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Relationship between plasma lipoprotein (a), apolipoprotein (a) phenotypes, and other coronary heart disease risk factors in a Melbourne South Asian population

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Abstract

Background: High plasma lipoprotein(a) [Lp(a)] level is a strong and important risk factor for cardiovascular disease (CVD). Small-sized apolipoprotein(a) [apo(a)] isoforms (F, B, S1, and S2) are inversely correlated with the high levels of Lp(a) in plasma and significantly associated with CVD. Although the effects of apo(a) phenotypes and various risk factors on Lp(a) status in South Asian population may have been studied in other countries, there are no reports involving these risk factors in Australia.

Methods and results: Factors contributing to variation in Lp(a) were surveyed in 402 (216 males and 186 females) South Asian Melburnians. There was a negative relationship between low alcohol beer per day and Lp(a) in men ($P < 0.05$). Approximately 21% of the variance of Lp(a) concentration in men and 6% in women were explained by age. Age was positively associated with Lp(a) concentrations in men but negatively in women. The most commonly occurring phenotype was apo(a) S3. In this phenotype, Lp(a) concentrations ranged from non-detectable to 811 mg/l. After adjusting for age, an inverse correlation was observed between Lp(a) concentration and apo(a) phenotypes ($P < 0.01$).

Conclusions: Although Lp(a) has been reported to be genetically determined, there are clearly other factors contributing to variations in Lp(a) concentrations in a South Asian population.

Keywords: Apolipoprotein(a) phenotypes; Heart risk factors; South Asian Australians lifestyle; Independent variables

Introduction

Lipoprotein (a) [Lp(a)] is a genetically controlled quantitative predictor of cardiovascular disease (CVD) in Caucasian populations [1–3]. Although it has been shown that Lp(a) levels are higher in black populations than in whites [4], the role of Lp(a) as a risk factor for CVD in black people is controversial [5–7].

South Asians have a higher median Lp(a) level compared with Caucasians [8] and it has been confirmed by Bhatnagar et al. [9] in migrants from the Indian subcontinent living in West London whose Lp(a) levels were significantly higher than those of white Europeans in Britain.

In the present study, Lp(a) concentrations and phenotypes were assessed in a representative population of ethnic Indians living in Melbourne, Australia, and the effect of age, gender, lifestyle, blood pressure, body mass index (BMI), waist circumference, and other lipids on plasma levels of Lp(a) also evaluated.

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Methods

Subjects

Recruitment was from Melbourne residents of ethnic Indian ancestry who had migrated to Australia from the Indian subcontinent, Africa, Fiji/Malaysia, and Singapore. A total of 402 healthy subjects (mean age was 43 ± 10 years) with South Asian-sounding surnames was randomly selected from the telephone directory and contacted by telephone to determine their eligibility for the study. Eligibility criteria included being over 25 years and being of Indian ancestry. A representative sample of 216 males and 186 females without any CVD, diabetes, and renal disease was assessed for Lp(a) concentration and apo(a) phenotype. All subjects recruited gave informed consent before the commencement of the study. The study protocols were approved by the Human Research and Ethics Committee at the Monash Centre, Australia.

Health Status and Lifestyle Assessment

Blood pressure levels were measured in the left arm on the day of interview using an 8 × 14 cm cuff of standard mercury sphygmomanometer and a random zero method [10]. The average of two readings for systolic and diastolic pressures was recorded for data analysis. Lifestyle characteristics including smoking, use of alcohol, and level of education were obtained using questionnaire responses from each participant. Smokers were categorized as current and ex-smokers, while subjects who had never smoked were classified as non-smokers. The number of cigarettes smoked per day and the length of smoking were recorded for smokers and ex-smokers. In this study, current smokers and non-smokers categories were used for the analysis. Amount of alcohol consumption was obtained from responses to the questionnaire. Drinkers included those who consumed any type of alcoholic beverage including beer, wine, and spirits. Teetotalers were categorized as non-drinkers.

Anthropometric Measurements

Anthropometric measurements included weight, height, body mass index (BMI), circumferences, and skinfold thickness. Body weight was measured on a calibrated electronic balance. Height was measured using a stadiometer with subjects standing upright, heels together, and looking horizontally ahead. BMI was used to assess overall adiposity and calculated using the formula: BMI = weight (kg)/height (m^2). Waist measurement was made 12 cm below the xiphisternal notch.

