Lipoprotein(a) Serum Concentrations and Apolipoprotein(a) Phenotypes in Mild and Moderate Renal Failure

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Abstract. High lipoprotein(a) (Lp(a)) serum concentrations and the underlying apolipoprotein(a) (apo(a)) phenotypes are risk factors for cardiovascular disease in the general population as well as in patients with renal disease. Lp(a) concentrations are markedly elevated in patients with end-stage renal disease. However, nothing is known about the changes of Lp(a) depending on apo(a) size polymorphism in the earliest stages of renal impairment. In this study, GFR was measured by iohexol technique in 227 non-nephrotic patients with different degrees of renal impairment and was then correlated with Lp(a) serum concentrations stratified according to low (LMW) and high (HMW) molecular weight apo(a) phenotypes. Lp(a) increased significantly with decreasing GFR. Such an increase was dependent on apo(a) phenotype. Only renal patients with HMW apo(a) phenotypes expressed higher median Lp(a) concentrations, *i.e.*, 6.2 mg/dl at GFR >90 ml/min per 1.73 m^2, 14.2 at GFR 45 to 90 ml/min per 1.73 m^2, and 18.0 mg/dl at GFR <45 ml/min per 1.73 m^2. These values were markedly different when compared with apo(a) phenotype-matched control subjects who had a median level of 4.4 mg/dl (ANOVA, linear relationship, $P < 0.001$). In contrast, no significant differences were observed at different stages of renal function in patients with LMW apo(a) phenotypes when compared with phenotype-matched control subjects. The elevation of Lp(a) was independent of the type of primary renal disease and was not related to the concentration of C-reactive protein. Multiple linear regression analysis found that the apo(a) phenotype and GFR were significantly associated with Lp(a) levels. Non-nephrotic-range proteinuria modified the association between GFR and Lp(a) levels. In summary, an increase of Lp(a) concentrations, compared with apo(a) phenotype-matched control subjects, is seen in non-nephrotic patients with primary renal disease even in the earliest stage when GFR is not yet subnormal. This change is found only in subjects with HMW apo(a) phenotypes, however.

Numerous retrospective and most of the prospective studies identified high lipoprotein(a) (Lp(a)) levels as a risk factor for atherosclerotic complications in the general population (reviewed in reference (1). This lipoprotein attracted considerable attention because of the high degree of genetic determination. A size polymorphism at the apo(a) gene locus (2) originating from a varying number of kringle-IV (K-IV) repeats (3–6) is the most important determinant of Lp(a) levels and exhibits marked interindividual variation by more than 1000-fold. Those subjects who express a low number of K-IV repeats (low molecular weight (LMW) apo(a) phenotypes) show on average markedly higher Lp(a) concentrations than those with a high number of K-IV repeats (high molecular weight (HMW) apo(a) phenotypes) who usually have low Lp(a) concentrations. Studies considering the apo(a) size polymorphism concluded that the apo(a) gene locus determines the risk for cardiovascular disease through its allelic control of Lp(a) concentrations (7–13).

Two prospective studies in hemodialysis patients identified Lp(a) concentrations and/or the apo(a) phenotype as one of the most important risk factors for cardiovascular disease in this high-risk group for atherosclerosis (14,15). Since cardiovascu-

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lar disease is by far the most frequent cause of death in these patients (16), this lipoprotein is a valuable diagnostic risk marker (17).

Genetic determination of Lp(a) concentrations was estimated to range from 70% to more than 90% (4–6,18). High genetic determination combined with the wide range of Lp(a) concentrations makes it difficult to assess the impact of nongenetic factors on Lp(a) levels. Recently, we provided mathematical proof that a substantial number of subjects are necessary to quantitatively assess nongenetic determinants of Lp(a) levels with reliability. This is especially the case when a study is not controlled for apo(a) isoforms (19,20). Two of the most important nongenetic factors influencing Lp(a) concentrations are nephrotic-range proteinuria and end-stage renal disease (19). Patients with nephrotic syndrome show the highest elevations of Lp(a) among all known pathologic conditions (21,22). Dialysis patients treated with continuous ambulatory peritoneal dialysis (CAPD) show significantly higher levels than those treated with hemodialysis (19,20). Large studies in hemodialysis patients found that only patients with HMW apo(a) phenotypes experience a relative increase of Lp(a) when compared to apo(a) phenotype-matched control subjects, but not those with LMW apo(a) phenotypes (20,23–25). Besides an increase of Lp(a) in CAPD patients with HMW apo(a) phenotypes, those with LMW types showed increased (24), or at least a tendency (20) to increased, levels when compared to phenotype-matched control subjects.

Studies in patients with advanced or predialytic stages of renal disease clearly described elevated Lp(a) concentrations (24,26–33). Investigations that included patients with early stages of renal disease were contrasting and described unchanged (30) or elevated Lp(a) levels (27). Some of these reports found a correlation between Lp(a) levels and serum creatinine concentrations or GFR (27,29), but these findings were not confirmed by others (26,30,31). Most studies were small, sometimes comprising no more than 10 to 20 patients in each subgroup, and only two consider the apo(a) size polymorphism as a confounding factor (24,34). One study investigated patients recruited for essential hypertension and described higher Lp(a) levels in hypertensive patients with renal impairment but the patients did not have primary renal disease (35). In a subgroup of these patients, a strong genetic association between LMW apo(a) phenotypes and the prevalence of end-organ damage was noted (34). Milionis *et al.* included patients with more advanced renal failure (creatinine clearance, 10 to 60 ml/min) and calculated creatinine clearance instead of measuring true GFR (24).

We investigated a group of 227 patients with primary renal disease excluding those with nephrotic syndrome. GFR was determined by iohexol clearance. We addressed the following questions: (*1*) Do Lp(a) concentrations change when GFR is reduced? (*2*) Does the type of primary renal disease influence Lp(a) levels? (*3*) Is Lp(a) elevated in all apo(a) phenotype groups? (*4*) Is an acute phase reaction necessary to observe elevated levels of Lp(a) in these patients?

