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Towards Modelling Genetic Kidney Diseases with Human Pluripotent Stem Cells

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Abstract
Background: Kidney disease causes major suffering and premature mortality worldwide. With no cure for kidney failure currently available, and with limited options for treatment, there is an urgent need to develop effective pharmaceutical interventions to slow or prevent kidney disease progression. ***Summary:*** In this review, we consider the feasibility of using human pluripotent stem cell-derived kidney tissues, or organoids, to model genetic kidney disease. Notable successes have been made in modelling genetic tubular diseases (e.g., cystinosis), polycystic kidney disease, and medullary cystic kidney disease. Organoid models have also been used to test novel therapies that ameliorate aberrant cell biology. Some progress has been made in modelling congenital glomerular disease, even though glomeruli within organoids are developmentally immature. Less progress has been made in modelling structural kidney malformations, perhaps because sufficiently mature metanephric mesenchyme-derived nephrons, ureteric bud-derived branching collecting ducts, and a prominent stromal cell population are not generated together within a single protocol. ***Key Messages:*** We predict that the field will advance significantly if organoids can be generated with a full complement of cell lineages and with kidney components displaying key physiological functions, such as glomerular filtration. The future economic upscaling of reproducible organoid generation will facilitate more widespread research applications, including the potential therapeutic application of these stem cell-based technologies.

Introduction

End stage renal disease (ESRD) affects millions of individuals worldwide. With dialysis and transplantation being costly life-saving treatments that are not always available in every country [1], there is an urgent need to find new treatments for primary kidney diseases. Up to a few years ago, researchers have been limited to studying the following: cell lines (mesangial cells, tubular cells, and podocytes) grown from native human kidneys; mutant mice; and experimental animals exposed to insults such

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as nephrotoxins, altered diets, renal ischaemia, or urinary flow obstruction. While these studies have provided considerable insights, another strategy would be to create diseased human kidneys in the laboratory. The models could then be used to study pathobiology and as test beds for identifying new treatments.

Human pluripotent stem cell (hPSC) technology provides an option towards this ideal, and we here review how far so-called "kidney organoids," the developing kidney-like tissues derived from hPSCs, can model certain genetic kidney diseases. Table 1 lists both the disease genes and models we detail in this review, as well as other reports of hPSC-kidney organoid disease models [2–15]. PSCs have the ability to indefinitely replicate themselves and also to differentiate into cells of the 3 main tissue layers found in the embryo (mesoderm, endoderm, and ectoderm) and then to specific cell types found in the mature organism. Two main sources of PSCs have emerged: embryonic stem cells (ESCs) and, more recently, induced pluripotent stem cells (iPSCs). Human ESCs are harvested from early human embryos that are produced by in vitro fertilization: these are generally excess embryos that are not used to initiate a pregnancy. HiPSCs are made by reprogramming adult cells, for example, those harvested from blood samples or skin biopsies.

Several differentiation protocols have been described that drive hPSCs to intermediate mesoderm (IM) and then to kidney-like tissues in culture [9, 11, 16–18]. Ultimately, these or similar hPSC protocols may be used to generate functional kidney tissues that could be implanted into patients with ESRD and the reader is referred to recent reviews that address whether organoids are fit for purpose for such regenerative medicine therapies [19–21].

The Causes of Congenital Kidney Disease

Renal tract (i.e., kidney and/or urinary tract) malformations (Fig. 1) account for around a third of all congenital birth defects [22]. Moreover, approximately half of all children [23] and a quarter of young adults [24] with ESRD are born with structurally abnormal kidneys. The most severe malformation is renal agenesis, when the embryonic kidney does not form [25]. The next most severe is renal dysplasia, where the kidney begins to form but contains immature and metaplastic tissues [25]. The mildest anatomic defect is renal hypoplasia, where the organ contains fewer glomeruli than normal, which predisposes individuals to hypertension and renal functional impairment in later life [26, 27]. In other young children with ESRD, the kidney appears anatomically intact but there is a failure of terminal differentiation of specific cell types. Examples include congenital nephrotic syndrome, where podocytes fail to mature, and early onset tubulopathies, where terminal differentiation of nephron tubules or collecting ducts is incomplete. It is known that some of these diseases are caused by mutations of specific genes expressed during normal kidney development and differentiation [28]. While it is possible that all individuals born with abnormal kidneys will be found to harbour mutations, studies of patient cohorts have detected convincing pathogenic gene variants in only a minority [29, 30]. Human epidemiological and rodent experimental studies reveal that a range of environmental perturbations can disturb metanephric development, including altered maternal diet, placental insufficiency/hypoxia, and teratogens such as retinoic acid and angiotensin inhibitors [31]. It remains to be established whether these harmful effects are mediated by direct toxicity or more subtle mechanisms such as by influencing epigenetic regulation [32]. As yet, human kidney organoid technology has not been used to model environmental perturbations and this would be an interesting direction for future research.

