Human Transcriptomic Validation of a Proximal Tubule–Fibrogenic Interstitial Pyrimidinergic Axis in Kidney Disease

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Article

Human Transcriptomic Validation of a Proximal Tubule–Fibrogenic Interstitial Pyrimidinergic Axis in Kidney Disease

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Simple Summary

Kidney fibrosis is a central process in chronic kidney disease and reflects harmful communication between injured kidney tubules and surrounding interstitial cells. In our recent experimental study, we showed that metabolic changes in proximal tubule cells can promote extracellular pyrimidinergic signaling and activate fibrogenic pathways through the P2Y6 receptor in renal fibroblast cells. The next question was whether the same biological pattern can also be detected in human kidney tissue. In this study, we analyzed publicly available human single-cell RNA-sequencing data from healthy kidneys, acute kidney injury, and diabetic kidney disease. We found that pyrimidine metabolism genes were more active in proximal tubule cells from diseased kidneys. At the same time, the fibrogenic interstitial compartment was expanded and showed increased P2RY6 positivity, with enrichment in myofibroblasts and other specific fibrogenic mesenchymal states. In addition, higher tubular pyrimidine activity was associated with stronger interstitial calcium signaling and extracellular matrix remodeling. Together, these findings provide human transcriptomic support for a proximal tubule–interstitial pyrimidinergic axis in kidney disease and strengthen the translational relevance of our previous mechanistic work.

Abstract

Background: Kidney fibrosis develops through sustained communication between injured tubular epithelial cells and surrounding interstitial populations. In our recent mechanistic study, we identified a pyrimidinergic pathway linking injury-associated proximal tubule metabolism to P2Y6-mediated fibroblast activation. Here, we sought to determine whether this biological axis is also detectable in human kidney disease transcriptomes. Methods: We analyzed publicly available human kidney single-cell RNA-sequencing (sc-RNA-seq) data spanning healthy reference tissue, acute kidney injury (AKI), and diabetic kidney disease (DKD). We quantified proximal tubule (PT) pyrimidine metabolism at the subject level and examined the abundance, P2RY6 expression, and subtype distribution of fibrogenic interstitial cells. We also evaluated calcium signaling and extracellular matrix (ECM)-related associations across compartments. Results: PT pyrimidine metabolism was significantly increased in diseased kidneys, with the strongest elevation observed in AKI. P2RY6 expression was elevated in the stromal compartment in both AKI and DKD, with the strongest signal in ACTA2+ myofibroblasts. The fibrogenic interstitial compartment expanded in disease and showed a significant increase in P2RY6 expression in DKD. Fibrogenic interstitial cells from AKI and DKD kidneys also displayed increased calcium signaling activity. At the subject level, higher PT pyrimidine module score was associated with stronger interstitial calcium signaling (rho = 0.395, p = 0.011), while greater abundance of P2RY6-positive fibrogenic interstitial cells correlated with ECM remodeling (rho = 0.434, p = 0.005). Conclusions: These findings provide human transcriptomic validation of a proximal tubule–fibrogenic interstitial pyrimidinergic axis in kidney disease. By extending our prior mechanistic observations into human single-cell data, this study

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strengthens the translational relevance of P2Y6-linked epithelial–interstitial communication as a candidate pathway in fibrogenic kidney remodeling.

Keywords: CKD; kidney fibrosis; proximal tubule; pyrimidine metabolism; pyrimidinergic signaling; P2Y6; fibroblasts; human RNA-seq; single-cell RNA-seq; validation

1. Introduction

Chronic kidney disease (CKD) is an escalating global health issue, currently positioned among the primary causes of mortality worldwide and significantly contributing to disability and cardiovascular risk [1]. In spite of significant advancements in renoprotective therapies and risk-factor control, the disease is advancing in a significant number of patients. The clinical reality is that CKD is frequently detected at a late stage, when structural damage has already occurred and is challenging to reverse.

Acute kidney injury (AKI) is not merely a transient complication of hospitalisation; it is a significant event with long-term repercussions and a significant entrance point into CKD. Globally, AKI is estimated to affect ~13 million people each year and is linked to up to ~1.7 million deaths annually, underscoring its population-level burden [2]. It is crucial to note that the return of serum creatinine to baseline does not necessarily equate to biological recovery. Survivors frequently experience persistent kidney dysfunction and progressive fibrosis, particularly following severe or recurrent episodes. A landmark systematic review and meta-analysis showed that AKI is associated with a markedly higher subsequent risk of developing CKD (pooled adjusted hazard ratio ~8.8) and end-stage kidney disease (pooled adjusted hazard ratio ~3.1), compared with patients without AKI [3]. Recent large-scale syntheses have confirmed that AKI is still independently associated with increased long-term risks of CKD incidence, CKD progression, and kidney failure in a variety of cohorts [4].