Materials and Methods

Patients

Patients were recruited during 1997 from eight nephrology departments in Germany (Göttingen, Greifswald, Heidelberg, Homburg/Saar, and Munich), Austria (Feldkirch, Innsbruck), and South Tyrol (Bozen), with nearly two-thirds of the patients from two departments (Heidelberg and Innsbruck). We included Caucasian patients ages 19 to 65 yr who had visited the outpatient department at least once during the preceding year. Exclusion criteria were serum creatinine >6 mg/dl; diabetes mellitus; malignancy; liver, thyroid, or infectious disease at the time of recruitment; nephrotic syndrome defined as daily proteinuria >3.5 g/1.73 m^2; organ transplantation; allergy against ionic contrast media; and pregnancy. Three hundred and forty patients fulfilled the criteria, of whom 28 were not within reach and 85 refused to participate in the study. The remaining 227 patients were included in the study. The study was approved by the institutional ethics committees, and subjects gave written informed consent.

To avoid interobserver differences, all renal patients were recruited by one physician (E.K.) who visited the participating centers. Each patient's history was recorded during an interview and compared with his or her records. All patients underwent a physical examination. The primary cause of renal disease was glomerulonephritis in 97 patients (biopsy confirmed in 90 cases), polycystic kidney disease in 37, chronic "pyelonephritis" in 24, other types of renal disease in 43, and unknown in 26.

Patients were compared with 227 age- and gender-matched Caucasian control subjects of the same ethnic origin without renal impairment or liver disease who were recruited in 1997 from one of the PROCAM study centers (36).

Laboratory Procedures

Serum and ethylenediaminetetra-acetic acid plasma were taken after a 12-h overnight fast. After low-speed centrifugation, samples were frozen and kept at −80°C before analysis (37). Depending on the serum creatinine level, two to three blood samples for the determination of GFR by the iohexol method (38) were obtained after infusion of iohexol during the same visit in the outpatient department. We calculated in 18 patients with mostly advanced impairment of renal function the GFR using the formula of Cockroft and Gault (39). Patients were carefully instructed about the collection of a 24-h urine sample for the determination of proteinuria.

Measurement of Lp(a), serum albumin, GFR, C-reactive protein (CRP), and apo(a) phenotyping were performed each centrally in a single laboratory, respectively, to avoid interlaboratory differences in measurements. At this time, the laboratory staff involved in the study was unaware of the patient's renal function and the patient/control status of the measured samples.

Lp(a) quantification was performed as described in detail (37) with a double-antibody enzyme-linked immunosorbent assay (ELISA), using an affinity-purified polyclonal apo(a) antibody for coating and the horseradish peroxidase-conjugated monoclonal 1A2 for detection. An Lp(a)-positive serum from Immuno (Vienna, Austria) with the same apo(a) isoforms served as standard throughout the study. Each sample was analyzed in duplicate, and intra- and interassay coefficients of variation were 2.7 and 6%, respectively. Serum albumin (bromcresol green method) was measured using a kit from Boehringer Mannheim (Mannheim, Germany). Measurements were made on microtiter plates as described previously (37). Adjustment of Lp(a) concentrations for hematocrit had no major impact on our findings (40). CRP was measured on a Behring BNA nephelometer, using reagents from

Behring Diagnostics (N Latex CRP mono; Behring Diagnostics, Marburg, Germany). The lower detection limit of this test was 0.02 mg/dl.

Apo(a) phenotyping was performed by sodium dodecyl sulfate-agarose gel electrophoresis (SDS-agarose) under reducing conditions as outlined (41) with slight modifications. Fifty nanograms of Lp(a) was applied to the gel when serum Lp(a) concentrations were above 4 mg/dl. With lower concentrations, a fixed volume of 1.5 μl of serum was applied to the gel. Electrophoresis was followed by immunoblotting (2), using the monoclonal antibody 1A2 for detection of apo(a) isoforms.

Calculation of the Lp(a) Concentration Derived from each Apo(a) Isoform

In subjects expressing two apo(a) isoforms, a single investigator (F.K.) estimated the percent relation of the two isoforms by densitometric scanning of the apo(a) bands of the immunoblots from SDS-agarose gel electrophoresis. We used the Lp(a) concentrations measured by ELISA to calculate the isoform-specific amount of Lp(a) derived from each isoform by using these densitometric relative estimates. Figure 1 represents an example of this procedure. When an individual with 21 and 33 K-IV repeats had an Lp(a) serum concentration of 40 mg/dl measured by ELISA, and the 21 K-IV isoform accounted for 85% of the staining in the SDS-agarose gel electrophoresis, we calculated the Lp(a) concentration originating from this isoform to be 34 mg/dl (40 $\times$ 0.85). The isoform with 33 K-IV repeats accounted for the remaining 6 mg/dl. The whole Lp(a) concentration counted for the expressed isoform in case a subject showed only one apo(a) band.

Statistical Analyses

Statistical analyses were performed with Statistical Package for the Social Sciences (SPSS) for Windows version 7.5.2. Univariate comparisons of continuous variables between control subjects and renal patients were done by unpaired *t* test or the nonparametric Wilcoxon rank sum test in the case of nonnormally distributed variables. Dichotomized variables were compared using Pearson's χ^2 test or the likelihood ratio χ^2 test. ANOVA was used to compare continuous variables between control subjects and renal patients subgrouped by the three tertiles of GFR. Non-normally distributed variables were logarithmically transformed before including them in the analysis. The Spearman correlation test was used to correlate proteinuria and GFR with concentrations of lipids, lipoproteins, and apolipoproteins, as well as CRP with Lp(a). Adjustment of Lp(a) serum concentrations for proteinuria and/or GFR in patients was done using linear regression analysis. Multiple regression analysis was used to investigate the association of different variables with Lp(a) serum concentrations.

