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Association Between Apolipoprotein(a) Phenotypes and Coronary Heart Disease at a Young Age

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Objectives. **The purpose of this study was to investigate lipoprotein(a) [Lp(a)] levels and apolipoprotein(a) [apo(a)] phenotypes in relation to age of onset of coronary heart disease (CHD).**

Background. **Although Lp(a) levels have been extensively analyzed in relation to age of CHD, apo(a) phenotypes have not.**

Methods. **Three hundred and thirty-five consecutive CHD patients were enrolled and grouped according to their age of CHD onset (<45 years; 45 to 54 years; ≥55 years).**

Results. **In each patient group Lp(a) levels were higher than in an age-matched control group, but among the patient groups no differences in Lp(a) levels were observed. Apolipoprotein(a) phenotype distributions showed significant differences between patients and age-matched control subjects. Among the patient groups the difference in percentage of subjects with two apo(a) isoforms of low molecular weight (MW) was highly significant ($p < 0.001$). Multivariate analysis showed that apo(a) phenotypes were the best predictors of early CHD ($p < 0.000001$). The age-specific odds ratios (ORs) of the presence of at least one apo(a) isoform of low MW for CHD declined with age; in particular apo(a) phenotypes had their highest predictive value in younger persons (OR: 14.62). The OR for the presence of two isoforms of low MW/presence of only isoforms of high MW was 40.88 in the younger age group, 27.17 in age group of 45 to 54 years and 15.83 in the older age group.**

Conclusions. **The present article reports the first evidence of a strong independent association of apo(a) phenotypes with the age of onset of CHD. Thus, if our data are confirmed by larger studies, apo(a) phenotypes might be used together with Lp(a) levels as powerful genetic markers in assessing the actual risk of developing CHD at a young age.**

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Lipoprotein(a) [Lp(a)] represents a plasma lipoprotein, which is similar in structure to low density lipoprotein (LDL), but has a specific apolipoprotein, called apolipoprotein(a) [apo(a)], linked by a disulfide bond to apolipoprotein B100 of LDL (1). Apolipoprotein(a) is characterized by a high degree of genetic polymorphism, with many isoforms in plasma (2,3). Lipoprotein(a) plasma concentrations are largely determined by apo(a) gene, and they are inversely related to the molecular weight (MW) of apo(a) isoforms (4). Many studies have shown that Lp(a) levels are closely associated with premature coronary heart disease (CHD) (5–12). Studies on the association between Lp(a) levels and CHD in older persons have reported quite contradictory results (5–7,13,14). Some investigations have suggested the importance of apo(a) polymorphism as a predictor of CHD (15–17), especially when apo(a) polymorphism is studied by high resolution methods (18–20). However, no specific studies have investigated apo(a) phenotypes as predictors of age of onset of CHD. Therefore, we analyzed the behavior of Lp(a) levels and apo(a) phenotypes in relation to the age of onset of CHD in patients, grouped according to their age of CHD onset.

Methods

Subjects. A total of 335 patients with CHD (295 men; 40 women; mean age: 52.0 ± 7.3 years) were consecutively recruited from among patients attending the outpatients' department of the Division of Cardiology. The criterion for inclusion was an established CHD with a clinical onset 1 to 6 months before the recruitment. To avoid variation in Lp(a) levels caused by acute events, we enrolled the patients at least 1 month after the acute cardiovascular event. However, all patients were enrolled within 6 months from the clinical onset of CHD. The mean time elapsed between the clinical onset of CHD and the enrollment was 2.1 ± 1.6 months. Coronary heart disease was defined as a documented myocardial infarction (diagnosed by elevation of cardiac enzyme levels and diagnostic change on their electrocardiogram); coronary artery

