Enhanced MCP-1 Release in Early Autosomal Dominant Polycystic Kidney Disease

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Introduction: Autosomal dominant polycystic kidney disease (ADPKD) causes kidney failure typically in adulthood, but the disease starts in utero. Copeptin, epidermal growth factor (EGF), and monocyte chemotactant protein-1 (MCP-1) are associated with severity and hold prognostic value in adults but remain unstudied in the early disease stage. Kidneys from adults with ADPKD exhibit macrophage infiltration, and a prominent role of MCP-1 secretion by tubular epithelial cells is suggested from rodent models.

Methods: In a cross-sectional study, plasma copeptin, urinary EGF, and urinary MCP-1 were evaluated in a pediatric ADPKD cohort and compared with age-, sex-, and body mass index (BMI)-matched healthy controls. MCP-1 was studied in mouse collecting duct cells, human proximal tubular cells, and fetal kidney tissue.

Results: Fifty-three genotyped ADPKD patients and 53 controls were included. The mean (SD) age was 10.4 (5.9) versus 10.5 (6.1) years (P = 0.543), and the estimated glomerular filtration rate (eGFR) was 122.7 (39.8) versus 114.5 (23.1) ml/min per 1.73 m² (P = 0.177) in patients versus controls, respectively. Plasma copeptin and EGF secretion were comparable between groups. The median (interquartile range) urinary MCP-1 (pg/mg creatinine) was significantly higher in ADPKD patients (185.4 [213.8]) compared with controls (154.7 [98.0], P = 0.010). Human proximal tubular cells with a heterozygous PKD1 mutation and mouse collecting duct cells with a PKD1 knockout exhibited increased MCP-1 secretion. Human fetal ADPKD kidneys displayed prominent MCP-1 immunoreactivity and M2 macrophage infiltration.

Conclusion: An increase in tubular MCP-1 secretion is an early event in ADPKD. MCP-1 is an early disease severity marker and a potential treatment target.

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DPKD is a common monogenic cause of kidney failure that is induced in the large majority by mutations in either PKD1 (~78%) or PKD2 (~15%), encoding for the proteins polycystin 1 (PC1) and polycystin 2 (PC2), respectively. Rarely, mutations in other genes, such as GANAB, DNAJB11, and ALG9, are causal. The underlying genetic defect initiates an incessant process of formation and growth of fluid-
filled cysts leading to the loss of functional kidney tissue. The rate of progression is highly variable, ranging from embryonic lethal to mild late adult phenotypes, but 70% of the patients reach kidney failure at a mean age of 58 years. However, because the genetic defect is present from conception and the poly cysts are expressed in the embryonic kidney, the disease process likely starts prenatally.

The sole specific treatment that is currently available, the vasopressin antagonist tolvaptan, has been validated in adults with rapidly progressive disease. Targeting factors essential for disease progression before significant morphologic damage to the kidneys has occurred could be an alternative strategy to preserve renal function long-term. For such an approach, 2 preconditions need to be fulfilled. First, preferentially noninvasive markers are needed to accurately predict the risk for end-stage kidney disease early in the disease course in order to identify high-risk patients who would benefit from such an intervention. The currently available markers have been studied in adult ADPKD patients with advanced disease stages. Whether they are affected by processes common to chronic kidney disease or by more ADPKD-specific processes remains uncertain. Second, better knowledge on the events that are driving cystogenesis and early disease progression is needed. Those events can only be detected in early-stage patients.

Copeptin, EGF, and MCP-1 remain unstudied in early ADPKD. Plasma copeptin, a surrogate for vasopressin, is associated with disease severity and predicts outcome in adults with ADPKD.

Urinary epidermal growth factor (uEGF), a marker of progression in both adults and children with generic chronic kidney disease, is decreased in adults with ADPKD compared with controls. uEGF is a marker of functional tubular cell mass and might be a sensitive indicator of kidney damage in ADPKD.

Finally, MCP-1 (also known as chemokine [C-C motif] ligand 2) is a chemotactic factor for circulating monocytes and a proinflammatory activator of macrophages. In adults with ADPKD, urinary monocyte chemoattractant protein-1 (uMCP-1) is increased, and uMCP-1 is associated with disease severity and progression. After the knockout of PC1 in tubuli, MCP-1 is upregulated, suggesting an important contribution for the renal epithelium. Moreover, renal macrophages are increased in animal models of ADPKD and in adults with advanced disease. They are associated with disease severity and stimulate cyst growth.

