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Research article

# Clinical implications of proximal tubular multicilia in glomerular diseases

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ABSTRACT

*Introduction:* Multiciliated cells (MCCs) have been identified in the proximal tubules of patients with kidney disease; however, their clinical significance is unknown. We aimed to investigate whether MCCs are associated with clinical outcomes in patients with glomerular diseases.
*Methods:* Between August 2012 and April 2021, 134 patients (including 126 with glomerular disease patients and 8 controls) who were hospitalized at Seoul National University Boramae Medical Center and Seoul National University Hospital were included in this study. The ratio of MCCs to total proximal tubular cells was calculated using immunohistochemistry. The relationship between the MCC ratio and kidney disease-related clinical features was then analyzed.
*Results:* MCCs were exclusively detected in patients with glomerular diseases (68.3 %), not those in the control group. Patients with diabetic kidney disease (88.9 %) or antineutrophil cytoplasmic antibody (ANCA)-associated glomerulonephritis (GN, 86.4 %) had higher MCC ratios. MCC-positivity and MCC ratios were significantly associated with increased age and proteinuria, and a decreased estimated glomerular filtration rate (eGFR). Patients with higher MCC ratios had a significantly higher risk of end-stage kidney disease (ESKD) and composite outcomes with death. In multivariable analysis, MCC ratios were significantly correlated with an increased risk of ESKD (hazard ratio [HR], 1.413; 95 % confidence interval [CI], 1.012–1.972) and composite outcome (HR, 1.401; 95 % CI, 1.028–1.909).
*Conclusion:* Higher MCC ratios were correlated with poorer prognosis; therefore, quantification of MCCs in the proximal tubules can serve as a valuable prognostic marker in clinical practice.

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## 1. Introduction

Glomerular diseases encompass a diverse spectrum of diseases, including primary glomerulonephritis (GN) [1,2] and secondary forms such as diabetic kidney disease (DKD), the most common cause of chronic kidney disease (CKD) worldwide [3,4]. These glomerular diseases exhibit a broad spectrum of clinical courses, ranging from rapidly progressive to mild and self-limiting to chronic and irreversible presentations. Nevertheless, owing to the overlapping clinical features between different disease groups and the diverse outcomes within the same disease group, predicting prognosis and choosing therapeutic options for glomerular disease often remain challenging even after diagnosis through biopsy [5]. However, the mechanisms underlying the development and progression of heterogeneous glomerular diseases remain unclear. Therefore, identifying appropriate prognostic indicators and novel therapeutic targets is crucial.

The cilium is an antenna-like organelle that protrudes from the plasma membrane and is composed of microtubules, which are nucleated from the basal body. Cilia are classified as either primary or multicilia, depending on the number of cilia present in the cell [6]. Primary cilia, which represent a single cilium per cell, are found in almost all cells, including renal epithelial cells. They sense mechanical and chemical stimuli from the lumen [7]. Multicilia or motile cilia, which refer to more than two cilia in a cell, are located in specific organs such as the bronchus, reproductive tracts, nasal cavity, and brain ventricles. They play crucial roles in controlling the movement of fluid or mucus [8].

Interestingly, multiciliated cells (MCCs) have been identified within the proximal tubules of some patients with kidney diseases [9–13]. However, the causes and functions of kidney MCCs remain unclear. Analyzing their association with clinical prognosis in glomerular diseases may provide potential insights into the pathophysiology of MCCs, which could facilitate their utilization as prognostic markers and targets for therapeutic intervention.

This study aimed to investigate the prevalence of proximal tubular MCCs in patients who had undergone biopsies for various glomerular diseases and to determine the association between MCC ratios and clinical outcomes across various disease types.

## 2. Methods

### 2.1. Study participants and specimens

Sample size calculation was performed using G*POWER statistical software (https://stats.oarc.ucla.edu/other/gpower/) to determine the minimum number of participants required to detect differences in characteristics between MCC-positive and MCC-negative groups. Based on an independent *t*-test analysis with the following parameters: medium effect size ($d = 0.50$), significance level ($\alpha = 0.05$), statistical power ($1\text{-}\beta = 0.80$), and two-tailed hypothesis testing, the minimum required sample size was estimated to be 128 participants.

Participants were selected from patients with glomerular disease who were hospitalized at Seoul National University Boramae Medical Center and Seoul National University Hospital and had undergone a kidney biopsy between August 2012 and April 2021. Kidney biopsies were performed based on the clinical decision to differentiate the pathological etiologies in patients with unexplained proteinuria, hematuria, or progressive renal dysfunction. Among them, a total of 602 participants (aged ≥17) voluntarily agreed to prospective enrollment of their clinical data and biospecimens including serum urine, and kidney tissues (Supplementary Table S1). To achieve the minimum required sample size of 128 participants, we aimed to recruit 20–30 subjects from each major glomerular disease category. Subject selection was conducted by chronologically screening patients from the study initiation date, with enrollment contingent upon the availability of both complete clinical data and adequate tissue specimens. All eligible cases meeting these criteria were consecutively included without exclusion. Patients with pathologically confirmed immunoglobulin A nephropathy (IgAN, N = 37), DKD (N = 27), minimal change disease (MCD, N = 22), primary focal segmental glomerulosclerosis (primary FSGS, N = 18), and antineutrophil cytoplasmic antibody-associated glomerulonephritis (ANCA-associated GN, N = 22) were finally included in the study group. Additionally, patients with kidney biopsies showing no remarkable pathology findings, with an estimated glomerular filtration rate (eGFR) ≥ 60 mL/min/1.73 $m^2$ and urine protein-to-creatinine ratio (uPCR) < 1.0 g/g, were assigned to the control group (N = 8). Therefore, 134 patients were included in the final analysis. Biopsy specimens and blood and urine samples were collected at the time of the kidney biopsy, and informed consent was obtained. The biopsy specimens were preserved in formalin-fixed paraffin-embedded blocks (FFPE).

