%, triglycerides by 22% and HDL-cholesterol (HDL-c) by 11% (30). However, the degree of such spontaneous variations in Lp(a) levels has not been determined, and factors related to these variations are also unknown.

In this study, Lp(a) levels were serially followed once a month for one year in patients with chronic disease(s) such as hypertension, hyperlipidemia and diabetes mellitus in a well controlled and stable state. The degrees of variation in Lp(a) in the same individuals were determined and factors related to these variations were also analyzed.

Subjects

The subjects included in this study were 16 outpatients (5 males and 11 females) whose average age was 67±10 years, among whom 5 patients had hypertension with hyperlipidemia, 5 had familial hypercholesterolemia, 2 hyperlipidemia, 1 each hypertension, hyperlipidemia and hyperuricemia, 1 hypertension with hyperlipidemia and glucose intolerance, 1 hypertension with familial hypercholesterolemia, and 1 Werner syndrome. Their conditions were well controlled and monitored by the outpatient clinic and all were in a stable state, except for an occasional common cold, minor gastric disorder or sleeplessness for short intervals during the study period.

These subjects were selected because they were regularly examined once a month at least 10 times between September 1992 and August 1993, and completed all 42 clinical tests, including physical signs, body height and weight, blood pressure, pulse rate, serum lipids, apoproteins and clinical chemistry tests examined as described below.

The instructions for diet therapy were not changed during the study period in these patients. None of the subjects were being treated with any nicotinic acid preparations that influence Lp(a). The types and doses of drugs used to treat the underlying disease were not changed.

Methods

The patients were asked to visit the clinic after an overnight fast once a month. After the body weight was checked, questions about their mental and physical conditions were asked by the same physician, and a physical examination including blood pressure measurement was performed.

Blood samples were then collected from the cubital vein. The whole blood was used for determination for red cell count (RBC) hemoglobin (Hb), hematocrit (Ht), white cell count (WBC), platelet count (PLT), and 1 hr and 2 hr erythrocyte sedimentation rates (ESR-1 and ESR-2). Serum was isolated by centrifugation at 2,000 G, 4°C for 10 min. Serum total cholesterol (TC), phospholipids (PL), triglycerides (TG), and free fatty acids (FFA) were determined by enzymatic methods. HDL was separated by the dextran-Ca sedimentation method, and HDL-c was determined in the supernatant by an enzymatic method (Hitachi 7450, Tokyo). Apoprotein A-I, A-II, B, C-II, C-III and E (Daichii Pure Chemical, Tokyo) were quantified by the immunoturbidity method (Cobas Mira S, Basel). Clinical chemistry was performed using an autoanalyzer (Hitachi 7450, Tokyo) for serum proteins (Alb, Gb, and A/G), liver functions (LDH, GOT, GPT, y-GPT, ALP, LAP), renal functions (BUN, creatinine, Na, K, Ca, Mg, P and Cl), blood sugar (FBS), amylase (AMY), creatinine phosphokinase (CPK) and uric acid (UA).

