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Cellular and Molecular Interrogation of Kidney Biopsy Specimens

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Abstract

Purpose of review—Traditional histopathology of the kidney biopsy specimen has been an essential and successful tool for the diagnosis and staging of kidney diseases. However, it is likely that the full potential of the kidney biopsy has not been tapped so far. Indeed, there is now a concerted worldwide effort to interrogate kidney biopsy samples at the cellular and molecular levels with unprecedented rigor and depth. This review examines these novel approaches to study kidney biopsy specimens and highlights their potential to refine our understanding of the pathophysiology of kidney disease and lead to precision-based diagnosis and therapy.

Recent findings—Several consortia are now active at studying kidney biopsy samples from various patient cohorts with state-of-the art cellular and molecular techniques. These include advanced imaging approaches as well as deep molecular interrogation with tools such as epigenetics, transcriptomics, proteomics and metabolomics. The emphasis throughout is on rigor, reproducibility and quality control.

Summary—While these techniques to study kidney biopsies are complementary, each on its own can yield novel ways to define and classify kidney disease. Therefore, great efforts are needed in order to generate an integrated output that can propel the diagnosis and treatment of kidney disease into the realm of precision medicine.

Keywords

Kidney biopsy; transcriptomics; large-scale imaging; tissue cytometry

Introduction

Since Nils Alwall performed the first aspiration needle kidney biopsy in 1944, traditional histopathology remains the cornerstone for biopsy-based diagnosis and classification of kidney disease (1, 2). Features of renal histopathology (e.g. fibrosis, crescents) can also guide therapy. Nevertheless, the response to therapy and clinical courses of individual

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patients are often unpredictable. Indeed, patients with identical diagnoses and similar pathologic features on their kidney biopsy specimens can have very dissimilar clinical courses. Therefore, a deeper examination of kidney tissue may reveal novel signatures at the cellular and molecular levels that allow a more detailed classification of renal disease. Such classification may explain the variable clinical courses of individual patients and allow for precision-based diagnostic and therapeutic approaches.

When properly handled and stored, a kidney biopsy specimen contains a wealth of information at the morphologic, cellular and molecular levels. Advances in large-scale imaging and tissue cytometry offer a novel view of kidney tissue that is both comprehensive and quantitative. In addition to classifying epithelial, stromal, immune and vascular cell types in situ, large-scale imaging techniques can also identify structural relationships in the complex architecture of the kidney. These structural relationships and their alteration in disease states can be key for a deeper understanding of renal pathophysiology. Similarly, molecular interrogation with tools such as single cell/nuclear/spatial transcriptomics, epigenetic modifications, regional proteomics and metabolomics will also refine our diagnostic spectrum and identify novel biomarkers and therapeutic targets (3**). It is hoped that the coordinated efforts of various consortia will lead to an integrated output that redefines the diagnosis and therapy of renal disease based on a spatially anchored multiplexed cellular and molecular reading of the kidney biopsy.

Text of Review

Novel technologies to interrogate kidney biopsy samples

A number of novel technologies have been utilized in kidney tissue to better understand the pathogenesis of kidney disease (Table 1). These technologies are summarized below, spanning from those which interrogate the kidney on the regional or structural level to the single cell level with and without the spatial context.

1. *Digital pathology*: Whole slide imaging and digitization is steadily transforming the field of pathology (4, 5). The availability of digital images on a cloud server for sharing and analysis have opened the way for image-based analytics and implementation of novel computational methods for automated segmentation and feature extraction. Some of the early efforts are focusing on segmenting structures such as glomeruli, tubules and the interstitium, which are also typically assessed by standard pathology (6–10). As novel machine learning tools are implemented, it is expected that sub-visual features could be automatically identified and linked with other molecular phenotypes. Hence, standard histopathology images could carry imaging-based biomarkers that will be potentially linked with molecular pathophysiology and clinical outcomes (5). Therefore, the field of pathology would further expand from diagnostics towards discovery.

2. *Single cell/ single nuclear RNA sequencing (Sc/snRNAseq)*: Driven by advances in microfluidics, cell-based RNA sequencing has revolutionized medicine and science by generating gene expression data at the level of the cells.