Because of the high number of detectable apo(a) isoforms (>30), many phenotypes were represented only in low numbers. To account for this problem, we decided *a priori* to combine apo(a) isoforms in steps of three K-IV repeats according to the molecular weight of the smaller apo(a) isoforms to have sufficient sample sizes in each category (42). Because subjects with 11 to 16 or >34 K-IV repeats were represented relatively rarely, we built one group by combining 11 to 19 and another by combining >31 K-IV repeats. Furthermore, we divided apo(a) phenotypes into two subgroups according to the molecular weight of the smaller apo(a) isoforms, as done in previous works (7,9,13,20,23,25,34,43–47). The LMW group included all subjects with at least one apo(a) isoform with 11 to 22 K-IV repeats (48); the HMW group comprised all subjects having only isoforms with more than 22 K-IV repeats. If two apo(a) isoforms were detectable, we used only the smaller apo(a) isoform for categorization, which we discussed recently in detail (47). In a subanalysis, however, we considered the effect of the second apo(a) isoform, if expressed, as described above.

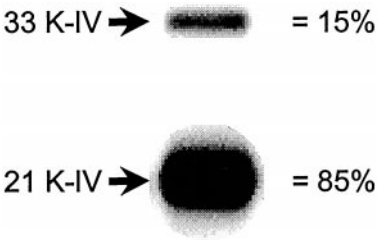

Figure 1. Calculation of the lipoprotein(a) (Lp(a)) concentrations deriving from each apoprotein(a) (apo(a)) isoform. The percent relation of the two isoforms from densitometric scanning of the immunoblots from sodium dodecyl sulfate-agarose gel electrophoresis was used to calculate the isoform-specific amount of Lp(a) originating from each apo(a) isoform. The total Lp(a) concentration was measured by enzyme-linked immunosorbent assay. The example provided in this figure expressed a high molecular weight (HMW) apo(a) isoform with 33 kringle IV (K-IV) repeats, which accounted for 15% of the total Lp(a) serum concentration (6 mg/dl). The low molecular weight (LMW) apo(a) isoform with 21 K-IV repeats accounted for 85% of the total Lp(a) serum concentration (34 mg/dl).

Results

Comparison of Renal Patients and Control Subjects

The average GFR in patients with renal disease was 70 $\pm$ 42 ml/min per 1.73 m^2, ranging from 10 to 209 ml/min per 1.73 m^2. Patients had a lower body mass index, lower serum albumin and total protein, and higher creatinine and urea concentrations compared with age- and gender-matched control subjects. Systolic and diastolic BP, as well as the frequency of drug-treated hypertension, were markedly higher in patients (Table 1).

Renal patients had significantly higher Lp(a) serum concentrations when compared to control subjects (mean $\pm$ SD; median: 29.5 $\pm$ 32.0; 17.9 *versus* 20.7 $\pm$ 32.8; 6.9, $P < 0.001$) (Table 2). Apo(a) phenotyping was performed to investigate whether the elevation of Lp(a) in renal patients is determined primarily by the apo(a) gene or renal disease. In case of the former, we expected an association of LMW apo(a) isoforms with renal disease. Because we observed in renal patients a similar frequency distribution of apo(a) alleles either in terms of apo(a) K-IV repeats or in terms of LMW and HMW apo(a) phenotypes as in control subjects (Table 2), we concluded that the elevation of Lp(a) is caused by glomerulotubular dysfunction.

Influence of Primary Renal Disease

Lp(a) serum levels were compared between patients with glomerulonephritis and patients with polycystic kidney disease as prototypes of inflammatory and noninflammatory renal disease, respectively. Crude and Lp(a) serum concentrations adjusted for proteinuria and GFR as well as the frequency of LMW apo(a) phenotypes were similar in both groups (Table 3). Therefore, no influence of the etiology of renal disease on the Lp(a) levels was observed.

Influence of GFR

The correlation of Lp(a) with GFR ($r = -0.18$, $P < 0.001$) was more pronounced than that of Lp(a) with non-nephrotic-

Table 1. Characteristics of patients with renal disease and age- and gender-matched control subjects[a]

Characteristic	Control Subjects (n = 227)	Renal Patients (n = 227)
Age (yrs)	45.8 ± 12.3	45.7 ± 12.6
Gender (F/M)	73/154	73/154
BMI	26.4 ± 3.6	25.2 ± 3.8[b]
GFR (ml/min per 1.73 m^2)		70 ± 42 [38, 63, 96]
Creatinine (mg/dl)	0.99 ± 0.18	2.02 ± 1.16[b]
Urea (mg/dl)	29 ± 7	60 ± 34[b]
Proteinuria (g/24 h per 1.73 m^2)		0.9 ± 0.9 [0.2, 0.6, 1.5]
Serum albumin (g/dl)	4.88 ± 0.49	4.57 ± 0.41[b]
Total protein (g/dl)	7.54 ± 0.55	7.01 ± 0.42
Hematocrit		0.41 ± 0.06
CRP (mg/dl)		0.37 ± 0.76 [0.07, 0.16, 0.41]
Systolic BP (mmHg)	129 ± 13	137 ± 21[b]
Diastolic BP (mmHg)	81 ± 9	87 ± 14[b]
Drug-treated hypertension (%)	10.6	78.9[b]
Smoker/exsmoker/nonsmoker	61/58/108	49/57/121

[a] Data are mean ± SD and [25th percentile, median, 75th percentile] where appropriate. BMI, body mass index; CRP, C-reactive protein.
[b] $P < 0.001$ for comparison with control subjects.