To investigate their potential as an early marker of severity or a disease-specific driver of progression, we studied plasma copeptin, uEGF, and uMCP-1 in a well-characterized and genotyped pediatric ADPKD cohort. Based on these findings, MCP-1 was further explored in human cell models and fetal kidneys.

**METHODS**

**Study Population**

Between June and October 2017, all consenting genotyped ADPKD patients attending the outpatient pediatric ADPKD clinic of the University Hospital of Leuven and age-, sex-, and BMI-matched healthy controls were included. Healthy controls were defined as children without a medical history and were recruited after electronic advertisements at University Hospital Leuven and KU Leuven University. Potential participants were selected based on age, sex, and BMI to be able to match them with our study population. At inclusion and after signing consent forms with their parents, they underwent a short clinical review and physical examination before blood and urine samples were provided. Serum and urine were collected and stored at −80 °C. Before the sampling, diet and water intake were ad libitum in all subjects. Clinical data were collected from the electronic medical files. The eGFR was computed using the Schwartz-Haycock formula. Proteinuria was measured on a urine spot and quantified in g/g creatinine. Proteinuria was defined as persistent protein/creatinine > 0.5 and > 0.2 for children younger than 2 years and older than 2 years, respectively. In ADPKD patients, total kidney volume was measured by 3-dimensional ultrasound and adjusted by height-corrected total kidney volume (htTKV), and the cyst score was determined as previously described. The ADPKD cohort was divided according to a tentative prognostic phenotype into 2 groups: those with very early onset (VEO) disease (defined as a radiologic diagnosis in utero or before the age of 18 months) or early symptomatic (defined as hypertension or early urologic event [gross hematuria, flank pain, and/or urinary tract infections]) before 18 years and those with asymptomatic disease. Informed consent was obtained from all participants, and all study procedures were approved by the ethical committee of UZ Leuven (S59500 and S51837).

**Immunooassays**

EGF and MCP-1 were measured using commercial immunoassays (Human Quantikine DEG00 and SCP00, respectively; R&D Systems, Minneapolis, MN) according to the manufacturer’s instructions. uMCP-1 and uEGF were referenced to the urine creatinine concentration. Copeptin was measured on EDTA plasma using an automated immunofluorescence assay (Copeptin-proAVP KRYPTOR; BRAHMS GmbH, Hennigsdorf, Germany).
Cell Lines and Fetal Kidney Tissue

Mouse inner medullary collecting duct cells IMCD-3 (ATCC CRL–2123; ATCC, Manassas, VA) were grown in DMEM:F-12 (Biowest, Riverside, MO) supplemented with 10% fetal bovine serum (FBS; Westburg, Leusden, Netherlands) and 1% Pen/Strep (Westburg). PC2 knockout, described previously, and PC1 knockout IMCD3s generated in a similar fashion were a kind gift from Drs. Y Cai and K Dong (Yale University, New Haven, CT).

Proximal tubular epithelial cells (PTECs) were obtained from the urine of young genotyped PKD1 ADPKD patients or healthy individuals. Cell lines were immortalized and subcloned as previously described.

In short, primary PTECs were conditionally immortalized with a retroviral construct containing SV40_LT_tsA58. It expresses a temperature-sensitive SV40 large T (LT) antigen caused by a point mutation in SV40 LT, which makes the SV40 LT unstable at 37°C. Hence, conditionally immortalized proximal tubular epithelial cells (ciPTECs) are incubated at 33°C to allow expansion and subcloning to obtain monoclonal cell lines. For experiments, monoclonal ciPTECs are incubated for 10 days at 37°C, which triggers SV40 LT breakdown, halts proliferation, and allows cells to differentiate (Supplementary Figure S1). The presence of the heterozygous germline mutation was confirmed by long-range quantitative polymerase chain reaction (Supplementary Table S1). Kidney tissue from 2 genetically confirmed fetal ADPKD cases and 2 age-matched controls without intrauterine growth restriction and without renal pathology who underwent termination of pregnancy was obtained from UZ Leuven Biobank.