Kidney Development

Given that the above diseases are caused by aberrant kidney development and that the generation of kidney organoids from hPSCs aims to recapitulate normal kidney development, it is worth here outlining the main developmental stages (Fig. 2). In mammals, the definitive kidney is called the metanephros. It initiates when the ureteric bud (UB) epithelial tube, itself arising from the anterior IM, sprouts from the Wolffian duct and grows into a zone of posterior IM that condenses around the bud to form the metanephric mesenchyme (MM) [16]. The MM forms nephrons, while the UB tip branches serially to form the collecting duct system (Fig. 2a). In humans, the metanephros initiates at 5 weeks of gestation, and new layers of nephrons form until 34 weeks of gestation [25]. Classic organ culture experiments using mouse metanephric kidneys suggested that reciprocal signalling between the MM and UB causes their initial growth and differentiation, and when either tissue was cultured on its own, development stopped and the cells died [33]. Studies with mutant mice have pinned down specific molecules that mediate these processes. For example, it is now known that the MM secretes growth

Table 1. hPSC-kidney organoid disease models

Affected gene	Associated disease	Mutant line generation	Organoid disease characteristics	Disease intervention	Ref.
CTNS	Cystinosis	Both patient-derived and CRISPR-Cas9 gene-edited biallelic mutant hiPSC lines	Cystine accumulation, lysosome enlargement, and reduced basal autophagy flux	Using cysteamine with everolimus more completely corrects the phenotype than monotherapy	[2]
HNF1B	Renal cysts and diabetes syndrome	CRISPR-Cas9 gene-edited biallelic mutant hiPSC line	Reduced HNF1B protein and reduced proximal tubule and thick ascending limb markers	Not reported	[3]
HNF1B	Renal cysts and diabetes syndrome	CRISPR-Cas9 gene-edited heterozygous mutant iPSC line	Reduced UB-derived tubule branching	Not reported	[4]
IFT140	Nephronophthisis	Patient-derived biallelic mutant hiPSC line	Short club-shaped cilia and perturbed expression of molecules associated with epithelial cell apicobasal polarity, proliferation, and cellular junctions	CRISPR-Cas9 corrected isogenic lines produce organoids with a rescued disease phenotype	[5]
MUC1	Tubulo-interstitial kidney disease	Patient-derived heterozygous mutant hiPSC lines	Misfolded mutant MUC1 protein shows intracellular accumulation in tubule epithelia instead of normal apical localization	Treatment with small molecule BRD4780 redirects misfolded MUC1 proteins to the lysosome for degradation	[6]
NPHS1	Congenital nephrotic syndrome	Patient-derived biallelic mutant hiPSC line	Reduced nephrin expression and the protein does not localize to the surface of podocyte-like cells. Implanted cells lack slit diaphragms	CRISPR-Cas9 corrected isogenic lines produce organoids with a rescued phenotype	[7]
		Patient-derived biallelic mutant hiPSC line	Reduced levels of nephrin and podocin slit diaphragm proteins and large hypertrophied podocyte bodies detected by scanning electron microscopy	Not reported	[8]
PAX2	Renal coloboma syndrome	TALEN-gene-edited biallelic mutant hiPSC line	Reduced UB branching morphology	Not reported	[9]
PKD1	ADPKD	CRISPR-Cas9 gene-edited biallelic mutant hESC line	Infrequent cyst-like structures formed from organoid proximal tubules with a higher frequency when organoids were maintained in suspension cultures	Not reported	[10, 11]
		Both heterozygous patient-derived lines and heterozygous and biallelic CRISPR-Cas9 gene-edited mutant hiPSC lines	Cysts formed from both nephron progenitor and UB/collecting duct tubules in organoids after cAMP stimulation	Not reported	[12]
		Both patient-derived heterozygous lines and heterozygous and biallelic CRISPR-Cas9 gene-edited mutant hiPSC lines	Cysts formed from proximal tubules following cAMP stimulation	Cystogenesis ameliorated when cAMP-stimulated mutant organoids were treated with CFTR inhibitor 172 or everolimus	[13]
PKD2	ADPKD	CRISPR-Cas9 gene-edited biallelic mutant hESC line	Infrequent cyst-like structures formed from organoid proximal tubules with a higher frequency when organoids were maintained in suspension cultures	Not reported	[10, 11]

Table 1 (continued)

Affected gene	Associated disease	Mutant line generation	Organoid disease characteristics	Disease intervention	Ref.
PKDH1	ARPKD	Patient-derived biallelic mutant hiPSC line	Cysts formed from proximal tubules following cAMP stimulation	CRISPR-Cas9 corrected isogenic lines produce organoids with ameliorated cystogenesis. Cystogenesis was reduced when cAMP-stimulated mutant organoids were treated with thapsigargin or a CFTR inhibitor	[14]
PODXL	Early-onset nephrotic syndrome	CRISPR-Cas9 gene-edited biallelic mutant hESC line	Mutants exhibited a lack of microvilli on apical and lateral podocyte cell membranes and reduced lateral spacing	Not reported	[15]
		CRISPR-Cas9 gene-edited biallelic mutant hESC line	Defective junctional organization and decreased gaps between adjacent podocytes	Not reported	[11]

ADPKD, autosomal dominant polycystic kidney disease; ARPKD, autosomal recessive polycystic kidney disease; cAMP, cyclic adenosine monophosphate; CFTR, cystic fibrosis transmembrane conductance regulator; *HNF1B, hepatocyte nuclear factor 1B;* hPSC, human pluripotent stem cell; iPSC, induced pluripotent stem cell; *MUC1, Mucin-1; PAX2, paired box 2; PKD, polycystic kidney disease*; *PODXL*, *podocalyxin*; UB, ureteric bud.