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Abbreviations and Acronyms

apo(a) = apolipoprotein(a)
BMI = body mass index
CHD = coronary heart disease
HDL = high density lipoprotein
LDL = low density lipoprotein
Lp(a) = lipoprotein(a)
MW = molecular weight
OR = odds ratio

bypass grafting or angioplasty, or a history of angina pectoris together with a positive exercise test and a stenosis of the lumen >70% in at least one major coronary vessel. Coronary heart disease patients were receiving nitrates, calcium channel blocking agents, beta-adrenergic blocking agents, angiotensin-converting enzyme inhibitors and antiaggregant agents. No patients received diuretics, digitalis, estrogens, lipid-lowering drugs or niacin. Diagnosis of diabetes mellitus was done according to the criteria of the National Diabetes Data Group (21). Diagnosis of hypertension was done according to WHO/ISH criteria (22). We also evaluated a family history of coronary heart disease (first degree relatives with documented myocardial ischemia or infarction) and smoking habits (as the percentage of smokers/nonsmokers and as smoking index = daily cigarettes × years of smoking). Exclusion criteria were: valvular heart disease, bundle branch block, congestive heart failure, cardiomyopathy and renal or liver diseases. Patients were divided into three groups according to their age of onset of CHD: 50 patients were 44 years old or younger, 152 patients were aged 45 to 54 years and 133 patients were 55 years old or older. As control groups, three groups of healthy subjects matched for age and gender were enrolled from blood donors, staff hospital and their relatives. In every control subject the medical history and an exercise stress test excluded the possibility of CHD (19,20,23). All subjects recruited were White Italians and gave their informed consent to participate in the study. A part of the database (21.7% of the patients and 60.2% of the control subjects) was the same as a previous report (20).

Laboratory procedures. Venous blood samples were taken from all subjects between 9 and 11 AM after fasting for 12 h. For the quantification of Lp(a) and the characterization of apo(a) isoforms we used plasma obtained by addition of ethylenediaminetetraacetic acid and low speed centrifugation at 4°C for 12 min. Immediately after centrifugation, the plasma samples were frozen and stored at −80°C. Total cholesterol, high density lipoprotein (HDL) cholesterol and triglycerides were enzymatically measured by an automatic analyzer Hitachi 737 (Tokyo, Japan). Low density lipoprotein cholesterol was calculated by Friedewald's formula (24), considering the contribution of Lp(a) on LDL levels (25). Lipoprotein(a) plasma concentrations were determined by a sandwich enzyme-linked immunosorbent assay method [Macra-Lp(a), SDI, Newark, Delaware]. To detect apo(a) isoforms, an immunoblotting technique was used, as previously described (26). Briefly, after an electrophoretic run performed on 1% sodium dodecyl sulfate–agarose submarine gel, the proteins separated were transferred onto a polyvinylidene difluoride membrane (Immobilon P, Millipore, Bedford, Texas), by a capillary blotting technique. Then, the proteins were tested with a polyclonal antihuman Lp(a) antiserum from sheep (Immuno AG, Vienna, Austria). Peroxidase-conjugate rabbit antisheep immunoglobulins (Dako, Glostrup, Denmark) were used as a second antibody. At the end, the polyvinylidene difluoride membrane was immersed in a developing solution. As standards, apo(a) isoforms F1 = 400 kDa, S1 = 520 kDa, S2 = 580 kDa and S3 = 640 kDa (27) (Immuno AG) were utilized.

Statistical analysis. By using an analysis of covariance, all data regarding lipid parameters were adjusted for gender, body mass index (BMI), smoking habits, drug intake and presence of diabetes, hypertension and menopause. To assess differences in cholesterol, LDL, HDL and BMI, the Student t test (comparison between two groups) or analysis of variance (comparison among three groups) was utilized. Because of the highly "skewed" distribution of Lp(a) and triglycerides levels, to compare Lp(a) and triglycerides values the Mann–Whitney U test (comparison between two groups) or Kruskall–Wallis test (comparison among three groups) was used. The Pearson chi-square test was exploited for frequency comparison, as apo(a) isoforms, smokers/nonsmokers and so on. The relationship between Lp(a) levels and apo(a) phenotypes was evaluated both by Pearson's correlation coefficient and analysis of variance. Stepwise regression analysis was used to predict age of CHD onset among patients. Gender, diabetes, hypertension, family history of CHD, smoking index, BMI, Lp(a), cholesterol, triglycerides, LDL, HDL and apo(a) phenotypes of patients were tested as potential predictors. A forward selection procedure was used. The forward selection starts with an empty model and adds independent variables in order of their ability to predict the dependent variable (age of onset of CHD). The criterion for inclusion into the model as an independent variable is a p value <0.05, as determined with the F test. Variables showing a nonnormal distribution were logarithmically transformed before the analysis to produce approximately normal distribution. To provide a measure of the association of high Lp(a) levels and apo(a) phenotypes with CHD, age-specific odds ratios (ORs) were estimated, and the results were given as ORs and 95% confidence interval. Data were presented as means ± SD, unless otherwise stated. A probability of $p < 0.05$ was considered significant.

