Elevated plasma concentrations of lipoprotein(a) in patients with end-stage renal disease are not related to the size polymorphism of apolipoprotein(a).

H Dieplinger, … , H Graf, P König

Research Article

Patients with terminal renal insufficiency suffer from an increased incidence of atherosclerotic diseases. Elevated plasma concentrations of lipoprotein(a) [Lp(a)] have been established as a genetically controlled risk factor for these diseases. Variable alleles at the apo(a) gene locus determine to a large extent the Lp(a) concentration in the general population. In addition, other genetic and nongenetic factors also contribute to the plasma concentrations of Lp(a). We therefore investigated Apo(a) phenotypes and Lp(a) plasma concentrations in a large group of patients with end-stage renal disease (ESRD) and in a control group. Lp(a) concentrations were significantly elevated in ESRD patients (20.1 +/- 20.3 mg/dl) as compared with the controls (12.1 +/- 15.5 mg/dl, P < 0.001). However, no difference was found in apo(a) isoform frequency between the ESRD group and the controls. Interestingly, only patients with large size apo(a) isoforms exhibited two- to fourfold elevated levels of Lp(a), whereas the small-size isoforms had similar concentrations in ESRD patients and controls. Beside elevated Lp(a) concentrations, ESRD patients had lower levels of plasma cholesterol and apolipoprotein B. These results show that elevated Lp(a) plasma levels might significantly contribute to the risk for atherosclerotic diseases in ESRD. They further indicate that nongenetic factors related to renal insufficiency or other genes beside the apo(a) structural gene locus must be responsible for the high Lp(a) levels.

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Elevated Plasma Concentrations of Lipoprotein(a) in Patients with End-stage Renal Disease Are Not Related to the Size Polymorphism of Apolipoprotein(a)

Hans Dieplinger, Carolin Lackner, Florian Kronenberg, Christoph Sandholzer, Karl Lhotta,* Fritz Hoppichler, Helmut Graf,‡ Paul König*

*Institute of Medical Biology and Human Genetics and *University Clinic of Internal Medicine, University of Innsbruck, A-6020 Innsbruck, Austria; and ‡Department of Nephrology, University Clinic of Internal Medicine III, A-1090 Vienna, Austria*

Abstract

Patients with terminal renal insufficiency suffer from an increased incidence of atherosclerotic diseases. Elevated plasma concentrations of lipoprotein(a) [Lp(a)] have been established as a genetically controlled risk factor for these diseases. Variable alleles at the apo(a) gene locus determine to a large extent the Lp(a) concentration in the general population. In addition, other genetic and nongenetic factors also contribute to the plasma concentrations of Lp(a).

We therefore investigated Apo(a) phenotypes and Lp(a) plasma concentrations in a large group of patients with end-stage renal disease (ESRD) and in a control group. Lp(a) concentrations were significantly elevated in ESRD patients (20.1±20.3 mg/dl) as compared with the controls (12.1±15.5 mg/dl, $P < 0.001$). However, no difference was found in apo(a) isoform frequency between the ESRD group and the controls. Interestingly, only patients with large size apo(a) isoforms exhibited two- to fourfold elevated levels of Lp(a), whereas the small-size isoforms had similar concentrations in ESRD patients and controls. Beside elevated Lp(a) concentrations, ESRD patients had lower levels of plasma cholesterol and apolipoprotein B.

These results show that elevated Lp(a) plasma levels might significantly contribute to the risk for atherosclerotic diseases in ESRD. They further indicate that nongenetic factors related to renal insufficiency or other genes beside the apo(a) structural gene locus must be responsible for the high Lp(a) levels. (*J. Clin. Invest.* 1993. 91:397–401.) Key words: lipoprotein(a) • apolipoprotein(a) phenotypes • terminal renal insufficiency • end-stage renal disease • atherosclerosis

Introduction

Patients with chronic renal insufficiency and end-stage renal disease (ESRD)[1] form a large group with a well-known high incidence of cardiovascular disease and accelerated atheroscle-

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Address correspondence to Dr. Hans Dieplinger, Institute of Medical Biology and Human Genetics, University of Innsbruck, Schoepfstrasse 41, A-6020 Innsbruck, Austria. C. Lackner's present address is Departments of Internal Medicine and Molecular Genetics, University of Texas Southwestern Medical School, Dallas, TX 75235.