factors (e.g., glial cell line-derived neurotrophic factor [GDNF]) that act in a paracrine manner to stimulate the initiation of the UB and its serial branching [34, 35]. Here, optimal signalling is facilitated by the correct composition of the extracellular matrix surrounding the UB [36, 37]. Meanwhile, signals from the arborizing bud, with Wnt9b being a key player, induce nephron formation from the MM [38]. MM cells near the branch tips condense to form aggregates which undergo a mesenchymal-epithelial transition to generate renal vesicles, consisting of a simple epithelium surrounding a lumen. The vesicles undergo morphogenesis via comma-shaped then S-shaped bodies to form capillary loop-stage nephrons (Fig. 2b). The proximal end comprises a collection of cells, the precursors of the podocytes, that become invaded by capillaries and mesangial cells to form the filtering tuft; the middle section forms the Bowman's capsule and proximal tubule; while the distal section of the nephron forms the loop of Henle and distal convoluted tubule, the latter fusing with UB-derived collecting ducts (Fig. 2c).

hPSC Technology to Model Kidney Malformations

Autopsies of human fetuses with renal agenesis typically fail to detect either a kidney or a ureter, and it has long been speculated that the cause is a failure of UB initiation and/or initial induction between the UB and the MM [25]. Mutations of genes expressed in the initiating metanephros have been identified in such individuals. They include *rearranged during transfection proto*-oncogene (*RET*) [39] that encodes the cell surface receptor tyrosine kinase for GDNF, itself made by MM cells; and *Fraser syndrome 1* (*FRAS1*) and *FRAS1-related extracellular matrix protein 2* (*FREM2*), which encode proteins that associate with and coat the surface of the UB and that facilitate the actions of MM-derived paracrine factors [36, 37]. Thus far, renal agenesis has not been studied using hPSC technology, and a potential pitfall here is that the majority of protocols tend to generate MM derivatives (e.g., glomeruli and proximal tubules) rather than the UB/collecting duct lineage as demonstrated by the powerful technique of analysing organoids with single-cell RNA sequencing [40]. Early progress, however, is being made in using hPSCs to generate organoid models containing branching UB-derived collecting ducts. Taguchi and Nishinakamura [9] employed a reverse induction approach using mouse fetal kidneys to refine their protocol, which exploited the distinct origin of MM and UB, whereby hPSCs were differentiated through anterior IM towards UB identities. In their UB/collecting duct organoids, dichotomous branching morphology was observed in epithelia expressing characteristic collecting duct-like markers. They also showed that experimental biallelic deletion of the *paired box 2* (*PAX2*) transcription factor