Table 2. Lp(a) serum concentrations and apo(a) size polymorphism in control subjects and patients with renal disease[a]

Variable	Control Subjects (n = 227)	Renal Patients (n = 227)
Lp(a) (mg/dl), mean ± SD	20.7 ± 32.8	29.5 ± 32.0[b]
[25th percentile, median, 75th percentile]	[2.2, 6.9, 19.4]	[4.9, 17.9, 42.5]
Apo(a) alleles, n (%)[c]		
11 to 19 K-IV repeats	17 (7.5)	20 (8.8)
20 to 22 K-IV repeats	39 (17.2)	43 (18.9)
23 to 25 K-IV repeats	33 (14.5)	30 (13.2)
26 to 28 K-IV repeats	47 (20.7)	51 (22.5)
29 to 31 K-IV repeats	45 (19.8)	52 (22.9)
>31 K-IV repeats	46 (20.3)	31 (13.7)
Apo(a) phenotypes[d]		
LMW apo(a) phenotypes, n (%)	56 (24.7)	63 (27.8)
HMW apo(a) phenotypes, n (%)	171 (75.3)	164 (72.2)

[a] Lp(a), lipoprotein(a); apo(a), apolipoprotein(a); K-IV, Kringle IV; LMW, low molecular weight; HMW, high molecular weight.
[b] $P < 0.001$ by Wilcoxon rank sum test for comparison of Lp(a) serum concentrations between patients and control subjects.
[c] Likelihood ratio χ^2 test comparing the frequencies of apo(a) alleles between patients and control subjects: $\chi^2 = 4.19$, df = 5, $P = 0.52$.
[d] Pearson's χ^2 test comparing the frequencies of LMW apo(a) phenotypes between patients and control subjects: $\chi^2 = 0.56$, df = 1, $P = 0.46$.

range proteinuria ($r = 0.14$, $P < 0.05$). We therefore grouped renal patients according to the tertiles of GFR, *i.e.*, >90 ml/min per 1.73 m^2, 45 to 90 ml/min per 1.73 m^2, and <45 ml/min per 1.73 m^2. ANOVA showed linearly increasing levels of Lp(a) with decreasing renal function (first rows of Table 4). Lp(a) concentrations tended to be elevated even in the group with GFR >90 ml/min per 1.73 m^2 when compared with control subjects; Lp(a) concentrations were increased significantly in the other two groups with more markedly reduced renal function. The increase of Lp(a) with decreasing renal function remained similar when Lp(a) levels were adjusted for proteinuria. Figure 2 shows the frequency distribution of Lp(a) concentrations in control subjects and in the three groups of patients according to the GFR. Approximately 60% of random control subjects had Lp(a) concentrations <10 mg/dl. This frequency was lower by half in renal patients with a GFR ≤90 ml/min per 1.73 m^2.

Changes of Lp(a) Serum Concentrations in Relation to the Apo(a) Size Polymorphism

We analyzed whether the relative increase of Lp(a) with decreasing renal function was related to the apo(a) size poly-

Table 3. Lp(a) serum concentrations, frequency of LMW apo(a) phenotypes, and renal function in patients with GN as prototype for inflammatory disease, and in patients with PCKD as noninflammatory type of renal disease[a]

Variable	Cause for Renal Failure		*P* Value
	GN (n = 97)	PCKD (n = 37)	
Lp(a) crude (mg/dl)	28.8 ± 34.9 [13.6]	25.8 ± 25.8 [16.8]	0.61
Lp(a) adjusted for proteinuria and for GFR (mg/dl)	28.8 ± 35.4 [15.1]	25.6 ± 25.0 [18.2]	0.68
LMW apo(a) phenotypes (%)	27 (27.8)	9 (24.3)	0.68
GFR (ml/min per 1.73 m^2)	81 ± 40 [77]	55 ± 45 [43]	0.001
Proteinuria (g/24 h per 1.73 m^2)	1.1 ± 1.0 [0.7]	0.6 ± 0.6 [0.4]	0.006
Serum albumin (g/dl)	4.52 ± 0.45	4.66 ± 0.34	0.10

[a] Values of continuous variables are presented as mean ± SD and [median] where appropriate. GN, glomerulonephritis; PCKD, polycystic kidney disease. Other abbreviations as in Table 2.

Table 4. Lp(a) serum concentrations in control subjects and patients with renal disease according to apo(a) phenotypes[a]

Group	Control Subjects		Renal Patients: GFR >90 ml/min per 1.73 m^2 120 ± 28 (n = 79)		Renal Patients: GFR 45 to 90 ml/min per 1.73 m^2 65 ± 13 (n = 76)		Renal Patients: GFR <45 ml/min per 1.73 m^2 28 ± 12 (n = 72)		*P* Value[b]
	(mg/dl)	(n)	(mg/dl)	(n)	(mg/dl)	(n)	(mg/dl)	(n)	
All subjects									
crude values	20.7 ± 32.8 [6.9]	227	22.8 ± 30.8 [9.7]	72	29.3 ± 30.4 [17.4]	76	35.7 ± 33.6 [24.3]	79	0.001
adjusted for proteinuria	20.7 ± 32.8 [6.9]	227	23.4 ± 30.9 [11.0]	72	29.0 ± 29.2 [18.4]	76	34.6 ± 33.2 [24.4]	79	0.001
Subjects with LMW apo(a) phenotypes									
crude values	58.2 ± 45.8 [47.2]	56	49.1 ± 46.7 [39.6]	17	54.9 ± 37.8 [57.9]	21	58.2 ± 37.1 [66.6]	25	0.75
adjusted for proteinuria	58.2 ± 45.8 [47.2]	56	51.3 ± 46.8 [39.0]	17	52.7 ± 34.0 [51.1]	21	58.8 ± 38.0 [59.6]	25	0.82
Subjects with HMW apo(a) phenotypes									
crude values	8.5 ± 11.8 [4.4]	171	14.6 ± 17.7 [6.2]	55	19.5 ± 20.1 [14.2]	55	25.3 ± 26.2 [18.0]	54	0.001
adjusted for proteinuria	8.5 ± 11.8 [4.4]	171	14.8 ± 16.8 [6.1]	55	19.9 ± 21.3 [13.3]	55	23.4 ± 23.8 [16.8]	54	0.001

[a] Results are given as mean ± SD [median].
[b] By ANOVA.

morphism. To avoid extensive subgrouping resulting in small group sizes and to render the results comparable with previous findings, we grouped apo(a) phenotypes into LMW and HMW apo(a) phenotypes. ANOVA showed no significant differences in the Lp(a) concentrations in control subjects and renal patients with LMW apo(a) phenotypes independently of the degree of renal impairment (Table 4). In striking contrast, Lp(a) concentrations were markedly increased with decreasing renal function in patients with HMW apo(a) phenotypes: Compared to control subjects, mean and median levels were even higher by 70 and 40%, respectively, in renal patients who still had a GFR >90 ml/min per 1.73 m^2 (Table 4). To investigate whether this increase in Lp(a) was associated with proteinuria rather than with GFR, we excluded in a subanalysis 11 of the 55 patients with HMW apo(a) phenotypes who had a daily proteinuria >1 g/1.73 m^2. This still resulted in significantly higher Lp(a) levels in the patient group with GFR >90 ml/min per 1.73 m^2 when compared with control subjects (11.8 ± 13.8 mg/dl [median 5.0] *versus* 8.1 ± 11.8 mg/dl [median 4.4], $P < 0.05$). With further impairment of renal function, Lp(a) increased by three- to fourfold in renal patients with HMW apo(a) phenotypes and a GFR <45 ml/min per 1.73 m^2 when compared with phenotype-matched control subjects (Table 4).