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1. *Abbreviations used in this paper:* CHD, coronary heart disease; ESRD, end-stage renal disease; Lp(a), lipoprotein(a).

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rosis (1). Although undoubtedly contributed by many other factors, several lipoprotein abnormalities have been suggested as a major cause of these diseases (2). Most studies have described hypertriglyceridemia as the most common lipid abnormality in ESRD patients (3). Interestingly, these patients are usually normo- or even hypocholesterolemic (4), which stands in marked contrast to patients with nephrotic syndrome who develop hypercholesterolemia (5). Increased hepatic synthesis rates for triglyceride- and cholesterol-rich lipoproteins to compensate for protein urinary losses have been suggested as the cause of the hyperlipemic state of patients with nephrotic syndrome. The reason for the rather normolipemic state in ESRD patients and their high incidence of atherosclerosis is less clear. More recently, an impaired reversed cholesterol transport system was described in ESRD patients (4, 6).

Numerous case-control studies in different ethnic groups have shown elevated plasma concentrations of lipoprotein(a) [Lp(a)] to be associated with various atherosclerotic diseases (7–9). At the moment, only one small nested case control study supports the atherogeneity of elevated Lp(a) levels (10).

Lp(a) consists of a LDL-like particle whose apo B-100 carries an additional protein, the apolipoprotein(a) (for a recent review, see reference 11). It is synthesized and secreted primarily by the liver (12). The physiological and pathophysiological functions including the sites and mechanisms of catabolism are largely unknown. Apo(a) is highly homologous to human plasminogen and contains a protease domain, one kringle 5 structure, and multiple repeats of kringle 4. This protein exhibits a genetically determined size polymorphism. Six different isoforms (designated F, B, S1, S2, S3, and S4, according to different electrophoretic mobilities) that vary in size from ~ 400 kD to > 800 kD were originally discovered and described in our laboratory (13). Meanwhile, improved techniques at higher resolution revealed > 20 protein isoforms (14). Quantitative Southern blotting and pulsed-field gel electrophoresis have shown that this polymorphism results from varying numbers of tandem kringle 4 repeats, leading to ≥ 19 different apo(a) alleles (15, 16). In the general population, the size of these isoforms is inversely related to plasma concentrations of Lp(a) (13, 16). Large isoforms are associated with low Lp(a) and small isoforms are associated with high Lp(a) concentrations in plasma.

The mechanism by which the apo(a) polymorphism determines Lp(a) concentrations in plasma is unknown. In healthy Caucasians, ~ 40% of the variability in Lp(a) levels is attributed to apo(a) size polymorphism (17). In patients with premature coronary heart disease (CHD), Sandholzer et al. demonstrated that through their effect on plasma Lp(a) concentrations alleles at the apo(a) locus determine these patients' risk for CHD (18). From this study, Lp(a) can be considered a primary genetic risk factor for CHD, even in the absence of any large prospective studies.

A variety of other genetic and nongenetic factors beside the allelic variation at the apo(a) gene locus have been described to explain the remaining influence on the variation in plasma Lp(a) levels. These include defective alleles at the LDL receptor gene (19) or acute phase reactions (20). Lp(a) levels could also be significantly elevated secondary to diseases with known increased risk for atherosclerosis (e.g., renal disease or diabetes mellitus) and might, therefore, at least partly explain this increased risk. On the other hand, increased Lp(a) concentrations might significantly contribute to the high risk state for atherosclerosis in the otherwise rather normolipemic patients with ESRD.

The present study, therefore, investigated apo(a) isoforms and plasma concentrations of Lp(a) in a large group of ESRD patients and controls. In the event of significantly elevated levels of Lp(a), comparison of the frequency distribution of the apo(a) isoforms of both groups would indicate whether or not the apo(a) structural gene locus determines these elevated Lp(a) levels.