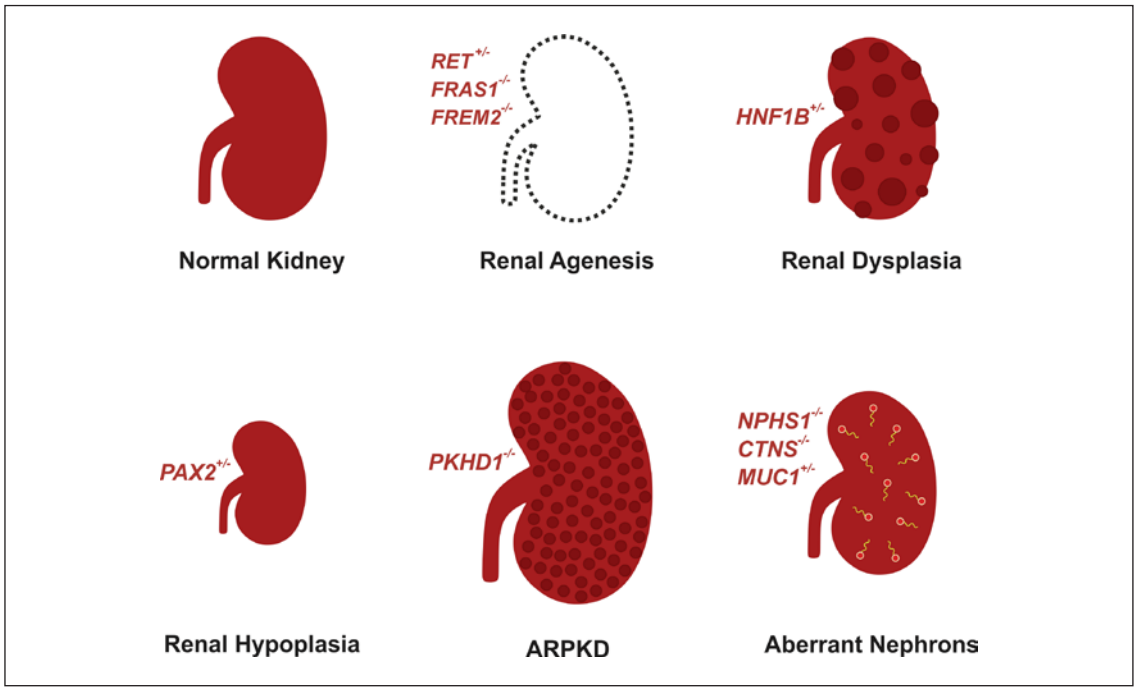

Fig. 1. Genetic kidney disease. A schematic representation of several genetic kidney malformations and examples of associated mutations that have been identified in a number of patients. In renal agenesis, the kidney does not form and has been associated with biallelic *FRAS1* and *FREM2* mutations and heterozygous *RET* mutations. Renal dysplasia includes multicystic kidneys, often caused by heterozygous *HNF1B* mutations. Renal hypoplasia is characterized by small kidneys with a reduced nephron number but with correct structural morphology and is often associated with heterozygous *PAX2* mutations. ARPKD is caused by biallelic mutations in *PKHD1* and manifests as an enlarged kidney containing multiple small cysts within tubules and collecting ducts. Other genetic disorders result in kidneys with aberrant terminal differentiation of glomeruli and tubules. Examples include congenital nephrotic syndrome, which disrupts the slit diaphragms of the glomerular filtration barrier and can be caused by biallelic *NPHS1* mutations; nephropathic cystinosis, which affects proximal tubule structure and function and is caused by biallelic mutations in *CTNS*; and *MUC1* disease, which affects tubule and collecting duct structures and is caused by heterozygous mutations in *MUC1*. ARPKD, *autosomal recessive polycystic kidney disease; FRAS1, Fraser syndrome 1; FREM2, FRAS1-related extracellular matrix protein 2; HNF1B, hepatocyte nuclear factor 1B; MUC1, Mucin-1; PAX2, paired box 2; RET, rearranged during transfection proto-oncogene.*

gene severely perturbed UB branching as had been observed in renal agenesis mice carrying biallelic mutation of this gene [41]. It is noteworthy that humans with biallelic *PAX2* mutations have never been recorded, probably because this is lethal in early embryogenesis; instead, a subset of people with renal hypoplasia carry heterozygous mutations of *PAX2* [42]. A further study utilized UB/collecting duct organoids to model autosomal dominant polycystic kidney disease caused by mutations in *polycystic kidney disease 1 (PKD1)* [12], which in patients features cystogenesis in both nephron and collecting duct tubules. Biallelic gene-edited mutant organoids displayed cyst formation following cyclic adenosine monophosphate (cAMP) activation; indeed, cAMP signalling is implicated in polycystic kidney disease (PKD) cystogenesis [43]. Cyst formation was also observed, albeit to a smaller degree, in cAMP-stimulated heterozygous *PKD1*-mutant organoids produced from gene-edited and patient hiPSCs [12]. Importantly, however, the UB-derived tubules that form in this protocol remain relatively undifferentiated and do not display a mature collecting duct molecular or physiological phenotype.

Using a similar approach by differentiating through anterior IM, Mae and colleagues [4] produced 3D branching UB/collecting duct organoids containing tubular lumens with overt apical and basal polarity. They used this method to generate a malformed UB tree from hPSCs with an experimentally-induced *hepatocyte nuclear factor 1B* (*HNF1B*) heterozygous mutation. This transcription factor is expressed in both fetal collecting ducts and nephron tubules [44], and heterozygous mutations are the most common known genetic cause of human cystic dysplastic kidneys [45]. The *HNF1B*$^{+/-}$ UB/collecting duct organoids displayed reduced budding regions and a loss