Tubulointerstitial fibrosis is a key histopathologic characteristic of CKD that progresses with irreversible deterioration. It is closely linked to loss of function and unfavourable long-term results and serves as a “final common pathway” across various aetiologies [5]. Fibrosis is becoming recognised as a tissue-level program driven by maladaptive interaction between injured epithelial compartments, especially proximal tubule (PT), and interstitial fibroblasts and perivascular stromal populations, despite the fact that it is frequently characterised as a stromal endpoint. In such paradigm, tubular stress signals actively influence the stromal response that maintains and intensifies scarring, rather than just accompanying fibrosis.

In our recent experimental study in animal models, we identified a mechanistic pathway linking injury-associated tubular pyrimidine metabolism to extracellular release of uridine diphosphate (UDP) and activation of the P2Y6 receptor (P2Y6R) in surrounding renal fibroblasts [6]. P2Y6R signaling triggered intracellular calcium responses and promoted fibroblast proliferation, migration, and induction of pro-fibrotic gene programs, consistent with a tubular–stromal axis that converts metabolic stress into a fibrogenic response [6]. These findings provided causal evidence in experimental systems. However, the translational question remains whether this pyrimidinergic program can also be detected in human kidney disease across clinically distinct contexts such as AKI and diabetic kidney disease (DKD), and whether it is accompanied by activation of the same fibrogenic interstitial remodeling program observed in our experimental study.

Human CKD spans heterogeneous causes, variable timing of injury, and mixed cellular niches that are difficult to capture with bulk tissue measurements alone. Recent scRNA-seq data now make it possible to test pathway conservation with cell-type resolution, including PT segment states and stromal subtypes positioned within disease-associated microenvironments. In particular, the single-cell datasets from Lake and colleagues provide a benchmark resource spanning healthy reference tissue and injured/diseased human kidneys, enabling direct assessment of epithelial–stromal patterns with high cellular resolution [7].

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Here, we perform a transcriptomic validation of the proximal tubule–

fibrogenic interstitial pyrimidinergic axis in human kidney disease. Using publicly available human kidney single-cell datasets from Lake et al., we quantify cell-type-resolved expression of pyrimidine metabolism and pyrimidinergic signaling genes across PT compartments and examine P2RY6 expression across a fibrogenic kidney interstitial cells in health and disease. We further assess whether disease-associated PT pyrimidine activity is accompanied by expansion, P2RY6 enrichment, and calcium signaling activation within this interstitial compartment, as well as by association with extracellular matrix remodeling.

By anchoring our analysis in independent human cohorts, we aim to strengthen the translational relevance of the pathway and provide a rationale for further work on P2Y6-related signaling as a biomarker and therapeutic entry point in kidney fibrosis [7].

2. Materials and Methods

2.1. Human Kidney Single-Cell RNA-Sequencing Dataset

Publicly available human kidney sc-RNA-seq data from the Lake atlas (GEO: GSE183276) were used for transcriptomic validation of the pyrimidinergic pathway identified in our previous experimental study. Raw count matrices and corresponding cell-level metadata were imported into R, and a Seurat object was generated directly from the raw counts with metadata attached at initialization. Gene expression values were normalized using Seurat's LogNormalize method with a scale factor of 10,000, yielding log-normalized expression values for downstream analyses.

All analyses were performed in R using the packages Seurat, BPCells, dplyr, ggplot2, ggpubr, purrr, tidyr, and tibble.

2.2. Cell population Selection

The analysis focused on two biologically relevant compartments: proximal tubule epithelial cells and an interstitial mesenchymal compartment linked to fibrogenic remodeling.

Proximal tubule (PT) cells were identified as cells annotated as "epithelial cells" with "Proximal Tubule" in the subclass.full metadata field. PT pyrimidine metabolism was assessed using a six-gene set consisting of CAD, DHODH, UMPS, UCK1, CDA, and DCTPP1.

For interstitial analyses, all cells annotated as "stroma cells" were used for stromal-level analyses. In addition, an operationally defined fibrogenic interstitial compartment was analyzed, comprising the following annotated cell states: subclass.full among Adaptive/Maladaptive/Repairing Fibroblast, Degenerative Fibroblast, Fibroblast, Medullary Fibroblast, Myofibroblast, and Vascular Smooth Muscle Cell/Pericyte. This combined compartment was selected to preserve the broader PDGFRB-associated interstitial program relevant to fibrogenic remodeling.

2.3. Expression Extraction and Positivity Definition

Raw counts and log-normalized expression values were extracted from the RNA assay using the counts and data layers, respectively. For analyses based on expression intensity, log-normalized values were used. For positivity-based analyses, a cell was considered positive when the relevant gene showed raw expression > 0.