### 2.2. Ethical considerations

The study was conducted in accordance with the principles of the Declaration of Helsinki and was approved by the Institutional Review Boards of the participating hospitals (Seoul National University Boramae Medical Center: 20-2022-102, Seoul National University Hospital: H-2304-030-1420), which included a prospective cohort of patients with kidney disease. The prospective cohort study was also approved by each Institutional Review Board (Seoul National University Boramae Medical Center: 06-2011-50 and 20-2019-48, Seoul National University Hospital: 1404–117-575). All participants gave written informed consent prior to kidney biopsy and study enrollment. The legal guardian of 17- and 18-year-old participants was also notified about the study and provided written informed consent simultaneously with the subject. All clinical characteristics and biospecimens were prospectively collected with the approval of the participants, and informed consent was obtained.

### 2.3. Clinical data collection and outcome assessment

Baseline clinical data, including sex, age, diagnosis, serum creatinine level, uPCR, and comorbidities such as diabetes and hypertension, were collected through a chart review of electronic medical records. The eGFR was calculated using the 2021 CKD-EPI Creatinine equation [14]. Outcomes were evaluated based on medical records, which included the initiation of renal replacement therapy (RRT), progression to end-stage kidney disease (ESKD), death, a 50 % decline in eGFR from baseline, and eGFR slope. Composite outcomes were defined as a combination of progression to ESKD and death. The eGFR decline and slope were calculated using serum creatinine levels at the last visit or immediately before the initiation of RRT. The eGFR changes were not evaluated in patients in whom dialysis was started within 3 days before or after the biopsy or those with a follow-up period of less than 50 days.

### 2.4. Immunohistochemistry (IHC)

To remove paraffin, FFPE slides were incubated at 60 °C for 30 min, followed by immersion in xylene for 7 min, repeated three times. The slides were then rehydrated using a series of diluted ethanol (100 %–70 %). For antigen retrieval, the slides were immersed in preboiled 10 mM sodium citrate buffer (pH 6) and subjected to boiling in a microwave for 15 min. After cooling, they were washed with phosphate buffered saline (PBS) twice and then incubated in a blocking buffer (1 % bovine serum albumin and 0.2 % Triton X-100 in PBS) for 30 min. The slides were then incubated with primary antibodies (acetylated alpha-tubulin, AcTub, T6793, Sigma-Aldrich, St. Louis, MO, USA; low-density lipoprotein receptor-related protein 2, LRP2, ab76969, Abcam, Cambridge, UK) in a humid chamber at 4 °C overnight. After washing with PBS, they were incubated with secondary antibodies (anti-mouse IgG conjugated with Alexa Fluor™ 594, A21203, Invitrogen, Waltham, MA, USA; anti-rabbit IgG conjugated with Alexa Fluor™ 488, A21206, Invitrogen) at 20 °C for 2 h. Unbound antibodies were removed by washing with PBS, and the nuclei were stained with 4′,6-diamidino-2-phenylindole (DAPI; D9542, Sigma-Aldrich). Anti-fade mounting medium (S3023; Dako, Glostrup, Denmark) was used when the slides were covered with coverslips. Representative images of the MCCs were acquired using a confocal microscope (LSM980; Zeiss, Oberkochen, Germany) equipped with a 63 × oil-immersion lens.

### 2.5. Calculation of MCC ratio

To count the total number of proximal tubular epithelial cells (PTECs), whole tissue images acquired by a slide scanner (Axioscan 7, Zeiss) were imported into Fiji open source image analysis software (NIH, Bethesda, MD, USA). Subsequently, the cell numbers were automatically counted as previously described [15]. The number of MCCs was manually counted by a researcher blinded to the

