

Apolipoprotein A-IV Predicts Progression of Chronic Kidney Disease: The Mild to Moderate Kidney Disease Study

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It has not been established firmly whether dyslipidemia contributes independently to the progression of kidney disease. Lipid and lipoprotein parameters, including levels of total, HDL, and LDL cholesterol; triglycerides; lipoprotein(a); apolipoprotein A-IV; and the apolipoprotein E and A-IV polymorphisms, were assessed in 177 patients who had mostly mild to moderate renal insufficiency and were followed prospectively for up to 7 yr. Progression of kidney disease was defined as doubling of baseline serum creatinine and/or terminal renal failure necessitating renal replacement therapy. In univariate analysis, patients who reached a progression end point ($n = 65$) were significantly older and had higher serum creatinine and proteinuria as well as lower GFR and hemoglobin levels. In addition, baseline apolipoprotein A-IV and triglyceride concentrations were higher and HDL cholesterol levels were lower. Multivariate Cox regression analysis revealed that baseline GFR (hazard ratio 0.714; 95% confidence interval [CI] 0.627 to 0.814 for an increment of 10 ml/min per 1.73 m²; $P < 0.0001$) and serum apolipoprotein A-IV concentrations (hazard ratio 1.062; 95% CI 1.018 to 1.108 for an increment of 1 mg/dl; $P = 0.006$) were significant predictors of disease progression. Patients with apolipoprotein A-IV levels above the median had a significantly faster progression ($P < 0.0001$), and their mean follow-up time to a progression end point was 53.7 mo (95% CI 47.6 to 59.8) as compared with 70.0 mo (95% CI 64.6 to 75.4) in patients with apolipoprotein A-IV levels below the median. For the apolipoprotein E polymorphism, only the genotype $\epsilon 2/\epsilon 4$ was associated with an increased risk for progression. In summary, this prospective study in patients with nondiabetic primary kidney disease demonstrated that apolipoprotein A-IV concentration is a novel independent predictor of progression.

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Without treatment, many kidney diseases tend to progress to renal failure. However, the rate of progression shows considerable interindividual variability and is dependent on multiple factors. Moorhead *et al.* (1) proposed in the early 1980s that abnormalities in lipoprotein metabolism cause glomerular and tubulointerstitial damage, promoting progression of chronic kidney disease (CKD). Experimental studies supported this concept that lipids contribute to progressive renal damage (2) by causing macrophage activation and infiltration in the kidney with resultant tubulointer-

stitial and endothelial cell injury. However, the extent, if any, to which dyslipidemia contributes to progression of renal disease in humans and the possible mechanisms by which this may occur have remained unclear (3). Data from the population-based Atherosclerosis Risk in Communities (ARIC) study support a role of altered lipid metabolism in developing renal dysfunction (4). Only a few prospective studies in patients with primary nondiabetic kidney disease have addressed this question (5–12), however, and the results have remained ambiguous. To establish and characterize a relationship between dyslipidemia and renal risk, long-term prospective follow-up studies with a reliable measurement of kidney function at baseline and well-defined renal function end points are required (13). The aim of this prospective 7-yr follow-up study in a cohort of patients without diabetes and with mostly mild to moderate primary kidney disease, therefore, was to evaluate the predictive value of lipoproteins for the progression of kidney disease.

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Materials and Methods

Patients and Baseline Investigations

We examined at baseline 227 white patients who were aged between 18 and 65 yr and had nondiabetic CKD and various degrees of renal impairment. These patients were recruited from eight nephrology departments in Germany, Austria, and South Tyrol as described earlier (14,15). The study was approved by the institutional Ethics Committees, and all patients gave written informed consent. They had stable renal function for at least 3 mo before entry into the study. Exclusion criteria were treatment with immunosuppressive agents, fish oil, or erythropoietin; serum creatinine >6 mg/dl; diabetes of any type; malignancy; liver, thyroid, or infectious disease; nephrotic syndrome (defined as proteinuria >3.5 g/1.73 m² per d); organ transplantation; allergy to ionic contrast media; and pregnancy. According to the National Kidney Foundation's classification of CKD, the 227 patients of our study showed the following stages of CKD: GFR ≥90 ml/min per 1.73 m² (stage 1) in 72 (31.7%) patients, GFR 60 to 89 ml/min per 1.73 m² (stage 2) in 49 (21.6%) patients, GFR 30 to 59 ml/min per 1.73 m² (stage 3) in 63 (27.8%) patients, GFR 15 to 29 ml/min per 1.73 m² (stage 4) in 27 (11.9%) patients, and GFR <15 ml/min per 1.73 m² (stage 5) in 16 (7.0%) patients. The primary cause of kidney disease was glomerulonephritis in 97 (biopsy-confirmed in 90) patients, adult polycystic kidney disease in 37 patients, interstitial nephritis in 24 patients, other types of kidney disease in 43 patients, and unknown in 26 patients.

For avoiding interobserver differences, all patients were recruited by one physician who visited all participating centers. Patient history, including smoking habits and antihypertensive treatment at baseline, was recorded by interview and confirmed by checking patient records. This was complemented by clinical examination, including assessment of body mass index (BMI) and BP. Hypertension was defined as BP >140/90 mmHg and/or the use of antihypertensive medication. We also calculated pulse pressure as the difference between systolic and diastolic BP. Antihypertensive medication (if present) was withheld on the day of the study to minimize interference with measurements of the GFR. Antihypertensive drugs were taken by 179 (79%) patients: Diuretics ($n = 83$; 37%), angiotensin-converting enzyme inhibitors ($n = 123$; 54%), calcium channel blockers ($n = 78$; 34%), β receptor blockers ($n = 67$; 30%), and α -1 receptor blockers ($n = 35$; 16%).

Prospective Follow-Up

After the baseline investigation, patients were followed prospectively until the primary study end point or the end of the observation period was reached. The primary end point was defined as doubling of baseline serum creatinine and/or terminal renal failure necessitating renal replacement therapy.