2.4. PT Pyrimidine Metabolism Score

To quantify PT pyrimidine metabolism, the mean log-normalized expression across the six PT pyrimidine genes (CAD, DHODH, UMPS, UCK1, CDA, DCTPP1) was calculated for each PT cell. These cell-level values were then averaged across all PT cells from the same subject to generate a subject-level PT pyrimidine score. Subjects with fewer than 20 PT cells were excluded from this summary analysis.

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2.5. Interstitial Compartment Analyses

To quantify disease-associated expansion of the interstitial mesenchymal compartment, the proportion of annotated fibroblast/pericyte-like cells within all stromal cells was calculated at the subject level. P2RY6 positivity was evaluated both in the broader stromal compartment and within the operationally defined fibrogenic interstitial compartment using the percentage of P2RY6-positive cells per subject.

For subtype-level interstitial analysis, stromal cells were additionally classified using marker-based module scores representing myofibroblast (ACTA2, TAGLN, MYL9, CNN1), matrix fibroblast (COL1A1, COL1A2, DCN, LUM, COL3A1), inflammatory fibroblast (CXCL12, IL6, CCL2, CXCL14), and pericyte-like (RGS5, PDGFRB, CSPG4, MCAM, DES) programs. Module scores were calculated with AddModuleScore, and each cell was assigned to the state with the highest score, provided the margin over the second-highest score exceeded 0.05. Cells with low-confidence assignment were labeled as "Other stroma" and excluded from the final subtype summary.

2.6. Calcium Signaling and Extracellular Matrix Programs

A calcium signaling program was evaluated within the fibrogenic interstitial compartment using the genes GNAQ, ITPR2, ITPR3, and ORAI1. For each cell, the mean log-normalized expression of these genes was calculated, followed by averaging across cells from the same subject to obtain a subject-level calcium signaling score.

Extracellular matrix remodeling was evaluated using an ECM gene set consisting of COL1A1, COL3A1, FN1, LUM, and DCN. Mean log-normalized expression across these genes was calculated at the cell level and then averaged across the cells within the fibrogenic interstitial compartment of the same subject.

2.7. Subject-Level Association Analyses

To assess epithelial–interstitial relationships, subject-level correlation analyses were performed between the PT pyrimidine score and interstitial readouts, including calcium signaling and the percentage of P2RY6-positive fibrogenic interstitial cells. Additional association analyses were performed between the abundance of P2RY6-positive fibrogenic interstitial cells and the ECM score.

2.8. Statistical Analysis and Visualization

All summary analyses were performed at the subject level, with each point in the boxplots representing one subject. Group comparisons were restricted to Normal Reference, Acute Kidney Injury (AKI), and Diabetic Kidney Disease (DKD) and were tested using the Wilcoxon rank-sum test. Associations between subject-level variables were assessed using Spearman correlation. Plots were generated using ggplot2 and ggpubr. Box-whisker plots display the median, the interquartile range (IQR) and the 1.5 x IQR with overlaid jittered subject-level points.

3. Results

3.1. Pyrimidine Metabolism Is Increased in Proximal Tubules in Human Kidney Disease

To determine whether the pyrimidine-related metabolic program could be detected in human kidney disease, we first examined a focused proximal tubule (PT) pyrimidine metabolism gene set in the Lake human scRNA-seq dataset. Subject-level analysis showed that PT pyrimidine metabolism was higher in diseased kidneys than in normal reference samples, with the strongest increase observed in acute kidney injury (AKI) and a more moderate elevation in diabetic kidney disease (DKD) (Figure 1).

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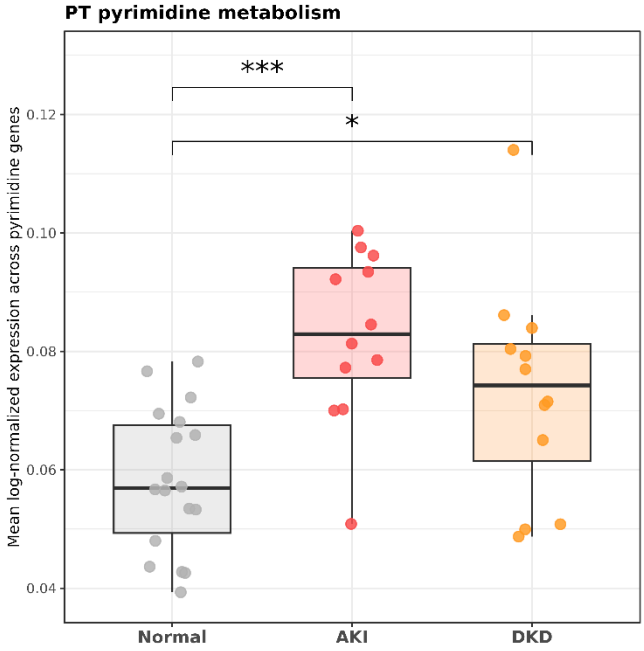

Figure 1. Proximal tubule pyrimidine metabolism is elevated in human kidney disease. Mean normalized expression of pyrimidine metabolism genes in proximal tubular epithelial cells across three conditions: normal kidney, acute kidney injury (AKI), and diabetic kidney disease (DKD). Expression values represent the mean log-normalized expression of a pyrimidine metabolism gene set calculated at the subject level. Each dot represents one individual sample, while boxplots indicate median values and IQR, with whiskers extending to 1.5 × IQR. Statistical comparisons between Normal and each disease group were performed using the Wilcoxon rank-sum test (*$p < 0.05$; ***$p < 0.001$).