Results

Table 1 reports the biological and clinical features of patients and control subjects, stratified by age. Among the patient groups, no differences in common cardiovascular risk factors were observed. The percentage of male, diabetic and hypertensive subjects significantly increased together with the age of the onset of CHD. Although Lp(a) levels were higher in younger patients, among the three patient groups the difference in Lp(a) levels did not achieve statistical significance.

Table 1. Biological and Clinical Characteristics of Patients and Control Subjects Stratified by Age

	Patients			
	< 45 Years	45 to 54 years	> 54 Years	p
n	50	152	133	
Age (yr)	40.6 ± 3.4	49.6 ± 2.7	59.1 ± 3.9	< 0.001
Age range (yr)	32 to 44	45 to 54	55 to 75	
Gender (M/F)	49/1	134/18	112/21	< 0.05
Family history of CHD	56.0%	50.6%	42.1%	NS
Diabetes	10.0%	20.3%	27.8%	< 0.05
Duration (yr)	8.2 ± 5.3	8.2 ± 6.0	6.6 ± 7.8	NS
Hypertension	14.0%	32.8%	45.8%	< 0.001
Duration (yr)	5.1 ± 3.6	7.4 ± 4.4	5.8 ± 5.2	NS
Smokers	74.0%*	66.4%*	60.9%*	NS
Smoking index	446.1 ± 397.5*	422.8 ± 405.4*	460.1 ± 510.5*	NS
BMI	26.6 ± 4.3*	25.5 ± 3.1	25.2 ± 3.1	NS
Triglycerides (mmol/liter)	1.8 ± 0.7†	1.7 ± 0.6*	1.7 ± 0.7	NS
Cholesterol (mmol/liter)	5.8 ± 1.4*	5.9 ± 1.1*	5.9 ± 1.2*	NS
LDL (mmol/liter)	3.8 ± 1.2*	3.8 ± 0.9*	3.9 ± 1.0*	NS
HDL (mmol/liter)	1.1 ± 0.2	1.1 ± 0.2	1.1 ± 0.2	NS
Lp(a) (mg/dl)				
Mean	29.8 ± 23.9	28.9 ± 22.3	27.9 ± 22.9	
Median	27.2*	24.0*	18.5*	NS
Range	0.5 to 88.5	0.5 to 92.5	0.5 to 97	
Percentage of subjects with Lp(a) > 30 mg/dl	42.0%*	33.5%*	36.8%*	NS
	Control Subjects			
	<45 Years	45 to 54 years	>54 years	p
n	69	158	143	
Age (yr)	40.5 ± 3.6	49.7 ± 2.8	59.0 ± 3.9	< 0.001
Age range (yr)	32 to 44	45 to 54	55 to 75	
Gender (M/F)	68/1	139/19	120/23	< 0.05
Family history of CHD	13.0%	28.4%	24.2%	< 0.05
Smokers	26.0%	30.3%	25.0%	NS
Smoking index	98.9 ± 123.6	166.3 ± 297.5	157.2 ± 300.5	< 0.001
BMI	22.5 ± 3.6	23.9 ± 3.1	24.4 ± 3.6	< 0.001
Triglycerides (mmol/liter)	1.4 ± 0.4	1.4 ± 0.5	1.5 ± 0.8	NS
Cholesterol (mmol/liter)	4.8 ± 0.6	5.1 ± 1.0	5.3 ± 1.1	< 0.05
LDL (mmol/liter)	2.9 ± 0.7	3.1 ± 0.8	3.2 ± 1.0	NS
HDL (mmol/liter)	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.3	NS
Lp(a) (mg/dl)				
Mean	15.0 ± 16.9	14.5 ± 15.9	14.0 ± 16.5	NS
Median	8.5	7.5	8.0	
Range	0 to 84	0 to 80	0 to 83.5	
Percentage of subjects with Lp(a) > 30 mg/dl	14.0%	13.9%	13.2%	NS