Lp(a) isoforms were determined by SDS/polyacrylamide gel electrophoresis and immunoblotting as described by Utterman et al. with modifications (31). Four μl of plasma was mixed with 10 μl of 10% SDS and 10 μl of sample treatment solution (2% SDS, 10% glycerol, 5% mercaptoethanol, 0.001% bromophenol blue, 62.5 mM Tris-HCl). The mixture was heated in a boiling water bath for 10 min and applied (10 μl/lane) to 4-15% gradient polyacrylamide gels. Electrophoresis was performed at a constant current of 40 mA until the bromophenol blue dye front migrated to the other end of the gel. After electrophoresis, proteins migrated on the gel were transferred onto nitrocellulose membranes by electroblotting at 25 volts and 4°C for 15 hrs. The membranes were rinsed for blocking with 5% non-fat dried milk for 60 min. The membranes were exposed to mouse anti-human apo(a) monoclonal antisera (Daichi Pure Chemical, Tokyo) for 60 min at room temperature. After 3 washes with phosphate buffer, the membranes were then incubated with the second antibody, HRP-rabbit anti-mouse IgG (Zymed, South San Francisco) for 60 min. The membrane was then washed 3 times and incubated with substrate solution (0.05% 3,3′-diaminobenzidine-HCl, 0.05%...
Lp(a) value was determined by enzyme-linked immunosorbent assay (TintElize Lp(a), Biopool AB, Umeå, Sweden) (32). All measurements of Lp(a) were performed at the same time for stored serum specimens. To prevent degradation of Lp(a), specimens from each patient were mixed with sodium azide (NaN₃ to a final concentration of 0.1% ; Sigma, St. Louis, Mo, USA), 0.01% ethylene diamine tetraacetic acid (EDTA; Sigma, St. Louis, Mo, USA) and 20 units per ml of aprotinin (Trasylol®, Bayer, Leverkusen, Germany) as an antiproteolytic agent. The tubes were tightly capped and stored at 4°C for 6 to 12 months. The effect of storage on Lp(a) in the sample was tested in a preliminary study (33).

The coefficients of variation (CV) throughout the study period were 2.3% for Lp(a), 1.0% for total cholesterol, 1.4% for triglycerides, and 3.3% for HDL-cholesterol.

To evaluate annual variations in Lp(a) and serum lipids in each patient, two indices were used: the amount of variation (SD mg/dl) and the percent variation (SD%). These were calculated as follows: the annual mean (mg/dl) and SD (mg/dl) were gained from whole 12-month Lp(a) and serum lipids of one patient, and this SD (mg/dl) was defined as the annual amount of variation (mg/dl) of one patient. Then, monthly values for each subject were converted to percentages of the annual mean, and percentile monthly values were averaged as 100×SD (%). This SD (%) was considered to be annual variation rate (%) of each subject, then each subject's annual SD (mg/dl) and SD (%) were averaged for the total subjects, respectively. The statistical significance of differences in the amount and the rate of variation from the annual mean level in Lp(a) and serum lipids each month were tested by ANOVA.

Relationships between Lp(a) levels and the rates of annual variation, and the amounts of variation were examined by univariate analysis. Factors related to the rates of variation in Lp(a) were also analyzed by multiple regression analysis. All Lp(a) values determined each month in each patient were treated as independent variables during analysis of factors related to Lp(a).

In general, the level of significance was considered to be below 5% by two-tailed test. However, since Lp(a) had a very skewed distribution curve tailing toward higher values, we considered the level of significance as below 0.1%, when statistical tests were performed between Lp(a) and other parameters.

Results

The clinical backgrounds of the 16 subjects are summarized in Table 1. Lp(a) averaged as 26.5 mg/dl. Six of the 12 patients with hyperlipidemia had phenotype IIa, five IIb and four IV. Isoform F was observed in one patient, S₁ in one, S₂ in one, S₃ in three, and S₄ in ten. As a whole, the subjects showed slightly elevated levels of total cholesterol, triglycerides and Lp(a).

In a preliminary study, the effect of storage (for 6-12 months with a mean of 8.9±1.8 months under the conditions described above) on Lp(a) were examined (n=17). As in Fig. 1, the mean Lp(a) level in the stored serum was 27.2±12.2 mg/dl (y-axis), while the mean Lp(a) level measured immediately after collection was 27.3±12.9 mg/dl (x-axis), with a correlation coefficient of r=0.983 (P<0.001). Thus, Lp(a) levels were confirmed to be unchanged even after storage at 4°C for 6-12 months in the presence of preservatives.

Annual changes in Lp(a), total cholesterol, triglycerides, and HDL-c in each patient are shown in Fig. 2. The variations of all lipids were smaller in patients with low lipid levels, but larger in those with high lipid levels. Figure 3 shows the variations for each lipid as a change in the mean variation rates in all patients. None of the variations in Lp(a) or serum lipids were significant.