This finding indicates that the PT pyrimidine program is not restricted to the experimental animal model, but is also present in human kidney injury. The stronger signal in AKI is consistent with the idea that acute epithelial stress is accompanied by a marked metabolic response, whereas DKD retains the same overall direction of change but in a less pronounced form.

Taken together, these data support disease-associated activation of pyrimidine metabolism in human kidney proximal tubule cells, identifying the proximal tubule as a potential source of UDP that could drive downstream P2Y6 receptor activation within the renal microenvironment.

3.2. Kidney Disease Is Associated with Increased P2RY6 Expression in Stroma Cells, Particularly in Myofibroblasts

We next examined whether the stromal compartment showed disease-related changes in P2RY6 expression. At the subject level, the percentage of P2RY6-positive stroma cells was significantly higher in both AKI and DKD compared to normal reference kidneys (Figure 2a). To determine whether this signal was distributed uniformly or concentrated in specific cell states, we classified stroma cells into marker-defined subtypes using module scores. This analysis revealed that P2RY6 expression was not uniformly distributed across all stromal populations (Figure 2b). Instead, the strongest signal was concentrated in the myofibroblast (ACTA2+) state, which showed notably higher P2RY6 positivity than other subtypes, particularly in DKD. Matrix fibroblast (COL1A1+) cells also showed elevated P2RY6 positivity compared to pericyte-like and inflammatory fibroblast states.

Taken together, these findings demonstrate that the increase in stromal P2RY6 positivity observed in kidney disease is not a nonspecific phenomenon but is concentrated in matrix-associated and contractile fibroblast states, particularly myofibroblasts. This subtype specificity is consistent with the fibrogenic cell populations most relevant to interstitial remodeling.

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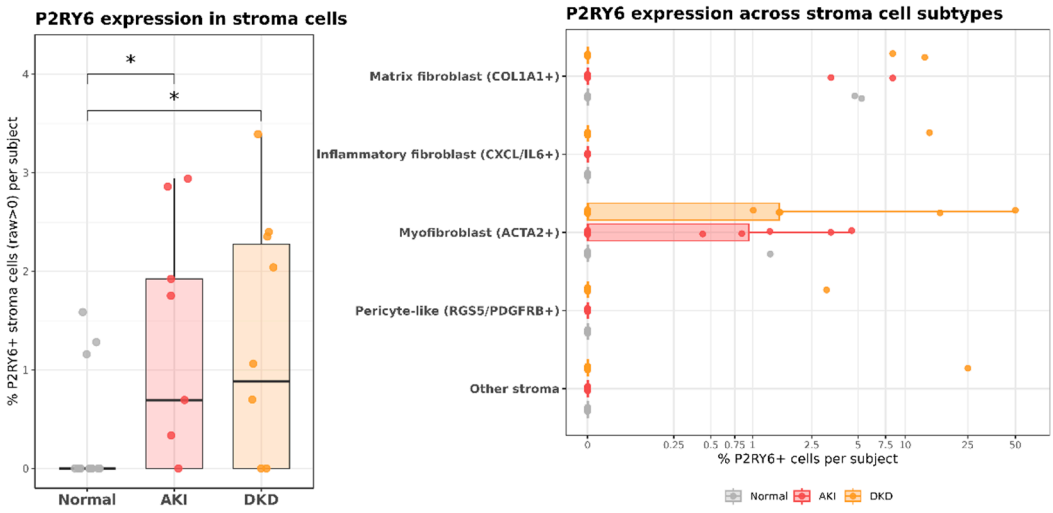
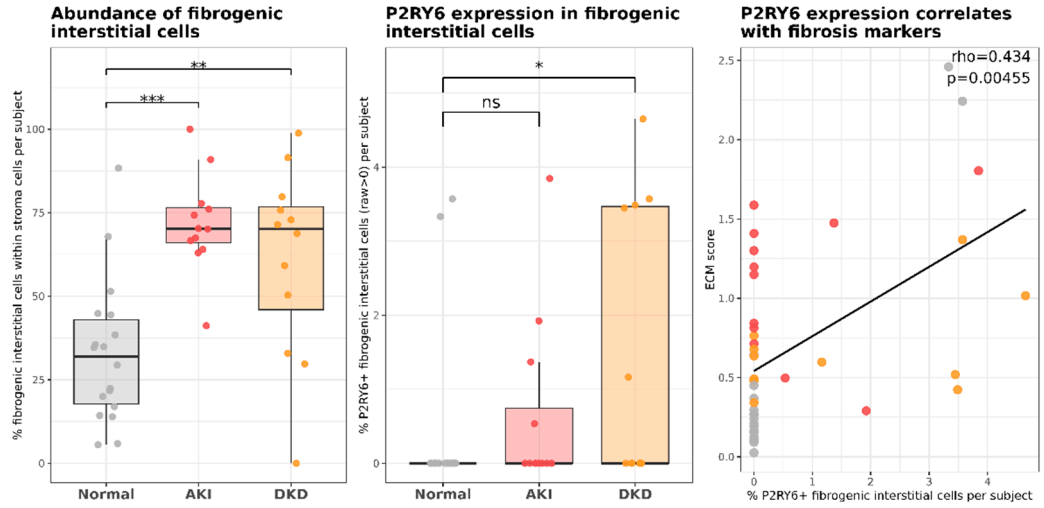