Biochemical Analysis

Following a 12-h fast, 30–40 ml of venous blood was taken from the antecubital vein into both evacuated plain tubes (serum) and tubes containing 1.6 mg ethylenediaminetetraacetic acid (EDTA) per every ml of blood. The amount of the blood was separated into three parts. One aliquot was kept at 4°C and used for immediate analyses, such as glucose, lipids, and Lp(a), the remaining two were held at −70°C for apo(a) phenotyping and repeat assays or additional investigations, which were thawed once only before processing. Total cholesterol (TC) was measured enzymatically using DuPont reagents on a Dimensin A.R. (DuPont, Wilmington, DE, USA). Triglycerides (TG) were measured enzymatically using DuPont CHOL Flex™ reagent cartridges on a Dimension A.R. (DuPont). High-density lipoprotein cholesterol (HDLC) was measured enzymatically using HDL Flex DuPont Reagent Cartridges on a DuPont Dimension A.R. analyzer. Low-density lipoprotein cholesterol (LDLC) was derived by calculated using the Friedewald formula adapted to SI units: LDLC (mmol/l) = TC − HDLC − TG/2.2 in subjects with triglycerides <400 mg/dl. Non-HDLC was estimated using formula: Non-HDLC = Total cholesterol − HDLC and direct measurement of the LDLC is performed in case of the triglyceride value was > 400 mg/dl. Blood glucose measurement was carried out on a Dimension A.R. using DuPont glucose-oxidize/peroxidase reagents. Insulin concentration was measured by radioimmunoassay using commercial kits (Pharmacia).

Measurement of Lp(a) Concentration

Measurements on the South Asian cohort had been done by an in-house immunoturbidimetry (ITA) method on a KONE Progress Selective Analyzer. (KONE Finland) with anti-human Lp(a) antiserum from Behring (Behringwerke AG, Marburg, Germany) and Lp(a) standards and controls from Incstar. The antibodies against apolipoprotein B (apoB) and plasminogen have been removed by solid-phase absorption. Before the commencement of these analyses, a comparison was made between the results obtained on the KONE and those on the COBAS FARA analyzers due to optimized parameters of the measurement of Lp(a) by ITA using a Roche COBAS FARA centrifugal analyzer in the Department of Clinical Biochemistry, Monash Medical Centre, the common reagents used on both the analyzers, a good agreement was shown ($r = 0.98$).

$$Y(\text{Kone}) = 1.79 \times (\text{CobasFara}) - 20.524$$

Plasma Lp(a) level of 300 mg/l is the generally accepted threshold for increased risk of atherosclerosis referred from Wong et al. [11].

Assessment of Apo(a) Phenotypes

Apolipoprotein (a) [apo(a)] phenotypes were assessed by SDS polyacrylamide gel electrophoresis (PAGE) using a

modification of the method by Huang et al. [12], based on the method of Utermann et al. [13]. SDS is an anionic detergent, which dissociates proteins into their polypeptide subunits and imparts a negative charge to these subunits. Protein denaturation is aided by heating the samples under investigation in buffer containing excess SDS and mercaptoethanol. Under these conditions, proteins are migrated in PAGE according to their molecular size. A discontinuous gel, consisting of a running (lower) gel and a stacking (upper) gel is used. The stacking gel concentrates large sample volumes, resulting in improved resolution of protein bands.

For statistical purposes, Lp(a) levels, which were below the limit of sensitivity of the assay used, have been recorded as zero. Similarly, when no visible banding pattern was seen on PAGE, the putative homozygous null phenotype was recorded as ''null''.

Statistical Analysis

Due to the highly skewed frequency distribution of Lp(a) values with mean values higher than the median in the population studied, which for statistical purposes is an abnormal distribution, data were summarized as mean, median, 25th, 75th, and standard error of mean (SEM) percentiles and necessitating use of non-parametric evaluation (Spearman's rank correlation coefficients) of the Lp(a) data were performed throughout the study. Comparisons between groups were made by using the Wilcoxon rank sum test. A P value < 0.05 was considered statistically significant. The SAS procedure PROC REG with MODEL-dependent option was used to perform multivar-

Table 1
Percentile distribution of Lp(a) concentrations in Melbourne South Asians