The annual mean levels of Lp(a) and serum lipids and the mean rates of annual variation are summarized in Table 2. The rate of annual variation was 16.6% for Lp(a), while that of total cholesterol was 7.3%, triglycerides 24.0% and HDL-c

Table 1. Clinical backgrounds of subjects.

| Number | 16(Male 5, Female 11) | A-I (mg/dl) | 136±34 (95-239) |
| Age (year) | 67±10 (44-81) | A-II (mg/dl) | 40±11 (23-61) |
| BMI | 21.6±2.0 (17.6-25.6) | B (mg/dl) | 110±25 (76-186) |
| SBP (mmHg) | 130±19 (97-168) | C-I (mg/dl) | 5.4±3.2 (1.8-13.4) |
| DBP (mmHg) | 75±9 (59-93) | C-II (mg/dl) | 16.1±12.8 (5.4-54.4) |
| TG (mg/dl) | 232±44 (151-320) | E (mg/dl) | 10.9±7.4 (5.9-31.2) |
| TG (mg/dl) | 200±267 (32-971) | Lp(a) (mg/dl) | 26.5±15.3 (7.8-65.0) |
| HDL-c (mg/dl) | 50±16 (31-104) | Lp(a) phenotype | F 1 |
| PL (mg/dl) | 260±72 (179-439) | | S₁ 1 |
| FFA (μEq/l) | 51±202 (235-917) | S₁S₄ 1 |
| UA (mg/dl) | 5.6±1.8 (3.3-10.8) | S₂ 3 |
| FBS (mg/dl) | 91±13 (75-128) | S₄ 10 |

BM1: Body mass index (Kg/m²), SBP and DBP: Systolic and diastolic blood pressure, TC: Total cholesterol, TG: Triglycerides, HDL-c: HDL-cholesterol, PL: Phospholipids, FFA: Free fatty acids, UA: Uric acid and FBS: Fasting blood Sugar
Variation Rate of Lp(a) 10.5%. According to the type of Lp(a) isoform, the rates were 18.6% for S3 and 18.8% for S4, as shown in the lower part of Table 2. The numbers of patients with other isoforms (F, S2, and S3S4) were small.

The relationships between the annual rates of variation and serum lipids including Lp(a) are shown in Fig. 4a. The amounts of variations (SD mg/dl) in total cholesterol, triglycerides and HDL-cholesterol increased with the lipid levels, as is generally the case in biological parameters. However, there was no significant correlation between Lp(a) level and the amount of variation ($r = -0.070$, NS). Rather, the variation of Lp(a) was almost constant at 3.8 mg/dl (2.2-7.8 mg/dl) regardless of the Lp(a) level. Therefore, a significant negative correlation was found between percent variation (%SD) and Lp(a) level ($r = -0.537$, $p < 0.05$) as shown in Fig. 4b.

The factors involved in Lp(a) variation were analyzed in three ways. Firstly, mean variation rates of Lp(a) and serum lipids were compared between WHO phenotypes of hyperlipidemia as shown in Fig. 5. Although there were no statistically significant differences in the annual variation rates of total cholesterol, triglycerides or HDL-c among the phenotypes, the mean variation rates of Lp(a) in normolipemia and type IIa patients were as low as 11.7% and 9.7%, respectively, while those for type IIb and type IV were both 26.7% as shown in the right most column of Fig. 5. Thus, the variation rates of Lp(a) were significantly higher in type IIb and type IV associated with triglyceride elevation.
Secondly, univariate correlation analysis was performed between Lp(a) and other laboratory findings. The results are presented in Fig. 6, where correlation coefficients are shown by bar graphs. The parameters that showed significant negative correlations with Lp(a) were C-II, followed by triglycerides, uric acid, phospholipids, apo A-II and C-III, in that order, while ESR-2 and ESR-1 showed significant positive correlations.

Thirdly, multiple regression analysis was performed by the stepwise method to detect factors related to Lp(a) variations. Lp(a) was used as an objective variable, and 17 independent parameters from 42 test items were selected as explanatory variables. The results are shown in Table 3. The factors that became apparent by primary analysis were C-II, E, PLT, B, and A-II. On secondary analysis using these five factors as explanatory variables, those that were significantly involved in the Lp(a) variation were C-II, followed by A-II, PLT and E, in that order. The multiple regression coefficient was 0.4399.