MCP-1 Stimulation

FBS induces MCP-1 expression and secretion, and MCP-1 protein levels were assessed at various time points in either full medium (FM) or FM without FBS (serum-free medium [SFM]) from ciPTECs and IMCD3 cell lines, similar to previously described. Cells were seeded and grown in 6 wells, after which 10 days of differentiation was allowed in case of ciPTECs. Confluence was between 60% and 80% for all cell lines. Next, after an incubation period of 24 hours in SFM, all medium (2 ml) was changed. After 8 and 16 hours (ciPTECs) or 24 hours (IMCD3) in SFM or FM, cell medium for MCP-1 measurements was snap frozen in liquid. FBS and medium from the same lot were used for all experiments. Data are expressed as the ratio of MCP-1 in FM versus the value in the SFM for the same duration.

Immunohistochemistry

Kidneys were fixed with 4% paraformaldehyde (pH = 7.4), embedded in paraffin, and stained with rabbit anti–MCP-1 (ab9669; Abcam, Cambridge, UK; 1:250 dilution) and anti–mannose receptor antibody (ab64693, Abcam; 1:1000 dilution) after antigen retrieval with citrate pH at 90°C for 60 minutes (MCP-1) or 95°C for 30 minutes (mannose receptor); biotinylated swine antirabbit secondary antibodies (Agilent, Santa Clara, CA; 1:400) and the DAB Substrate Kit (Vector Laboratories, Burlingame, CA) were used. Kidney sections were counterstained by hematoxylin. Images were created with the Axio Scan.Z1 (Zeiss, Oberkochen, Germany). The expression levels of MCP-1 were graded by 2 independent observers.

Statistics

When descriptive statistics were used, data are presented as mean ± SD or as median (range) if not normally distributed. Wilcoxon-signed rank tests, McNemar tests, and their extension for larger tables were used to compare cases and controls. Plasma copeptin, uEGF, and uMCP-1 values were compared between cases and controls using a linear model with a random effect to handle the correlation within each pair. The residual variability was allowed to differ between cases and controls. The resulting model is a linear mixed model. All values were log transformed to obtain a more symmetric distribution of the (marginal) model residuals. For these variables, model means on the log scale were back transformed to the original scale, yielding geometric means (and 95% confidence intervals [CIs]).

The ratios of MCP-1 in FM versus the value in SFM were calculated. An unpaired t test for unequal variances on the log-transformed ratios was used to compare unstimulated (SFM) and the serum-dependent release (FM) between both groups. This was done separately for the values after 8 and 16 hours. Log-transformed ratios of 3 IMCD3 cell lines were compared with a repeated measurement 1-way analysis of variance using the Geisser-Greenhouse correction. All analyses were performed using SAS software (Version 9.4; SAS Institute, Cary, NC).

RESULTS

Study Population

The characteristics of 53 patients and 53 controls are summarized in Table 1. Participants were on average 10.5 years old. Baseline characteristics including age, sex, BMI, and eGFR were not statistically different between groups. Urine osmolality (570.2 vs. 757.9 mmol/kg, \( P = 0.003 \)), plasma osmolality (285.1 vs. 285.1 mmol/kg, \( P = 0.003 \)), plasma creatinine (2.2 vs. 2.3 mmol/L, \( P = 0.003 \)), and serum sodium (136.8 vs. 136.8 mmol/L, \( P = 0.003 \)) were significantly different between groups. The characteristics of 53 patients and 53 controls are summarized in Table 1. Participants were on average 10.5 years old. Baseline characteristics including age, sex, BMI, and eGFR were not statistically different between groups. Urine osmolality (570.2 vs. 757.9 mmol/kg, \( P = 0.003 \)), plasma osmolality (285.1 vs. 285.1 mmol/kg, \( P = 0.003 \)), plasma creatinine (2.2 vs. 2.3 mmol/L, \( P = 0.003 \)), and serum sodium (136.8 vs. 136.8 mmol/L, \( P = 0.003 \)) were significantly different between groups.
significantly lower uMCP-1 compared with controls (82.6 vs. 135.2; 0.009; Figure 1c). The difference remained after correction for age, sex, and BMI (Figure 1b). Those with VEO or symptomatic disease had significantly higher median uMCP-1 (interquartile range) compared with asymptomatic patients (265.2 [272.4] vs. 144.5 [129.8]; P = 0.009; Figure 1c). The difference remained after correction for age, sex, and BMI (264.5 [95% CI, 202.6–345.2] vs. 161.1 [95% CI, 112.3–231.2; P = 0.035]).