Methods

Cases and controls. A total of 138 center-based patients (57% male and 43% female) with ESRD and undergoing hemodialysis were selected from the Centers of Clinical Nephrology at the University Hospitals in Innsbruck and Vienna. They had been treated for ESRD for an average of 27.8 mo (range of 1–157 mo). Dialysis was performed three times weekly for 4 h using fiber dialyzers (GF 120 series; Gambro, Hechingen Germany). The cause of ESRD was chronic glomerulonephritis in 90, chronic pyelonephritis in 36, and polycystic kidney disease in 12 cases. Patients with polycystic kidney disease had an average residual glomerular filtration rate of 8 ml/min and a daily urine output of 0.5–1.5 l. Glomerular filtration rate and urine output of all other patients was zero.

Dietary recommendations for all patients included a low carbohydrate (35% of total dietary caloric input) and fat-enriched (55% of total) diet. A daily food intake of 35 kcal/kg body wt including 1 g protein/kg body wt was chosen to compensate for the loss of amino acids during hemodialysis and for increased protein catabolism.

All patients received phosphate-binding medication. Of the patients, 38% received antihypertensive β-blocking therapy, Ca^{++} channel blocking agents and angiotensine-converting enzyme inhibitors.

The control group consisted of 236 individuals free of liver and renal disease (59% males and 41% females) selected from a group of consecutive blood donors in Innsbruck. ESRD patients and controls were comparable with regard to sex distribution. Mean age, however, was higher in the ESRD patients as compared with the controls (Table I).

In the hemodialysis group, blood was drawn immediately before dialysis and heparinization. Plasma was obtained by addition of

Table I. Mean Age and Plasma Concentration of Lp(a), Cholesterol, and Apo B in ESRD Patients and Controls

	ESRD patients (n = 138)	Controls (n = 236)	P
Age (yr)	51.2±13.8	39.2±12.8	<0.01
Lp(a) (mg/dl)	20.1±20.3	12.1±15.5	<0.001
Total cholesterol (mg/dl)	166.8±49.3	205.0±47.9	<0.001
Apolipoprotein B (mg/dl)	52.4±23.9	86.1±21.5	<0.001

Na$_2$EDTA and low speed centrifugation at 4°C. For quantification of plasma concentrations of cholesterol, Lp(a) and apo B and for phenotyping of apo(a), samples were frozen immediately after centrifugation and kept at −20°C before analysis.

Laboratory procedures. Lp(a) quantification was performed, essentially as described (21), with a double-antibody ELISA using an affinity-purified polyclonal anti-apo(a) antibody for coating and the horseradish peroxidase–conjugated monoclonal antibody 1A2 for detection. This antibody does not crossreact with plasminogen. A commercially available Lp(a) positive plasma from Immuno (Vienna) served as a standard. Lp(a) concentrations were expressed as total Lp(a) lipoprotein mass.

Apo(a) phenotyping was performed with SDS PAGE of plasma under reducing conditions followed by immunoblotting as previously described (13) with some minor modifications. Briefly, 2 µl of plasma was added to 50 µl 5% (wt/vol) SDS, 0.02 mmol/liter ethylmorpholine, pH 8.6, and 2 µl β-mercaptoethanol, and the solution was heated for 3 min in a microwave oven. Thereafter, 4 µl 1.5% (wt/vol) bromophenolblue in 10% (vol/vol) glycerol was added. A 5-µl aliquot was applied to a 6.6% polyacrylamide gel, prepared, and run according to Neville (22) in a 10 × 10-cm electrophoresis chamber from Biometra (Göttingen, Germany). Immunoblotting was performed as described using the monoclonal antibody 1A2 (23). A goat anti–mouse immunoglobulin conjugated with peroxidase was used as second antibody.

To keep the analytical conditions comparable to various previous studies, we used the same methodology and the same apo(a) isoform standards as recently described (13). Isoforms that did not exactly comigrate with the standards were binned with the closest respective isoform (18). The apparent mol mass of apo(a) isoforms was as follows: B ~ 460 kD, S1 ~ 520 kD, S2 ~ 580 kD, S3 ~ 640 kD, and S4 > 700 kD.