**Discussion**

The results of this study are discussed in terms of the following three points: i) problems related to collective measurement of Lp(a) in stored specimens; ii) evaluation of spontaneous variation in Lp(a) and serum lipids; and iii) clinical factors related to Lp(a) variations.

**Problems related to determining annual variations in Lp(a) using stored specimens**

In this study, annual variation in Lp(a) was determined collectively using stored serum specimens. When serum specimens are stored long term, they are usually kept frozen at -20°C or below. However, apo B-containing lipoproteins such as VLDL and LDL are known to degrade and give values as low as 22%, when stored at -20°C or below for only one month (34). Therefore, the serum specimens including apo B-containing lipoprotein of Lp(a) were not frozen in this study, but the preservative sodium azide, the bivalent cation chelating agent EDTA, and the proteolytic enzyme inhibitor aprotinin

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**Table 2. Annual mean levels of serum lipids and Lp(a), and the mean amounts and mean rates of annual variations.**

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SD (mg/dl)</th>
<th>Δ (mg/dl)</th>
<th>%Δ</th>
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</thead>
<tbody>
<tr>
<td>Total Cholesterol</td>
<td>234 ± 32</td>
<td>17 ± 7</td>
<td>7.3 ± 2.3</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>190 ± 181</td>
<td>52 ± 67</td>
<td>24.0 ± 12.1</td>
</tr>
<tr>
<td>HDL-cholesterol</td>
<td>51 ± 15</td>
<td>5 ± 3</td>
<td>10.5 ± 4.3</td>
</tr>
<tr>
<td>Lp (a)</td>
<td>28.8 ± 14.5</td>
<td>3.8 ± 1.7</td>
<td>16.6 ± 11.1</td>
</tr>
<tr>
<td>S₄</td>
<td>25.6 ± 10.4</td>
<td>4.0 ± 1.9</td>
<td>18.8 ± 12.5</td>
</tr>
<tr>
<td>S₆</td>
<td>19.4 ± 5.1</td>
<td>3.5 ± 1.1</td>
<td>18.6 ± 4.6</td>
</tr>
<tr>
<td>S₆S₄</td>
<td>30.6</td>
<td>2.5</td>
<td>8.1</td>
</tr>
<tr>
<td>S₁</td>
<td>51.4</td>
<td>4.6</td>
<td>9.0</td>
</tr>
<tr>
<td>F</td>
<td>65.0</td>
<td>2.9</td>
<td>4.4</td>
</tr>
</tbody>
</table>

Fig. 3. Changes in the mean rate of annual variation (%) in serum total cholesterol, triglyceride, HDL-cholesterol and Lp(a) levels. None of the changes were statistically significant.
Fig. 4a. Relationship between the annual mean level and the amount of annual variation (mg/dl) in serum lipids and Lp(a) of each patient.

Fig. 5. Serum lipid levels and mean rate of annual variation of Lp(a) according to WHO phenotype of hyperlipidemia.
Fig. 4b. Relationship between the annual mean Lp(a) level and the rate of annual variation (%) in serum lipids and Lp(a) of each patient.

Fig. 6. Simple correlation of the mean rate of annual variation of Lp(a) with clinical parameters.
were added as preservatives. Tubes containing the mixtures were tightly stoppered, then stored at 4°C.

The appropriateness of the collective measurement of stored specimens was confirmed by a preliminary study(33). Lp(a) and other serum lipids in the specimens collected from subjects during clinic visits were compared with those of the same specimen treated with the preservatives described above and stored at 4°C for at least 6-12 months (8.9 ± 1.8 months, n=17). There were no significant difference between the two measurements in terms of the mean and correlation coefficient (33) as in Fig.1.