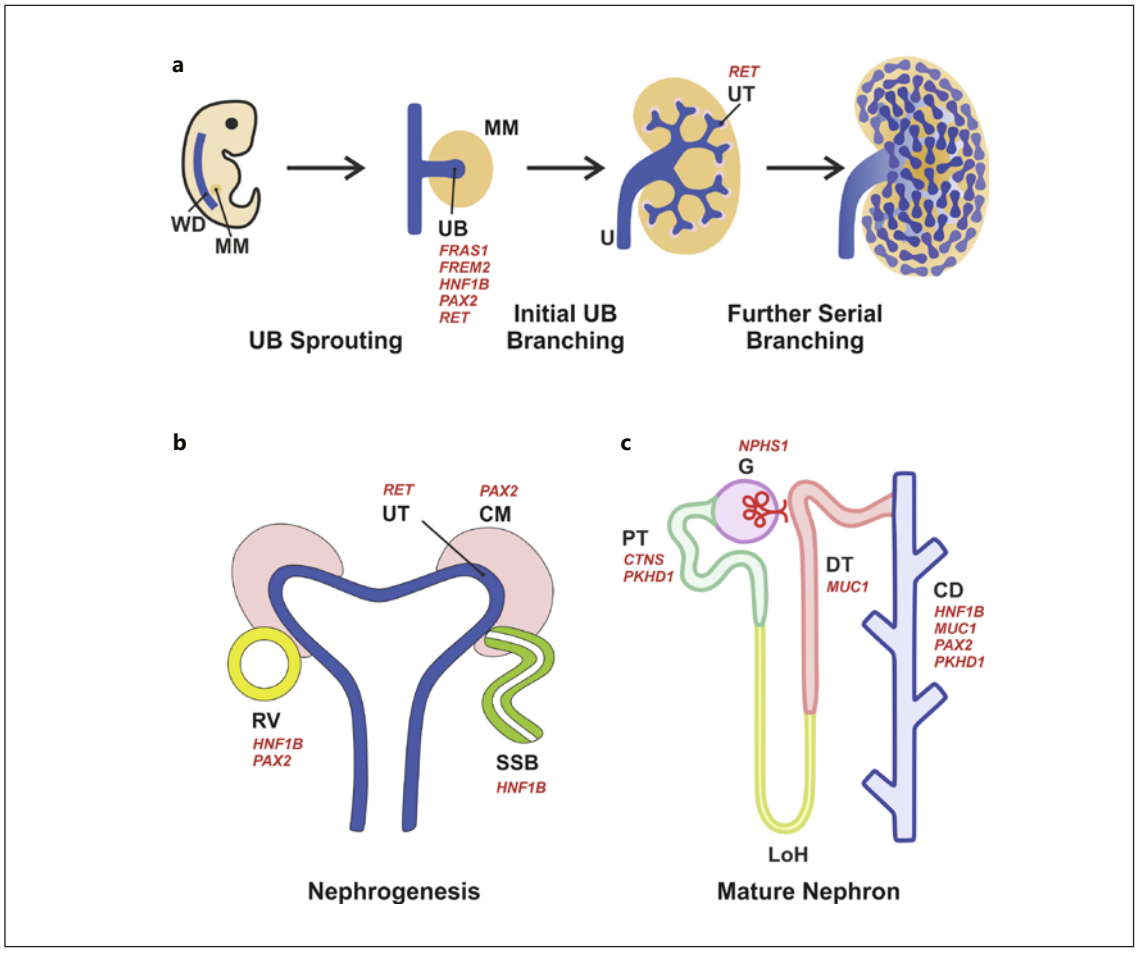

Fig. 2. Human metanephric kidney development. All frames are annotated with the normal expression pattern of selected genes with disease producing mutations. **a** UB/collecting duct branching morphogenesis. At week 5 of human gestation, the UB sprouts from the WD into the adjacent MM. The UB then undergoes serial branching up until week 34 of gestation. The rightmost diagram depicts the UTs within a 3-dimensional metanephros. The genes *FRAS1, FREM2, HNF1B, PAX2*, and *RET* are expressed by the UB, and *RET* expression is retained in the UTs. **b** The UTs supports both the CM, which contains nephron progenitor cells, and nephrogenesis. A RV and SSB nephron precursor structures are shown. *RET* is expressed in the UT, *PAX2* is expressed in the CM and RV, and *HNF1B* is expressed by the nephron precursors. **c** A healthy mature nephron containing a vascularized G, PT, LoH, DT, and a CD. Podocyte cells in the glomeruli express *NPHS1,* the PT expresses *CTNS* and *PKHD1,* the DT expresses *MUC1* and the CD expresses *HNF1B*, *MUC1*, *PAX2*, and *PKHD1*. UB, ureteric bud; WD, Wolffian duct; MM, metanephric mesenchyme; *FRAS1, Fraser syndrome 1; FREM2, FRAS1-related extracellular matrix protein 2; RET, rearranged during transfection proto-oncogene; HNF1B, hepatocyte nuclear factor 1B; PAX2, paired box 2;* U, ureter; UT, ureteric bud branch tips; CM, cap mesenchyme; RV, renal vesicle; SSB, S-shaped body; G, glomerulus; *NPHS1, Nephrin*; PT, proximal tubule; LoH, loop of Henle; DT, distal tubule; *MUC1, Mucin-1*; CD, collecting duct.

of apicobasal polarity. No cysts were seen in this model although the group did not investigate the effects of cAMP stimulation. Another group showed that biallelic *HNF1B* mutations caused dysmorphic nephrons in kidney organoids containing predominately MM derivatives [3]. However, in humans, biallelic *HNF1B* mutations have never been described, and whether mutation of a single allele results in malformed nephrons in MM kidney organoids awaits further study. A more complete organoid model of human *HNF1B*-associated kidney disease would ideally simultaneously differentiate both nephrons and collecting ducts thus addressing pathobiology in both using heterozygous mutant cells.

Efforts to combine separately derived UB and MM populations from mouse PSCs, along with mouse stromal cells, have yielded promising results, whereby kidney or-