A total of 177 (78%) patients from the baseline cohort could be assessed during the follow-up. Patients who were lost to follow-up ($n = 50$) had significantly better renal function than patients not lost for follow-up, *i.e.*, a higher mean GFR (91 ± 44 versus 64 ± 39 ml/min per 1.73 m²; $P < 0.01$). According to the National Kidney Foundation's classification, in the 177 patients with follow-up, we found GFR ≥90 ml/min per 1.73 m² (stage 1) in 43 (24.3%) patients, GFR 60 to 89 ml/min per 1.73 m² (stage 2) in 40 (22.6%) patients, GFR 30 to 59 ml/min per 1.73 m² (stage 3) in 57 (32.2%) patients, GFR 15 to 29 ml/min per 1.73 m² (stage 4) in 24 (13.6%) patients, and GFR <15 ml/min per 1.73 m² (stage 5) in 13 (7.3%) patients. However, both groups did not differ significantly with respect to age and gender. Patients who were lost to follow-up had moved away or were not referred by their private physicians for follow-up visits in the renal units.

Laboratory Measurements

Blood samples for measurement of routine chemistry, high-sensitivity C-reactive protein (hsCRP), and lipid parameters were taken after

an overnight fast of at least 12 h. The samples were immediately centrifuged at $1500 \times g$ and 4°C for 10 min, and the supernatants were stored in aliquots at -80°C until further use.

Serum apolipoprotein A-IV (apoA-IV) concentrations were determined with an ELISA (16). Lp(a) quantification and apo(a) phenotyping were performed as described in detail (14) with a double-antibody ELISA and by SDS-agarose gel electrophoresis, respectively. Measurements of serum albumin, total and HDL cholesterol, triglycerides, and hsCRP were performed using routine laboratory tests. LDL cholesterol was calculated according to the Friedewald formula. In addition, GFR was assessed in all patients using the iothalamate clearance technique as described in detail elsewhere (17). Genotyping of the apolipoprotein E (apoE) and apoA-IV (T347S and Q360H corresponding to rs675 and rs5110, respectively) was performed with 5' nuclease allelic discrimination (Taqman) assays. Genotypings were performed within the Genotyping Unit of the Gene Discovery Core Facility at the Innsbruck Medical University.

Statistical Analyses

Statistical analysis was performed with SPSS for Windows 12.01 (SPSS, Inc., Chicago, IL). Univariate comparisons of continuous variables between various groups were performed using an unpaired *t* test or the nonparametric Wilcoxon rank sum test in case of nonnormally distributed variables. Dichotomized variables were compared using Pearson χ^2 test. Differences were considered as significant at $P < 0.05$. Data are presented as mean \pm SD or as median and interquartile range for skewed variables. Univariate correlation analysis was performed by Spearman correlation analysis. Kaplan-Meier time-to-event curves were generated for patients with serum apoA-IV concentrations above and below the median. Multivariable adjusted risk estimates for progression end points were calculated using a Cox proportional hazards regression analysis (short Cox regression). A forward likelihood ratio procedure was used to identify variables that were associated with progression end points over time. Verification of the results was done by a backward likelihood ratio procedure.

Results

Univariate Association of Lipoprotein Parameters and Progression of CKD

Baseline clinical characteristics and laboratory data of the patients with follow-up are reported in the first data column of Table 1. The median follow-up after completion of the baseline investigation was 53 mo (3 to 84). During the follow-up, 65 patients had progressed to a renal end point: 36 patients had doubled their serum creatinine concentration, and 29 patients had reached terminal renal failure necessitating renal replacement therapy. Table 1 further summarizes data of patients with and without progression during the follow-up period. Patients who had reached a progression end point were significantly older and had higher baseline serum creatinine levels and protein excretion rates as well as lower GFR and hemoglobin levels. In addition, they had higher triglyceride and apoA-IV concentrations and lower HDL cholesterol levels. Lp(a) was slightly but not significantly higher in patients who reached a progression end point, but the frequency of low molecular weight (LMW) apo(a) phenotypes was similar in the two patient groups. There were no differences for surrogate parameters of nutritional (BMI and serum albumin) and inflammatory status (hsCRP). Analogous results were obtained using univariate Cox regression analysis: GFR and apoA-IV had the stron-

Table 1. Baseline clinical and laboratory data of 177 patients with completed follow-up with further stratification of those with and without progression of kidney disease during the follow-up period^a

	All Patients (n = 177)	Nonprogressors (n = 112)	Progressors (n = 65)
Gender (male/female)	118/59 (67%/33%)	74/38 (66%/34%)	44/21 (68%/32%)
Age (yr)	46.4 ± 12.2	44.8 ± 12.6	49.1 ± 11.1 ^b
BMI (kg/m ²)	25.2 ± 3.7	24.8 ± 3.5	25.7 ± 3.9
Current smokers (n [%])	34 (19%)	18 (16%)	16 (25%)
Serum creatinine (mg/dl)	2.15 ± 1.22	1.54 ± 0.61	3.21 ± 1.31 ^c
GFR (ml/min per 1.73 m ²)	64 ± 39	79 ± 38	38 ± 25 ^c
Proteinuria (g/24 h per 1.73 m ²)	1.01 ± 0.92	0.87 ± 0.95	1.25 ± 0.83 ^c
Serum albumin (g/dl)	4.56 ± 0.40	4.57 ± 0.43	4.53 ± 0.36
Systolic BP (mmHg)	137 ± 20	136 ± 22	137 ± 17
Diastolic BP (mmHg)	87 ± 13	86 ± 14	88 ± 12
Pulse pressure (mmHg)	50 ± 15	50 ± 16	50 ± 14
Hemoglobin (g/dl)	13.6 ± 1.8	14.1 ± 1.5	12.6 ± 1.9 ^c
hsCRP (mg/L)	0.28 ± 0.31	0.28 ± 0.32	0.29 ± 0.31
Total cholesterol (mg/dl)	216 ± 43	215 ± 42	217 ± 46
LDL cholesterol (mg/dl)	138 ± 39	137 ± 36	138 ± 43
HDL cholesterol (mg/dl)	44 ± 15	46 ± 15	40 ± 13 ^d
Non-HDL cholesterol (mg/dl)	172 ± 42	169 ± 39	177 ± 46
Triglycerides (mg/dl)	172 ± 95	159 ± 93	194 ± 96 ^e
Lp(a) (mg/dl)	29 ± 31	27 ± 30	32 ± 33
LMW apo(a) phenotypes (n [%])	48 (27%)	30 (27%)	18 (28%)
ApoA-IV (mg/dl)	26 ± 8	23 ± 8	31 ± 6 ^c
ApoA-I (mg/dl)	121 ± 23	123 ± 25	117 ± 21
ApoB (mg/dl)	107 ± 26	106 ± 26	109 ± 27
Use of statins (n [%])	26 (14.7%)	16 (14.3%)	10 (15.4%)
Use of fibrates (n [%])	9 (5.1%)	6 (5.4%)	3 (4.6%)