The above subgrouping into LMW and HMW apo(a) phenotypes in a given individual is based on the apo(a) isoform with the lowest molecular weight. It does not consider that many subjects with an LMW apo(a) phenotype have not only one LMW, but also one HMW apo(a) isoform. To control for the influence of the second apo(a) isoform, we calculated based on the measured Lp(a) concentrations in each subject sepa-

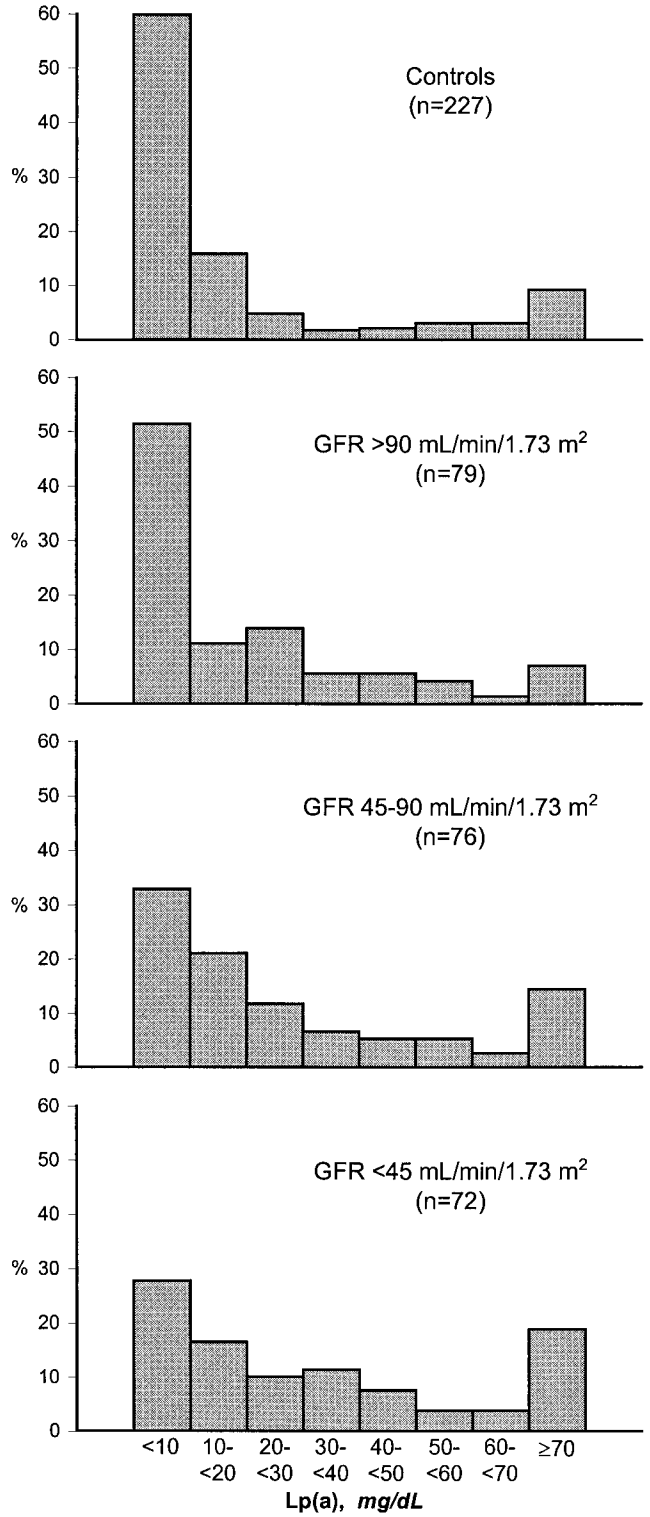

Figure 2. Distribution of Lp(a) serum concentrations in control subjects and three groups of renal patients with different levels of GFR (>90 ml/min per 1.73 m^2, 45 to 90 ml/min per 1.73 m^2, and <45 ml/min per 1.73 m^2).

rately the Lp(a) concentrations arising from the first and the second apo(a) isoforms by estimating the relative proportion of the two isoforms in the SDS-agarose gel electrophoresis. The term "first" simply refers to the isoform with the smaller number of K-IV repeats in case of two expressed isoforms or the expressed isoform in subjects who show only one isoform. The "second" isoform means the isoform with the higher number of K-IV repeats in case of two expressed isoforms. (For details about the calculation of isoform-specific Lp(a) concentrations, see Materials and Methods.) When the first apo(a) isoform was of low molecular weight (≤22 K-IV repeats), we observed that the Lp(a) concentrations arising from these LMW apo(a) isoforms were similar in control subjects and all three groups of patients (Table 5). We found highly significant increasing Lp(a) concentrations with decreasing renal function in case the first isoform was already of high molecular weight (>22 K-IV repeats). A similar but less pronounced increase was observed for the second apo(a) isoform in renal patients who expressed both isoforms of high molecular weight. We did not calculate the apo(a) isoform-specific concentrations of Lp(a) for the second isoform in case it was of low molecular weight, since this constellation was only met by one or two patients in each subgroup of renal function.