**Table 1**
Baseline characteristics based on the presence of MCC.

| | Total | | MCC(+) | | MCC(−) | | Missing | p-value |
|---|---|---|---|---|---|---|---|---|
| **No. of participants, n(%)** | 134 | | 86 | | 48 | | | |
| **MCC ratio (%)** | 0.100 | (0.025) | 0.156 | (0.238) | – | | 0 | |
| **Age (year)** | 49.2 | (19.4) | 52.5 | (18.9) | 43.3 | (19.2) | 0 | **0.008** |
| **Sex, male, n(%)** | 85 | (63.4) | 60 | (69.8) | 25 | (52.8) | 0 | **0.042** |
| **Diagnosis, n(%)** | | | | | | | 0 | |
| **Control** | 8 | (6.0) | 0 | (0.0) | 8 | (16.7) | | |
| **IgAN** | 37 | (27.6) | 18 | (20.9) | 19 | (39.6) | | |
| **DKD** | 27 | (20.1) | 24 | (27.9) | 3 | (6.3) | | |
| **MCD** | 22 | (16.4) | 12 | (14.0) | 10 | (20.8) | | |
| **Primary FSGS** | 18 | (13.4) | 13 | (15.1) | 5 | (10.4) | | |
| **ANCA-associated GN** | 22 | (16.4) | 19 | (22.1) | 3 | (6.3) | | |
| **Underlying disease, n(%)** | | | | | | | | |
| **DM** | 48 | (35.8) | 39 | (45.3) | 9 | (18.8) | 0 | **0.002** |
| **HTN** | 54 | (40.3) | 38 | (44.2) | 16 | (33.3) | 0 | 0.219 |
| **Baseline laboratory findings** | | | | | | | | |
| **Serum Cr (mg/dL)** | 2.12 | (2.18) | 2.68 | (2.49) | 1.12 | (0.80) | 0 | **<0.001** |
| **eGFR (mL/min/1.73m$^2$)** | 66.3 | (42.0) | 53.2 | (40.45) | 89.6 | (34.19) | 0 | **<0.001** |
| **Urine PCR (g/g)** | 4.41 | (4.42) | 4.99 | (4.37) | 3.38 | (4.38) | 0 | **0.043** |
| **Outcomes** | | | | | | | | |
| **ESKD, n(%)** | 23 | (17.2) | 22 | (25.6) | 1 | (2.1) | 0 | **0.001** |
| **RRT, n(%)** | 26 | (19.4) | 25 | (29.1) | 1 | (2.1) | 0 | **<0.001** |
| **eGFR slope (mL/min/1.73m$^2$*yr)** | −4.66 | (20.19) | −5.31 | (24.18) | −3.54 | (10.49) | 23 | 0.596 |
| **50 % decline of eGFR, n(%)** | 12 | (9.0) | 12 | (15.4) | 0 | (0.0) | 9 | **0.003** |
| **Death** | 7 | (5.2) | 7 | (8.1) | 0 | (0.0) | 0 | 0.050 |

ANCA-associated GN, antineutrophil cytoplasmic antibody-associated glomerulonephritis; Cr, creatinine; eGFR, estimated glomerular filtration rate; ESKD, end-stage kidney disease; IgAN, immunoglobulin A nephropathy; DKD, diabetic kidney disease; DM, diabetes mellitus; FSGS, focal segmental glomerulosclerosis; HTN, hypertension; MCC, multiciliated cell; MCD, minimal change disease; PCR, protein-to-creatinine ratio; RRT, renal replacement therapy.
P-value was computed by independent *t*-test for continuous variables and chi-square test or Fisher's exact test for categorical variables.
Data was reported as mean (SD; standard deviation) for continuous variables and n (%) for categorical variables.

diagnosis and prognosis of the enrolled patients. The MCC ratio was calculated as follows: total number of MCCs/total number of PTECs × 100.

### 2.6. Statistical analysis

Baseline characteristics were analyzed based on the presence of MCCs, as determined by IHC. Continuous variables were compared using an independent *t*-test and presented as mean ± standard deviation (SD), and categorical variables were compared using a chi-squared test and presented as numbers and percentages.

Prior to comparing MCC ratios based on clinical characteristics, natural logarithmic transformation, denoted by ln(MC%) was applied because the original distribution was skewed to the left. For MCC-negative cases, an arbitrary value (0.0020 %) below the minimum value (0.0026 %) was assigned for log-transformation. Owing to the high frequency of MCC(−) cases, the MCC ratio did not present a normal distribution, even after log transformation. Therefore, non-parametric tests, such as the Mann–Whitney test and Kruskal–Wallis test, were used to compare the MCC ratios between groups. Linear regression analysis for continuous variables was conducted only for MCC(+) cases after confirming their normal distribution.

To evaluate the relationship between MCC ratios and kidney disease outcomes, the Cox proportional hazards model was employed to analyze the influence of the MCC ratio on the initiation of RRT, progression to ESKD, and the composite outcome of ESKD and death. Adjusted variables included age, sex, baseline eGFR, uPCR, and history of diabetes. Additionally, the eGFR slope according to the MCC ratio was analyzed using linear regression.

Furthermore, within each diagnostic subgroup, linear regression was performed to assess the association between the MCC ratio and baseline eGFR, uPCR, age, and eGFR slope. Cox regression analyses were performed to assess the kidney disease outcomes. All statistical analyses were performed using SPSS software 25.0 (SPSS Inc., Chicago, IL, USA). A $p < 0.05$ indicated statistical significance.

## 3. Results

### 3.1. Baseline characteristics

This study included 134 patients, including 8 patients as controls (mean age: 49.2 years; 63.4 % were men). The baseline clinical characteristics based on the presence of MCCs are summarized in Table 1. The median follow-up period was 20.3 months (interquartile range [IQR]: 18.6). The mean eGFR and the mean uPCR were 66.3 mL/min/1.73 $m^2$ and 4.41 g/g, respectively. The baseline eGFR and uPCR values based on the diagnostic groups are presented in Supplementary Table S2. Progression to ESKD during the follow-up period was reported in 17.2 % of patients.