^aapo, apolipoprotein; BMI, body mass index; hsCRP, high-sensitivity C-reactive protein; LMW, low molecular weight.

^b*P* < 0.05, ^c*P* < 0.001, ^d*P* < 0.01, ^e*P* < 0.005, progressors versus nonprogressors.

gest influence on progression-free survival (Table 2). When we constructed Kaplan-Meier curves of the progression-free survival comparing patients with supramedian and inframedian (25.9 mg/dl) serum apoA-IV concentrations (Figure 1), patients with the apoA-IV levels above the median had a worse prognosis and significantly faster progression to the end point compared with those with apoA-IV levels below the median (log-rank test, *P* < 0.0001): The mean follow-up time to a progression end point was 53.7 mo (95% confidence interval [CI] 47.6 to 59.8) compared with 70.0 mo (95% CI 64.6 to 75.4) in the two groups of patients. There was no difference in the frequency of the two apoA-IV polymorphisms T347S and Q360H between progressors and nonprogressors of kidney disease (Table 3).

For evaluation of the significance of apoA-IV as predictor for the progression of kidney disease, the receiver operating characteristic (ROC) analysis was performed for apoA-IV in comparison with GFR (Figure 2). The area under the curve (AUC) was only slightly larger for GFR (AUC = 0.842; 95% CI 0.780 to 0.904; *P* < 0.001) than for apoA-IV (AUC = 0.792; 95% CI 0.726 to 0.859; *P* < 0.001).

Multivariate Association of Lipoprotein Parameters and Progression of CKD

To identify lipoprotein parameters that are associated with progression over time after adjustment for other variables, we performed a multiple Cox regression analysis. Besides age and gender, we added to the model variables that showed *P* < 0.2 in the univariate Cox regression analysis (Table 2). Only baseline GFR (hazard ratio [HR] 0.714; 95% CI 0.627 to 0.814; *P* < 0.0001) and serum apoA-IV concentrations (HR 1.062; 95% CI 1.018 to 1.108; *P* = 0.006) were significantly associated with progression during the follow-up period. HDL cholesterol and triglycerides did not enter into the model as independent factors irrespective of whether we added these variables as a continuous or categorical variable defined by the median of each variable. ApoA-IV remained in the model (HR 1.045; 95% CI 1.001 to 1.092; *P* = 0.046), even when we adjusted for asymmetric dimethylarginine (ADMA), which was shown recently as a significant predictor for progression of kidney disease (18). The type of renal disease as well as the use of antihypertensive medications and especially angiotensin-converting enzyme inhibitors or calcium channel blockers had no

Table 2. The association of different variables with progression of kidney disease during the observation period using univariate and multiple Cox proportional hazards regression models^a

Variable	Coefficient	SEM	χ^2	HR (95% CI)	P
Univariate Cox regression analysis					
age (1 yr)	0.021	0.011	3.419	1.021 (0.999 to 1.045)	0.064
gender (0 = men, 1 = women)	0.103	0.268	0.147	1.108 (0.656 to 1.873)	0.701
GFR (10 ml/min per 1.73 m ²)	-0.389	0.059	42.976	0.678 (0.603 to 0.761)	<0.0001
24-h proteinuria (1g/24 h)	0.264	0.121	4.742	1.302 (1.027 to 1.652)	0.029
apoA-IV (1 mg/dl)	0.112	0.019	34.433	1.118 (1.077 to 1.161)	<0.0001
triglycerides (1 mg/dl)	0.002	0.001	2.008	1.002 (0.999 to 1.004)	0.157
HDL cholesterol (1 mg/dl)	-0.018	0.010	3.270	0.982 (0.964 to 1.001)	0.071
Lp(a) (1 mg/dl)	0.002	0.004	0.205	1.002 (0.994 to 1.009)	0.651
Multiple Cox regression analysis ^b					
GFR (10 ml/min per 1.73 m ²)	-0.336	0.066	25.627	0.714 (0.627 to 0.814)	<0.0001
apoA-IV (1 mg/dl)	0.060	0.022	7.697	1.062 (1.018 to 1.108)	0.006

^aCI, confidence interval.

^bVariables not in the equation: age ($P = 0.63$), gender ($P = 0.97$), proteinuria ($P = 0.42$), triglycerides ($P = 0.98$), HDL cholesterol ($P = 0.26$).

influence on the progression of kidney disease when adjustment for baseline GFR was performed (data not shown).

