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Urinary Monocyte Chemoattractant Protein-1 in Patients With Alport Syndrome

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In patients with Alport syndrome, the rate of kidney function decline varies with genotype, chromosomal sex, and possibly other factors.[1] We hypothesized that certain urinary biomarkers can predict the rate of kidney function decline in patients with Alport syndrome. We collected urine samples from 76 patients with Alport syndrome (39 male, 37 female) recruited through the Alport Syndrome Treatments and Outcomes Registry (ClinicalTrials.gov identifiers NCT00481130 and NCT01602835) and from 40 healthy adult and 11 healthy pediatric volunteers. The samples were assayed for the concentrations of 18 proteins and corrected for urine creatinine concentration (Supplementary Methods). In a subset of 28 patients with Alport syndrome, both baseline and 5-year urine samples were collected. Data from medical records (serum creatinine levels and height) were used to calculate estimated glomerular filtration rates (eGFRs) and the slope of decline in eGFR (ml/min per 1.73 m^2 per year) from baseline to 5-year follow-up, using the CKiDs formula for pediatric patients and the unbiased Chronic Kidney Disease Epidemiology Collaboration formula for adults (Supplementary Methods).[S1,S2] Regression analysis was used to evaluate the relationship between baseline urine biomarker levels and the slope of eGFR decline (Supplementary Methods). All study procedures were approved by the Institutional Review Board of the University of Minnesota. Written consent was obtained from patients at baseline and at follow-up sample collection.

The 28 patients with Alport syndrome with baseline and 5-year data included 19 males and 9 females (Table 1). The males were younger at baseline and had higher baseline and 5-year eGFRs than the females; these differences were statistically significant at baseline but did not reach statistical significance at 5 years. Of the 28 patients, 27 received treatment with an angiotensin-converting enzyme inhibitor or angiotensin receptor

Table 1. Characteristics of patients with baseline and 5-year urine samples

Patient characteristics	Males (n = 19)	Females (n = 9)	Significance
Age			0.0001
Mean	14	36	
SD	10	15	
Range	6–51	11–52	
X-linked AS	17	7	NS
Hearing loss	11	5	NS
ACEi/ARB	19	8	NS
Baseline eGFR			0.0063
Mean	126	83	
SD	33	24	
Range	4–190	36–114	
eGFR at second time point			0.0684
Mean	104	76	
SD	31	23	
Range	24–135	35–110	
eGFR slope			NS
Mean	−2	−1	
SD	5	2	
Range	−16 to 5	−5 to 2	
Baseline MCP-1/Cr			NS
Mean	6	3.6	
SD	0.8	1.0	
Range	0.4–12.7	1.0–9.4	
MCP-1/Cr at second time point			0.0099
Mean	6	3.4	
SD	0.9	0.45	
Range	1.3–14.5	1.6–6.0	

ACEi, angiotensin-converting enzyme inhibitor; ARB, angiotensin receptor blocker; AS, Alport syndrome; Cr, creatinine; eGFR, estimated glomerular filtration rate; MCP-1, monocyte chemoattractant protein-1; NS, not significant.