Thus, storage of serum specimens without freezing did not result in degradation of Lp(a), and collective determination of specimens at one time minimizes the errors in measurements due to deterioration of samples, technical fluctuation, instrumental instability, and differences in reagent lot. These results justify the methodology for observing long-term variations of Lp(a) used in this study.

**Evaluation of annual spontaneous variation of Lp(a) and serum lipids**

The rates of spontaneous variation in Lp(a) and serum lipids for one year are shown in Table 2. The mean variation rate for Lp(a) was 16.6%, total cholesterol 7.3%, triglycerides 24.0% and HDL-c 10.5%. The observed variations in total cholesterol, triglycerides and HDL-c levels were very close to those reported previously (30). The variation in Lp(a) level in this study, 16.6%, is larger when compared with those of total cholesterol and HDL-c, but smaller than that of triglyceride.

This is related to the following three characteristics in Lp(a) variation: i) presence of isoforms, ii) different relationship of the rate, and iii) the amount of variation to Lp(a) levels.

Different investigators have reported different numbers of isoforms of Lp(a): 6 (31, 35), 12 (36) or 23 types (37). In this study, we investigated the variation of Lp(a) using the six isoforms classified by Utermann et al. (31). The variation

**Table 3.** Multivariate stepwise analysis of factors related to annual variation rate of Lp(a). The upper panel is the first step analysis using 17 independent factors. The lower panel gives the result of second step analysis using 5 factors determined in the first step to be as significantly related.

<table>
<thead>
<tr>
<th>Factor</th>
<th>Partial-regression parameter</th>
<th>Standard error</th>
<th>t-Value</th>
<th>P</th>
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<td>0</td>
<td>27.2750</td>
<td>3.7557</td>
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</tr>
<tr>
<td>1 Body mass index</td>
<td>0.2528</td>
<td>0.3890</td>
<td>0.652</td>
<td>0.5155</td>
</tr>
<tr>
<td>2 Systolic blood pressure (mmHg)</td>
<td>0.0523</td>
<td>0.0680</td>
<td>0.769</td>
<td>0.4429</td>
</tr>
<tr>
<td>3 Total cholesterol (mg/dl)</td>
<td>0.0485</td>
<td>0.0417</td>
<td>1.162</td>
<td>0.2468</td>
</tr>
<tr>
<td>4 Triglycerides (mg/dl)</td>
<td>-0.0293</td>
<td>0.0157</td>
<td>1.863</td>
<td>0.0642</td>
</tr>
<tr>
<td>5 HDL-cholesterol (mg/dl)</td>
<td>0.0707</td>
<td>0.1024</td>
<td>0.690</td>
<td>0.4910</td>
</tr>
<tr>
<td>6 Free fatty acids (μmol/l)</td>
<td>-0.0070</td>
<td>0.0044</td>
<td>1.577</td>
<td>0.1167</td>
</tr>
<tr>
<td>7 Phospholipids (mg/dl)</td>
<td>-0.0584</td>
<td>0.0431</td>
<td>1.355</td>
<td>0.1772</td>
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<tr>
<td>8 apoA-I (mg/dl)</td>
<td>0.3058</td>
<td>0.0540</td>
<td>1.073</td>
<td>0.2846</td>
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<tr>
<td>9 apoA-II (mg/dl)</td>
<td>-0.5744</td>
<td>0.1658</td>
<td>3.464</td>
<td>0.0007***</td>
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<tr>
<td>10 apoB (mg/dl)</td>
<td>0.1828</td>
<td>0.0521</td>
<td>3.509</td>
<td>0.0006***</td>
</tr>
<tr>
<td>11 apoC-II (mg/dl)</td>
<td>-4.8257</td>
<td>0.6782</td>
<td>7.115</td>
<td>0.0000***</td>
</tr>
<tr>
<td>12 apoC-III (mg/dl)</td>
<td>0.8209</td>
<td>0.3231</td>
<td>2.521</td>
<td>0.0563</td>
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<tr>
<td>13 apoE (mg/dl)</td>
<td>1.8174</td>
<td>0.4311</td>
<td>4.216</td>
<td>0.0000***</td>
</tr>
<tr>
<td>14 Uric acid (mg/dl)</td>
<td>-0.0675</td>
<td>0.6596</td>
<td>1.499</td>
<td>0.1356</td>
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<tr>
<td>15 Fasting blood sugar (mg/dl)</td>
<td>0.0409</td>
<td>0.0475</td>
<td>0.862</td>
<td>0.3896</td>
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<tr>
<td>16 Platelet (×10³/µl)</td>
<td>0.6921</td>
<td>0.1929</td>
<td>3.588</td>
<td>0.0004***</td>
</tr>
<tr>
<td>17 Erythrocyte segmentation rate-2h (mm)</td>
<td>0.0971</td>
<td>0.0526</td>
<td>1.845</td>
<td>0.0667</td>
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Multiple correlation coefficient (r)=0.7331 (p<0.0001)