	Lp(a) concentration (mg/l)									
	n	Mean	SEM	Percentiles						
				Min	10	25	50	75	90	Max
Men	216	310	321	0	24	80	227	426	700	2320
Women	186	340	289	0	39	109	282	486	720	1414
Total	402	324	306	0	30	96	239	451	719	2320
Men										
<40 years	82	276	25.9	0	24	76	214	414	638	922
40–60 years	111	332	35.8	0	25	80	228	424	784	2320
>60 years	23	328	60.5	0	36	75	280	498	607	1272
Women										
<40 years	93	322	28.5	0	33	99	283	426	689	1414
40–60 years	81	370	34.0	0	53	127	296	542	810	1365
>60 years	12	281	80.8	1	1	25	198	475	701	793
Total										
<40 years	175	301	19.4	0	27	95	238	426	670	1414
40–60 years	192	348	25.1	0	42	105	238	476	810	2320
>60 years	35	312	47.9	0	17	66	270	498	622	1272

Based on the generally accepted Lp(a) risk threshold of 300 mg/l.

Table 2
Gender-based Spearman correlation coefficients (r) of Lp(a) concentration and other parameters

	Lp(a) concentration		
	Men	Women	Total
Cigarette smoking per day	−0.24	1.0	−0.18
Low alcohol beer per day	−0.83†	−0.03	−0.83†
Wine per day	0.19	0.14	0.2
Spirits or liqueurs per day	−0.01	−0.45	−0.08
Systolic blood pressure	−0.02	−0.11	−0.08
Diastolic blood pressure	0.03	−0.08	−0.03
Weight	−0.02	−0.11	−0.08
Height	−0.05	−0.07	−0.07
BMI	0.03	−0.07	−0.02
Waist circumference	−0.027	−0.107	−0.073
Total Cholesterol	0.16†	0.01	0.07
Triglycerides	−0.18†	−0.23§	−0.22¶
HDL cholesterol	0.02	−0.07	−0.01
LDL cholesterol	0.27#	0.12	0.19#
LDL/HDL ratio	0.15†	0.14	0.13†
Insulin	−0.17†	−0.05	−0.11†
Glucose	−0.18†	−0.02	−0.12†

† Significantly different from zero: $P < 0.05$.
§ Significantly different from zero: $P < 0.005$.
# Significantly different from zero: $P < 0.001$.
¶ Significantly different from zero: $P < 0.0001$.

Table 3
Gender based association of Lp(a) concentrations with various other factors

Independent variable	Dependent variable Lp(a) concentration (mg/l)		
	Regression coefficient*		Partial $R^2 \times 100$
	Parameter estimate	Standard error	
Men (n = 216)			
Age (year)	11.52#	2.99	21‡
Glucose (mmol/l)	−2.55†	1.06	7
% Variation explained by the model (model $R^2 \times 100$)			28%
Women (n = 186)			
Age (year)	−14.36‡	4.88	6†
Trig (mmol/l)**	−376.79¶	71.48	12†
% Variation explained by the model (model $R^2 \times 100$)			18%
Total (M + W) (n = 402)			
Age (year)	4.97	3.27	3
Waist circ (cm)***	−11.82§	3.53	15§
Trig (mmol/l)**	−92.77†	37.84	6†
% Variation explained by the model (model $R^2 \times 100$)			24%

* Significant at the 0.15 level and variables met the significance level for entry into the model.
** Trig = triglycerides.
*** Waist circ = waist circumference.
† Significantly different from zero for F test: $P < 0.05$.
‡ Significantly different from zero for F test: $P < 0.01$.
§ Significantly different from zero for F test: $P < 0.005$.
# Significantly different from zero for F test: $P < 0.001$.
¶ Significantly different from zero for F test: $P < 0.0001$.