*p < 0.001 (vs. age-matched control subjects). †p < 0.05 (vs. age-matched control subjects). ‡p < 0.01 (vs. age-matched control subjects). BMI = body mass index; CHD = coronary heart disease; HDI = high density lipoprotein; LDI = low density lipoprotein; Lp(a) =lipoprotein(a).

Moreover, no differences in the percentage of subjects with Lp(a) levels >30 mg/dl were observed. However, each patients group showed Lp(a) levels and a percentage of subjects with Lp(a) levels >30 mg/dl higher than age-matched control subjects. We have also evaluated the percentages of subjects with Lp(a) levels >50 or 70 mg/dl, but no differences were found.

Analysis of apo(a) polymorphism. Out of the 705 subjects recruited, we identified 30 different apo(a) isoforms with apparent MW varying from 280 to 835 kDa. The detection method gave good sensitivity, specificity and reproducibility, as previously described (26,28). Among the patients, 166 (49.5%) were heterozygotes (29 in the group of patients younger than 45 years; 82 in that of patients aged 45 to 54 years; 55 in that of patients 55 years old or older), 169 (50.5%) showed only one apo(a) isoform (21 in the group of patients younger than 45 years; 70 in that of patients aged 45 to 54 years; 78 in that of patients 55 years old or older) and none had the so-called "null-phenotype" (no bands detectable by immunoblotting). Among the control subjects, 177 (47.9%) were heterozygotes (25 in the group of control subjects younger than 45 years; 82 in that of control subjects aged 45 to 54 years; 70 in that of

Table 2. Number and Percentage of Several Apolipoprotein(a) Phenotypes Found in Patients and Control Subjects Stratified by Age

	Patients			
apo(a) Phenotypes	< 45 Years (n = 50)	45 to 54 Years (n = 152)	> 54 Years (n = 133)	p
Subjects with at least one apo(a) isoform of low MW	44 (88.0%)*	131 (86.2%)*	98 (73.7%)*	< 0.02
Subjects with two apo(a) isoforms of low MW	16 (32.0%)*	32 (21.1%)*	11 (8.3%)	< 0.001
Subjects with one apo(a) isoform of low MW	28 (56.0%)†	99 (65.1%)*	87 (65.4%)*	NS
Subjects with only apo(a) isoforms of high MW	6 (12.0%)*	21 (13.8%)*	35 (26.3%)*	< 0.02
	Control Subjects			
apo(a) Phenotypes	< 45 Years (n = 69)	45 to 54 Years (n = 158)	> 54 Years (n = 143)	p
Subjects with at least one apo(a) isoform of low MW	23 (33.2%)	51 (32.3%)	48 (33.6%)	NS
Subjects with two apo(a) isoforms of low MW	3 (4.3%)	6 (3.8%)	2 (1.4%)	NS
Subjects with one apo(a) isoform of low MW	20 (28.9%)	45 (28.5%)	46 (32.2%)	NS
Subjects with only apo(a) isoforms of high MW	46 (66.8%)	107 (76.7%)	95 (66.4%)	NS

*$p < 0.001$ (vs. age-matched control subjects). †$p < 0.05$ (vs. age-matched control subjects). apo(a) = apolipoprotein(a); MW = molecular weight.