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Consequently, these technologies allow cataloging of molecularly defined cell populations and subpopulations in health, and the discovery of novel cell states induced by injury (11–15**). For example, recent data from the Kidney Precision Medicine Project consortium (KPMP) suggest the presence of various cell states (e.g. altered, degenerative, repairing) of proximal and thick ascending limb cells that could be associated with kidney disease progression (15**). Such important information at the cell level is generated from dissociated tissue, but can also be spatially anchored using other technologies, thereby enhancing our understanding of the pathogenesis of kidney disease, particularly in the heterogenous microenvironments within the human kidney (14–16).

3. *Large scale 3D imaging and tissue cytometry:* This is an imaging-based technology that relies on label free imaging with second harmonic generation for detecting fibrosis and confocal multiplexed fluorescence microscopy with optical sectioning to perform 3D imaging of a thick kidney section (17–21). Such imaging is followed by tissue cytometry analysis to survey and classify all cells based on fluorescent labels or other parameters such as spatial coordinates or neighborhood membership. Tissue cytometry is typically performed using a variety of specialized software. We have recently developed a custom open-source software tool (Volumetric Tissue Exploration and Analysis, VTEA) that allows the interactive quantitative exploration of 3D imaging data (17, 20, 21). VTEA analysis can provide quantitative measures about cell abundance and distribution, and offers spatial anchoring of cell-cell or cell- structure interactions based on spatial proximity. This could be performed in a supervised and unsupervised manner (18). In addition, the 3D aspect of the imaging allows better recognition of morphological changes and classification of injury, with a number of surveyed cells ranging between 100,000 to 250,000 per kidney thick section. Consequently, the data output with all the captured features results in a big data set, yielding findings with high confidence at the tissue level (17, 18). With advancement in enhanced tissue clearing techniques and light sheet microscopy, it is possible to image thicker sections of kidney tissue, which would allow a better capture of the morphological changes in kidney disease (20, 22). However, systematic implementation of such an approach will require specialized efforts to integrate segmentations of structures and morphometric analysis in 3D, and it will likely be costly from a computational standpoint.

4. *Co- detection by indexing (CODEX):* This technology expands the ability to multiplex markers on 10 μm-thick tissue sections using DNA-conjugated antibodies (23). With CODEX imaging, these antibodies are revealed three at a time by the reversible binding of fluorescent oligonucleotide reporters. Following imaging, the fluorescent reporters are stripped from the tissue and replaced with a second set of three probes and imaged again. This process is repeated until all the antibodies in the tissue have been revealed. Images of DAPI-labeled nuclei are collected in each round to enable registration of images into a single highly multiplexed image (23). More than 40 antibodies can be used in this process, and

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the analysis can be quite informative to determine cell type and subtype, albeit in the 2D space.

5. *Imaging Mass Cytometry (IMC):* This approach relies on chemical imaging whereby antibodies against specific targets are conjugated to various heavy metals which can be ionized using laser ablation at a 1 μm diameter spot size. Measuring the amount of ions is performed using time of flight mass spectrometry (24). Therefore, the metal isotopes associated with each spot are simultaneously measured and indexed against the location of each spot. Line scanning is performed across the tissue, and sequential scan lines will yield an intensity map of all target proteins throughout the tissue or the region of interest. This technology is performed on formalin fixed paraffin embedded tissue and has been successfully used in kidneys by Singh et al. using 22 different markers (25). IMC can also provide a powerful multiplexing potential for > 40 markers and be very useful in detecting cell types and subtypes spatially within the kidney.