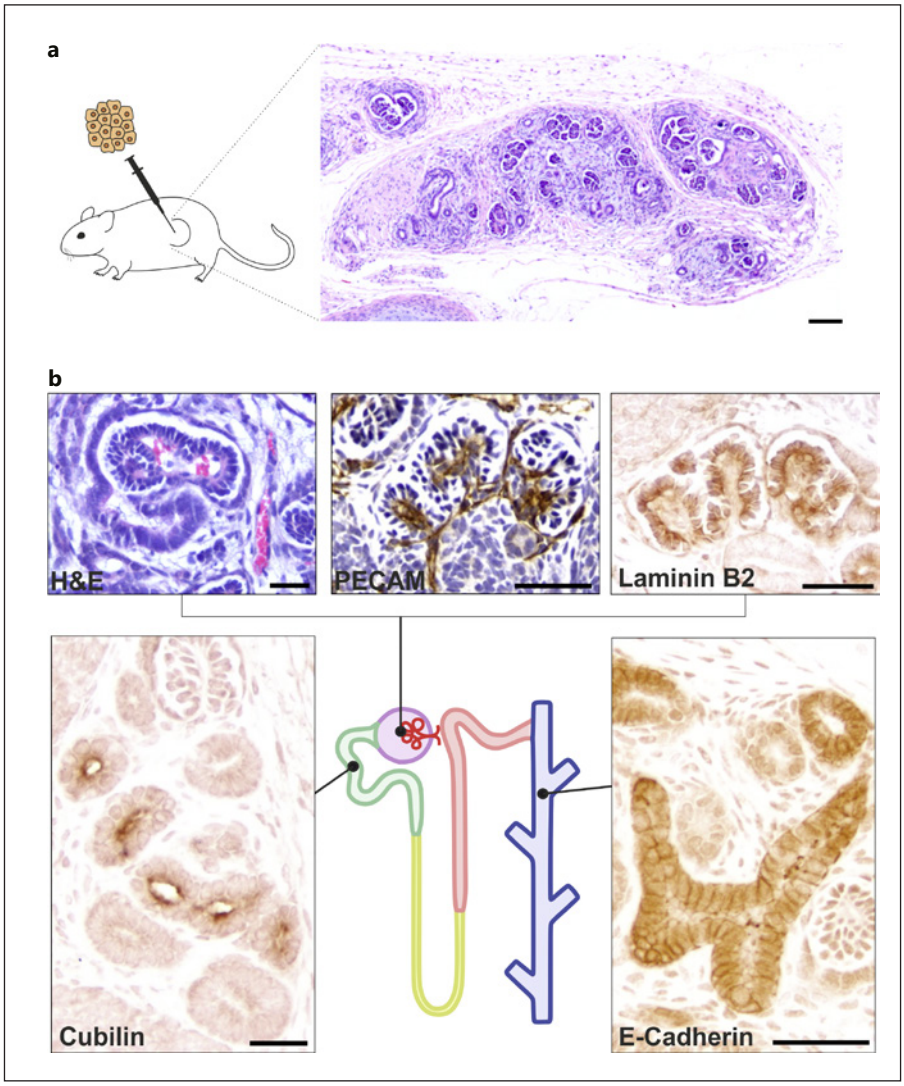

Fig. 3. Human kidney organoid implants. **a** A low power image of a H&E-stained section of a 3-month subcutaneous hPSC-kidney tissue implant. **b** High-power images of the implants show maturation of glomeruli with vascular tufts. The top left to right images, respectively, show the following: H&E staining of red blood cells in a glomerular tuft and in a vessel between tubules; PECAM immunostaining of glomerular capillaries; and Laminin B2 immunostaining of the glomerular basement membrane. Cubilin and E-cadherin immunostaining highlights proximal and distal tubules/collecting duct structures respectively. Scale bars, 100 μm (**a**) and 50 μm for all images in (**b**). H&E, haemotoxylin and eosin; hPSC, human pluripotent stem cell. Images are adapted from Bantounas et al. [47] via Creative Commons Attribution License (CC BY).

ganoids contain an extensively branched UB/collecting duct lineage that supported both the maintenance of nephron progenitor cell populations and nephrogenesis [9]. Combination of separately derived UB and MM populations from hPSCs, however, has been less successful and did not result in UB branching morphogenesis [9], even following in vivo implantation under the kidney capsule of immunodeficient mice [46]. In the future, a similar combination approach and further investigation into the signals generating human renal stromal cells and the signals that emanate from them may enable the study of impaired UB and MM interactions in hiPSCs derived from individuals with renal agenesis who carry, as examples, *RET, FRAS1,* or *FREM2* mutations.

hPSC-Derived Organoids to Investigate Congenital Glomerular Disease

As alluded to above, the majority of hPSC-kidney organoid protocols result in the generation of an abundance of MM derivatives, including nephrons comprising glomeruli and proximal tubules [11, 16–18]. The organoid glomeruli contain podocytes that themselves make some of the characteristic proteins found in mature native glomeruli, including the slit diaphragm proteins nephrin, synaptopodin, and podocin [17, 47]. Transmission electron microscopy showed organoid podocytes exhibit primary and secondary foot processes but tertiary processes and specialized slit diaphragm connections as found in native glomeruli, were not present [17]. Within organoids, rudimentary vascular networks surround but do not

invade glomeruli, and this lack of podocyte-endothelial interaction may explain why other mature glomerular proteins, such as basement membrane collagen IV α3 and α4, are not detected [8]. Indeed, a mature glomerular basement membrane does not form in hPSC organoids unless they are implanted in vivo and even then it is unclear whether this is uniform across all glomeruli [47]. It has been established that when hPSC-derived kidney tissues were implanted into mice (Fig. 3), patent capillary loops formed in non-mutant glomeruli and this was accompanied by upregulation of collagen IV α3. Electron microscopy reveals the presence of a trilaminar glomerular basement membrane abutted by foot processes, with slit diaphragm structures detected between these processes [47]. Implanted organoids also demonstrate the ability to filter fluorescently labelled low molecular weight dextran injected into the host vasculature [47]. It remains to be determined if the ultrafiltation barrier of these glomeruli has an overall negative charge as occurs in native glomeruli and which is thought to limit permeability to proteins such as albumin.