**MCP-1 Secretion in IMCD3 and ciPTECs**

MCP-1 was studied in wild-type IMCD3 cells and in isogenic PC1 knockout and PC2 knockout cells. Reduced polycystin protein expression was validated by Western blot (Figure 2a). The median (interquartile range) ratios of MCP-1 concentration after 24 hours in

### Table 2. Genetics

| Genetic analysis (N = 53) | PKD1 | Truncating | Nontruncating | PKD2 | Ganab |
|---------------------------|------|------------|---------------|------|-------|
| PKD1                      | 49 (92%) | 37 (70%) | 12 (23%) | 3 (6%) | 1 (2%) |

287.9 mmol/kg, P = 0.008), and plasma sodium (139.4 vs. 140.1 mmol/l, P = 0.040) were significantly lower in the patient group. The fractional excretion of sodium was 0.57% in patients and 0.45% in controls (P = 0.073). The mean htTKV was 186.5 ml/min in ADPKD patients, and the median cyst score was 2.0. All patients were genotyped, and the majority had a PKD1 truncating mutation (Table 2). Twenty (38%) patients had asymptomatic disease, whereas 33 (62%) had VEO and/or early symptomatic disease. In the asymptomatic group, 13 (65%) had truncating PKD1, 4 (20%) nontruncating PKD1, and 3 (15%) PKD2 and a GANAB mutation. In the VEO and symptomatic patients (n = 33), 24 (73%) had truncating PKD1, 8 (24%) nontruncating PKD1, and 1 (3%) a PKD2 mutation.

### EGF, Copeptin, and MCP-1

Although uEGF and plasma copeptin were not statistically different in patients and controls, the median (interquartile range) uMCP-1 (pg/mg) was significantly higher in patients (185.4 [213.8]) compared with controls (154.7 [98.0]; P = 0.010; Table 3). A subanalysis revealed that patients with a PKD1 mutation had a significantly higher uMCP-1 compared with controls (192.2 [204.9] vs. 159.7 [104.6]; P = 0.004; Figure 1a). In contrast, subjects with a PKD2 mutation had a significantly lower uMCP-1 compared with controls (82.6 [38.8] vs. 135.2 [29.9]; P = 0.02). In both patients and controls, a negative correlation of uMCP-1 with age and a positive correlation with eGFR was observed. No significant correlations were found with BMI or urine osmolality and uMCP-1. There was no significant correlation with htTKV or cyst score and uMCP-1 in patients (Supplementary Table S2).

When uMCP-1 was analyzed in the ADPKD cohort, no differences were observed between subjects with truncating and nontruncating PKD1 mutations, whereas uMCP-1 was significantly higher in patients with PKD1 mutations compared with those with PKD2 and/or GANAB mutations. This difference remained after correction for age, sex, and BMI (Figure 1b). Those with VEO or symptomatic disease had significantly higher median uMCP-1 (interquartile range) compared with asymptomatic patients (265.2 [272.4] vs. 144.5 [129.8]; P = 0.009; Figure 1c). The difference remained after correction for age, sex, and BMI (264.5 [95% CI, 202.6–345.2] vs. 161.1 [95% CI, 112.3–231.2; P = 0.035]).

### MCP-1 Secretion in IMCD3 and ciPTECs

MCP-1 was studied in wild-type IMCD3 cells and in isogenic PC1 knockout and PC2 knockout cells. Reduced polycystin protein expression was validated by Western blot (Figure 2a). The median (interquartile range) ratios of MCP-1 concentration after 24 hours in