control subjects 55 years old or older), 185 (50.0%) showed only one apo(a) isoform (42 in the group of control subjects younger than 45 years; 73 in that of control subjects 45 to 54 years old; 70 in that of control subjects 55 years old or older) and 8 (2.1%) showed the "null-phenotype" (2, 3 and 3, respectively). As done in previous studies (19,20,28–30), we used a cutoff between 640 and 655 kDa to divide apo(a) isoforms of low MW (280-400-430-445-460-490-505-520-535-550-565-580-595-610-625-640 kDa) and high MW (655-670-685-700-715-730-745-760-775-790-805-820-835 kDa). This cutoff seems to discriminate well apo(a) isoforms associated with high cardiovascular risk (19,20). In every patient group the percentage of apo(a) isoforms of low MW was greater than that of isoforms of high MW. In particular, the percentage of apo(a) isoforms of low MW was 75.8% in the group of patients younger than 45 years, 69.6% in patients aged 45 to 54 years and 58.0% in patients 55 years old or older. On the contrary, in every control group the percentage of apo(a) isoforms of high MW was higher than that of isoforms of low MW. In particular, the percentage of apo(a) isoform of high MW was 72.2% in the group of control subjects younger than 45 years, 76.1% in control subjects aged 45 to 54 years and 76.3% in control subjects 55 years old or older. Among the three patient groups, the difference in the percentages of apo(a) isoforms of low and high MW was significant (chi-square = 7.818; df = 2; $p < 0.02$). In addition, the difference in apo(a) isoforms between each patient group and age-matched control group was highly statistically significant ($p < 0.001$). In our study population Lp(a) levels were inversely related to apo(a) isoforms MW in both patients ($r = -0.289966$; $p = 0.000000$) and control subjects ($r = -0.295531$; $p = 0.000553$). This analysis was performed between each apo(a) isoform (expressed as a MW) and Lp(a) levels log-transformed. In subjects showing two electrophoretic bands, the band with lower MW was considered for the expression of the phenotype, because the band with lower MW was well correlated with Lp(a) in double-band phenotype (31). Analysis of variance shows that apo(a) phenotypes explained 25% of the variability in Lp(a) levels in control subjects and 33% in patients.

Analysis of apo(a) phenotypes. Table 2 reports number and percentage of subjects with several apo(a) phenotypes in groups of patients and control subjects, stratified by age. First of all, we divided subjects of each group into two subgroups: subjects with at least one apo(a) isoform of low MW (low phenotypes) and subjects with only apo(a) isoforms of high MW (high phenotypes). In each patient group the percentage of subjects with low phenotypes is higher than the percentage of subjects with high phenotypes. On the contrary, in each control group subjects with high phenotypes are more prevalent. The difference in apo(a) phenotypes between each patient group and age-matched control group is highly significant ($p < 0.001$). Moreover, among the three groups of patients the difference in apo(a) phenotypes is significant (chi-square = 8.999; df = 2; $p < 0.02$).

Then, subjects with at least one apo(a) isoform were subdivided into two subgroups: subjects with two apo(a) isoforms of low MW and subjects with one apo(a) isoform of low MW. As shown in Table 2, among CHD patient groups, whereas no differences in the percentage of subjects with one apo(a) isoform of low MW were observed, the difference in the percentage of subjects with two apo(a) isoforms of low MW is highly significant (chi-square = 16.372; df = 2; $p < 0.001$).

Multivariate analysis. In CHD patients the common cardiovascular risk factors, including Lp(a) concentration, were tested as potential predictors of age of CHD onset (dependent variable) in a stepwise regression analysis. This analysis showed that only hypertension entered into the model as a significant independent variable (step = 1; F value = 9.563399; beta = 0.167084; $p = 0.002153$). When apo(a) phenotypes (presence of two isoforms of low MW; presence of one isoform of low MW; presence of only isoforms of high MW) were added to the list of potential predictors, regression analysis showed that apo(a) phenotypes (step = 1; F value = 37.53338; beta = −0.325534; $p = 0.000000$), male gender (step = 2; F

Table 3. Age-Specific Odds Ratios and 95% Confidence Intervals of High Lipoprotein(a) [Lp(a)] Levels (> 30 mg/dl) and Low Apolipoprotein(a) [apo(a)] Phenotypes (Presence of at Least One Isoform of Low Molecular Weight) for Coronary Heart Disease

Age (yr)	High Lp(a) Levels	Low apo(a) Phenotypes
< 45	4.27 (1.78 to 10.24)	14.62 (5.45 to 39.45)
45 to 54	3.40 (1.94 to 5.96)	13.00 (7.41 to 23.11)
≥ 55	3.80 (2.04 to 6.92)	5.54 (3.29 to 9.34)

value = 10.60775; beta = −0.120434; p = 0.026956) and hypertension (step = 3; F value = 4.89960; beta = 0.119076; p = 0.027515) are independent variables predicting age of onset of CHD.