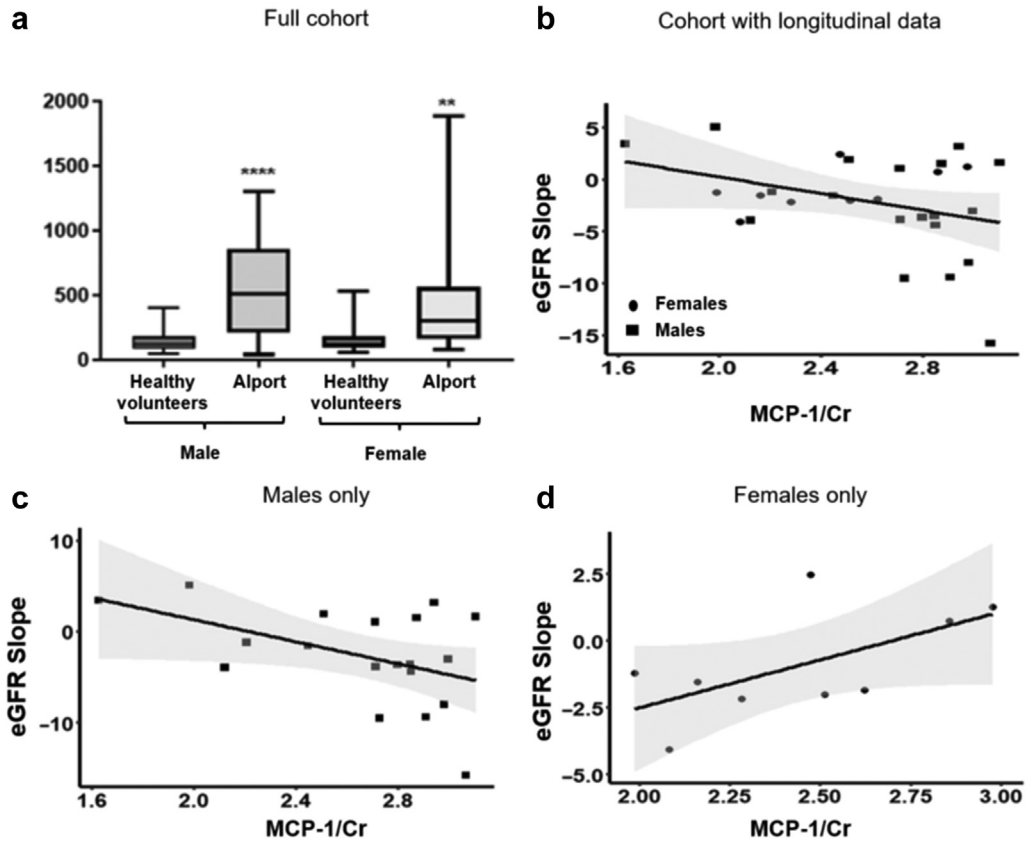

Figure 1. MCP-1/Cr levels in subjects with Alport syndrome and healthy volunteers and correlation with slope of decline in eGFR. (a) MCP-1/Cr levels (pg/mg) were determined in male subjects with Alport syndrome ($n = 39$) and healthy volunteers ($n = 25$) and in female subjects with Alport syndrome ($n = 37$) and healthy volunteers ($n = 21$). The significance of the difference in MCP-1/Cr levels compared with healthy volunteers was analyzed using one-way ANOVA followed by Bonferroni multiple comparison test: $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$. (b) In the subset of the cohort with Alport syndrome with longitudinal data, the correlations of MCP-1/Cr levels (log 10) with eGFR slope (ml/min per 1.73 m^2 per year) were tested using Pearson correlation analysis and linear modeling. Baseline MCP-1/Cr level was negatively correlated with eGFR slope in the full cohort, but this correlation was not statistically significant ($r = -0.35$, $P = 0.072$). (c) In males with Alport syndrome with longitudinal data, baseline MCP-1/Cr level was negatively and significantly correlated with eGFR slope ($r = -0.46$, $P = 0.047$). (d) In females with Alport syndrome with longitudinal data, baseline MCP-1/Cr level was positively correlated with eGFR slope, but this correlation was not statistically significant ($r = 0.61$, $P = 0.084$). ANOVA, analysis of variance; Cr, creatinine; eGFR, estimated glomerular filtration rate; MCP-1, monocyte chemoattractant protein-1.

blocker. The rate of decline in eGFR was similar in males and females. Baseline urinary monocyte chemoattractant protein-1 (MCP-1)/creatinine (Cr) levels were higher in males than in females, but this difference was not statistically significant. Urinary MCP-1/Cr levels at 5 years were higher in males, and this difference was statistically significant (Supplementary Figure S1).