| Variable | ADPKD (N = 53) | Controls (N = 53) | P value |
|----------|----------------|-------------------|---------|
| n (%)    | Mean (SD)      | Median (IQR)      |         |
| Age (years) | 10.4 (5.9)    | 11.4 (8.8)        | 0.543   |
| Male/female | 25/28 (47.2) | 25/28 (47.2) |         |
| BMI SDS  | -0.1 (1.3)     | 0.1 (1.9)         | 0.471   |
| Tanner score | 2.3 (1.7)     | 1.0 (3.0)         | 0.850   |
| SBP SDS  | 0.1 (1.0)      | 0.0 (1.0)         | 0.484   |
| DBP SDS  | 0.1 (1.0)      | 0.1 (1.3)         | 0.475   |
| Antihypertensive medication | 2/53 (3.8) | 0/53 (0) | 0.285   |
| Creatinine (mg/dl) | 0.6 (0.2)     | 0.5 (0.3)         | 0.294   |
| eGFR (ml/min per 1.73 m²) | 122.7 (40.5) | 120.9 (22.9) | 0.177   |
| Plasma osmolality (mmol/l) | 285.1 (4.5) | 284.0 (7.0) | 0.008   |
| Plasma sodium (mmol/l) | 139.4 (1.4)   | 139.6 (1.7) | 0.040   |
| Urine osmolality (mmol/kg) | 570.2 (336.1) | 539.0 (638.0) | 0.003   |
| Urine sodium (mmol/l) | 102.4 (71.2) | 83.3 (127.7) | 0.239   |
| Fractional excretion sodium (%) | 0.57 (0.3) | 0.49 (0.35) | 0.073   |
| Proteinuria | 0/53 (0)      | 0/53 (0)         | NA      |
| htTKV (ml/m) | 166.5 (98.7) | 161.6 (104.1) | NA      |
| Cyst score | 2.3 (2.1)     | 2.0 (3.0)         | NA      |
Table 3. Biomarkers

| Variable       | ADPKD | Control | Mean (95% CI) | Ratio (95% CI) | P value |
|----------------|-------|---------|---------------|----------------|---------|
| uEGF (ng/mg)   | 43.5 (40.0) | 43.7 (35.0–54.6) | 1.0 (0.9–1.2) | 0.714   |
| Serum copeptin (pmol/l) | 5.2 (4.3) | 5.7 (5.0–6.6) |       |         |
| uMCP-1 (pg/mg) | 185.4 (213.8) | 217.8 (176.5–268.8) | 1.4 (1.1–1.7) | 0.010   |

ADPKD, autosomal dominant polycystic kidney disease; CI, confidence interval; IQR, interquartile range; uEGF, creatinine-corrected urinary epidermal growth factor; uMCP-1, creatinine-corrected urinary monocyte chemoattractant protein-1.

FM versus 24 hours in SFM, reflecting the serum-dependent release, were significantly higher in PC1 knockout (18.0 [37.0], P = 0.049) and lower in PC2 knockout cells (2.2 [1.0], P ≤ .0001) compared with wild-type IMCD3 cells (11.4 [6.4], Figure 2b). Similar experiments were performed in ciPTECs from 3 young ADPKD patients with PKD1 truncating mutations and 3 young healthy controls. The ratios of MCP-1 concentration in FM versus SFM after 8 and 16 hours of incubation reflect the serum-dependent release. After 8 hours of incubation, the ratio (95% CI) of MCP-1 in FM/SFM was 3.1 (95% CI, 1.4–6.8) in control and 7.2 (95% CI, 1.3–38.9) in patient cell lines (P = 0.151); after 16 hours, the ratio was 3.5 (95% CI, 2.5–5.0) versus 10.8 (95% CI, 3.2–36.9; P = 0.0495; Figure 2c and d).

MCP-1 and Macrophages in VEO ADPKD

IHC was performed on kidney tissue in 2 cases of biallelic fetal PKD1 ADPKD. Termination of pregnancy was performed at 30 weeks 5 days’ and 27.5 weeks’ gestational age, respectively. The first case was caused by a truncating mutation c.5627C>G (p.Ser1876*) and a c.1010>G, p.(Ile3367Met) variant of unknown significance in PKD1 inherited from the mother and a missense variant c.9499A>T, and p.(Ile3167Phe) in PKD1 inherited from the father. The second case carried a mutation c.9829C>T p.(Arg3277Cys) in PKD1, a hypomorph mutation that is associated with the ADPKD phenotype when homozygous,30 inherited from the mother and a mutation c.12706T>G (p.(Tyr4236Asp) in PKD1 from the father. Mutations in other ciliopathy genes, including PKDHD1 and HNF1β, were excluded in both. Autopsy revealed bilateral enlarged kidneys without macroscopically visible cysts (Supplementary Figure S2). As controls, renal tissue from fetuses of 31 weeks 0 days’ and 27 weeks 0 days’ gestational age without intrauterine growth restriction and without renal pathology was obtained.