Lp(a) Serum Concentrations in Relation to Acute Phase Reactant CRP

We investigated whether the apo(a) phenotype-specific elevation of Lp(a) is related to an acute phase reaction (diagnosed by elevated CRP levels), as recently suggested for patients with advanced impairment of renal function (33) or hemodialysis patients (25). None of our patients was investigated during obvious acute infection or illness. Only 41 of the renal patients (18%) had CRP concentrations ≥0.5 mg/dl. We found no evidence that the elevation of Lp(a) was associated with elevated CRP concentrations. No correlation was observed between the concentration of CRP and Lp(a) ($r = 0.05$, $P = 0.47$). Figure 3 shows that the elevation of Lp(a) levels was specific for HMW apo(a) phenotypes regardless of whether we analyzed the whole patient group or whether we excluded those patients with elevated CRP serum concentrations. This analysis revealed similar results when either crude Lp(a) concentrations or Lp(a) levels adjusted for proteinuria and GFR were considered in the calculations.

Correlation of Variables with Lp(a) Serum Concentrations by Multiple Regression Analysis

We finally investigated in a multiple regression analysis which variables were associated with Lp(a) serum concentrations in renal patients (Table 6). The apo(a) phenotype classification in LMW and HMW apo(a) phenotypes and GFR were significantly associated with Lp(a) serum concentrations. Proteinuria was not independently associated with Lp(a) but modified the association between GFR and Lp(a) with borderline statistical significance, as shown by an interaction term of proteinuria and GFR. When the analysis was repeated including only patients with HMW apo(a) phenotypes, we found that GFR was significantly associated with Lp(a) levels and that the interaction term of proteinuria and GFR showed again a marginally significant association. Both GFR and the interaction

Table 5. Apo(a) isoform-specific mean ± SD [median] Lp(a) serum concentrations in control subjects and patients with renal disease after controlling for the other isoform in case of two expressed isoforms[a]

Group	Control Subjects		Renal Patients: GFR >90 ml/min per 1.73 m^2 120 ± 28 (n = 79)		Renal Patients: GFR 45 to 90 ml/min per 1.73 m^2 65 ± 13 (n = 76)		Renal Patients: GFR <45 ml/min per 1.73 m^2 28 ± 12 (n = 72)		P Value[b]
	(mg/dl)	(n)	(mg/dl)	(n)	(mg/dl)	(n)	(mg/dl)	(n)	
Calculated Lp(a) concentration for the first LMW apo(a) band									
crude values	44.5 ± 35.3 [41.4]	56	43.2 ± 46.5 [39.6]	17	41.9 ± 33.4 [46.3]	21	46.9 ± 34.3 [55.8]	25	0.80
adjusted for proteinuria	44.5 ± 35.3 [41.4]	56	43.9 ± 44.3 [39.0]	17	40.0 ± 30.5 [39.1]	21	47.3 ± 34.9 [47.7]	25	0.83
Calculated Lp(a) concentration for the first HMW apo(a) band									
crude values	6.5 ± 9.9 [3.4]	170	11.4 ± 14.6 [4.5]	55	14.4 ± 16.5 [8.7]	55	18.0 ± 17.0 [14.6]	54	0.001
adjusted for proteinuria	6.5 ± 9.9 [3.4]	170	11.5 ± 14.3 [5.3]	55	14.9 ± 17.9 [8.8]	55	16.6 ± 15.7 [14.4]	54	0.001
Calculated Lp(a) concentration for the second HMW apo(a) band									
crude values	7.7 ± 11.1 [3.4]	127	6.8 ± 7.8 [3.5]	39	11.5 ± 12.4 [7.5]	48	13.4 ± 13.4 [10.9]	48	0.005
adjusted for proteinuria	7.7 ± 11.1 [3.4]	127	7.4 ± 8.3 [3.5]	39	11.2 ± 11.7 [7.5]	48	12.6 ± 12.3 [11.3]	48	0.010

[a] In subjects expressing two apo(a) isoforms, the percent relation of the two isoforms was estimated by densitometric scanning of the apo(a) bands of the immunoblots from sodium dodecyl sulfate-agarose gel electrophoresis. The Lp(a) concentrations measured by enzyme-linked immunosorbent assay were used to calculate the isoform-specific amount of Lp(a) derived from each isoform by using these densitometric relative estimates. The whole Lp(a) concentration counted for the expressed isoform in case a subject showed only one apo(a) band. For exact procedure, see Materials and Methods and Results.

[b] By ANOVA.

term of proteinuria and GFR did not contribute to the model in patients with LMW apo(a) phenotypes.

Discussion

This study provides strong evidence that Lp(a) increases in very early stages of non-nephrotic primary renal disease (GFR >90 ml/min per 1.73 m^2). This increase of Lp(a) is apo(a) phenotype-specific, meaning that only renal patients with HMW apo(a) phenotypes exhibit an increase of Lp(a) compared to apo(a) phenotype-matched control subjects. The increase of Lp(a) was not related to the acute phase reactant CRP, and the association of GFR with Lp(a) was modified by proteinuria.

In a first step, we excluded that the higher levels of Lp(a) in renal patients were caused by a higher frequency of LMW apo(a) phenotypes in renal patients when compared with control subjects. This would have indicated that the LMW apo(a) phenotype predisposes to specific renal diseases, as noted for example for coronary artery disease (7–13) or end-organ damage in patients with essential hypertension (34). Dialysis patients with LMW apo(a) phenotypes have a two- to threefold higher prevalence (47,49) as well as incidence (15) of major cardiovascular events than those with HMW apo(a) types. Although we demonstrated already in previous studies (20,23) that dialysis patients have a similar apo(a) phenotype distribution than control subjects, we considered that we could not completely exclude a potential confounding survivor effect in dialysis patients in view of the enormous risk of atherosclerosis in those patients with LMW apo(a) phenotype (15,45,47). We can now exclude a primary genetic cause for the elevated Lp(a) levels with very high probability because patients and matched control subjects had a similar apo(a) phenotype distribution and because the markedly shorter disease duration should have minimized a confounding survivor effect. Furthermore, a primary reason for an elevation of Lp(a) in these patients seems to be less likely due to heterogeneity of renal disease. Indeed, we did not see major differences in the apo(a) phenotype distribution between patients who suffered from glomerulonephritis and polycystic kidney disease.