Table 4
Age based association of Lp(a) concentrations with various other factors

Independent variable	Dependent variable Lp(a) concentration (mg/l)		
	Regression coefficient*		Partial $R^2 \times 100$
	Parameter estimate	Standard error	
Age <40 years (n = 175)			
Age (year)	−33.23§	10.24	21‡
Trig (mmol/l)**	−177.07#	39.56	38§
LDL chol (mmol/l)***	95.98	49.55	7
% Variation explained by the model (model $R^2 \times 100$)			66%
Age ≥ 40–< 60 years (n = 192)			
Waist circ (cm)****	−10.24	5.13	28§
% Variation explained by the model (model $R^2 \times 100$)			28%
Age ≥60 years (n = 35)			
No variable met the 0.15 significance level for entry into the model			

* Significant at the 0.15 level and variables met the significance level for entry into the model.
** Trig = triglycerides.
*** LDL chol = LDL cholesterol.
**** Waist circ=waist circumference.
† Significantly different from zero for F test: $P < 0.05$.
§ Significantly different from zero for F test: $P < 0.005$.
‡ Significantly different from zero for F test: $P < 0.01$.
# Significantly different from zero for F test: $P < 0.001$.

iate stepwise regression analyses and the STEPWISE option was selected to determine predicted values of independent variables. A significance level of 0.15 was set for both entry and removal of variables from the model. All models were performed with or without logarithmically transformed Lp(a) concentration as an independent variable. All statistical analyses were performed using an SAS software package.

Results

A total of 402 ethnic Melbourne South Asians (216 men, 186 women) were studied. Distribution statistics of Lp(a) concentrations in this population are presented in Table 1. The frequency distribution for Lp(a) concentration in South Asians was skewed to low levels with a median of 239 mg/l and a mean of 324 mg/l and with only 25.6% (14.7% men, 10.9% women) of the population having Lp(a) concentrations ≤ 100 mg/l. Mean and median Lp(a) concentrations were higher in women (mean 340, median 282) than in men (mean 310, median 227) but the differences were not statistically significant. The proportion of individuals with Lp(a) values above the generally accepted risk threshold of 300 mg/l was 44% (36% men, 46% women). In women, Lp(a) concentrations were low in old age group than in young and middle-age groups.

Spearman correlation coefficients describing relationships of Lp(a) concentrations with age, health status, lifestyle, anthropometry and biochemistry are shown in Table 2. Lp(a) concentrations were negatively correlated with daily intake of low alcohol beer, triglycerides, insulin, and glucose and positively correlated with total cholesterol, LDLC, and LDL to HDL ratio only in men. There was a negative relationship between Lp(a) and triglycerides in women.

The results of a stepwise multiple regression analysis of various factors and Lp(a) concentration in men and women, and in young (men = 82, women = 93), middle (men = 111, women = 81), and old (men = 23, women = 12) age groups are shown in Tables 3 and 4. A multiple linear regression

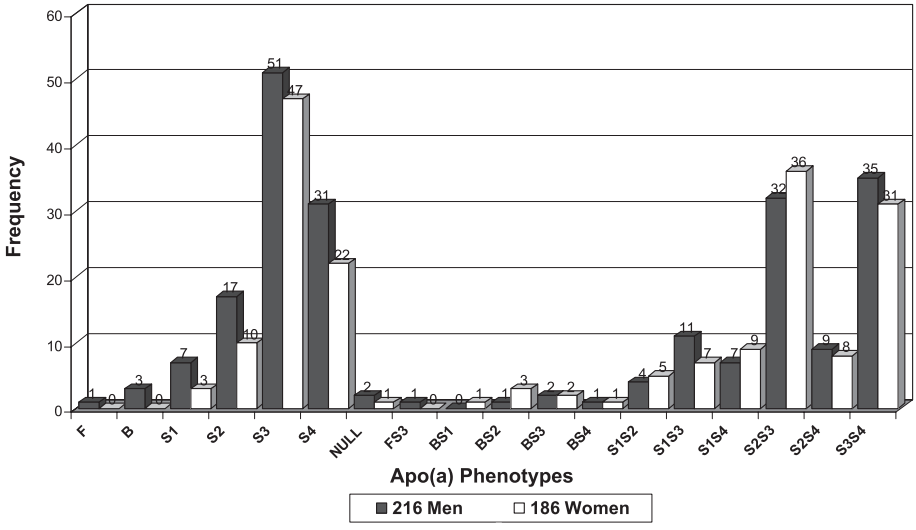

Fig. 1. Gender based distribution of Apo(a) phenotypes in 402 South Asian Melburians.