ApoE Polymorphism and Progression of CKD

Table 3 shows the distribution of apoE genotypes in renal patients with and without progression to a renal end point during the follow-up period. There were no major differences between the two groups with the exception of apoE genotype $\epsilon 2/\epsilon 4$. Of the six patients who showed an $\epsilon 2/\epsilon 4$ genotype, five progressed to the end point. When formally tested against the combined number of other genotype carriers, this difference in frequency reached significance ($P = 0.027$). When we offered a variable describing the $\epsilon 2/\epsilon 4$ carrier status as an additional covariate to the Cox regression model, those with that genotype had a significantly higher probability for a progression of kidney disease (HR 2.68; 95% CI 1.06 to 6.75; $P = 0.037$). In this analysis, the estimates for GFR and apoA-IV remained almost identical as in the model above.

Discussion

Lipid and Lipoprotein Profile and Progression of Kidney Disease

There is little doubt that kidney disease is associated with abnormalities in lipoprotein metabolism. As reviewed in Table 4, however, it has remained controversial whether dyslipidemia plays an independent role in the progression of primary CKD. The basic pathophysiologic concept is derived from the hypothesis that dyslipidemia, among other factors, causes glomerular injury that leads to glomerulosclerosis (19). It has been suggested that glomerulosclerosis and atherosclerosis share common pathophysiologic pathways (20). However, little is known about the exact mechanism (21).

This prospective study had a follow-up time of adequate duration and used exact measurements of GFR, and primary end points were reached by more than one third of the participants. No significant association was found between baseline

classical lipid or lipoprotein parameters and the progression of renal insufficiency. These results are consistent with another prospective study in 138 patients with moderate renal insufficiency (8). Locatelli *et al.* (5) also found no correlation between the baseline lipid profile and the end point of ESRD in 311 patients who did not have diabetes or nephrosis and had moderate renal insufficiency and were followed for 2 yr. Conversely, Samuelsson *et al.* (7) described in 73 patients with mild to severe renal insufficiency total and LDL cholesterol as well as apoB to be significantly associated with a rapid decline in renal function but not triglycerides or HDL cholesterol. The Modification of Diet in Renal Disease (MDRD) Study demonstrated lower HDL cholesterol levels as an independent predictor of a decline in GFR in patients with kidney disease; however, triglyceride levels were not measured (6). In our study, lower HDL cholesterol showed only a borderline association with progression of disease, which was no longer significant after adjustment for GFR in the multiple Cox regression analysis. Syrjanen *et al.* (11) found hypertriglyceridemia as an independent risk factor for progression of IgA nephropathy.

These nonuniform data illustrate that, so far, it has remained uncertain which lipoproteins are the best predictors for a progression of kidney disease (Table 4). The finding of low HDL cholesterol levels as predictors of progression is in line with studies performed in the general population (4,22). The ARIC study reported that higher triglyceride levels and lower HDL cholesterol were associated with a higher relative risk for worsening renal function over 3 yr (4). The prospective, randomized Helsinki Heart Study investigated patients who had dyslipidemia and non-HDL cholesterol >200 mg/dl and were at baseline free of renal disease and found lower HDL cholesterol and an elevated LDL/HDL cholesterol ratio to be independent predictors of an increase of serum creatinine during study follow-up; LDL cholesterol and triglycerides were not independent predictors. However, these variables explained only a very small fraction (at best 1%) of

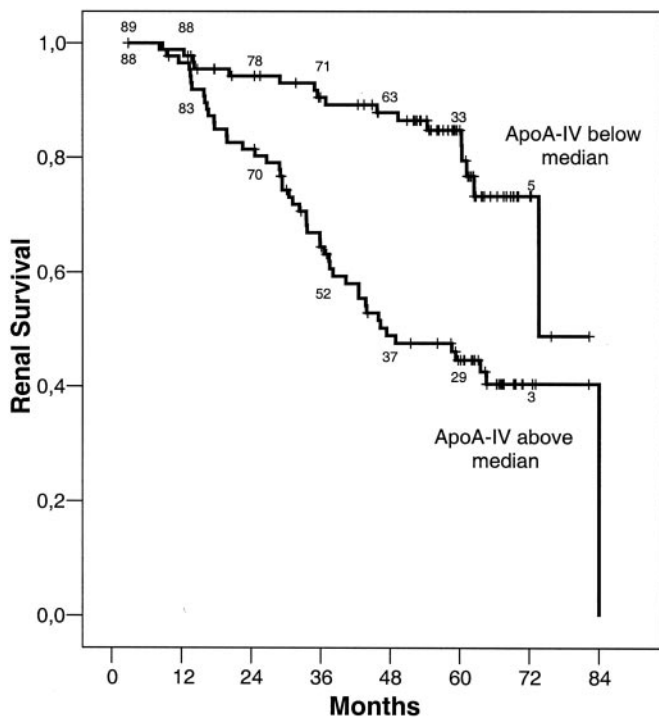


Figure 1. Kaplan-Meier curves of renal end points in patients with infra- and supramedian plasma apolipoprotein A-IV (apoA-IV) concentrations. In patients with supramedian plasma apoA-IV concentrations (i.e., >25.9 mg/dl), progression was significantly faster (log-rank test, $P < 0.0001$). Numbers near the survival curves represent the number of patients at risk with apoA-IV levels below and above the median at 0, 12, 24, 36, 48, 60, and 72 mo.

the variance in creatinine increase (22). Such small contribution can be detected only in large studies. Further explanations for differences to our and other studies in patients with kidney disease might be that studies in the general population investigated individuals without primary kidney disease at baseline or with mild impairment of kidney function at most. Another explanation might be the definition of the progression end point, which was only a minor increase in serum creatinine in some of these studies (4).