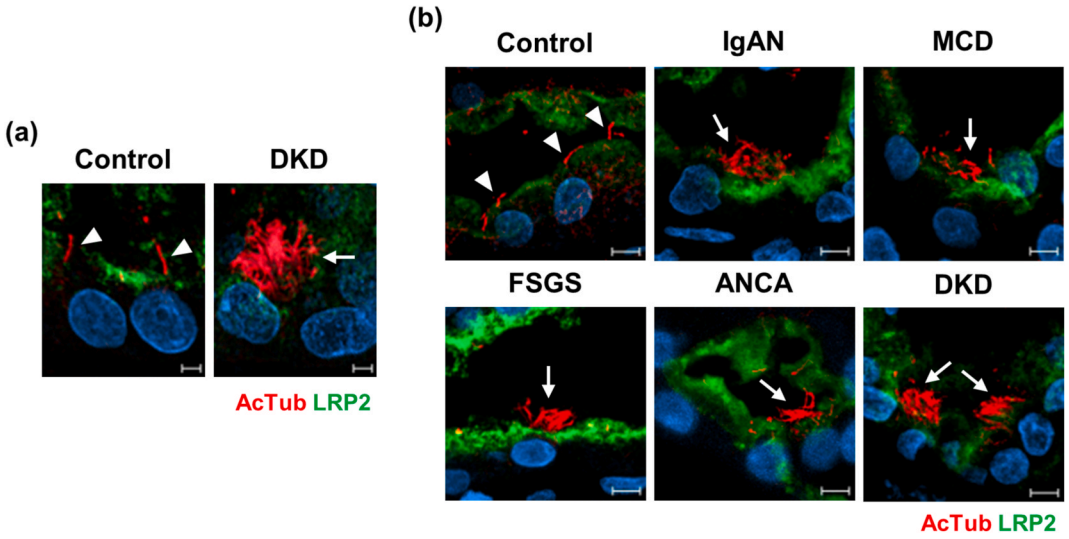


**Fig. 1.** The presence of kidney multiciliated cells (MCCs) in proximal tubules of glomerular diseases.
(a) Representative images of kidney primary cilia and MCCs in proximal tubules from a control and a diabetic kidney disease (DKD) patient, respectively. Cilia were stained with an anti-acetylated alpha tubulin (AcTub) antibody, and the brush border of proximal tubules were marked with an anti-low-density lipoprotein receptor-related protein 2 (LRP2) antibody. Scale bar, 2 μm(b) Representative images of kidney MCCs in proximal tubules from each disease. Scale bar, 10 μm. Arrow head, primary cilia; arrow, MCCs. ANCA, anti-neutrophil cytoplasmic autoantibody-associated glomerulonephritis; IgAN, immunoglobulin A nephropathy; FSGS, primary focal segmental glomerulosclerosis; MCD, minimal change disease; DKD, diabetic kidney diseases.

### 3.2. Detection of proximal tubular MCCs in patients with glomerular diseases

To observe the ciliary phenotype, kidney biopsy tissues were stained with the ciliary marker (AcTub) and proximal tubule marker (LRP2). In contrast to the control group, MCCs within the proximal tubules were exclusively detected in patients with glomerular disease (Fig. 1). Of the 134 patients, MCC-positivity was confirmed in 86, with an average MCC ratio of 0.100 % (SD: 0.025) (Table 1). In the MCC-positive groups, DKD accounted for 27.9 %, and ANCA-associated GN accounted for 22.1 %. Conversely, all patients in the control group were MCC-negative. The participants in the MCC-positive group were older, with a higher proportion of men, and had a lower eGFR.

### 3.3. Differences in MCC ratios based on clinical characteristics

MCC ratios were higher in men than in women ($p = 0.014$) (Fig. 2a). Additionally, individuals with diabetes exhibited higher MCC ratios ($p < 0.001$) (Fig. 2b). Patients with hypertension only showed a trend towards higher MCC ratios, with no statistical significance ($p = 0.066$) (Fig. 2c). In terms of disease groups, MCC ratios were significantly higher in the DKD and ANCA-associated GN groups than in the control, IgAN, and MCD groups (Fig. 2d). Moreover, MCC ratios increased with a decrease in kidney function and an increase in uPCR (Fig. 2e and f). The median and IQR for each variable are presented in Supplementary Table S3.