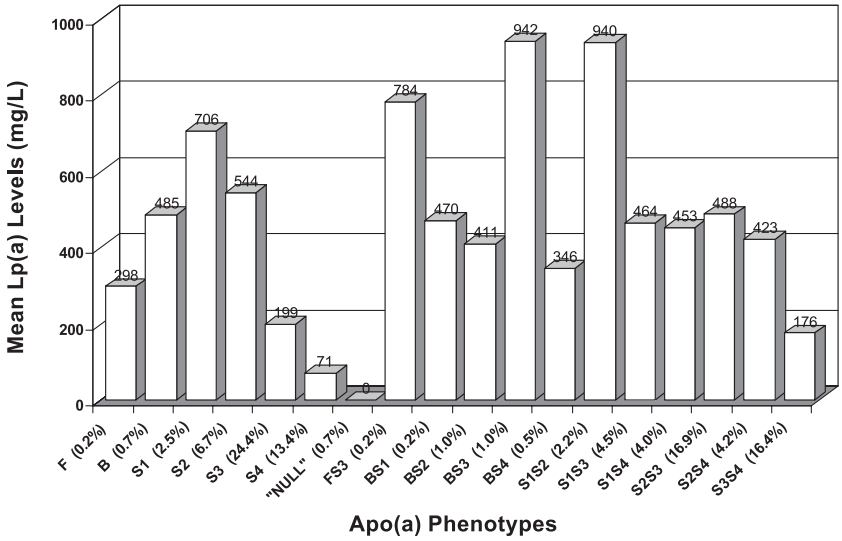

Fig. 2. Relationships between Apo(a) phenotypes and Lp(a) concentrations in 402 South Asian Melburians.

analysis was first performed using Lp(a) lipoprotein level as a dependent variable as described by Rosby and Berg [14] and Trommsdorff et al. [15], the independent quantitative variables included in the model were age, waist circumferences, glucose, triglycerides, and LDLC. Table 3 showed that 28% of the variance of Lp(a) concentration in men and 18% in women were explained by age, waist circumferences, glucose, and triglycerides. Age was positively associated with Lp(a) concentrations in men, but negatively in women. Table 4 showed the results of stepwise regression analyses of Lp(a) concentrations based on three age groups. The variables included in the model were age, waist circumference, triglycerides, and LDLC. In young people, age, triglycerides, and LDLC were found to be determinants of Lp(a) concentration. Twenty-one percent (21%) of the Lp(a) variance was accounted for by age ($P < 0.005$) and 38% by triglycerides ($P < 0.001$).

The distribution of apo(a) phenotypes is shown in Fig. 1. The S3 phenotype (24.4%) occurred more frequently than S4 (13.4%) in this population.

After adjusting for age ($P < 0.01$) or when Lp(a) was logarithmically transformed ($P < 0.001$), an inverse relationship between Lp(a) concentration and single-banded apo(a) phenotypes, except for B and "null", were identified in only three people, respectively, and F which occurred only in one person, too small a number from which to draw conclusions. BS3 and S1S2 phenotypes were associated with the highest mean Lp(a) concentrations in the population (Fig. 2).

Discussion

The present study investigated the distributions of Lp(a) concentrations and apo(a) phenotypes and their relationship. Sandholzer et al. [16] indicated that in people of Indian ancestry, the distribution of Lp(a) levels is intermediate in shape between Caucasians and blacks. The distribution of Lp(a) levels in the present study was skewed towards lower levels, which is similar to the distribution described for Indians living in Singapore [17].

Epidemiological studies have shown that Lp(a) plays an independent role in elevated risk of CVD in Indian population [11]. In our investigation, 40% of the population had Lp(a) levels greater than 300 mg/l. The results indicated that the population of South Asians with Indian ancestry living in Melbourne seem to be at a much greater risk of developing CVD, although definition of the risk threshold value for Lp(a) is clearly dependent of analytical method and ethnicity. However, the reason why Lp(a) concentration is so high in this population is not yet understood. Further study in South Asians of Indian ancestry with CVD is necessary.

We did not find differences in the mean and median plasma Lp(a) concentrations between men and women. The result is consistent with other studies [18–20]. However, there was a general trend in women to have higher Lp(a) values than men. An observation was confirmed by some investigators [21–23], but not by others, [24] that women have a lower risk of CVD compared with men. After menopause, the risk of CVD was reached in women more than in men [25].