6. *Spatial transcriptomics*: Spatial transcriptomics technologies provide whole transcriptome mRNA expression with localization. Sections of frozen or formalin-fixed paraffin embedded kidney tissue are permeabilized on a specialized slide. RNA diffuses to barcoded beads underlying the tissue, after which a cDNA library is prepared and sequenced. The barcodes allow reconstruction of the spatial distribution of mRNA, aligning each unique molecular identifier to a “spot”. Multiple spatial transcriptomic platforms are available. The Visium spatial gene expression platform facilitates the mapping of over 20,000 genes detected per sample and up to 3,000 genes per spot directly over a histologic image stained with hematoxylin and eosin. This allows direct correlation of mRNA expression with renal pathology (26). Presently, the spot sizes are 55 μm in diameter. Thus, multiple cell types contribute to the expression signature of each spot. In contrast, Slide-seqV2 has greater resolution with spot sizes of 10 μm diameter or nearly single cell resolution (27*), but histology is obtained from a consecutive section. Both technologies can localize clusters and cell types derived from single cell technologies by integrating their transcriptomic signature with that of spatial transcriptomics.

7. *Regional transcriptomics*: This technology uses laser microdissection (LMD) to capture spatially defined regions in the kidney and perform transcriptomic analysis with RNA sequencing (28, 29). Using markers such as fluorescently conjugated small molecules or antibodies targeting specific cell types, LMD can faithfully dissect specific nephronal segments such as glomeruli, proximal tubules, thick ascending limbs, distal tubules/collecting duct, and the interstitium. A pipeline for RNA extraction, cDNA library generation and sequencing has been established leading to high depth sequencing data that can be used to uncover the transcriptomic signature of each of these renal regions in health and disease (28, 29). Such information could provide a spatial anchor to data obtained by dissociative technologies such as Sc/snRNA seq.

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8. *Metabolomics* : Kidney molecular imaging with spatial metabolomics can be achieved using matrix-assisted laser desorption/ionization (MALDI) mass spectrometry imaging (MSI) (30). Fresh-frozen kidney sections are coated with an organic matrix that facilitates desorption and ionization of endogenous molecules, and serially probed with a laser to attain mass spectral information at predefined locations. Molecular annotation coupled with data visualization and co-registration with other optical images is an important component of data processing and can be accomplished by software tools such as the METASPACE platform (developed by the EMBL) (31).

9. *Regional proteomics*: This technology delivers detailed characterization of the kidney proteome in health and disease, identifying protein markers that reflect each segment, matrix, and cell type within the tissue. LMD followed by liquid chromatography tandem mass spectrometry (LC-MS/MS) allows for direct analysis of complex protein mixtures to generate an agnostic global profile of glomerular and tubulointerstitial (TI) proteins (32–34). Both cellular and extracellular proteins are identified. The glomeruli and TI are obtained after structural identification by light microscopy on the LMD instrument. After protein extraction, peptide identification is done using mass spectrometry. Regional proteomics offers deep protein signatures at compartment level resolution that extends other spatially anchored technologies. When combined with technologies such as spatial transcriptomics, simultaneous gene and protein expression changes can be determined in the same sample, thereby identifying high confidence targets in health and disease (35).

10. Epigenetic interrogation methods – Gene expression is tightly regulated and adapts to physiological stresses and injury in kidney disease. Activation or silencing of gene transcription can be regulated by changes in the chromatin landscape. Three epigenomic technologies which facilitate the measurement of active, silent, or poised chromatin include Whole Genome Bisulfite Sequencing (WGBS), Cleavage Under Targets and Release Using Nuclease (CUT&RUN), and Assay for Transposase-Accessible Chromatin using sequencing (ATAC-seq). WGBS measures DNA methylation across the genome. Methylation signatures can be altered by developmental programming in utero (36, 37), metabolic or environmental insults (38), or kidney injury and disease (39). CUT&RUN lends itself to kidney biopsy sample interrogation as it can define the chromatin landscape with genome-wide mapping in a small amount of tissue as compared to Chromatin immunoprecipitation with sequencing (ChIP-seq). Chromatin profiles with histone modifications are assessed such as those of H3K27me3 (often silencing) and H3K27ac (often activating). Finally, single cell ATAC-seq identifies areas of open chromatin in specific cell clusters (15, 40). These regions represent potentially active regulatory regions; however, silenced regulatory regions often remain unidentified due to a lack of reads in these regions. Each of these three technologies can help to explain gene expression regulation within the kidney.