Immunohistochemistry images (Figure 3.1 and 3.2) showed nephrogenic zones with ongoing

Figure 1. Urinary monocyte chemoattractant protein-1 (uMCP-1) levels according to genotype and phenotype. Genotype, phenotype, and monocyte chemoattractant protein-1. (a) uMCP-1 in young autosomal dominant polycystic kidney disease (ADPKD) patients and controls, indicating that urinary monocyte chemoattractant protein-1 (uMCP-1) is elevated in patients with PKD1 mutations and decreased in patients with PKD2 mutations compared with matched healthy controls. The ratio (95% confidence interval [CI]) of controls versus PKD1 was 1.4 (95% CI, 1.1–1.8) and controls versus PKD2 was 1.9 (95% CI, 1.2–3.1). (b) In the patient group, patients with PKD1 truncating and PKD1 nontruncating mutations have similar uMCP-1, whereas patients with PKD2 and GANAB mutations have a lower uMCP-1. The ratios (95% CI) of PKD1 T versus PKD1 NT was 0.9 (95% CI, 0.6–1.6), PKD1 T versus PKD2 and Ganab was 2.9 (95% CI, 1.5–5.8), and PKD1 NT versus PKD2 and Ganab was 3.1 (95% CI, 1.4–6.5). (c) ADPKD patients with very early onset (VEO) or early symptomatic disease have a higher uMCP-1 compared with asymptomatic subjects. The ratio (95% CI) was 0.61 (95% CI, 0.4–1.0). aS, asymptomatic; NT, non truncating; S, early symptomatic; T, truncating. The P values are after correction for age, sex, and body mass index in b and c.
nephrogenesis in all kidneys and multiple dilated tubuli and cysts predominantly in the medulla of the ADPKD kidneys. MCP-1 immunoreactivity was present in glomeruli (graded 0–1+) and more pronounced (2+–3+) in both proximal and distal tubuli of the normal kidney as well as in dilated tubuli and cysts walls (3+) in case of ADPKD. This was associated with interstitial M2 macrophage infiltration in the ADPKD cases but not in the controls. In the ADPKD cases, multiple mannose receptor–positive cells were found in the medullary region, including in the walls of and adjacent to dilated tubuli and cysts.

**DISCUSSION**

The current study demonstrates in a pediatric ADPKD cohort that uMCP-1 secretion increases before kidney function declines and before significant cystic deformation occurs and that it correlates with both genotype and phenotype. Our translational data in cell models are in line with the findings in the patient cohort and corroborate with the recent findings from a mouse model that show the key role of the tubular epithelium. The finding of an increased level of MCP-1 excretion in case of PKD1 mutations is consistent in murine and human cells, in proximal tubule and collecting duct models, and in cases of heterozygous or homozygous loss of PC1. In addition, although fetal ADPKD situates at the extreme of the ADPKD spectrum, we could demonstrate for the first time that tubular structures and cyst walls are the primary source of immunoreactivity to MCP-1 antibodies in fetal kidneys and that macrophage infiltration can be observed already in utero.