Age-specific ORs of high Lp(a) levels and apo(a) phenotypes. Table 3 reports age-specific ORs of high Lp(a) levels and low apo(a) phenotypes for CHD. High Lp(a) levels significantly increased the risk for CHD in every age, even if Lp(a) levels did not seem to show important differences in ORs in relation to age of CHD onset. On the contrary, the age-specific ORs of low apo(a) phenotypes declined with the age; in particular low apo(a) phenotypes conferred the highest cardiovascular risk in subjects <45 years old. Age-specific ORs for the presence of two isoforms of low MW and for the presence of one apo(a) isoform of low MW were also estimated. The age-specific ORs for presence of two isoforms of low MW/presence of only isoforms of high MW were 40.88 (9.14 to 182.91) in the younger age group, 27.17 (10.10 to 73.09) in the age group of 45 to 54 years and 15.83 (3.33 to 75.18) in the older age group. Therefore, subjects with two isoforms of low MW have a risk of developing CHD before 45 years 40.88-fold higher than subjets with only isoforms of high MW. The risk related to this phenotype decline with age. The age-specific ORs for the presence of one apo(a) isoform of low MW/presence of only apo(a) isoforms of high MW were 10.73 (3.84 to 29.95) in the younger age group, 11.20 (6.24 to 20.13) in the age group of 45 to 54 years and 5.13 (3.03 to 8.69) in the older age group.

Discussion

The present report shows a strong association between Lp(a) levels and CHD. This finding is in agreement with many case–control studies (5–8,10,12,15–20,27) and some prospective studies (9,11,32–34). However, in some of the large-scale prospective studies no strong association between Lp(a) levels and CHD has been identified (35–37). Different experimental designs, different number and characteristics of subjects recruited, ethnical differences, medical treatments (aspirin, beta carotene, lipid-lowering drugs), lack of standardization of methods for the determination of Lp(a) levels and different ways in which plasma samples are handled might at least partially explain the differences among the studies. Only one prospective study analyzed both Lp(a) levels and apo(a) phenotypes as predictors of CHD (38). In that study Lp(a) levels appeared a strong independent risk factor for CHD in men, but apo(a) phenotypes did not (38). This contradictory result might be explained not only by the relatively small number of case–control pairs and the high number of apo(a) isoforms (38) but also by the fact that a cutoff of apo(a) polymorphism was not used (20).

Lp(a) levels, apo(a) phenotypes and age of onset of CHD. Lipoprotein(a) levels do not seem to be reliable predictors of age of onset of CHD. Indeed, several studies documented elevated Lp(a) levels in patients with premature CHD (5–12), whereas conflicting results have been reported on the role of Lp(a) levels in the development of CHD in older persons (5–7,13,14). In our survey we found significant differences in Lp(a) levels between each patient group and age-matched control group; moreover, among patient groups no differences were observed both in univariate and in multivariate analysis. When apo(a) phenotypes were analyzed, we observed that significant differences in the percentage of subjects with only one apo(a) isoform of low MW exist between each patient group and age-matched control group; nevertheless, no differences in the distribution of this phenotype were present among patient groups. Therefore, Lp(a) levels and the presence of one apo(a) isoform of low MW have the same behavior in relation to age of CHD onset. This suggests that subjects with high Lp(a) levels and one apo(a) isoform of low MW have a high genetic risk of developing CHD, but for these subjects it is not possible to predict the age of occurrence. However, the presence of one apo(a) isoform of low MW appears to be a genetic risk factor independent of Lp(a) levels. Therefore, in a primary prevention setting it might be useful to take into consideration both Lp(a) levels and apo(a) phenotypes to identify the highest number of subjects with high cardiovascular risk linked to apo(a) gene. Indeed, if only Lp(a) levels are considered, subjects with normal Lp(a) levels but near to cutoff (for example: 15 to 30 mg/dl) usually are not considered at high risk for CHD. In reality, some of these subjects may have an isoform of low MW, considering that persons with the same apo(a) phenotype often show widely diffent Lp(a) levels and vice versa (4,39). Moreover, to evaluate well the genetic cardiovascular risk related to apo(a) gene, the study of apo(a) polymorphism is useful in subjects with genetic or environmental factors that can affect Lp(a) levels (40). The interesting new finding of this investigation regards the presence of two apo(a) isoforms of low MW. Indeed, a strong association between this apo(a) phenotype and CHD at a young age is present. Whereas the presence of two isoforms of low MW in control groups is quite uncommon (4.3% or less), this phenotype has a high prevalence in CHD patients <45 years old (32%); in addition, its prevalence declines significantly with age. The strong association between the presence of two isoforms of low MW and CHD at a young age is confirmed by ORs; indeed, subjects with two isoforms of low MW have a risk of developing CHD before 45 years 40.88-fold higher than subjects with only isoforms of high MW. Therefore, the presence of two apo(a) isoforms of low MW could represent an efficient discriminator of subjects with high genetic risk for early onset CHD, in particular of subjects with high genetic risk of