Cholesterol concentrations in plasma were determined using a commercially available kit from Boehringer Mannheim GmbH (Mannheim, Germany).

Apolipoprotein B concentrations in plasma were analyzed with a double-antibody ELISA using an affinity-purified polyclonal antibody against apo B for coating and the same antibody, labeled with peroxidase, for detection. The assay was performed in detail as follows: After coating microtiter plates with 4 µg/ml anti-apo B and blocking with a 0.1% solution of casein in PBS, plasma samples and standards were incubated for 2 h at 37°C. Plasma samples of patients and controls were diluted 1:25,000 in casein solution. A calibrated standard in a dilution series from 1:7,500–1:240,000 (Calibration Serum Apolipoprotein; Boehringer Mannheim), served as secondary standard. After incubation of samples and standards and several washing steps, the plate was incubated with the conjugated antibody for 2 h at 37°C, followed by incubation with the substrate o-phenylene diamine. This ELISA was established by comparison of the same plasma samples from patients and controls with a routine nephelometric method (Tina-quant Apolipoprotein B; Boehringer Mannheim) which gave reasonable correlation coefficients of 0.72 for the ESRD group and 0.85 for the controls.

Statistical analysis. Pearson's χ^2 statistic was used to test the independence of apo(a) phenotype frequencies between the samples of ESRD patients and controls. Because of the highly skewed distribution of Lp(a) plasma concentrations, the nonparametric Wilcoxon rank sum test was applied to discriminate for differences of Lp(a) levels between the groups of ESRD patients and controls, as well as in the most frequent phenotype groups. Multiple regression analysis was used to adjust apo B levels for the linear effects of sex and age. Student's *t* test was applied to assess differences of plasma cholesterol and plasma apo B concentrations between both groups. The Kruskal-Wallis analysis of variance by ranks was used to test the hypothesis that average phenotypic Lp(a) concentrations were significantly different among individuals with different apo(a) phenotypes. Spearman's rank test was used to correlate Lp(a) concentrations with the duration of dialysis, as well as apo B concentrations with age. Generally, $P > 0.05$ was considered NS.

Results

Apo(a) phenotypes and Lp(a) concentrations in ESRD patients and controls. Table I shows the mean plasma concentrations of Lp(a), apoB cholesterol, as well as the mean age of the ESRD patient group in comparison to the control group. The average Lp(a) plasma concentration of the patients (20.1±20.3 mg/dl) was 1.7-fold higher than in the control group (12.1±15.5 mg/dl) ($P < 0.001$). These increased levels are also demonstrated by the frequency distribution graph in Fig. 1. Lp(a) levels did not correlate with the duration of dialysis treatment (Spearman's rank test, $r = -0.02$, $P = 0.815$) in the overall ESRD group or in subgroups with individual apo(a) phenotypes (S2: $r = 0.07$, $P = 0.72$; S3: $r = -0.014$, $P = 0.94$; S4: $r = -0.09$, $P = 0.53$).

The frequency distribution of the different apo(a) isoforms shown in Table II, did not, however, vary significantly between ESRD patients and controls ($\chi^2 = 11.015$, $P > 0.2$). When both groups were subdivided into two subgroups with at least one low molecular weight isoform and with only high molecular weight isoforms, no difference was observed between the subgroups of either study group. Table III shows the average plasma Lp(a) plasma concentrations for each phenotype in both groups. These data clearly demonstrate that the well-known inverse correlation between isoform protein size and plasma concentrations of Lp(a) is virtually the same in patients and controls. Furthermore, comparison of the most frequent isoforms (S2, S3, and S4) in both groups revealed that Lp(a) levels were elevated 200–400% in the patients with the high molecular weight isoforms S3 and S4, whereas the S2 isoform exhibited Lp(a) concentrations in the ESRD group that did not statistically differ from those in the controls. This also held true when groups with all S2-containing isoforms were compared between patients and controls. These patients had a mean Lp(a) plasma concentration of 24.8±21.6 mg/dl, which was not significantly different from the respective control subgroup (23.3±20.3 mg/dl, Wilcoxon test: NS).