Urinary MCP-1/Cr levels were significantly higher in patients with Alport syndrome than in healthy volunteers (Figure 1a). In the 28 patients followed for 5 years, correlation and univariate regression analysis revealed that baseline urinary MCP-1/Cr was negatively correlated with the slope of eGFR decline (Figure 1b and Supplementary Table S1), but this relationship did not reach statistical significance ($P = 0.072$). Urine MCP-1/Cr was found to have a significant negative correlation with the slope of eGFR decline in the 19 male patients with Alport syndrome ($P = 0.0473$), but it was not significantly correlated with the slope of eGFR decline in the 9 female patients with Alport syndrome (Figure 1c and d). In male patients with Alport syndrome, after age adjustment, the negative association between baseline urinary MCP-1/Cr and eGFR slope was marginally significant ($P = 0.056$) (Supplementary Table S1).

MCP-1 is a powerful regulator of monocyte migration and infiltration that has been implicated in the progression of chronic kidney diseases.[2] Malhotra et al. found that baseline urinary MCP-1 levels were associated with a significantly increased risk of 50% decline in eGFR or kidney failure in participants with nondiabetic chronic kidney disease in the SPRINT trial,[3] whereas Puthumana *et al.*[4] observed that higher urinary MCP-1 levels were associated with greater eGFR decline after hospitalization.

Angiotensin II is a known inducer of MCP-1 expression in the kidney, and there is ample evidence of

increased angiotensin II activity in experimental and human Alport syndrome.[5,6] MCP-1 has been found to be up-regulated in the kidneys of mice with Alport syndrome, and elevated kidney levels of MCP-1 precede kidney function decline.[7] Induction of kidney MCP-1 activity was found to be a downstream effect of tumor necrosis factor-α in a mouse with Alport syndrome model.[8] Falcone *et al*.[7] studied strain-specific differences in rate of kidney function decline in mice with ARAS because of a *col4a4* point mutation. These investigators found that strain-determined inflammatory activity in kidneys, including MCP-1, correlated with the rate of kidney function decline and suggested that urinary MCP-1 could be a useful biomarker of Alport kidney disease progression.

The finding of elevated urinary MCP-1/Cr levels in male and female patients with Alport syndrome suggests the presence of inflammatory activity in the kidneys of these patients, consistent with findings in animal models and human Alport kidney biopsies.[9] Male patients with Alport syndrome in our study had, as a group, normal eGFR level, indicating kidney inflammation may be present early in the course of Alport syndrome. Amelioration of angiotensin II-mediated increases in MCP-1 activity may be one of the beneficial effects of early blockade of the renin-angiotensin-aldosterone system in Alport syndrome.

There are several limitations to our study. The number of subjects with baseline and 5-year samples, and at least 3 eGFR determinations, is relatively small. As this was a hypothesis-generating study, we evaluated multiple proteins in a relatively small patient cohort. We could not confirm *COL4A3*/*COL4A4*/*COL4A5* genotype in all patients, although most were determined to have X-linked disease based on genotype or family pedigree. We were also unable to determine MCP-1 genotype, which has been correlated with chronic kidney disease risk in a previous study.

Our findings suggest that urinary MCP-1/Cr levels at baseline may be associated with a more rapid subsequent rate of kidney function loss in male patients with Alport syndrome. Monitoring of urinary MCP-1/Cr during clinical trials may provide useful information regarding the effects of therapeutic interventions on inflammatory pathways in the kidneys of patients with Alport syndrome.[S3]

DISCLOSURE

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SUPPLEMENTARY MATERIAL

Supplementary File (PDF)

Supplementary Methods.

Figure S1. Spaghetti plot of baseline and 5-year urinary MCP-1/Cr levels in male and female patients with Alport.

Table S1. Association of urinary MCP-1/Cr levels and eGFR slope using linear regression modeling.

Supplementary References.

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