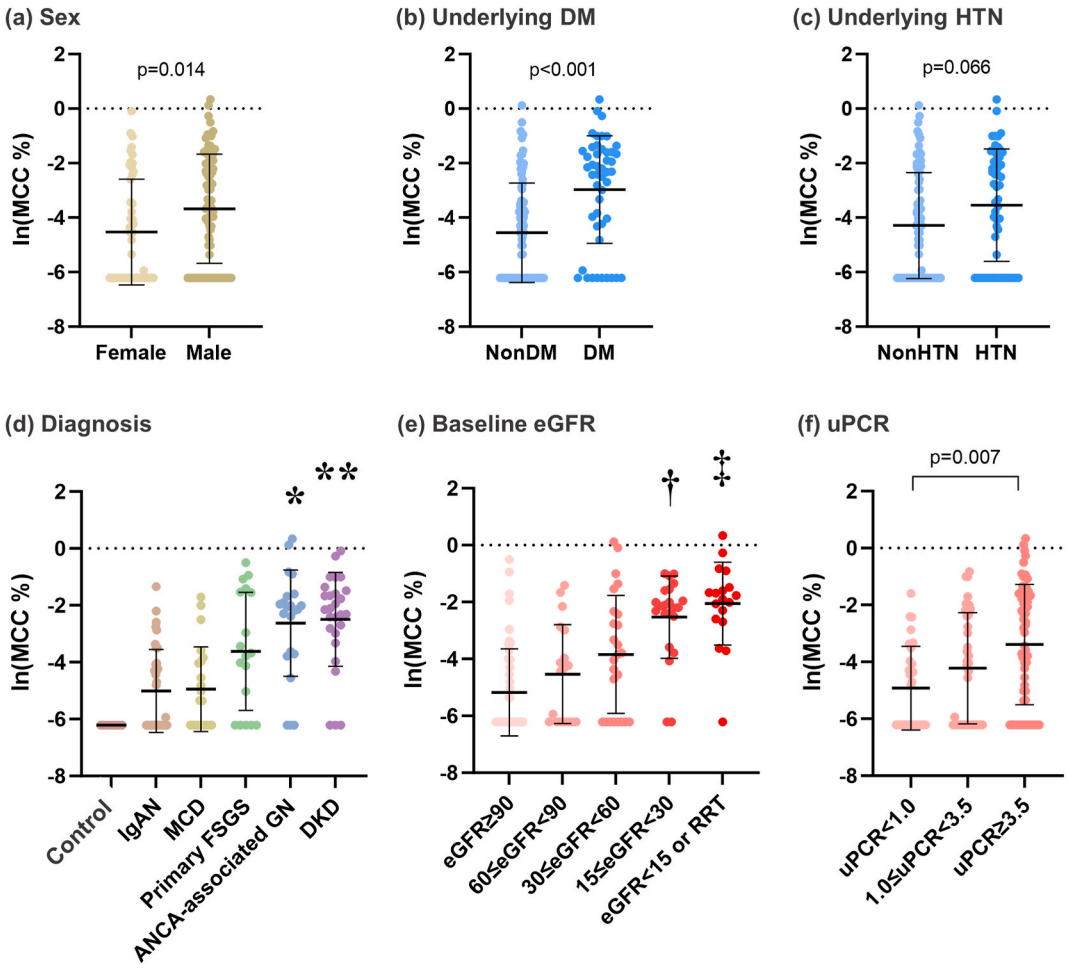


**Fig. 2.** Comparison of multiciliated cell (MCC) ratios according to baseline clinical characteristics.
The mean comparison of MCC ratios was assessed using Mann–Whitney test for (a) sex, (b) underlying diabetes mellitus (DM), and (c) underlying hypertension (HTN). Kruskal–Wallis test was employed to compare MCC ratios based on (d) diagnosis, (e) baseline estimated glomerular filtration rate (eGFR), and (f) urinary protein-to-creatinine ratio (uPCR), and p-values were adjusted for multiple testing using Bonferroni correction. MCC ratios were transformed using the natural logarithm for the analysis. For MCC-negative cases, a log-transformed value of an arbitrary percentage (0.002 %) below the minimum value was assigned. * vs. IgAN ($p < 0.001$); vs. MCD ($p < 0.005$); vs. controls ($p < 0.05$), ** vs. IgAN, MCD ($p < 0.001$); vs. controls ($p < 0.005$), † vs. eGFR≥90 ($p < 0.001$); vs. 60≤eGFR<90 ($p < 0.05$), ‡ vs. eGFR≥90 ($p < 0.001$); vs. 60≤eGFR<90 ($p < 0.005$); 30≤eGFR<60 ($p < 0.05$). ANCA, antineutrophil cytoplasmic autoantibody-associated glomerulonephritis; IgAN, immunoglobulin A nephropathy; FSGS, primary focal segmental glomerulosclerosis; MCD, minimal change disease; DKD, diabetic kidney diseases.

Linear regression analysis conducted only for MCC-positive cases (N = 86) elucidated a significant association between the MCC ratios and clinical variables (Supplementary Fig. S1). An increase in MCC ratios was significantly associated with an increase in age (β = 0.023; p = 0.005) and uPCR (β = 0.084; p = 0.017), and a decrease in eGFR (β = −0.018; p < 0.001).

### 3.4. Correlation between MCC ratios and kidney disease outcomes

Of the 134 patients, 26 underwent RRT, with 23 of them progressing to ESKD. Among patients with progression to ESKD, 44.4 % (N = 12) and 40.9 % (N = 9) were in the DKD and ANCA-associated GN groups, respectively (Table 2a). Further analysis of kidney disease outcomes based on MCC categories revealed that the majority (39.5 %) of ESKD cases were observed in patients with high MCC ratios (Table 2b). The log-rank test confirmed that the risks of ESKD and death were significantly higher in patients with high MCC ratios. The Kaplan–Meier plot for survival analysis is shown in Fig. 3.

The Cox proportional hazard model was used to evaluate the impact of MCC ratios on the risk of RRT, ESKD, and the composite outcome (Table 3). In the unadjusted model, an increase in MCC ratios was associated with an elevated risk of RRT, ESKD, and the composite outcome. Even after adjusting for age, sex, baseline eGFR, uPCR, and diabetes, the increase in MCC ratios remained significantly correlated with an increased risk of ESKD (HR, 1.413; 95 % CI, 1.012–1.972; p = 0.042) and the composite outcome (HR, 1.401; 95 % CI, 1.028–1.909; p = 0.033). However, MCC ratios and eGFR slope were not significantly correlated (Supplementary Fig. S2).

### 3.5. Subgroup analysis

Given the significant variations in MCC ratios and kidney disease outcomes within each disease group, separate analyses were conducted within each diagnostic group (Table 4). Within the ANCA-associated GN group, the decrease in baseline eGFR and the increase in uPCR were significantly associated with an increase in MCC ratios (β = −0.087; p = 0.009, β = 0.208, p = 0.043, respectively) (Table 4a, b). Furthermore, a decrease in baseline eGFR was correlated with an increase in MCC ratios in the IgAN (β = −0.016; p = 0.028) and DKD (β = −0.031; p = 0.002) groups (Table 4a).

Cox regression analysis for the composite outcome of ESKD and death (Supplementary Table S4) revealed no statistically significant impact of the MCC ratios within each disease group.