Even if the evidence for an association between lipid parameters and the progression of renal disease in humans is not consistent, several studies investigated the effect of lipid lowering on the progression of renal insufficiency. In a number of animal models, lipogenic diets worsen whereas cholesterol-lowering medications ameliorate renal injury (23–27). Fried *et al.* (28) performed a meta-analysis that examined the role of lipid-lowering therapy on renal function in humans. The data were derived from lipid-lowering trials that commonly performed *post hoc* analysis of subgroups with renal insufficiency for renal end points that were not predefined. They suggested that lipid-lowering therapy may slow progression, but this conclusion was derived from a few trials with small numbers and generally short follow-up times (28). In case these agents indeed show a progression-retarding effect, it remains to be

Table 3. Influence of the apoA-IV and the apoE polymorphism on the progression of kidney disease during the follow-up period in patients with mild and moderate kidney disease^a

Genotype	No Progression		Progression	
	<i>n</i>	%	<i>n</i>	%
ApoA-IV T347S				
AA	73	66.4	41	65.1
AT	32	29.1	18	28.6
TT	5	4.5	4	6.3
ApoA-IV Q360H				
AA	1	0.9	0	0.0
AC	19	17.3	8	12.7
CC	90	81.8	55	87.3
ApoE				
2/2	1	0.9	1	1.5
2/3	17	15.5	7	10.8
3/3	69	62.7	36	55.4
3/4	22	20.0	15	23.1
4/4	0	0.0	1	1.5
2/4	1	0.9	5	7.7

^aApoA-IV and apoE genotypes are available in 173 and 175 of 177 patients, respectively.

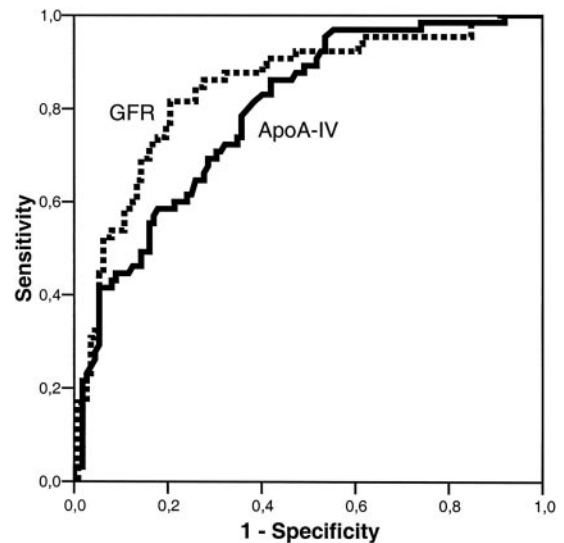


Figure 2. Receiver operating characteristic (ROC) curve of GFR and apoA-IV levels with progression of kidney disease as status variable. The area under the curve (AUC) is only slightly larger for GFR than for apoA-IV (AUC 0.842 and 0.792, respectively).

proved whether this retardation derives from lipid-lowering or from pleiotropic effects of statins.

It is also interesting to note that Lp(a) concentrations were not predictive for the progression of kidney disease, although *in vivo* experiments pointed to a progression-advancing effect. Atherogenic lipoproteins such as LDL cholesterol or Lp(a),

Table 4. Summary of prospective studies in patients with nondiabetic kidney disease examining the relationship of dyslipidemia and the progression of kidney disease^a

Author, Year	Patients	Duration of Follow-up	Definition of Progression	Associations with Progression of Kidney Disease
Hunsicker <i>et al.</i> , 1997 (6)	$n = 840$; GFR 15 to 55 ml/min per 1.73 m ²	Up to 3.5 yr	Rate of decline in GFR (GFR slope ml/min per yr)	Lower HDL cholesterol independent predictor of a faster decline in GFR No significant effect by TC, LDL cholesterol, apoA-I, apoB
Locatelli <i>et al.</i> , 1996 (5)	$n = 311$; Cr 119 to 619 mmol/L and CCr <60 ml/min	2 yr	Need for dialysis, doubling of baseline Cr	No correlation with TC
Syrjanen <i>et al.</i> , 2000 (11)	$n = 223$ patients with IgA nephropathy; 81% normal Cr levels, 19% elevated Cr levels	Median 10 yr (0.2 to 17 yr)	Cr >125 μmol/L (men) or >105 μmol/L (women) and >20% elevation from baseline	Hypertriglyceridemia independent risk factor for progression of disease No significant correlation with TC, LDL cholesterol, HDL cholesterol, LDL/HDL cholesterol ratio
Massy <i>et al.</i> , 1999 (8)	$n = 138$ (including 2 to 3% patients with type 2 diabetes); CCr 20 to 70 ml/min	Mean ~7 yr	ESRD	No correlation with TG, HDL cholesterol, TC, apoA-I, apo-B, Lp(a)
Washio <i>et al.</i> , 1996 (10)	$n = 104$ (including 9 patients with type 2 diabetes); Cr 0.5 to 5.0 mg/dl	Mean 4.1 yr	(-1) × (the slope of the 1/Cr plotted against the observation time)	Positive correlation with TC level No significant correlation with TG
Samuelsson <i>et al.</i> , 1997 (7)	$n = 73$; GFR 15 to 75 ml/min per 1.73 m ²	Mean 3.2 yr	Annual change in GFR	Significant association with TC, LDL cholesterol, and apoB (in univariate analysis) No significant relation with TG, HDL cholesterol, VLDL cholesterol, apoA-I, apoA-II, apoC-III, apoE
Cappelli <i>et al.</i> , 1992 (9)	$n = 52$; CCr 20 to 60 ml/min	12 mo	Slope of regression line of 1/Cr negative at end of follow-up period	Significant relationship with apoA/apoB ratio No association with TC, HDL cholesterol, LDL cholesterol, VLDL cholesterol, TG, apoB
Mazouz <i>et al.</i> , 1999 (12)	$n = 49$ (including 7 patients with type 2 diabetes); mean CCr 24 ± 14 ml/min	At least 2 yr before the start of dialysis ^b	ΔCCr	No significant association with TC and TG

^aThe table lists mostly adjusted results unless otherwise stated. Cr, creatinine; CCr, creatinine clearance; TC, total cholesterol; TG, triglycerides.