For adult ADPKD patients, several prognostic factors have been identified, which are often also markers of disease severity (htTKV and GFR), but the prediction of end-stage kidney disease in the individual adult ADPKD patient remains imprecise. We evaluated a selection of noninvasive markers in a cross-sectional pediatric ADPKD cohort to investigate their potential as early markers of disease progression. First, uEGF, a marker of functional tubular cell mass, was not statistically different from controls, further corroborating
Figure 3. Fetal kidney tissue of autosomal dominant polycystic kidney disease (ADPKD) and controls at (3-1) 31 weeks’ gestational age and (3-2) 27 weeks’ gestational age. (a–c) Hematoxylin and eosin (H&E) staining of ADPKD fetal kidney. (a) Overview. (b) Cortex. (c) Medulla. The majority of cysts (*) are medullary. The nephrogenic zone with ongoing nephron formation (arrows). (d–f) H&E staining of normal fetal kidney. (d) Overview. (e) Cortex. (f) Medulla. The nephrogenic zone with ongoing nephron formation (arrows). (g–i) Monocyte chemoattractant protein-1 (MCP-1) staining of ADPKD fetal kidney. (g) Overview. (h) Cortex. (i) Medulla. Immunoreactivity (brown) in glomeruli graded 0 to 1+ (arrows), nondilated tubuli (2+ to 3+, crosses), and cyst lining cells (2 to 3+, *). (j–l) MCP-1 staining of a normal fetal kidney. (j) Overview. (Continued)
Figure 3. (Continued) (k) Cortex. (l) Medulla. Immunoreactivity (brown) in glomeruli (arrows) graded 0 to 1+ and more pronounced (2+–3+) in proximal (cross) and distal tubuli (*). (m–o) Mannose receptor staining of an ADPKD fetal kidney. (m) Cortex. (n and o) Medulla. Scattered positive cells (brown dots, arrows) in the interstitium around cysts signifying M2 macrophage infiltration. (p–r) Mannose receptor staining of normal fetal kidney. (p) Overview. (q and r) Medulla. There was an absence of mannose receptor immunoreactive cells.
that we observe an early stage of ADPKD with unaffected kidney function. Second, it has been demonstrated that copeptin correlates with disease severity and disease progression in adult patients. Although a lower urine osmolality was observed in patients, this was accompanied by a lower plasma osmolality, whereas plasma copeptin was not statistically different. We hypothesize that the observed alterations are due to increased water drinking that is routinely advised to patients in our ADPKD pediatric clinic. Finally, although others have similarly reported an increase in uMCP-1 in adult cohorts, our findings show a rise in uMCP-1 before significant destruction and fibrosis have arisen. In addition, uMCP-1 secretion correlated with early genotype (PKD1 mutation) and phenotype (early hypertension, early urologic events, and VEO) characteristics that can be reliably assessed during childhood and that have been associated with a worse prognosis. Of note, although all patients underwent routine prenatal ultrasound, it cannot be formally excluded that subjects with a family history were more intensively screened. In contrast, we observed a lower uMCP-1 in patients with PKD2 mutations. Given the small number (n = 3), this information should be considered with caution.

Interestingly, we observed the same trend in IMCD3 cells that exhibited less MCP-1 secretion in case of loss of PC2 compared with wild-type cells. Few data on the interaction between PC2 and MCP-1 have been published. Our finding of decreased MCP-1 secretion in cases of loss of PC2 is in line with the observation by Flores et al that shear stress–induced MCP-1 mRNA expression was impaired in PC2-deficient IMCD3 cells. We did not find a correlation between uMCP-1 and eGFR and htTKV in children, although a correlation has been described in adults. This can be explained by the fact that eGFR is still in the normal range, and TKV is measured by ultrasound in a small cohort with still growing kidneys. Of note, circulating MCP-1 levels were not evaluated. However, urinary MCP-1 levels are considered a reflection of local renal production independent of circulating MCP-1. The current findings present a strong rationale to study uMCP-1 as an early prognostic marker that could help identify rapid progressors at a young age in larger longitudinal ADPKD cohorts.