## 4. Discussion

The MCC ratios in various glomerular diseases were calculated in this study to elucidate the association between clinical characteristics, kidney outcomes, and the MCC ratios in patients with glomerular diseases. The MCC ratios identified in the proximal tubules of glomerular diseases were significantly higher in patients with DKD and ANCA-associated GN than in controls. Moreover, old age, male sex, diabetes, a decline in baseline renal function, and increased proteinuria were positively associated with MCC ratios. Survival analysis revealed that higher MCC ratios were associated with an elevated risk of ESKD after adjusting for age, sex, baseline eGFR, uPCR, and underlying diabetes. A significant association was observed between a decline in kidney function and an increase in the MCC ratios in patients with IgAN, DKD, and ANCA-associated GN.

**Table 2**
Frequency of kidney disease outcome events based on diagnosis and MCC category.

(a) Diagnosis

| | Total | RRT | | ESKD | | Death (all-cause) | | ESKD + death | |
|---|---|---|---|---|---|---|---|---|---|
| | N | N | % | N | % | N | % | N | % |
| **Control** | 8 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **IgAN** | 37 | 1 | 2.7 | 1 | 2.7 | 0 | 0.0 | 1 | 2.7 |
| **DKD** | 27 | 12 | 44.4 | 12 | 44.4 | 1 | 3.7 | 12 | 44.4 |
| **MCD** | 22 | 1 | 4.5 | 0 | 0.0 | 0 | 0.0 | 0 | 0.0 |
| **Primary FSGS** | 18 | 1 | 5.6 | 1 | 5.6 | 1 | 5.6 | 2 | 11.1 |
| **ANCA-associated GN** | 22 | 11 | 50.0 | 9 | 40.9 | 5 | 22.7 | 10 | 45.5 |
| **Total** | 134 | 26 | | 23 | | 7 | | 25 | |

(b) MCC category

| | Total | RRT | | ESKD | | Death (all-cause) | | ESKD + death | |
|---|---|---|---|---|---|---|---|---|---|
| | N | N | % | N | % | N | % | N | % |
| **No MCC** | 48 | 1 | 2.3 | 1 | 2.3 | 0 | 0.0 | 1 | 2.3 |
| **MCC low** | 43 | 6 | 14.0 | 5 | 11.6 | 1 | 2.3 | 6 | 14.0 |
| **MCC high** | 43 | 19 | 44.2 | 17 | 39.5 | 6 | 14.0 | 18 | 41.9 |
| **Total** | 134 | 26 | | 23 | | 7 | | 25 | |

ANCA-associated GN, antineutrophil cytoplasmic antibody-associated glomerulonephritis; DKD, diabetic kidney disease; FSGS, focal segmental glomerulosclerosis; IgAN, immunoglobulin A nephropathy; MCC, multiciliated cell; MCD, minimal change disease.
The MCC has been categorized into a total of three groups, with MCC low and MCC high determined based on the median MCC level within the MCC-positive group.

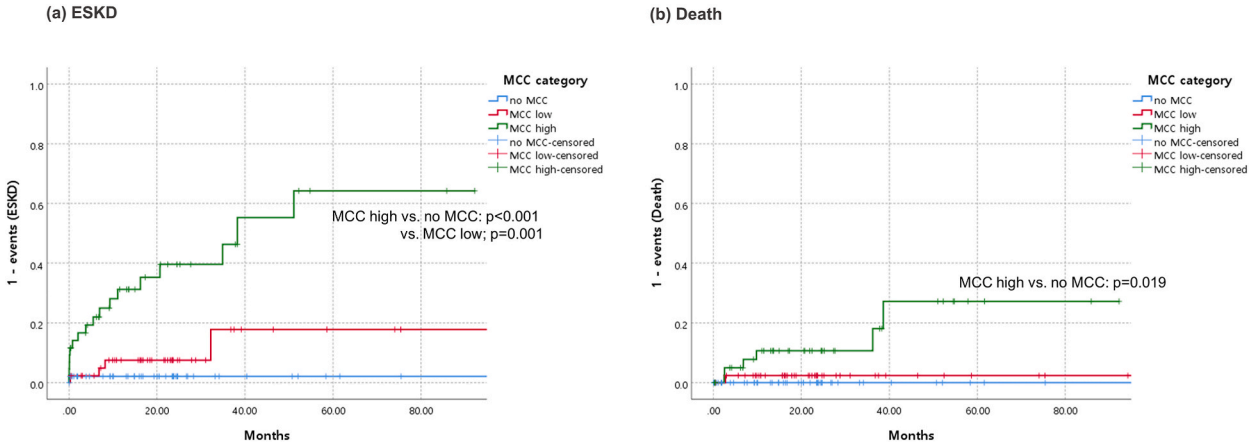


**Fig. 3.** Kaplan–Meier plot for kidney outcomes according to multiciliated cell (MCC) category.
A log-rank test was conducted to compare the incidence of (a) end-stage kidney disease (ESKD) and (b) death among different MCC categories. The MCC ratios have been categorized into a total of three groups, with MCC low and MCC high determined based on the median MCC ratios within the MCC-positive group.