Coefficient of determination (r²)=0.5375

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<td>0</td>
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<td>1 apoC-II (mg/dl)</td>
<td>-3.8696</td>
<td>0.4393</td>
<td>8.663</td>
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<td>2 apoE (mg/dl)</td>
<td>1.4234</td>
<td>0.2844</td>
<td>5.005</td>
<td>0.0000***</td>
</tr>
<tr>
<td>3 Body Mass Index (kg/m²)</td>
<td>0.8553</td>
<td>0.1682</td>
<td>5.084</td>
<td>0.0000***</td>
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<tr>
<td>4 apoB (mg/dl)</td>
<td>0.0107</td>
<td>0.0434</td>
<td>0.247</td>
<td>0.8052</td>
</tr>
<tr>
<td>5 apoA-II (mg/dl)</td>
<td>-0.6401</td>
<td>0.1086</td>
<td>5.895</td>
<td>0.0000***</td>
</tr>
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Multiple correlation coefficient (r)=0.6633 (p<0.0001)

Coefficient of determination (r²)=0.4399.
rate was 18.8% in 10 patients with isoform S4 and 18.6% in 3 patients with S3. The rates in patients with S3, S4, and F were 8.1%, 9.0% and 4.4%, respectively, although only one patient had all of these isoforms. The annual variation in Lp(a) of 16.6% is therefore a composite value of these isoform variations, mainly reflecting those of low Lp(a) levels which are present at high incidence in the population.

The rate of variation (SD%) in Lp(a) showed a negative correlation with Lp(a) concentration as demonstrated in Fig. 4b. When the molecular weight of an Lp(a) isoform is low, the serum Lp(a) concentration is known to be higher (35, 36). Therefore, in patients with a high serum Lp(a) level and low molecular weight isoforms like F, the variation rate tended to be smaller. The rate was higher in patients with low serum Lp(a) level and isoforms with high molecular weight such as S3 and S4. There was a reciprocal relationship between the rate of variation and Lp(a) level or isoform molecular weight (Fig. 4b).

However, the mean annual variation (SD mg/dl) in Lp(a) was approximately constant at 3.8 ± 1.7 mg/dl (2.2-7.5 mg/dl) even at different serum Lp(a) levels or in different isoforms as can be seen in Fig. 4a. This is in contrast to the variations in other serum lipids such as total cholesterol, triglycerides and HDL-c which were proportional to the lipid levels as shown in Fig. 4a. When the amount of variation was constant, the rate of variation remained proportional to the level of Lp(a), since the rate was obtained from a calculation in which the variation rate = amount of variation/serum Lp(a) x 100 (%). Thus, the variation rate was high at low Lp(a) concentrations and vice versa. The important point therefore is that the amount of annual variation in Lp(a) was approximately 3.8 mg/dl regardless of differences in serum Lp(a) level or isoform type as can be seen in Fig. 4a. At present, the actual cause of this phenomenon has yet to be determined. However, the implication of our findings is that this kind of phenomenon can occur only when data are gained from a sample population consisting of several subgroups whose distribution curves are all normal and whose spontaneous variations are close to each other. This statistical implication corresponds to the observation that Lp(a) has six or more subgroups of isoforms (31, 35-37).