(**a**) (**b**)

Figure 2. P2RY6 expression is increased in stroma cells in kidney disease, with enrichment in myofibroblasts. (a) Percentage of P2RY6-positive stroma cells per subject (raw expression > 0) across Normal, AKI, and DKD conditions. Each dot represents one subject; boxplots indicate the median and IQR. Statistical comparisons are against the Normal group (Wilcoxon rank-sum test; *p < 0.05). (b) P2RY6 positivity across module-score-defined stroma subtypes (Matrix fibroblast COL1A1+, Inflammatory fibroblast CXCL/IL6+, Myofibroblast ACTA2+, Pericyte-like RGS5/PDGFRB+, Other stroma) per subject, plotted on a log10 scale. Colors indicate condition (grey = Normal, red = AKI, orange = DKD).

3.3. Kidney Disease Is Associated with Expansion of the Fibrogenic Interstitial Compartment and Increased P2RY6 Expression in Fibrogenic Interstitial Cells

We then examined the fibrogenic interstitial compartment specifically, defined by the annotation-based cell states most closely associated with fibrogenic remodeling. At the subject level, the proportion of fibrogenic interstitial cells within all stromal cells was significantly higher in both AKI and DKD, indicating clear disease-associated expansion of this compartment (Figure 3a). P2RY6 expression within fibrogenic interstitial cells was significantly elevated in DKD, while the difference in AKI did not reach statistical significance (Figure 3b).



(**a**) (**b**) (**c**)

Figure 3. Expansion of the fibrogenic interstitial compartment, P2RY6 enrichment, and correlation with extracellular matrix remodeling in kidney disease. (a) Percentage of fibrogenic interstitial cells within all stromal cells per subject across Normal, AKI, and DKD conditions. (b) Percentage of P2RY6-positive fibrogenic interstitial cells (raw expression > 0) per subject across conditions. Each dot represents one subject; boxplots

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indicate the median and IQR. Statistical comparisons are against the Normal group (Wilcoxon rank-sum test; ns = not significant; *p < 0.05; **p < 0.01; ***p < 0.001). (c) Spearman correlation between the percentage of P2RY6-positive fibrogenic interstitial cells per subject and the ECM program score (rho = 0.434, p = 0.005). Each dot represents one subject, colored by condition (grey = Normal, red = AKI, orange = DKD).

These findings show that fibrogenic interstitial cells not only expand in number in diseased kidneys, but also display increased P2RY6 expression, particularly in the context of DKD. The compartment expansion observed in both disease conditions, combined with the selective P2RY6 enrichment in DKD, is consistent with a progressive remodeling trajectory in which the P2RY6-positive fibrogenic program becomes more prominent as fibrotic injury evolves.

To determine whether the degree of P2RY6 positivity in fibrogenic interstitial compartment was associated with a functionally relevant downstream output, we examined its correlation with the ECM program score at the subject level. The percentage of P2RY6-positive fibrogenic interstitial cells correlated positively with the ECM score (rho = 0.434, p = 0.005; Figure 3c), indicating that subjects with a higher proportion of P2RY6-expressing interstitial cells also displayed stronger extracellular matrix gene expression. This finding links the P2RY6-positive interstitial signaling state to active fibrogenic tissue remodeling, consistent with a functional role of pyrimidinergic receptor signaling in matrix deposition within the renal interstitium.