Plasma concentrations of cholesterol and apolipoprotein B. To address the question of a possible link between LDL and Lp(a) metabolism in these patients, plasma levels of cholesterol and apo B were measured and compared with those in the control group (Table I). Both parameters were significantly lower in the plasma from patients. Since plasma concentrations of LDL are age dependent, and the two study groups had different mean ages, we calculated the correlation between apo
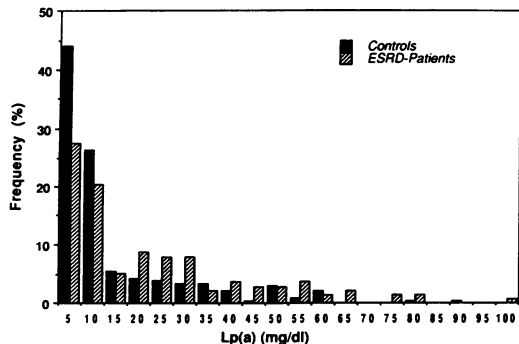

Figure 1. Distribution of plasma concentrations of Lp(a) in ESRD patients (*n* = 138, mean = 20.1 mg/dl) and controls (*n* = 236, mean = 12.1 mg/dl).

Table II. Frequencies (%) of Apo(a) Phenotypes in ESRD Patients and Controls

Phenotype	ESRD patients (*n* = 138)	Controls (*n* = 236)
B	2.2	1.7
S1	0.7	0.8
S2	15.9	13.6
S3	20.3	24.1
S4	35.5	29.7
0	5.8	5.1
BS2	—	0.4
BS3	—	0.4
BS4	0.7	—
S1S3	2.9	—
S2S3	1.5	5.5
S2S4	3.6	8.5
S3S4	10.9	10.2
B + BS2 + BS3 + BS4 + S1 + S1S3 + S2 + S2S3 + S2S4	27.5	30.9
S3 + S4 + 0 + S3S4	72.5	69.1

Pearson χ^2 test: No significant difference in apo(a) phenotype frequencies between ESRD patients and controls ($\chi^2 = 11.015$; 8 degrees of freedom; $P = 0.2$).

B concentrations and age in both study groups. The ESRD group showed no correlation ($y = 0.165\,x + 44.05$, $r = 0.089$, $P = 0.3887$), whereas the controls demonstrated a positive correlation between these two parameters ($y = 0.489\,x + 66.96$, $r = 0.290$, $P < 0.0001$). When apo B concentrations were corrected for their age dependency in both groups, the differences between ESRD patients and controls were even more pronounced ($P < 0.0001$).

Discussion

The association of elevated plasma concentrations of Lp(a) with premature CHD has been demonstrated in many case control and one small prospective study (7, 10).

The major findings of our study were as follows. First, Lp(a) plasma concentrations were significantly elevated in patients with end-stage renal disease who are considered to be at high risk for atherosclerotic diseases (1). Second, only those patients with high molecular weight isoforms had elevated Lp(a) plasma levels. Third, these elevations were not caused by overrepresentation of apo(a) alleles coding for high Lp(a) levels. Finally, this study showed significantly decreased plasma concentrations of cholesterol and apo B.

Our study shows for the first time elevated Lp(a) levels in a large group of ESRD patients, therefore, possibly contributing to the high risk for CHD in these patients. Since the apo(a) isoform frequency was not different from the control group, these elevations are unlikely to be explained by variations at the structural apo(a) gene locus. The age difference between the cases and the controls would certainly not affect the Lp(a) levels in the two groups, because Lp(a) concentrations have been reported to be independent of age (24).