These models have shown utility in studying genetic congenital glomerular disease. For example, one study made hiPSCs from a patient who carried biallelic missense mutations of *NPHS1*, the gene encoding nephrin [7]. In control in vitro organoids, nephrin was detected by immunostaining in lateral membranes, perhaps constituting pre-slit diaphragm structures. In the patient-derived organoids, nephrin expression was low and immunostaining failed to detect nephrin at the podocyte cell surface. In contrast to controls, electron microscopy showed that adjacent podocyte-like cells in mutant organoids lacked intervening rod-like connections. By implanting the tissue, it was found that while *NPHS1* mutant podocytes formed foot processes, they did not form slit diaphragm structures as were detected in non-mutant control implants. Importantly, the disease model phenotype of patient organoids was rescued following gene editing to correct the *NPHS1* mutation. Another study used single-cell RNA sequencing to demonstrate that a subset of podocytes within non-mutant organoids have a gene expression profile similar to that found in biopsies from a cohort of patients with chronic kidney disease (CKD). Indeed, expression of organoid podocyte transcripts found in this subset – *LY6/plaur domain-containing protein* (*LYPD1*), *serine protease 23* (*PRSS23*), and *cadherin 6* (*CDH6*) which decline with organoid maturation – correlate with proteinuria in people with CKD [48]. This suggests that developmental gene expression profiles are reactivated in acquired glomerular disease, highlighting the potential of kidney organoids as an effective model for studying pathological mechanisms not limited to congenital nephropathies.

hPSC-Derived Organoids for Studying Tubular Disease

Genetic diseases of the kidney can also manifest as incomplete tubule differentiation. In these cases, tubules may carry on proliferating, resulting in PKD, or may fail to acquire normal physiological functions resulting in, for example, a Fanconi syndrome. In 2019, Low and colleagues [14] reported the generation of hiPSCs from an individual with autosomal recessive PKD (ARPKD) carrying a biallelic mutation of *PKHD1*. ARPKD is characterized by enlarged kidneys with 2 waves of cystogenesis. Postnatally, kidney histology is dominated by collecting duct cysts, whereas proximal tubule cysts are prominent in fetal ARPKD kidneys [49]. Low and colleagues [14] used a protocol that produces organoids containing MM derivatives. The arising mutant kidney organoids tended to form cysts, but a more complete cystic phenotype was achieved by adding chemicals that stimulated cAMP signalling. In this model, cysts arose in the proximal tubule structures as identified by the binding of the proximal tubule marker *Lotus tetragonolobus* lectin (LTL). The investigators then CRISPR-Cas9 gene edited the mutant cells to rescue the *PKHD1* mutation, and the arising isogenic control organoids demonstrated substantially reduced cyst formation. In mutant organoids, a loss of the glomerular protein nephrin and reduced binding of LTL was observed as cysts developed, indicating nephron cell de-differentiation. An assessment of proximal tubular function following cAMP activation revealed that ARPKD mutant organoids did not take up 70 kDa dextran whereas tubules in control organoids did. As proof of principle of the suitability of this disease model for the identification of novel therapies, the group showed that both thapsigargin (a drug that induces release of Ca^{2+}) and an inhibitor of the cystic fibrosis transmembrane conductance regulator (CFTR; a chloride channel that facilitates trans-epithelial fluid secretion) inhibited cAMP-induced cystogenesis. Future experiments will be needed to model the second, collecting duct-derived, wave of cystogenesis that dominates the later stages of development of ARPKD in native kidneys.

hiPSC-kidney organoids have also been used to model genetic tubulopathies, and here we highlight 2 examples. Nephropathic cystinosis is a lysosomal storage disorder caused by biallelic mutations in *CTNS* that encodes the

lysosomal cystine transporter cystinosin. Cystine accumulates within lysosomes and leads to proximal tubule dysfunction and a Fanconi syndrome, in addition to more widespread damage to extra-renal tissues. Nephropathic cystinosis is currently treated with cysteamine, which cleaves cystine allowing it to be removed from the lysosome. Cysteamine slows disease progression but does not provide a cure. Hollywood and colleagues [2] generated *CTNS*-mutant hiPSCs and showed that these undifferentiated cells exhibited cystine accumulation, lysosome enlargement and defective basal autophagy as seen in the diseased organs. It was further shown that combined treatment with cysteamine and everolimus, an mTOR pathway inhibitor, rescued the disease phenotype in the mutant hiPSCs [2]. The authors validated the effectiveness of their proposed combined treatment in patient-derived and CRISPR-Cas9 mutant kidney organoids, which exhibited a similar disease phenotype to the mutant hiPSCs. Everolimus was particularly important, as it activated basal autophagy, which was not seen with cysteamine treatment alone.

Mucin-1 (MUC1) heterozygous mutations give rise to a form of medullary cystic kidney disease manifesting in adulthood with slowly progressive renal failure [6]. Histology shows tubular atrophy and interstitial fibrosis [50]. Healthy distal tubules and collecting ducts express *MUC1* that encodes an apical membrane protein; in contrast, the missense *MUC1* mutation generates a protein retained within the epithelial cell, and this build up damages the tubules. Genetically engineered mice that carry the mutant human allele in the *MUC1* locus, immortalized patient tubular cells, and patient-derived hiPSC-kidney organoids were together used to study the pathobiology of this disease. In all 3 models, misfolded MUC1 protein accumulated within transmembrane emp24 domain-containing protein 9 (TMED9)-positive vesicles in the early secretory pathway, and treatment with the small molecule BRD4780 markedly reduced levels of misfolded MUC1 by trafficking it to lysosomes for degradation. Together these studies show that kidney organoids can be effectively used for the assessment of therapeutics and that they provide a useful and relevant complement to other disease models.