3.4. Fibrogenic Interstitial Cells Acquire an Activated Calcium Signaling Phenotype in AKI and DKD

As our previous work linked pyrimidinergic signaling to calcium-dependent fibroblast activation, we next tested whether a similar functional pattern could be detected in the human dataset. We therefore quantified a calcium signaling module within fibrogenic interstitial cells across conditions, based on GNAQ, ITPR2, ITPR3, and ORAI1, which together represent a calcium signaling program compatible with P2Y6R-mediated signaling.

This analysis showed that fibrogenic interstitial cells from diseased kidneys had substantially higher calcium signaling scores than those from normal reference samples, with both AKI and DKD demonstrating an activated profile (Figure 4). The result extends the descriptive abundance findings into a functional direction, suggesting that interstitial remodeling in disease is accompanied by a shift toward a more activated signaling state rather than reflecting simple cell accumulation alone.

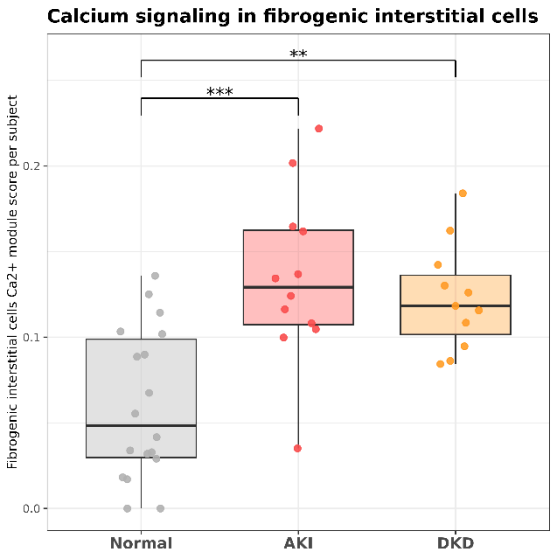


Figure 4. Calcium signaling is activated in fibrogenic interstitial cells in human kidney disease. Ca2+ module score (mean log-normalized expression of GNAQ, ITPR2, ITPR3, ORAI1) in fibrogenic interstitial cells per subject across Normal, AKI, and DKD conditions. Each dot represents one subject; boxplots indicate the median and IQR, with whiskers extending to 1.5 × IQR. Statistical comparisons are against the Normal group (Wilcoxon rank-sum test; **p < 0.01; ***p < 0.001).

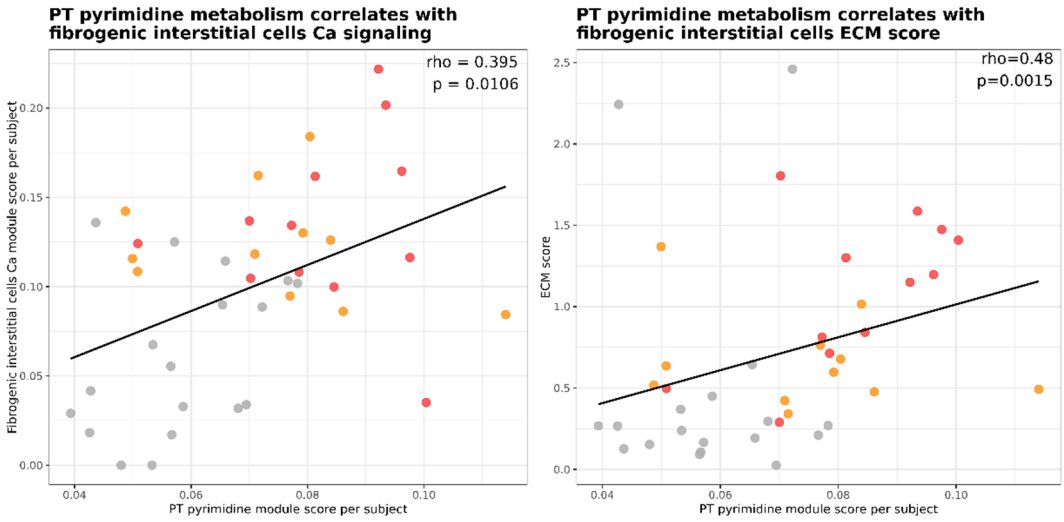
 doi:10.20944/preprints202603.1435.v1

In biological terms, these data strengthen the interpretation that the interstitial compartment is not just expanded in diseased kidneys, but is also transcriptionally primed toward calcium-linked activation programs that are relevant to fibrogenic remodeling.

3.5. Kidney Proximal Tubule Pyrimidine Activity Is Associated with Interstitial Activation and Extracellular Matrix Remodeling

Finally, we asked whether the epithelial and interstitial signals identified above were linked at the subject level. Spearman correlation analysis showed that higher PT pyrimidine program activity was associated with stronger calcium signaling in fibrogenic interstitial cells (rho = 0.395, p = 0.011; Figure 5a). This relationship supports the idea that tubular metabolic reprogramming and interstitial activation are coordinated features of diseased human kidneys rather than independent events.