^bForty-nine patients selected of 173 presently treated hemodialysis patients on the basis of availability of a quarterly follow-up for 2 yr before starting dialysis.

especially when oxidized, induce the formation of oxygen radicals in arteries, in glomeruli, and in juxtaglomerular cells, which results in an inhibition of nitric oxide-mediated vasodilation (29), stimulation of renin release, and modulation of mesangial cell growth and apoptosis (30–33). However, Lp(a) concentrations in our and two other studies (8,34) were not related to the progression of kidney disease.

ApoA-IV

This study was the first to investigate the association between a novel lipid parameter: ApoA-IV concentration and progression of renal insufficiency. We found that higher baseline plasma apoA-IV concentrations were one of the best predictors for the progression of kidney disease apart from baseline GFR. This association was independent of other lipoprotein parameters; of ADMA, which was shown recently to be a highly significant predictor of kidney disease progression (18); and of proteinuria, BP, and nutritional and inflammatory status. However, this does not necessarily suggest that apoA-IV *per se* is pathogenic. It is conceivable that apoA-IV reflects catabolism of this apolipoprotein in the kidney not entirely reflected by the changes in GFR. It is known that ESRD affects apoA-IV serum concentrations. We and others reported a pronounced increase of apoA-IV concentrations in hemodialysis patients (35–38). We could even demonstrate that in patients with kidney disease, a significant increase in apoA-IV concentrations is found even when GFR is still within the normal range (15). It therefore seems that apoA-IV is an early marker of renal impairment. This is supported by our recent findings of apoA-IV immunoreactivity in kidney tubular cells, suggesting a direct role of the human kidney in apoA-IV metabolism (39). A granular staining pattern in those cells probably represents lysosomes degrading apoA-IV. It is interesting that a high correlation was found between apoA-IV and GFR ($r = -0.62$) (15). When we compare GFR and apoA-IV in the univariate Cox regression model, both parameters predict similarly the progression-free survival ($\chi^2 = 43$ versus 34; Table 2). Nevertheless, apoA-IV still significantly contributes to the prediction of disease progression after adjustment for GFR, which supports the additional value of apoA-IV as a kidney function parameter.

The association of higher apoA-IV levels with progression of kidney disease is unexpected, given the physiologic functions of apoA-IV. According to *in vitro* studies, apoA-IV participates in several steps of the reverse cholesterol transport pathway, which removes cholesterol from peripheral cells and directs it to liver and steroidogenic organs for metabolism (40–42). Furthermore, other studies documented antioxidative properties of apoA-IV (43). One therefore might have anticipated that the increased apoA-IV levels in impaired kidney function would have resulted in improved removal of cholesterol from mesangial cells and, together with the antioxidative properties, slower progression. The observation of an opposite effect would be compatible with the assumption that apoA-IV is not fully functionally active or that high apoA-IV levels reflect an aspect of renal impairment that is not parallel to GFR.

ApoE Polymorphism

Up to now, numerous case-control and cross-sectional studies investigated the apoE polymorphism in patients with kidney disease compared with control subjects with contrasting results. Only a few of them were designed to investigate an association between the apoE polymorphism and the progression of renal insufficiency by a follow-up observation (44–46). Araki *et al.* (45) found in Japanese patients with type 2 diabetes that the 31 patients who developed or who showed a progression of nephropathy during an average observation period of 4.4 yr were more often $\epsilon 2$ carriers. The apo $\epsilon 4$ allele was found to be an independent protective factor for the progression to ESRD in a retrospective follow-up study in Japanese patients with type 2 diabetes (44). Results from the prospective ARIC study in the general population without severe renal dysfunction at baseline recently showed that $\epsilon 2$ moderately increased and $\epsilon 4$ decreased risk for renal disease progression (46). In contrast to these studies, we could not see a negative effect of the $\epsilon 2$ allele or a protective effect of the $\epsilon 4$ allele. When we used the allele-counting method, we observed that progressors showed a very similar frequency of the $\epsilon 2$ allele (10.8 versus 9.1%) and a higher rather than a lower frequency of the $\epsilon 4$ allele (16.9 versus 10.8%), which did not reach significance, however. It is not clear whether this finding can be explained by differences in study design, patient selection, or definition of kidney disease progression. The above-mentioned prospective studies either investigated patients with type 2 diabetes considering albuminuria or proteinuria as progression end point (45) or focused on patients at the population level without kidney disease at baseline and an end point mostly defined by an increase in creatinine of at least 0.4 mg/dl (46). Our study, however, included patients with primary kidney disease at study entry. Progression was defined as doubling of baseline serum creatinine or necessity of renal replacement therapy during follow-up. The only noticeable observation concerning the apoE polymorphism in our study was that five of the six carriers of apoE genotype $\epsilon 2/\epsilon 4$ experienced a progression of the disease. However, we are aware of the low frequency of this genotype and of the need that the finding be confirmed by future large studies.

Limitations of the Study

One of the limitations of our study is that we do not have sequential GFR measurements by iohexol technique during the follow-up period. We believed, however, that it was more important to have an exact measurement of GFR at baseline because a simple measurement of creatinine or a GFR calculation by a formula would have been imprecise. Doubling of serum creatinine is an accepted specific end point (47).

The applied exclusion criteria in our study resulted in a selected group of patients. Therefore, the association of increased apoA-IV and renal disease progression may not be applicable to other types of CKD, such as diabetic nephropathy or nephrotic forms of kidney diseases. This selection, however, might reduce confounding and increase the power of our study. Furthermore, whether these findings observed in white individuals can be extrapolated to other ethnic groups has to be

investigated in further studies. Finally, the sample size of our study might have been too small to investigate the influence of various subgroups of glomerulonephritis or tubulointerstitial renal diseases and the influence of various antihypertensive medications on the progression of kidney diseases.

Conclusion

In our prospective study of patients with nondiabetic primary kidney disease, we found no significant and independent association between the classical lipid parameters and the progression of kidney disease to an end point. We identified, however, apoA-IV as a novel predictor for progression of kidney disease. Our study failed to confirm an association between apoE polymorphism and progression of CKD that had been described in previous studies.