developing CHD before 45 years. However, the importance of apo(a) phenotypes in identifying subjects with high risk for early CHD is emphasized both by regression analysis and by ORs for low phenotypes. It is interesting to note that stepwise regression analysis showed a strong independent correlation between apo(a) phenotypes and age of CHD onset among patients recruited. The regression analysis was performed, including as the dependent variable the age of each patient and not a categorization of data (for example, in relation to age groups). Therefore the differences in apo(a) phenotypes found in univariate analysis among patient groups are not influenced by the age cutoff points chosen to divide subjects. However, we used some cutoff points, to permit a clinical interpretation of results. In particular, a cutoff point (55 years) is helpful to identify subjects with premature CHD (5,6,9,17); moreover, among patients with premature CHD, another cutoff point (45 years) was fixed to identify subjects with CHD at a young age.

Apolipoprotein(a) isoforms and atherothrombosis. Obviously it is important to understand why two apo(a) isoforms of low MW are more strongly associated with early CHD than the presence of one apo(a) isoform of low MW. This may be explained by a possible additional direct role of apo(a) isoforms in atherothrombosis independently of corresponding Lp(a) plasma concentrations. Indeed, recently it has been hypothesized that different apo(a) isoforms may differ in their pathogenicity by a mechanism that is independent of corresponding Lp(a) level in plasma (41). In addition, it is generally accepted that the majority of Lp(a)–substrate interactions are mediated by the apo(a) component of Lp(a) (42). Finally, a recent study has shown that apo(a) isoforms may display polymorphism-linked functional heterogeneity with regard to cell binding, which may explain the higher association with cardiovascular risk of small apo(a) isoforms (43). Our study seems to provide in vivo evidence of these findings. Therefore, it is reasonable to hypothesize that in subjects with high Lp(a) levels and both isoforms of low MW, coronary atherosclerosis may be earlier and more accelerated; moreover, thrombotic mechanisms due to Lp(a) (44) may be more active. Thus CHD might occur at a younger age.

Clinical implications. The present study may have important clinical implications. Indeed, if our data are confirmed by larger studies, apo(a) phenotypes could be used together with Lp(a) levels as powerful markers to assess the genetic risk of developing CHD at a young age. Indeed, the analysis of apo(a) polymorphism in subjects with high Lp(a) levels might permit discriminating subjects with a high genetic risk for CHD (when one isoform of low MW is present), and those at high risk for CHD before 45 years (when both isoforms have low MW). This analysis may have great importance both in patients having diseases with high cardiovascular risk (diabetes, hypertension, familial hypercholesterolemia) and in subjects with other cardiovascular risk factors. Indeed, in these persons, when a high risk for early onset CHD linked to apo(a) gene is present, more intensive treatment of modifiable cardiovascular risk factors, and early and frequent diagnostic checks could be suitable.

Conclusions. The present article reports the first evidence of a strong independent association of apo(a) phenotypes with CHD at a young age. Accordingly, apo(a) phenotypes might be used together with Lp(a) levels as reliable genetic markers in assessing more precisely the risk of an early onset CHD.

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