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Kidney Precision Medicine Project (KPMP) –

One of the important challenges when applying various state of the art technologies on kidney tissue samples is to integrate the data obtained in a meaningful and interpretable manner that could provide actionable information (3**, 15**, 35, 41). For example, novel cell types and cell states uncovered by one technology should be spatially anchored and linked to a logical site within the kidney microenvironment that could explain the changes induced by disease (15). Also, these findings are optimally connected to changes in pathology and possibly others such as injury markers, signaling pathways and metabolites. Ultimately, changes at the molecular, cell and tissue levels should be linked with enhanced clinical phenotypes of the disease. The array of technologies that are used in the KPMP consortium allows Integration at various biological units: at the single cell, structure and biopsy levels (Figure 1). Integration can also occur in various ways, and this is facilitated by the fact that many technologies are performed from contiguous sections of the same biopsy core specimen (15**). Therefore, integration theoretically can occur 1) by spatial registration, which superimposes information from one technology to another based on sharing the same tissue space; 2) by alignment in the analytical space, which could allow data from various technologies to be analyzed concurrently once they are converted to a common denominator (if feasible) and 3) by a systems biology approach where dynamic pathway enrichment analyses and network mapping to biological processes critical to renal pathophysiology can be done concurrently with various technologies. A big challenge in this area is the required domain expertise in multiple technologies and in computational methods of integration across various technologies. For this reason, a large consortium such as KPMP is indeed needed, where collaborative expertise from various domains converge with clinical and pathology expertise (3**, 41).

Consortium contributions

Other important consortia and groups have sought to better understand the pathogenesis of kidney disease through biopsy specimen or nephrectomy interrogation. Often, each has a specific disease focus or uses different approaches to acquire and study kidney tissue. The Chan-Zuckerberg initiative’s Human Cell Atlas (HCA) and the NIH’s Human BioMolecular Atlas Program (HuBMAP) both seek to establish a reference atlas of all cells (HCA) and organs (HuBMAP) within the body. Several groups have adopted multi-omics approaches to study diabetic kidney disease, including the Transformative Research In DiabEtic NephropaThy (TRIDENT) consortia, the European cDNA bank, and new molecular studies in the Pima Indian diabetic kidney disease cohort. Biopsy interrogation of glomerulonephritidies has been undertaken by the Nephrotic Syndrome Study Network (NEPTUNE), Cure Glomerulonephropathy Network (CureGN), the European cDNA bank, and the Accelerating Medicines Partnership (AMP)-RA/SLE consortia. It is not possible to summarize all of the major contributions these groups have made to the understanding of renal disease pathogenesis here. However, brief introductions and key findings are provided below to help orient the reader to the immense body of literature available (Table 2).

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Conclusion

The novel interrogation techniques described above are ushering in a new era in the diagnosis and treatment of kidney disease. At this early stage, these interrogation techniques are best implemented and validated though organized large consortia. This is essential for rigor, validation, quality control and integration. It is hoped that the output from these consortia will redefine readouts from kidney biopsies and propel nephrology into the realm of precision medicine.

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Key points:

1. Kidney biopsy samples have and untapped potential that can be realized through novel interrogation techniques.
2. These imaging, cellular and molecular techniques are best investigated through organized consortia that allow for validation and integration
3. Novel interrogation techniques of the kidney tissue will reshape the diagnosis and classification of kidney disease and point the way to precision renal medicine.