The 16.6% annual variation of Lp(a) was corroborated by evaluation of isoform distribution and the relation between the variation and Lp(a) concentration. Since variation rates of Lp(a) were related to isoform type and serum Lp(a) concentration, the rates can be estimated from the isoform distribution. In this study, S3 and S4 isoforms were present in 13 of 16 (81%) subjects, and the total mean annual variation rate was 16.6% as shown in Table 2. In general, the isoforms most frequently observed in all races are S3 and S4 (31, 35). Judging from the variation rates in these isoforms, that in the general population was estimated to be 18.6-18.8%.

The variation rate of Lp(a) in the general population can also be evaluated from the relationship between Lp(a) level and the rate of variation as shown in Fig. 4b, where a regression formula has been obtained between variation rates (y%) and Lp(a) levels (xmg/dl): y = -0.461x + 29.8. Since the mean level of Lp(a) for the Japanese population was found to be around 15 mg/dl (9), this value was substituted into the formula and the variation rate was calculated as 22.9%.

These results gained from both observations and corroborations lead to the conclusion that the variation rate in Lp(a) will generally range from 16-23%. Determining the variation rate of such a genetically determined factor as Lp(a) may provide clues to estimating the degree to which environmental factors can influence a genetic factor.

**Factors related to spontaneous variations in Lp(a)**

Factors related to variation in Lp(a) can be determined from three types of analysis: i) comparison of the variation between the WHO phenotypes of hyperlipidemia, ii) univariate correlation analysis, and iii) multivariate regression analysis.

When the variation of Lp(a) was compared among WHO phenotypes of hyperlipidemia, type IIb and IV patients have a greater Lp(a) variation of 26.7%, while normolipidemia and type IIa show a smaller variation of approximately 10%. Since type IIb and IV are accompanied by elevation in triglycerides, the variation in Lp(a) is considered to be influenced by the levels of triglycerides. This relationship is shown in Fig. 6.

Univariate analysis revealed that the parameters yielding significant negative correlations were triglycerides, phospholipids, Apo C-II, C-III, E, A-II and uric acid, as can be seen in the lower bars of Fig. 6. The parameters showing positive correlation were ESR-1 and ESR-2 as shown in the upper bars of Fig. 6.

Multivariate analysis conducted on 17 independent factors of 42 test items gave the following five factors of apo C-II, A-II, E and platelet count as independent explanatory variables for Lp(a) variation.

Taken together, Lp(a) variations were considered to be influenced by three groups of factors. The first group consisted of triglycerides, phospholipids, Apo C-II, E, A-II and uric acid. The second was platelet count, and the third ESR-1 and ESR-2. Thus, although the basal level of Lp(a) is a genetic trait, the following are thought to be involved in Lp(a) variation: i) factors related to the structure and metabolism of VLDL-TG (39, 40), ii) factors related to thrombus formation centering on platelets (9, 41-46), and iii) factors related to acute phase reactions represented by the blood sedimentation rate (47).

Taking these factors and their actions into consideration, we have drawn the schematic diagram shown in Fig. 7. The individual basal level (on the y-axis) is genetically determined by the isoforms expressed (31), a level which varies between individuals by at least 16-19% (18). The factors leading to a decrease within the range of variation (left, oblique downward in Fig. 7) are related to VLDL and triglyceride metabolism, while those leading to an increase with in the range of variation (right, oblique upward in Fig. 7) are related to throm-
bus formation and/or acute phase reactions.

Details of the mechanism by which these factors cause Lp(a) to decrease or increase within a certain range are currently unknown (41). However, this may be in part due to the fact that not only the total mechanisms of synthesis and catabolism of Lp(a), but also the physiological functions of Lp(a) are not yet fully understood. Under these circumstances, it would be useful to determine the rate of intrindividual variation of Lp(a), because determining the spontaneous variation rate will distinguish the effects of external causes from spontaneous variations within individuals.

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