Age is also important as a predictor of CHD. In our study, using a stepwise regression analysis, 21% of the variance of Lp(a) concentration in men was attributable to age. In women, the figure was only 6%. Increased age was positively associated with Lp(a) concentrations in men and negatively in women. When age was classified to three groups, 21% of the variance of Lp(a) concentration could be accounted for by the <40 age group ($P < 0.005$). This result is consistent with those of our Melbourne Chinese, Anglo-Celtic, and Pilbara Aboriginal [26–28] populations

studies and suggested that age may be an affected factor for Lp(a), although Heinrich et al. [29] and Steinmetz et al. [30] found a lack of relationship of Lp(a) concentration to age.

Moderate alcohol intake decreases the degree of CVD and can benefit health [31]. The results of the present study showed an inverse relationship between daily drinking of low alcohol beer and Lp(a) concentrations. It supports the beneficial effects of alcohol in the reduction of Lp(a) levels thereby reducing the risk of CVD. This association could be because beer contains phytoestrogenic substance [32] which may affect the Lp(a) metabolism. However, the mechanism of this effect of phytoestrogens upon Lp(a) *is not clear*. Another surprising result is the positive correlation of daily wine intake and Lp(a) concentration. This could indicate that excessive alcohol consumption might cause Lp(a) levels increased. How alcohol interferes with Lp(a) metabolism is *unknown*. It has recently been demonstrated that Lp(a) was synthesized in liver [33]. Therefore, damage of the liver resulting from heavy alcohol intake could affect Lp(a) synthesis [34].

Lp(a) is a constituent of human plasma similar to LDLC in its composition. The results of the present study showed that Lp(a) concentrations and LDLC are positively correlated in men ($P < 0.001$). It supports the view that the structure of Lp(a) is similar to LDLC, that is, both are rich in cholesterol, contains apoB (B-100), and have a phospholipid content [35–37]. It is suggested that they likely share a similar mechanism. However, this relationship, which did not exist in women, is possibly explained by metabolic differences [1].

Triglycerides were inversely correlated with Lp(a) levels in both men and women. The negative relationship between Lp(a) concentrations and plasma triglycerides detected in the present study is in agreement with our findings in Aboriginal and Anglo-Celtic Melburnian populations [27,28] but differ from the results obtained in the Chinese Melburnian [26]. One possible reason for this could be that increased plasma triglycerides is associated with decreased Lp(a) synthesis in the liver or those plasma triglycerides may play a role in regulating Lp(a) synthesis.

Based on bands and molecular weight of the phenotypes, subjects were classified as single- and double-banded and large and small molecular weight (MW) apo(a) phenotypes. Strong statistically significant differences were found Lp(a) concentrations between single- and double-banded and large and small MW phenotypes. In agreement with the earlier studies of Helmhold et al. [38], we also found that Lp(a) levels of the small MW were higher than those of the large molecular weight ($P < 0.0001$). Moreover, we found that Lp(a) concentrations of the double-banded were higher than those in single-banded phenotypes in men ($P < 0.001$) and women ($P < 0.0001$). After adjusting for age ($P < 0.01$) or when Lp(a) was logarithmically transformed ($P < 0.001$), an inverse association between Lp(a) levels and the apo(a) isoform mass was observed in accordance with previous data [26,27,30,38]. From the results of ectrophoretic mobility, the six types of apo(a) isoforms were identified based on their Kringle 4 repeat numbers such as 21, 23, 40, etc. According to Kraft et al. [39], although we did not attribute the corresponding number of Kringle 4 repeats to each isoform. These phenotypes can be inter-converted into the six different phenotypic groups as F (with 11–15 repeats), B (15–19), S1 (19–23), S2 (23–27), S3 (27–31), and S4 (31–35) [40], although Albers and Marcovina [41] provide evidence that variation in the number of Kringle 4 repeats influences results in methods using anti apoB-100 recognition antibodies. Therefore, the number of Kringle 4 repeats being inversely correlated to Lp(a) lipoprotein level.

Conclusions

This study confirmed the major influence of the apo(a) size on plasma Lp(a) concentrations and investigated other factors including age, lifestyle, and other lipids, which may partly contributed to variance of Lp(a) levels. Further studies are necessary to define the mechanism in which the Lp(a) level is regulated based on a large sample of patients with CVD and to evaluate the contribution of Lp(a) concentrations and apo(a) phenotypes in the pathogenesis of CVD.

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