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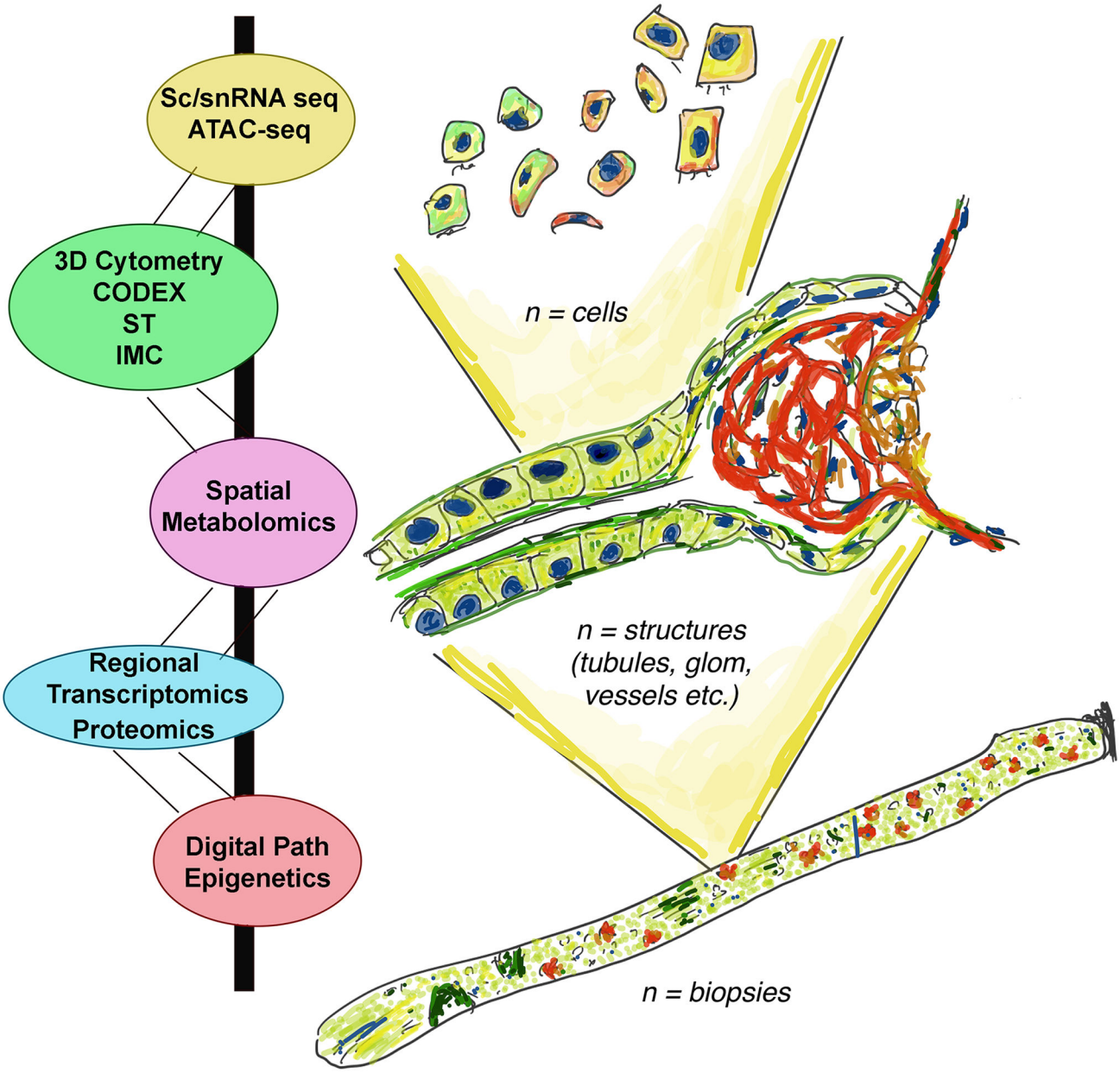

Figure 1:

Technology integration in the KPMP.

Schematic of integration of various technologies used in the KPMP consortium at the various biological units within the kidney: cells, structures, entire biopsy specimens. Single cell single nuclear RNA sequencing: Sc/snRNA seq; Assay for Transposase-Accessible Chromatin using sequencing: ATAC-seq; Digital Pathology: Digital Path; Co-detection by Indexing: CODEX; Spatial transcriptomics: ST; Imaging mass cytometry: IMC.