Human ciPTECs with loss of heterozygosity of PC1 were more prone to MCP-1 secretion than ciPTECs from healthy controls, indicating that the human tubular epithelium is a source of MCP-1, potentially already at the stage of cyst initiation. Several potential mechanisms by which the polycystins could regulate the expression of MCP-1 have been reported. The fact that we observed no statistical difference in uMCP-1 between patients with truncating and nontruncating PKD1 mutations could imply a mechanism that can be affected by both type of mutations (e.g., G protein signaling). Its function remains unknown, but PC1 is a transmembrane protein that resembles the adhesion G protein–coupled receptor family. The C-terminal tail of PC1 is capable of recruiting G protein α and βγ subunits that sequester free βγ subunits. When the G protein–binding function is lost, the pool of free βγ subunits increases, resulting in hyperactivity of G protein α- and βγ-dependent signaling. It has been proposed that the imbalance in G protein signaling occurs when PC1 is in close proximity to other G protein–coupled receptors. The loss of PC1 function could then increase the available G proteins, allowing other G protein–coupled receptors to recruit them and become overactivated. Thus, the loss of PC1 function could both directly affect MCP-1 expression or indirectly potentiate physiological inducers of MCP-1 secretion. Indeed, it has been shown that the C-terminal tail of PC1 can modulate the activity of transcription factors such as activator protein 1 and nuclear factor kappa B, which are important for both the constitutive and induced expression of the MCP-1 promoter. One could postulate that a loss of inhibition in PC1-deficient tubular cells leads to an increased expression and secretion of MCP-1. Alternatively, signaling of an unknown physiological stimulus for MCP-1 could be potentiated in patients with PKD1 mutations. We observed an FBS-dependent increase in MCP-1 secretion in human PTEC cells. FBS is the standard supplement of cell culture media to promote cell growth and differentiation. It contains a mix of growth factors, hormones, transport proteins, cofactors, and essential minerals and trace elements, many of them unknown. MCP-1 release can be enhanced by various physiological inducers such as trypsin, thrombin, tumor necrosis factor-α, transforming growth factor-β, interferon-γ, and interleukin-1β, some of them via G protein–coupled receptor signaling. It remains unknown which physiological factors are key regulators of MCP-1 secretion by the kidney epithelium and what their relation is with polycystins.

Other explanations for increased MCP-1 secretion could be given consideration. So-called third-hit triggers, such as ischemia reperfusion and nephotoxicity, can stimulate cyst formation. Hyperfiltration is a feature of early PKD, and proteinuria is a common finding in more advanced ADPKD. It has been demonstrated that albumin uptake and overload of albumin endocytosis in the proximal tubules lead to interstitial inflammation and infiltration of inflammatory cells via upregulation of proinflammatory...
cytokines such as MCP-1. Increased albumin exposure of proximal tubular cells in ADPKD might be an additional (third-hit) driver for MCP-1 production in tubular cells in more advanced disease stages, as could be tubular crystal deposition. None of these potential mechanisms are mutually exclusive.

A body of evidence confirms that an increase in MCP-1 is associated with increased chronic M2 macrophage infiltration with detrimental effects. In addition, MCP-1, through unknown receptors, has a direct inflammatory effect on renal epithelial cells, and an intrinsic increase in MCP-1 secretion due to the loss of polycystin in the tubular epithelium could thus initiate a vicious circle and potentially play a role in cyst initiation. Although macrophage infiltration has been described in renal tissue from adult patients with advanced disease, we demonstrate that macrophage infiltration can be a feature early in the disease process.

The present findings add to a growing body of evidence that suggest that MCP-1 is a potential therapeutic target in ADPKD. In a mouse model, double knockout of both \textit{PKD1} and \textit{MCP1} resulted in attenuation of the disease phenotype. In addition, treatment with a CCR2 antagonist was protective. In human ADPKD, no trials targeting MCP-1 signaling have yet been undertaken. The present study provides a rationale for clinical trials targeting MCP-1 in early ADPKD.

The limitations of this study include the single-center and the cross-sectional nature. The sample size is relatively small, and in case of the absence of statistically significant differences, a lack of power cannot be excluded. In addition, the CI contains small differences in uMCP-1, and additional larger studies are needed to confirm a potential clinical utility. Moreover, the number of the kidney specimens is also small. However, these are very rare cases.

The strengths of this study include the fact that we present, to our knowledge to this date, the largest published cohort of early-stage genotyped and thoroughly characterized patients. Furthermore, the cohort wasstringently age and sex matched with healthy subjects. In addition, the clinical findings were reproduced in 2 cell models, including a human cell model from early disease stage. These findings can lead to novel mechanistic insights on early ADPKD.

In conclusion, the present study demonstrates that an increase in uMCP-1, intrinsically linked to \textit{PKD1} mutations in the tubular epithelium, is an early event in human ADPKD independent from tissue destruction. Future studies on its value as an early prognostic biomarker and as a treatment target are warranted.

**DISCLOSURE**

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**SUPPLEMENTARY MATERIAL**

Supplementary File (PDF)

Figure S1. (A) Left and (B) right kidney of ADPKD fetus case number 1.

Figure S2. Characterization of human PTEC cells.

Table S1. Genotype of human PTECs.

Table S2. Correlations of uMCP-1.

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