One of the most interesting findings in this study population is the apo(a) phenotype-specific elevation of Lp(a), which we and others also found in hemodialysis patients (20,23–25). This means that Lp(a) in hemodialysis patients is only elevated in patients with HMW apo(a) phenotypes when compared to control subjects with the same apo(a) phenotypes. Patients with LMW apo(a) phenotypes have nearly identical Lp(a) levels as apo(a) phenotype-matched control subjects. The observation in hemodialysis patients is based on the investigation of more than 1000 patients and about 650 control subjects in four studies (20,23–25), but was not confirmed by three smaller studies in different populations using different phenotyping methods, Lp(a) assays, and cut-points for categorization of LMW and HMW apo(a) phenotypes (50–52). Recently, Milionis *et al.* studied a group of 68 patients with more advanced

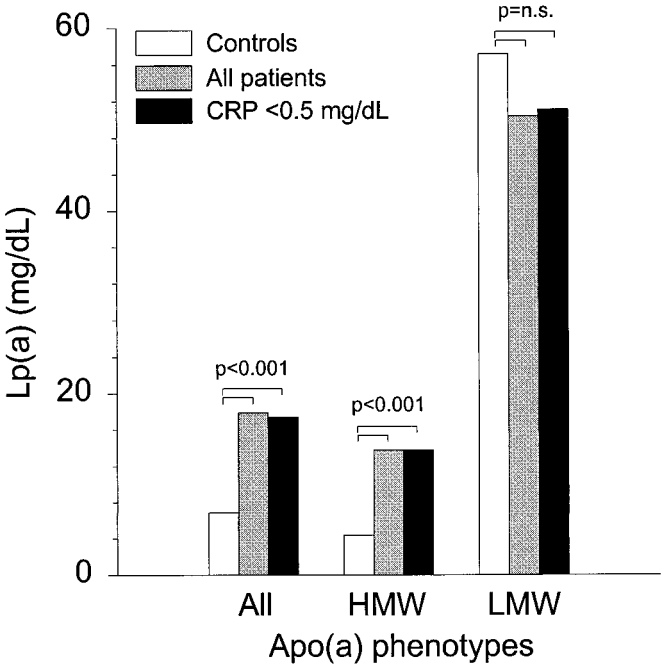

Figure 3. Influence of C-reactive protein (CRP) on the apo(a) phenotype-specific elevation of Lp(a). Similar results were obtained whether all patients ($n = 227$) or only those with normal CRP levels (*i.e.*, <0.5 mg/dl) ($n = 186$) were included in the analysis.

Table 6. The association of variables with logarithmically transformed Lp(a) serum concentrations in patients with mild and moderate renal failure determined by multiple regression analysis[a]

Group/Variable	Coefficient	SEM	*P* Value
Total patient group ($n = 227$)			
apo(a) phenotype[b]	−1.270	0.189	0.001
ln GFR	−0.306	0.124	0.015
interaction term ln proteinuria × ln GFR	0.108	0.106	0.094
Patients with HMW apo(a) phenotypes ($n = 164$)			
ln GFR	−0.312	0.145	0.032
interaction term ln proteinuria × ln GFR	0.150	0.082	0.069
Patients with LMW apo(a) phenotypes ($n = 63$)			
ln GFR	−0.310	0.248	0.22
interaction term ln proteinuria × ln GFR	0.030	0.099	0.77

[a] Variables and interaction terms that did not significantly contribute to the multiple regression model: ln proteinuria, ln CRP, interaction term ln GFR × apo(a) phenotype, and interaction term ln proteinuria × apo(a) phenotype. Cause of renal disease was offered to the model in a subanalysis but did not show a significant contribution (data not shown). "ln" means that variables are logarithmically transformed.

[b] Apo(a) phenotype: 1 = low molecular weight (LMW); 2 = high molecular weight (HMW).

renal failure (creatinine clearance 10 to 60 ml/min) and also observed an apo(a) phenotype-specific elevation of Lp(a) (24). Patients and control subjects, however, had an unusually high frequency of LMW apo(a) phenotypes (45.2 and 52.9%, respectively) that was never observed in any other Caucasian population studied thus far (6). In the present study, we observed such phenotype-specific elevation of Lp(a) in renal patients with non-nephrotic proteinuria at all levels of GFR even in the earliest stage of disease when GFR is still normal (>90 ml/min per 1.73 m^2). We therefore conclude that this phenomenon is not restricted to hemodialysis patients. One investigation studying 168 CAPD patients showed a trend toward elevated Lp(a) levels in LMW apo(a) phenotypes besides the markedly elevated levels in HMW apo(a) phenotypes (20). A smaller study including only 47 CAPD patients reported elevated levels in both phenotype groups (24). Patients with nephrotic syndrome have a clear elevation of Lp(a) in all apo(a) phenotype classes (21). It therefore seems that a high degree of proteinuria or of protein loss through the peritoneal membrane is related to an increased hepatic synthesis of Lp(a) of all apo(a) isoform classes. We therefore excluded in our analysis all patients with nephrotic-range proteinuria to minimize the risk of confounding the results by heterogeneity of the causes of Lp(a) elevation. We furthermore analyzed the data with and without adjustment of Lp(a) levels for the magnitude of proteinuria. Adjustment did not significantly change the findings. Even exclusion of the patients with a daily proteinuria >1 g/1.73 m^2 in the group of patients with HMW apo(a) phenotypes and a GFR >90 ml/min per 1.73 m^2 resulted in higher Lp(a) levels in patients when compared to apo(a) phenotype-matched control subjects. We cannot determine with certainty whether this small amount of the remaining proteinuria or the renal disease itself is responsible for the elevation of Lp(a) in this earliest stages of renal disease. Multiple regression analysis in the entire patient group, however, revealed that besides the apo(a) size polymorphism, GFR was significantly associated with Lp(a) serum concentrations. This association between GFR and Lp(a) was marginally modified by non-nephrotic-range proteinuria, but proteinuria itself did not significantly contribute to the model. This is in contrast to nephrotic syndrome, which has a tremendous influence on Lp(a) levels (21,22). A certain amount of proteinuria therefore might be necessary to show an independent association with Lp(a) levels. In the case of non-nephrotic proteinuria, proteinuria at the very most modifies the association between GFR and Lp(a) levels.