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References

- Moorhead JF, Chan MK, El Nahas M, Varghese Z: Lipid nephrotoxicity in chronic progressive glomerular and tubulo-interstitial disease. *Lancet* 2: 1309–1311, 1982
- Keane WF: Lipids and the kidney. *Kidney Int* 46: 910–920, 1994
- Keane WF: The role of lipids in renal disease: Future challenges. *Kidney Int* 57[Suppl 75]: S27–S31, 2000
- Muntner P, Coresh J, Smith JC, Eckfeldt J, Klag MJ: Plasma lipids and risk of developing renal dysfunction: The Atherosclerosis Risk in Communities study. *Kidney Int* 58: 293–301, 2000
- Locatelli F, Marcelli D, Comelli M, Alberti D, Graziani G, Bucciati G, Redaelli B, Giangrande A; the Northern Italian Cooperative Study Group: Proteinuria and blood pressure as causal components of progression to end-stage renal failure. *Nephrol Dial Transplant* 11: 461–467, 1996
- Hunsicker LG, Adler S, Caggiula A, England BK, Greene T, Kusek JW, Rogers NL, Teschan PE: Predictors of the progression of renal disease in the Modification of Diet in Renal Disease Study. *Kidney Int* 51: 1908–1919, 1997
- Samuelsson O, Mulec H, Knight-Gibson C, Attman PO, Kron B, Larsson R, Weiss L, Wedel H, Alaupovic P: Lipoprotein abnormalities are associated with increased rate of progression of human chronic renal insufficiency. *Nephrol Dial Transplant* 12: 1908–1915, 1997
- Massy ZA, Khoa TN, Lacour B, Descamps-Latscha B, Man NK, Jungers P: Dyslipidaemia and the progression of renal disease in chronic renal failure patients. *Nephrol Dial Transplant* 14: 2392–2397, 1999
- Cappelli P, Evangelista M, Bonomini M, Palmieri PF, Albertazzi A: Lipids in the progression of chronic renal failure. *Nephron* 62: 31–35, 1992
- Washio M, Okuda S, Ikeda M, Hirakata H, Nanishi F, Onoyama K, Yoshimura T, Fujishima M: Hypercholesterolemia and the progression of the renal dysfunction in chronic renal failure patients. *J Epidemiol* 6: 172–177, 1996
- Syrjanen J, Mustonen J, Pasternack A: Hypertriglyceridaemia and hyperuricaemia are risk factors for progression of IgA nephropathy. *Nephrol Dial Transplant* 15: 34–42, 2000
- Mazouz H, Kacso I, Ghazali A, El Esper N, Moriniere P, Makdassi R, Hardy P, Westeel PF, Achard JM, Pruna A, Fournier A: Risk factors of renal failure progression two years prior to dialysis. *Clin Nephrol* 51: 355–366, 1999
- Crook ED, Thallapureddy A, Migdal S, Flack JM, Greene EL, Salahudeen A, Tucker JK, Taylor HA Jr: Lipid abnormalities and renal disease: Is dyslipidemia a predictor of progression of renal disease? *Am J Med Sci* 325: 340–348, 2003
- Kronenberg F, Kuen E, Ritz E, Junker R, Konig P, Kraatz G, Lhotta K, Mann JFE, Muller GA, Neyer U, Riegel W, Riegler P, Schwenger V, Von Eckardstein A: Lipoprotein(a) serum concentrations and apolipoprotein(a) phenotypes in mild and moderate renal failure. *J Am Soc Nephrol* 11: 105–115, 2000
- Kronenberg F, Kuen E, Ritz E, Junker R, Konig P, Kraatz G, Lhotta K, Mann JFE, Muller GA, Neyer U, Riegel W, Riegler P, Schwenger V, Von Eckardstein A: Apolipoprotein A-IV serum concentrations are elevated in mild and moderate renal failure. *J Am Soc Nephrol* 13: 461–469, 2002
- Kronenberg F, Lobentanz E-M, Konig P, Utermann G, Dieplinger H: Effect of sample storage on the measurement of lipoprotein(a), apolipoproteins B and A-IV, total and high-density lipoprotein cholesterol and triglycerides. *J Lipid Res* 35: 1318–1328, 1994
- Bostom AG, Kronenberg F, Ritz E: Predictive performance of renal function equations for patients with chronic kidney disease and normal serum creatinine levels. *J Am Soc Nephrol* 13: 2140–2144, 2002
- Fliser D, Kronenberg F, Kielstein JT, Morath C, Bode-Boger SM, Haller H, Ritz E; for the MMKD Study Group: Asymmetric dimethylarginine and progression of chronic kidney disease: The Mild to Moderate Kidney Disease Study. *J Am Soc Nephrol* 16: 2456–2461, 2005
- Diamond JR: Analogous pathobiologic mechanisms in glomerulosclerosis and atherosclerosis. *Kidney Int* 39[Suppl 31]: S29–S34, 1991
- Kasiske BL: Relationship between vascular disease and age-associated changes in the human kidney. *Kidney Int* 31: 1153–1159, 1987
- Diamond JR, Karnovsky MJ: A putative role of hypercholesterolemia in progressive glomerular injury. *Annu Rev Med* 43: 83–92, 1992
- Manttari M, Tiula E, Alikoski T, Manninen V: Effects of hypertension and dyslipidemia on the decline in renal function. *Hypertension* 26: 670–675, 1995
- Kasiske BL, O'Donnell MP, Schmitz PG, Kim Y, Keane WF: Renal injury of diet-induced hypercholesterolemia in rats. *Kidney Int* 37: 880–891, 1990
- Wellmann KF, Volk BW: Renal changes in experimental hypercholesterolemia in normal and in subdiabetic rabbits. II. Long term studies. *Lab Invest* 24: 144–155, 1971
- Kasiske BL, O'Donnell MP, Keane WF: Pharmacological treatment of hyperlipidemia reduces glomerular injury in the rat 5/6 nephrectomy model of chronic renal failure. *Circ Res* 62: 367–374, 1988