**Table 3**
Analysis of the impact of MCC ratios on kidney disease outcome using Cox proportional hazard model.

| | RRT | | | ESKD | | | ESKD + death | | |
|---|---|---|---|---|---|---|---|---|---|
| | HR | 95 % CI | p-value | HR | 95 % CI | p-value | HR | 95 % CI | p-value |
| **Unadjusted** | 1.716 | 1.357–2.169 | **<0.001** | 1.773 | 1.370–2.295 | **<0.001** | 1.746 | 1.367–2.230 | **<0.001** |
| **Model 1** | 1.729 | 1.354–2.207 | **<0.001** | 1.792 | 1.373–2.341 | **<0.001** | 1.759 | 1.363–2.269 | **<0.001** |
| **Model 2** | 1.311 | 0.990–0.990 | 0.059 | 1.486 | 1.072–2.059 | **0.017** | 1.465 | 1.080–1.988 | **0.014** |
| **Model 3** | 1.248 | 0.937–1.663 | 0.130 | 1.413 | 1.012–1.972 | **0.042** | 1.401 | 1.028–1.909 | **0.033** |

CI, confidence interval; ESKD, end-stage kidney disease; HR, hazard ratio; RRT; renal replacement therapy.
MCC was utilized in the analysis after being transformed using the natural logarithm. For MCC-negative cases, a log-transformed value of an arbitrary percentage (0.002 %) below the minimum value was assigned.
Model 1: adjusted for age, sex.
Model 2: adjusted for age, sex, baseline eGFR, uPCR.
Model 3: adjusted for age, sex, baseline eGFR, uPCR, underlying DM.

**Table 4**
Correlation between baseline eGFR, uPCR, and MCC ratios within disease subgroups.

| (a) Baseline eGFR | | | |
|---|---|---|---|
| Diagnosis | $R^2$ | β-coef. | p-value |
| **IgAN** | 0.131 | −0.016 | **0.028** |
| **DKD** | 0.321 | −0.031 | **0.002** |
| **MCD** | 0.133 | −0.014 | 0.095 |
| **Primary FSGS** | 0.078 | −0.016 | 0.262 |
| **ANCA-associated GN** | 0.297 | −0.087 | **0.009** |
| (b) uPCR | | | |
| Diagnosis | $R^2$ | β-coef. | p-value |
| **IgAN** | 0.055 | 0.160 | 0.161 |
| **DKD** | 0.114 | 0.144 | 0.084 |
| **MCD** | 0.001 | −0.008 | 0.897 |
| **Primary FSGS** | 0.115 | 0.178 | 0.169 |
| **ANCA-associated GN** | 0.189 | 0.208 | **0.043** |

ANCA-associated GN, antineutrophil cytoplasmic antibody-associated glomerulonephritis; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; IgAN, immunoglobulin A nephropathy; MCC, multiciliated cell; MCD, minimal change disease; uPCR, urine protein-to-creatinine ratio.
Within each disease subgroup, the correlation between MCC and (a) baseline eGFR and (b) uPCR was analyzed using univariable linear regression analysis. MCC was utilized in the analysis after being transformed using the natural logarithm. For MCC-negative cases, a log-transformed value of an arbitrary percentage (0.002 %) below the minimum value was assigned.

Developing valuable biomarkers to predict kidney disease-related outcomes in glomerular diseases is important. Patients with glomerular disease exhibit diverse clinical courses, ranging from isolated proteinuria or hematuria alone with a well-maintained eGFR over an extended period to progressive eGFR decline leading to ESKD requiring RRT [16–18]. Decreased eGFR and increased proteinuria are well-established biomarkers of kidney diseases [19]. In general, the prognostic relevance of blood and urinary biomarkers related to tubular injury (kidney injury molecule 1, monocyte chemoattractant protein 1, neutrophil gelatinase-associated lipocalin, and interleukin 18) and tubular dysfunction (α1-microglobulin, uromodulin, and epidermal growth factor) in acute kidney injury and chronic kidney disease has been actively investigated [20]. The most well-known serological biomarker of glomerular disease is the phospholipase A2 receptor antibody in membranous nephropathy [21]. In addition, the deposition of IgA antibodies in the kidney mesangial tissue in IgAN, glomerular deposition of complements (C3, C5, C6, C7, C8, and C9) in C3 glomerulonephritis, and fibrillar deposits in fibrillary GN are histological biomarkers with diagnostic significance [22]. However, in glomerular diseases, the prognostic relevance of histological biomarkers determined by pathological examination is relatively lacking.

In this study, the MCC ratios differed in various glomerular diseases depending on the type and severity of the disease. Among the 126 patients with glomerular diseases, the MCC-positivity rate was 68.3 %, with relatively higher positivity rate observed in DKD (88.9 %) and ANCA-associated GN (86.4 %). In addition, the presence of MCCs and MCC ratios were significantly associated with an increase in age, proteinuria, and a decrease in eGFR. Patients with observed MCC expression or higher MCC ratios showed significantly higher rates of mortality and ESKD. Eymael et al. demonstrated MCC expression in proximal tubules of various kidney diseases, including not only FSGS, DKD, and IgAN as identified in our study, but also membranous nephropathy and tubulointerstitial disease [23]. They reported that this MCC expression was associated with markers of tubular injury and interstitial fibrosis, such as KIM-1 and αSMA. Further research is needed to determine whether MCC expression itself is a pathophysiological cause related to the development and progression of various kidney diseases, or serves as a surrogate biomarker associated with prognosis. Additionally, larger-scale studies are required to establish whether MCC expression can function as a prognostic factor independent of patients' clinical factors, type of kidney disease, or disease severity.