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Table 1:

Renal biopsy specimen interrogation technologies

Technology	DNA, RNA, Protein, metabolite	Tissue preservation	Spatial data (2D/3D)	Advantages	References
Sc/snRNA seq	RNA	OCT Frozen or Cryostor preservative	No	single cell gene expression at high resolution	[11–13, 15]
ATAC-seq	DNA	OCT Frozen	No	Regulation of gene expression at cell level	[15, 40]
Regional transcriptomics	RNA	OCT frozen	Yes (2D)	High sequencing depth for specific renal region	[28, 29, 35]
Regional proteomics	Protein	OCT frozen	Yes (2D)	Agnostic proteomics 3000–5000 proteins for specific renal regions	[35]
Spatial transcriptomics	RNA	OCT frozen	Yes (2D)	High sequencing depth with spatial resolution	[14–16, 27]
Spatial metabolomics	metabolites	Liquid nitrogen	Yes (2D)	Regional distribution of metabolites	[35]
3D tissue Cytometry	Protein	OCT frozen	Yes (3D)	8-plex, cell distributing and abundance, 3D neighborhood analysis	[15, 17, 21]
CODEX	Protein	OCT frozen	Yes (2D)	Multiplexing 10–60 probes	[14, 35]
IMC	Protein	FFPE	Yes (2D)	Multiplexing 10–60 probes	[25]
WGBS	DNA	OCT frozen	Yes (2D)	DNA methylation regulates gene expression across the genome	[3, 38]
CUT & RUN	DNA	OCT frozen or Liquid nitrogen	No	Assesses active, poised, and silent chromatin regions	[42]

Single cell single nuclear RNA sequencing: Sc/snRNA seq; Assay for Transposase-Accessible Chromatin using sequencing: ATAC-seq; Digital Pathology: Digital Path; Co-detection by Indexing: CODEX; Spatial transcriptomics: ST; Imaging mass cytometry: IMC.

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Table 2:

Major consortia dedicated to renal biopsy specimen interrogation

Kidney biopsy specimen interrogation group	Disease state focus	Unique features	References
Kidney Precision Medicine Project (KPMP)	AKI, CKD	Prospective study using protocol biopsies in subjects with AKI and CKD who may not have clinical indications for a biopsy.	[3, 41]
Transformative Research In DiabEtic NephropaThy (TRIDENT)	Diabetic Kidney Disease	Prospective observational cohort study of patients with diabetic kidney disease undergoing clinically indicated biopsies interrogated with multi-omics approaches.	[43–46]
Nephrotic Syndrome Study Network (NEPTUNE)	MCD, FSGS, MN	A prospective cohort of subjects with nephrotic syndrome with molecular kidney biopsy interrogation and long-term observation of 3–10 years.	[13, 47, 48]
PIMA Indian studies	Diabetic Kidney Disease	An extensively characterized cohort of diabetic kidney disease followed for over 50 years.	[49, 50]
Cure Glomerulonephropathy Network (CureGN)	MCD, FSGS, MN, IgA	A multi-center consortium that recruits a diverse population of glomerular disease patients. The study established a database of patients and follows clinical outcomes linked with histopathology.	[51–53]
Accelerating Medicines Partnership (AMP)-RA/SLE	Lupus Nephritis	An NIH and pharmaceutical partnership dedicated to define disease-specific pathways of rheumatoid arthritis and lupus. Molecular interrogation of circulating and resident kidney cells is performed.	[54, 55]
European cDNA bank	Diabetic Kidney Disease and other disease states	A multicenter European archive of human kidney biopsies specially processed for transcriptomic analysis.	[56, 57]
Human BioMolecular Atlas Program (HuBMAP)	Healthy reference	HuBMAP aims to develop a molecular map of the human body at the cellular level, with spatial context.	[58, 59]
Studies in transplantation[1]	Acute and chronic rejection	While not a single consortium, these studies sought to understand the pathogenesis of allograft failure	[60–65]
Chan-Zuckerberg Initiative (Human Cell Atlas)	Healthy reference	CZI seeks to create a comprehensive, open-reference map of every cell type in the body.	[66]

[1] Studies in transplantation does not refer to a consortium, but recognizes seminal work in renal transplant biopsy specimen interrogation.

AKI – acute kidney injury, CKD – chronic kidney disease, MCD – minimal change disease, FSGS – focal segmental glomerulosclerosis, MN – membranous nephropathy, IgA – Immunoglobulin A nephropathy, RA – rheumatoid arthritis, LSE – systemic lupus erythematosis.