26. Kasiske BL: Risk factors for accelerated atherosclerosis in renal transplant recipients. *Am J Med* 84: 985-992, 1988
27. Harris KPG, Purkerson ML, Yates J, Klahr S: Lovastatin ameliorates the development of glomerulosclerosis and uremia in experimental nephrotic syndrome. *Am J Kidney Dis* 15: 16-23, 1990
28. Fried LF, Orchard TJ, Kasiske BL: Effect of lipid reduction on the progression of renal disease: A meta-analysis. *Kidney Int* 59: 260-269, 2001
29. Galle J, Bengen J, Schollmeyer P, Wanner C: Impairment of endothelium-dependent dilation in rabbit renal arteries by oxidized lipoprotein(a)—Role of oxygen-derived radicals. *Circulation* 92: 1582-1589, 1995
30. Wanner C, Greiber S, Kramer-Guth A, Heinloth A, Galle J: Lipids and progression of renal disease: Role of modified low density lipoprotein and lipoprotein(a). *Kidney Int* 52[Suppl 63]: S102-S106, 1997
31. Greiber S, Kramer-Guth A, Pavenstadt H, Gutenkunst M, Schollmeyer P, Wanner C: Effects of lipoprotein(a) on mesangial cell proliferation and viability. *Nephrol Dial Transplant* 11: 778-785, 1996
32. Mondorf UF, Piiper A, Herrero M, Olbrich HG, Bender M, Gross W, Scheuermann E, Geiger H: Lipoprotein(a) stimulates growth of human mesangial cells and induces activation of phospholipase C via pertussis toxin-sensitive G proteins. *Kidney Int* 55: 1359-1366, 1999
33. Galle J, Schneider R, Heinloth A, Wanner C, Galle PR, Conzelmann E, Dimmeler S, Heermeier K: Lp(a) and LDL induce apoptosis in human endothelial cells and in rabbit aorta: Role of oxidative stress. *Kidney Int* 55: 1450-1461, 1999
34. Samuelsson O, Attman P-O, Knight-Gibson C, Larsson R, Mulec H, Wedel H, Weiss L, Alaupovic P: Plasma levels of lipoprotein(a) do not predict progression of human chronic renal failure. *Nephrol Dial Transplant* 11: 2237-2243, 1996
35. Nestel P, Noakes M, Belling B, McArthur R, Clifton P, Janus E, Abbey M: Plasma lipoprotein lipid and Lp[a] changes with substitution of elaidic acid for oleic acid in the diet. *J Lipid Res* 33: 1029-1036, 1992
36. Seishima M, Muto Y: An increased apo A-IV serum concentration of patients with chronic renal failure on hemodialysis. *Clin Chim Acta* 167: 303-311, 1987
37. Dieplinger H, Lobentanz E-M, Konig P, Graf H, Sandholzer C, Matthys E, Rosseneu M, Utermann G: Plasma apolipoprotein A-IV metabolism in patients with chronic renal disease. *Eur J Clin Invest* 22: 166-174, 1992
38. Kronenberg F, Konig P, Neyer U, Auinger M, Pribasniq A, Lang U, Reitingner J, Pinter G, Utermann G, Dieplinger H: Multicenter study of lipoprotein(a) and apolipoprotein(a) phenotypes in patients with end-stage renal disease treated by hemodialysis or continuous ambulatory peritoneal dialysis. *J Am Soc Nephrol* 6: 110-120, 1995
39. Haiman M, Salvenmoser W, Scheiber K, Lingenhel A, Rudolph C, Schmitz G, Kronenberg F, Dieplinger H: Immunohistochemical localization of apolipoprotein A-IV in human kidney tissue. *Kidney Int* 68: 1130-1136, 2005
40. Stein O, Stein Y, Lefevre M, Roheim PS: The role of apolipoprotein A-IV in reverse cholesterol transport studied with cultured cells and liposomes derived from another analog of phosphatidylcholine. *Biochim Biophys Acta* 878: 7-13, 1986
41. Dvorin E, Gorder NL, Benson DM, Gotto AM Jr: Apolipoprotein A-IV. A determinant for binding and uptake of high-density lipoproteins by rat hepatocytes. *J Biol Chem* 261: 15714-15718, 1986
42. Steinmetz A, Barbaras R, Ghalim N, Clavey V, Fruchart J-C, Ailhaud G: Human apolipoprotein A-IV binds to apolipoprotein A-I/A-II receptor sites and promotes cholesterol efflux from adipose cells. *J Biol Chem* 265: 7859-7863, 1990
43. Qin XF, Swertfeger DK, Zheng SQ, Hui DY, Tso P: Apolipoprotein AIV: A potent endogenous inhibitor of lipid oxidation. *Am J Physiol* 274: H1836-H1840, 1998
44. Kimura H, Suzuki Y, Gejyo F, Karasawa R, Miyazaki R, Suzuki S, Arakawa M: Apolipoprotein E4 reduces risk of diabetic nephropathy in patients with NIDDM. *Am J Kidney Dis* 31: 666-673, 1998
45. Araki S, Koya D, Makiishi T, Sugimoto T, Isono M, Kikkawa R, Kashiwagi A, Haneda M: APOE polymorphism and the progression of diabetic nephropathy in Japanese subjects with type 2 diabetes: Results of a prospective observational follow-up study. *Diabetes Care* 26: 2416-2420, 2003
46. Hsu CC, Kao WH, Coresh J, Pankow JS, Marsh-Manzi J, Boerwinkle E, Bray MS: Apolipoprotein E and progression of chronic kidney disease. *JAMA* 293: 2892-2899, 2005
47. Rossing P: Doubling of serum creatinine: is it sensitive and relevant [Editorial]? *Nephrol Dial Transplant* 13: 244-246, 1998