Because most subjects express two apo(a) isoforms and because HMW apo(a) isoforms are more common than LMW ones, LMW apo(a) phenotypes express according to our definition (see Materials and Methods) a second, mostly less intensive HMW apo(a) isoform in about 60% of the cases. To exclude that this second isoform could have influenced our findings in patients with LMW apo(a) phenotypes, we calculated the amount of Lp(a) originating from each isoform based on the percent distribution of the two isoforms on SDS-agarose

gel electrophoresis. This more sophisticated analysis demonstrated that Lp(a) concentrations derived from the LMW apo(a) isoform are not influenced by renal disease. However, there are two possible pitfalls. First, chemiluminescence analysis for visualizing apo(a) bands might not be linear over a wide range. This could indeed be a problem if we would apply from each person the same amount of serum to the gel as done in many phenotyping methods. In contrast, we first measure Lp(a) and apply for each person the individual amount of serum resulting in the same amount of Lp(a) on the gel. This amount was determined to be markedly below the maximum intensity of the signal. By always applying the same amount of Lp(a), we avoid reaching the maximum signal and narrow the range in the signal and therefore the problem of linearity. The second pitfall involves the immunoreactivity of apo(a) antibodies directed against repetitive epitopes of apo(a). When an antibody such as our 1A2 is directed against the repetitive K-IV, it does not necessarily mean that the antibody recognizes one molecule of apo(a) several times. This cannot be expected due to steric hindrance, and this was also not observed in a recent publication (53). It is even conceivable that an antibody, although directed against a repetitive antibody, detects Lp(a) isoform-independent when the steric configuration allows only the binding of one antibody to one molecule of Lp(a). We do not have evidence that our antibody recognizes one molecule of apo(a) several times. It must be further pointed out that we did not use the densitometric scanning of apo(a) bands to measure Lp(a) concentrations of each isoform. We used this method simply to determine the relative proportion of the isoforms in heterozygote subjects. This proportion was then used to calculate the Lp(a) serum concentration for each apo(a) isoform based on the exact Lp(a) concentrations measured by ELISA. This approach, reported here for the first time, was an attempt to minimize the confounding of results by the second apo(a) isoform. In case the above discussed pitfalls still played a role, serum samples of both patients and control subjects were exposed to them in the same way, which makes this attempt nevertheless reliable.

Recently, Stenvinkel and colleagues investigated a group of patients immediately before the start of renal replacement therapy with a creatinine clearance of 9 ± 1 ml/min. They found elevated levels of Lp(a) and suggested that uremia causes an increase of Lp(a) by an unknown mechanism (33). We conclude from our data that a uremic status is not mandatory for elevated Lp(a) levels in patients with renal disease. We even observed an elevation of Lp(a) in patients with HMW apo(a) phenotypes who suffered from a primary renal disease but still had a normal GFR (>90 ml/min per 1.73 m^2). The GFR in these very early stages of renal disease, however, may mask nephron loss because of a compensatory increase of single-nephron GFR (54). This would be compatible with the notion that the increase of Lp(a) in these patients does not result from diminished filtration *per se*, but from diminished parenchymal and metabolic function (*e.g.*, related to Lp(a) degradation). Degradation of Lp(a) in the kidney is suggested by large concentration differences of Lp(a) between the aorta and the renal vein (55) and the finding of apo(a) fragments in urine (56,57). Because these fragments have a molecular weight of up to more than 200 kD (56,57), at least the larger apo(a) fragments must have entered the postglomerular space via tubular mechanisms, mostly via active transport. With further progression of renal disease and the accompanying decrease of GFR, Lp(a) increases further. We propose that impaired catabolism of Lp(a) is the cause of increased Lp(a) concentrations in non-nephrotic renal patients. Turnover studies with stable isotope technique are necessary to confirm this hypothesis and exclude increased hepatic synthesis as an alternative possibility. Using this technique, increased hepatic synthesis of Lp(a) independent of the apo(a) isoform size has been demonstrated in patients with nephrotic syndrome (58).

Kario *et al.* reported in a small group of hemodialysis patients that high levels of CRP, sialic acid, and interleukin-6 are closely related to the elevated Lp(a) levels, however, apo(a) size polymorphism was not taken into consideration (59). Nevertheless, such a relation would be intriguing and mechanistically plausible since several interleukin-6-responsive elements were described in the 5′ flanking regulatory region of the apo(a) gene (60). Some studies described that Lp(a) concentrations behave as an acute phase reactant (61,62). Zimmermann and colleagues recently suggested that the apo(a) phenotype-specific elevation of Lp(a) in hemodialysis patients is explained by a state of microinflammation, also reflected by elevated CRP and serum amyloid A levels (25). This cannot be the entire explanation, however, because an elevation of Lp(a) was also seen in hemodialysis patients with HMW apo(a) phenotypes and normal CRP and normal serum amyloid A levels. Their Lp(a) concentration was still significantly higher than in control subjects, but lower than in hemodialysis patients with elevated CRP and serum amyloid A levels (25). When we analyzed our data in relation to CRP levels measured with a highly sensitive assay, we observed that only 18% of the renal patients had CRP levels in the pathologic range (≥ 0.5 mg/dl). An apo(a) phenotype-specific elevation of Lp(a) was noted even when we excluded these patients. We therefore conclude that an acute phase reaction measured by elevated CRP levels at the very most modifies Lp(a) concentrations, but fails to explain the apo(a) phenotype-specific elevation of Lp(a) at least in patients with mild and moderate impairment of renal function.

In summary, our data show that apo(a) phenotype-specific elevation of Lp(a) occurs in patients with renal disease and non-nephrotic proteinuria even when GFR is still normal. How such moderate damage to the kidney impairs Lp(a) metabolism requires additional studies.

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