We further examined whether the PT pyrimidine module score was directly associated with extracellular matrix remodeling at the subject level. Spearman correlation between the PT pyrimidine score and the ECM program score in fibrogenic interstitial cells revealed a positive association (Figure 5b), suggesting that stronger tubular metabolic reprogramming is accompanied by a broader fibrogenic response that extends to matrix gene expression within the interstitial compartment.



(**a**) (**b**)

Figure 5. PT pyrimidine metabolism is associated with fibrogenic interstitial cell activation and extracellular matrix remodeling. (a) Spearman correlation between subject-level PT pyrimidine module score and fibrogenic interstitial cells Ca2+ module score (rho = 0.395, p = 0.011). (b) Spearman correlation between subject-level PT pyrimidine module score and ECM program score in fibrogenic interstitial cells. Each dot represents one subject, colored by condition (grey = Normal, red = AKI, orange = DKD).

Overall, these integrative analyses support a disease-associated axis in which altered proximal tubule pyrimidine metabolism is linked to activation of a fibrogenic interstitial program characterized by calcium signaling and extracellular matrix remodeling.

4. Discussion

Chronic kidney disease progresses through a complex network of epithelial–stromal communication that ultimately drives fibrotic remodeling of the renal interstitium. In this validation study, we provide human single-cell transcriptomic evidence supporting a conserved proximal tubule–fibrogenic interstitial pyrimidinergic signaling axis previously identified in our experimental work [6]. Specifically, we observed two coordinated patterns across human kidney transcriptomes: (i) increased expression of pyrimidine metabolism genes within proximal tubular epithelial cells in diseased kidneys and (ii) expansion of the fibrogenic interstitial compartment together with increased P2RY6 expression within interstitial/stromal populations. Together, these observations support the

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concept that metabolic stress within injured tubules may be translated into purinergic signaling events that influence stromal activation and extracellular matrix production.

The proximal tubule is particularly susceptible to metabolic stress because it relies heavily on mitochondrial oxidative metabolism to meet its high energy demands, and tubular injury is accompanied by pronounced metabolic reprogramming involving fatty acid oxidation, oxidative phosphorylation, mitochondrial homeostasis, and redox balance [8–10]. Several studies have shown that metabolic responses initiated in stressed tubular cells are not purely intracellular adaptations, but can also generate extracellular signals that shape interaction within the renal microenvironment through purinergic pathways [11,12]. In this broader framework, injury-associated nucleotide release has been linked to inflammatory and profibrotic responses through activation of purinergic receptors on immune, vascular, and stromal cells [11,12]. Our data extend this concept by linking pyrimidine metabolic activation in proximal tubules with increased abundance of P2RY6-positive cells and enhanced calcium signaling within the fibrogenic interstitial compartment in human kidney disease, thereby validating in human transcriptomic data the mechanism we previously defined experimentally [6].

P2Y6R is a G-protein-coupled receptor whose endogenous agonist is extracellular UDP [13]. Purinergic signaling is widely acknowledged as an important regulator of renal inflammation and fibrosis. However, the exact cellular architecture of pyrimidinergic signaling in the injured human kidney remains inadequately characterized [11,12]. The present analysis addresses that gap. Rather than simply showing that P2RY6 is present in diseased tissue, our results indicate that P2RY6-positive cells become more prominent within the fibrogenic interstitial compartment during AKI and DKD, which is consistent with our earlier mechanistic work and suggests that the pathway identified experimentally is detectable in human disease at single-cell resolution [6].

An important aspect of our results is that the disease-associated stromal P2RY6 signal was not uniformly distributed, but was concentrated in ACTA2+ myofibroblasts and, to a lesser extent, matrix fibroblast (COL1A1+) states. This is biologically significant because it argues against a nonspecific stromal increase and instead points to selective enrichment within contractile and matrix-producing mesenchymal populations that are directly relevant to fibrogenic injury responses. The pattern fits well with emerging single-cell evidence showing that fibroblasts in kidney disease do not exist as a single terminal phenotype, but rather occupy multiple functional states. The enrichment of P2RY6 in myofibroblasts and matrix fibroblasts suggests that pyrimidinergic signaling may be especially relevant in mesenchymal populations engaged in active tissue remodeling.

Another important observation was the increase in calcium signaling activity within fibrogenic interstitial cells in AKI and DKD. This is especially relevant in light of our previous experimental observations, where P2Y6R activation triggered intracellular calcium responses and promoted fibroblast activation [6]. Although transcriptomic data cannot directly confirm receptor activation or real-time calcium flux, the fact that a calcium-related transcriptional program was elevated in the corresponding interstitial compartment of diseased human kidneys strengthens the biological continuity between the mechanistic and transcriptomic layers of the study.