The finding of elevated Lp(a) levels in ESRD patients confirms earlier reports of similar studies in much smaller patient

Table III. Mean Plasma Concentrations of Lp(a) (mg/dl)
of Different Apo(a) Phenotypes in ESRD Patients and Controls

Phenotype	ESRD patients* (n = 138)	Controls[‡] (n = 236)	P (Wilcoxon test)
B	32.3±14.0	41.8±24.6	
S1	52.0	32.5±0.7	
S2	28.0±23.5	25.4±20.9	P = 0.737, NS
S3	17.3±17.1	7.5±8.9	P < 0.01
S4	19.5±20.1	5.2±4.7	P < 0.01
0	1.8±1.8	1.2±0.6	
BS2	—	60.0	
BS3	—	23.0	
BS4	19.5	—	
S1S3	34.4±24.6	—	
S2S3	15.5±4.3	27.2±25.0	
S2S4	14.7±11.7	17.5±14.9	
S3S4	19.3±23.7	9.5±8.0	

Kruskal-Wallis test: * P < 0.005. [‡] P < 0.001.

collectives with diabetic nephropathies (25) and chronic renal failure (26). Our study, however, is the first to show elevated Lp(a) levels in a large group of ESRD patients.

A similar study showed that in CHD patients, the apo(a) phenotype frequency distribution was significantly shifted toward the isoforms associated with the higher Lp(a) levels (18), from which it was concluded that the apo(a) gene locus determining the apo(a) type is directly responsible for the increased Lp(a) levels and susceptibility to CHD.

In the ESRD patients, apo(a) isoform distribution was not significantly different from that of the controls, which suggests that renal insufficiency is the primary cause of the high Lp(a) levels (and not the other way around). This adds ESRD to the list of nongenetic factors for elevated Lp(a) levels, including hormones (27) and acute phase (20). There are principally two mechanisms that now provide possible explanations for the higher Lp(a) levels in ESRD patients: First, increased Lp(a) synthesis and/or secretion by the liver, or second, the physiological function of the normal human kidney as a catabolic organ of Lp(a). Increased lipoprotein synthesis caused by protein loss has been generally assumed to be the reason for hyperlipidemias in various renal diseases including the nephrotic syndrome (5). It has been hypothesized that the liver responds to a decrease in serum albumin or oncotic pressure by increased synthesis and secretion of plasma proteins including the lipoproteins. Indeed, Karádi et al. described increased levels of Lp(a) that correlated with proteinuria in a very small group of patients with different renal diseases (28). This general mechanism can hardly be effective in the ESRD patients on dialysis, since they do not suffer from any substantial protein loss. On the other hand, we have no experimental evidence of a catabolic function of the human kidney with respect to Lp(a). Lp(a) turnover studies or immunohistochemical studies are presently not known to support this mechanism.

An interesting and surprising detail in our results was the selective elevation of Lp(a) levels in the respective isoform groups. The ESRD patients with the high molecular weight isoforms S3 and S4 showed two- to fourfold higher Lp(a) levels as compared with the respective control subgroups, whereas

the small apo(a) isoforms were in the same concentration range both in ESRD patients and controls. This was observed in the single-band S2 subgroup, as well as after inclusion of all double-band S2 isoforms. The reason for these selectively elevated Lp(a) levels remains unclear at the moment.

The discrepancy seen in ESRD patients between plasma levels of Lp(a) on the one hand and cholesterol and apolipoprotein B on the other hand sheds new and interesting light on the ongoing discussion surrounding the (different?) catabolism of LDL and Lp(a). The residence time in plasma for Lp(a) is significantly longer than that of LDL (29). Numerous investigations, mostly in vitro but also in a transgenic animal model, permit us to assume that Lp(a) is taken up via the LDL receptor pathway, although with a somewhat lower efficiency (30, 31). The data showing elevated Lp(a) levels in patients with familial hypercholesterolemia also advocate this concept (19). Our results, among others, however, indicate that there must be two different mechanisms controlling LDL and Lp(a) levels.

From this study we conclude that elevated plasma Lp(a) concentrations of large size isoforms in patients with ESRD are probably nongenetic and secondary to the disease and might significantly contribute to the high incidence for atherosclerotic diseases in these patients.

Acknowledgments

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