Perspectives

Although kidney organoids provide a much needed model for early human kidney development, we consider that there are several limitations to hPSC-derived organoid technology that, if overcome, will allow for their more widespread and efficacious use in disease modelling. One challenge will be in developing methods that facilitate improved organoid maturation and long-term maintenance to provide more accurate models of human disease, especially for disease that manifests long after the nephrogenic period.

A significant barrier to in vitro organoid maturation is the lack of a mature and perfusable vasculature. While capillaries are present, they do not invade the glomerular tufts as in native developing organs. However, in vivo-implanted, hPSC-derived glomeruli acquire capillary loops in association with the expression of more mature glomerular markers [47, 51]. Recent studies have described manipulations to enhance hPSC-derived kidney organoid vasculature in vitro. For example, experimentally downregulating the activity of the microRNA-199a/214 cluster leads to an increase in organoid capillary density between tubules although vessels remain excluded from glomerular tufts [52]. Moreover, capillary loops form in in vitro organoids when the surface of the organoid is exposed to high fluid flow on a millifluidic chip [53]. These conditions, however, do not fully mimic the shear stresses from a high pressure renal arterial supply found in native kidneys, nor the delivery of blood cells, oxygen, nutrients, and bioactive molecules occurring in vivo. Achieving the normal vessel blood flow in these systems will be challenging and future approaches may make use of bioprinting strategies for engineering a perfusable vasculature within in vitro organoids [54, 55]. Such strategies may enable fuller functionality, especially active glomerular filtration, and will be needed to model later pathology found in diseased kidneys.

Also key to improving organoid maturity will be the generation of organoids containing a fuller array of renal cell types. For instance, differentiation of ureter-like epithelium is not seen in any hPSC-organoid system and prevents the modelling of genetic renal malformations that affect ureter function. Progress has been seen with mouse PSCs, where transplantation of mouse UB/collecting duct organoids into peri-Wolffian mesenchyme of ex fetu mouse kidneys encouraged ureter-like epithelium generation, surrounded by contractile smooth muscle cells [56]. Understanding the key signals at play here may inform human cultures. Other renal cell types may not be present within organoids and a detailed comparative characterization using single-cell RNA sequencing to confirm what cell types are present or absent across different protocols will reveal if little-explored cells develop under particular conditions. This will inform adaptation

of methods to differentiate hPSCs towards absent or under-represented cells. As an example, the renal lymphatic system has been overlooked in organoid studies and while its developmental role remains unclear, it is known that lymphatics undergo dynamic changes in development. Indeed, a recent study has shown distorted lymphatics in PKD mutant mice before large cysts were formed, suggesting an early role in normal development and disease [57]. Furthermore, most protocols produce organoids containing either MM or UB derivatives and therefore do not have a regionalization comparable to the native kidney. Generating human organoids with a branching UB combined with developing nephrons is critical to enable investigation of early UB and MM interactions. Insights from mouse PSC-derived organoids indicate that a mature stromal population may lead to progress in this area [9]. The importance of a mature stromal population is further supported by recent research that revealed the dynamic and spacially heterogeneous nature of the developing murine renal interstitium and the possibility that these cells create niches facilitating specific differentiation [58]. This finding correlated with single-cell RNA sequencing data from week 17 human fetal kidneys [58]. It is not known if this is mimicked in hPSC organoids, but attention to the often-ignored interstitial tissue may be critical to understanding many types of kidney disease of developmental origin.

In addition to advances in organoid development and maturity, utilizing multiple cell lines will be required to establish internally validated disease models. Single-cell RNA sequencing studies reveal that the composition of hPSC-derived wild-type kidney organoids are not entirely reproducible between batches of the same clones [59]. Notably, implantation of different lines of wild-type organoids appears to increase their similarity, perhaps by diminishing the proportions of non-renal cells sometimes found in these tissues in culture [60]. Variation is noted in organoid maturation rates, nephron segmentation, and the abundance of off-target cell types that are present between different hPSC lines. This is unsurprising from lines of diverse genetic background, but it does necessitate caution when interpreting disease modelling studies using organoids generated from a single hPSC line.

Finally, there are a number of considerations for the therapeutic application of kidney organoids. At present organoids are limited to the development of hundreds of nephrons, while approximately 1 million are required for a functioning human kidney. Current organoid protocols do not result in physiological polarity with cortical and medullary regions with extended loops of Henle in vitro or after transplantation. This will certainly limit our ability to study later consequences of human genetic kidney disease. Scale up of organoid culture using bioreactors may help here as already initiated although there is a long way to go before this technology can be widely adopted [3]. Use of organoids in drug discovery and toxicity testing has been suggested for some time [61] and the kidney organoid lends itself to high throughput formats. But routine use awaits establishment of further maturation and rudimentary physiological function, together with more economical scale up. If the discussed technological advances are successful, we may also reach a stage where self-hiPSCs could be used to generate authentic immunocompatible hPSC-kidney tissue implants to supplement or replace ailing kidneys.

Note Added in Proof

Progress continues to be made in advancing the development of more distal parts of the nephron in MM hPSC-kidney organoids [62].

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Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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Author Contributions

All 3 authors envisaged and wrote the paper and approved the final version.

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