Taken together, the present results support a unified model across our two studies. Our previous experimental study established the mechanistic framework, showing that tubular pyrimidine metabolism, extracellular UDP release, and P2Y6-mediated fibroblast activation form a causal fibrogenic pathway in experimental systems [6]. The present analysis provides the human validation layer, demonstrating that the same proximal tubule– fibrogenic interstitial pyrimidinergic axis is reflected in human kidney transcriptomes across disease contexts [6,7]. This study should be seen as a translational extension of our earlier work—it does not offer a new mechanism, but rather verifies that the fundamental pathway logic found experimentally is preserved in human disease.

The present study also underscores the value of human single-cell transcriptomic resources for validating mechanistic findings derived from experimental models. By using the human kidney atlas from Lake and colleagues, we were able to examine epithelial-stromal relationships with cell-type resolution in healthy and diseased kidneys, moving beyond bulk tissue associations and testing

 doi:10.20944/preprints202603.1435.v1

whether a specific signaling concept is supported within the relevant cellular compartments [7]. In this case, the answer is affirmative: human data support both the epithelial metabolic arm of the pathway and a disease-associated interstitial response centered on P2RY6-positive fibrogenic cells.

Several limitations should be acknowledged. Transcriptomic analyses capture gene expression levels rather than direct functional signaling activity, therefore our findings should be viewed as evidence of pathway consistency and relevance to human diseases rather than direct verification of extracellular UDP dynamics or receptor activation in vivo. Furthermore, currently available human single-cell kidney cohorts have a limited sample size and are clinically heterogeneous, and cross-sectional datasets cannot fully clarify the time sequence linking tubular damage, nucleotide release, interstitial activation, and matrix accumulation. Future studies integrating transcriptomics, metabolomics, and functional perturbation approaches in human tissue will be important to define this axis more precisely.

Despite these limitations, the consistency of our experimental findings with the current human transcriptomic data strongly supports the idea that pyrimidine metabolism and purinergic signaling are a previously overlooked link between tubular stress and stromal activation in kidney disease. From a translational perspective, this is especially relevant because P2Y6 is a G-protein-coupled receptor and therefore belongs to a therapeutically tractable class of targets. The present validation study thus strengthens the rationale for future work exploring P2RY6-related signaling both as a biomarker of fibrogenic activity and as a potential target for anti-fibrotic intervention. Overall, our findings support a model in which proximal tubular metabolic stress and stromal purinergic signaling are functionally connected components of the fibrotic response in human kidney disease. Most crucially, they provide independent human transcriptome support for our recently described experimental pathway, validating our conclusion that the proximal tubule-fibrogenic interstitial pyrimidinergic axis is a conserved and therapeutically relevant aspect of kidney fibrogenesis.

5. Conclusions

In conclusion, this study provides independent human transcriptomic support for the proximal tubule–fibrogenic interstitial pyrimidinergic axis that we recently identified in our experimental work. In that earlier study, we established a mechanistic link between injury-associated tubular pyrimidine metabolism, extracellular UDP signaling, and P2Y6-mediated fibroblast activation as a driver of renal fibrogenesis. The present analysis extends those findings into the human setting by showing that the same core biological pattern is detectable in diseased human kidneys at single-cell resolution. Specifically, we observed increased pyrimidine metabolism in proximal tubular cells together with expansion of the fibrogenic interstitial compartment, increased P2RY6 expression concentrated in ACTA2+ myofibroblasts and matrix fibroblasts, and enhanced calcium signaling within this mesenchymal niche, thereby providing translational validation of the pathway in human kidney disease.

Importantly, the consistency between our prior mechanistic experiments and the current transcriptomic analysis strengthens both the biological plausibility and the clinical relevance of this signaling axis. Rather than representing an isolated observation in experimental models, the proximal tubule–interstitial pyrimidinergic pathway appears to reflect a conserved component of kidney injury and fibrotic remodeling in humans. This convergence between experimental causality and human transcriptomic validation is particularly significant, because it suggests that pyrimidinergic signaling is not only mechanistically meaningful, but also potentially actionable in the context of disease progression. Taken together, our findings position the P2Y6-centered proximal tubule–interstitial signaling axis as a promising framework for future biomarker development, therapeutic targeting, and deeper investigation of fibrogenic cell–cell communication in kidney disease.

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Conflicts of Interest: The authors declare no conflicts of interest.

Abbreviations

The following abbreviations are used in this manuscript:

AKI Acute Kidney Injury
CKD Chronic Kidney Disease
DKD Diabetic Kidney Disease
ECM Extracellular Matrix
GEO Gene Expression Omnibus
PT Proximal Tubule
scRNA-seq Single-cell RNA Sequencing
UDP Uridine Diphosphate
P2Y6R P2Y6 Receptor
PDGFRB Platelet-Derived Growth Factor Receptor Beta
IQR Interquartile Range

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