Although our study highlighted the presence of MCCs in the proximal tubules in glomerular diseases and their impact on kidney disease-related outcomes, the occurrence of MCCs in the human kidney is uncommon. Nevertheless, MCCs have been observed in various renal conditions such as kidney sarcoidosis, congenital nephrotic syndrome, ANCA-associated GN, FSGS, lupus nephritis, and membranoproliferative GN [9–13]. The reasons for their restriction to the proximal tubules remain elusive. Tubular damage and regression of tubular cells to a primitive or less differentiated state are possible explanations for MCCs in human kidney disorders. MCCs in patients with kidney diseases have been reported to originate from scattered proximal tubular cells (STCs), which are stem cell-like cells derived from dedifferentiated PTECs [15,23,24]. Given that the factors that differentiate STCs to MCCs remain unclear, the mechanisms underlying MCC differentiation in lower vertebrates are relevant. In the zebrafish pronephros, which represents an immature form of the kidney in vertebrates, MCCs are primarily located in the proximal straight tubules, and their cell fates are determined by a balance of Notch, retinoic acid, and prostaglandin E2 signals [25–27]. Loss-of-function studies have demonstrated that retinoic acid and prostaglandin E2 promote MCC development, whereas activation of Notch receptors suppresses it [25,28]. Exploring whether these signals are correlated with the incidence of MCCs in humans could provide valuable insights into the underlying mechanisms.

In glomerular diseases, urinary conditions such as flow speed and viscosity often deviate from the normal range. A compensatory response may occur, leading to the generation of MCCs for restoring these parameters [23]. However, to investigate the exact role of kidney MCCs, mouse models that exhibit MCCs in the proximal tubules are necessary. Although ciliary phenotypes were screened in some mouse models, including those with unilateral ureteral obstruction, bilateral ischemia-reperfusion injury, or streptozotocin-induced diabetic nephropathy, kidney MCCs were not detected (data not shown). The exclusive presence of kidney MCCs in patients presents a challenge for further investigation; however, alternative experimental techniques such as kidney-on-a-chip or kidney organoids may be beneficial.

To our knowledge, this is the first clinical study to quantify kidney MCCs. Our study highlights that the MCC ratio is associated with the risk of ESKD, independent of clinical status such as baseline kidney function, proteinuria, and diabetes. However, this study had certain limitations. First, in terms of statistical power, statistical significance was not confirmed within the diagnostic subgroups or between the MCC ratio and eGFR slope. This could be attributed to the small sample size, which resulted from the exclusion of patients with a short follow-up period or the initiation of dialysis shortly before or after biopsy. These small sample sizes can lead to potentially overestimating or underestimating effects due to increased variability, reduced statistical power, and limited generalizability. Therefore, our findings require cautious interpretation and validation through larger confirmatory studies. Second, because MCCs were identified using IHC, an invasive kidney biopsy at diagnosis is inevitable. Other glomerular diseases require biopsy for diagnosis, but DKD is usually diagnosed based on proteinuria in patients with diabetes [29]. For ANCA-associated GN, clinical presentations and ANCA serology are often sufficient for diagnosis, although kidney biopsy remains the gold standard [30]. Our study should not be interpreted as indicating an absolute need for kidney biopsy in these two diseases, but rather as recommending additional MCC staining when kidney biopsy is already scheduled, especially for critical differential diagnosis. Identifying urinary markers specific to kidney MCCs is imperative to facilitate non-invasive quantification of MCCs. Axonemal proteins unique to motile cilia, such as dynein arms or radial spokes, are potential candidates because ciliary axonemes are easily released into the lumen by decapitation or shedding [31]. However, owing to their limited concentrations in urine, the development of an ultrasensitive detection method is required for these markers.

In conclusion, this study confirmed the presence of kidney MCCs in glomerular diseases and demonstrated their correlation with clinical presentation and outcomes. Kidney MCCs in the proximal tubules are found in several glomerular diseases; however, their detection ratios vary depending on the type of disease and clinical presentation including mortality and end-stage kidney disease

(ESKD). The significant association between MCC ratios and the poor prognosis of kidney disease outcomes, including mortality and ESKD, suggests a potential role for kidney MCCs as a prognostic marker. To utilize kidney MCC ratios as useful clinical indicators and further elucidate their mechanism and role, additional experimental or translational research is warranted.

## CRediT authorship contribution statement

**Bohye Kim:** Writing – original draft, Investigation, Data curation, Conceptualization. **Boram Weon:** Writing – original draft, Investigation, Formal analysis, Data curation, Conceptualization. **Evonne Kim:** Writing – review & editing, Validation. **Sohee Park:** Writing – review & editing, Validation. **Wencheng Jin:** Investigation, Data curation. **Nayeon Shin:** Investigation, Data curation. **Yun Kyu Oh:** Writing – review & editing, Supervision. **Chun Soo Lim:** Writing – review & editing, Supervision. **Jung Pyo Lee:** Writing – review & editing, Supervision. **Obin Kwon:** Writing – review & editing, Validation, Supervision, Conceptualization. **Jeonghwan Lee:** Writing – review & editing, Validation, Supervision, Conceptualization.

## Ethics statement

This study was reviewed and approved by the Institutional Review Boards of the participating hospitals (Seoul National University Boramae Medical Center: 20-2022-102, Seoul National University Hospital: H-2304-030-1420), which included a prospective cohort of patients with kidney disease. The prospective cohort study was also approved by each Institutional Review Board (Seoul National University Boramae Medical Center: 06-2011-50 and 20-2019-48, Seoul National University Hospital: 1404–117-575). All participants gave written informed consent prior to kidney biopsy and study enrollment. The legal guardian of 17- and 18-year-old participants was also notified about the study and provided written informed consent simultaneously with the subject. All clinical characteristics and biospecimens were prospectively collected with the approval of the participants.

## Disclosures

Nothing to disclose.

## Data availability statement

The data that support the findings of this study are not publicly available due to their containing information that could compromise the privacy of research participants but are available except for the private information from the corresponding author Jeonghwan Lee (jeonghwan@snu.ac.kr) upon reasonable request.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2025.e42416.

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