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Role of Renal Urothelium in the Development and Progression of Kidney Disease

Ashley R. Carpenter[1,2] and **Kirk M. McHugh**[2,3]

[1]Biomedical Sciences Graduate Program, The Ohio State University

[2]Center for Molecular and Human Genetics, The Research Institute at Nationwide Children's Hospital

[3]Division of Anatomy, The Ohio State University

Abstract

The clinical and financial impact of chronic kidney disease (CKD) is significant, while the progression and prognosis of CKD is variable and often poor. Studies using the megabladder ($mgb^{-/-}$) model of CKD have shown that renal urothelium plays a key role in modulating the early injury responses following the development of congenital obstruction. The aim of this review is to examine the role that urothelium has in normal urinary tract development and pathogenesis. We discuss normal morphology of renal urothelium and then examine the role that uroplakins (Upks) play in its development. Histologic, biochemical and molecular characterization of $Upk1b^{RFP/RFP}$ mice indicated Upk1b expression is essential for normal urinary tract development, apical plaque/AUM formation and differentiation and functional integrity of the renal urothelium. Our studies provide the first evidence Upk1b is directly associated with the development of congenital anomalies of the urinary tract (CAKUT), spontaneous age-dependent hydronephrosis and dysplastic urothelia. These observations demonstrate the importance of proper urothelial differentiation in the normal development and pathogenesis of the urinary tract, and provide a unique working model to test the hypothesis that the complex etiology associated with CKD is dependent upon predetermined genetic susceptibilities that establish pathogenic thresholds for disease initiation and progression.

Keywords

Urothelium; uroplakin; chronic kidney disease; congenital anomaly of the kidney and urinary tract; development; dysplasia

Introduction

Chronic kidney disease (CKD) is defined as the progressive loss of renal function over time. The financial impact of CKD is significant with expenditures exceeding 41 billion dollars

Address correspondence to: Kirk M. McHugh, The Research Institute at Nationwide Children's Hospital, Columbus, OH 43205, F | (614) 722-2817, P | (614) 355-2817, kirk.mchugh@nationwidechildrens.org.

Conflict of interest

The authors declare no conflict of interest

annually [1]. A leading cause of CKD in children is congenital obstructive nephropathy [2]. Children with CKD are at the greatest risk for developing life-long medical and social complications of the disease [3]. Despite clinical intervention, the prognosis for patients with CKD often remains uncertain with the morbidity and mortality rates associated with end stage renal disease (ESRD) remain high [4].

Studies using the *mgb*$^{-/-}$ mouse model of congenital obstructive nephropathy indicate that these animals develop CKD and undergo significant renal remodeling as a consequence of progressive hydronephrosis [5, 6]. Transcriptome analysis of *mgb*$^{-/-}$ kidneys identified an urothelial expression signature associated with the development and progression of CKD in these animals. The renal urothelium showed alterations in morphology that included changes in cellular organization, increased proliferation and thickening, and sub- urothelial collagen deposition in affected *mgb*$^{-/-}$ kidney. These changes represent the earliest histopathologic events observed in *mgb*$^{-/-}$ kidneys and suggest a potential role for renal urothelium in the initiation of CKD and its progression to end stage renal disease (ESRD).

In this review, we will examine the unique morphologic features associated with the renal urothelium and discuss new data utilizing the uroplakin 1b-RFP knock- in mouse (*Upk1b*$^{RFP/RFP}$) of urothelial dysmorphogenesis. These studies indicate that Upk1b expression is essential for normal urothelial development, homeostasis and structural integrity and provide the first evidence that Upk1b is involved in the development of congenital anomalies of the kidney and urinary tract (CAKUT). This work provides evidence that CAKUT and the later development of urinary tract diseases and disorders can have a common genetic linkage that creates a series of overlapping susceptibilities. We hypothesize that these susceptibilities define threshold events for the pathogenic initiation of CKD and subsequent progression to ESRD.

Renal Urothelium

Urothelium is a specialized epithelium that lines most of the urinary tract including the renal pelvis, ureter, bladder, and proximal urethra. Urothelium is known to play a key role in membrane permeability, modulating surface area and mediating host response to infection [7–10]. The embryonic origin of the urothelium is regionally distinct, with proximal urethra and bladder arising from endoderm and ureter and renal pelvis from mesoderm. Whether these developmental differences have any functional or pathogenic significance remains to be determined.

In contrast to the well-characterized bladder urothelium, much less is known regarding the development and pathogenesis of renal urothelium. Murine renal urothelium can be subdivided into five morphologically distinct regions that are summarized in Figure 1 and include: 1) the inner medulla lining the renal papillae, 2) the inner medullary band of the fornix, 3) the outer medullary band of the fornix, 4) the renal cortex and 4) the renal pelvis [11].

Starting at the papillary tip, the renal urothelium is composed of columnar cells with microvilli that transition to a simple cuboidal epithelium on the remainder of the medullary

papilla. The papillary urothelium is contiguous with that lining the larger collecting tubules and is generally devoid of Upk expression, although occasional apical plaques are detected on individual columnar cells within this region. The inner medulla possesses pseudostratified epithelia with variable microvilli and limited Upk expression and plaques, while the outer medulla transitions to a 2–3 cell thick layer that has limited Upk expression and apical plaques with occasional microvilli. Cortical urothelium is three layers thick with strong Upk expression and the presence of superficial umbrella cells that possess an asymmetric unit membrane (AUM). Transition to the renal pelvis and ureter is marked by urothelial expansion to a 3–5 cell thick epithelia that closely resembles that observed in the bladder with well-defined basal, intermediate and superficial umbrella cells, robust Upk expression and a well-developed AUM [11].

Prior studies suggest that the progressive increase in urothelial complexity from the renal papillae to cortex is a byproduct of the osmolarity of the underlying renal tissue [11]. At the renal medulla, interstitial fluid osmolarity is similar to that of urine resulting in a neutral osmotic pressure and making the presence of an AUM and its corresponding blood:urine barrier unnecessary. In contrast, as one approaches the renal cortex, the osmolarity of the interstitial fluid decreases rapidly to almost one-quarter of that observed at the medulla. This decrease in interstitial osmolarity produces significant osmotic pressure on the underlying renal tissue necessitating the need for the development of a blood:urine barrier that corresponds to an increase in cellular complexity within the urothelium and development of an AUM. These observations clearly suggest that the functional integrity of the urothelium is intimately associated with its morphologic complexity, Upk expression and proper development of the AUM.

Upks and Apical Plaques

Urothelium possesses properties that render it flexible and yet impermeable. Superficial umbrella cells elaborating a rigid apical plaque that plays a key role in modulating membrane permeability, integrity, homeostasis and host response to pathogens [7–10]. Apical plaques are principally composed of Upk protein particles arranged into 2D crystals (Figure 2) [12]. Upk proteins are highly conserved among mammals and to date five Upk proteins have been identified as constituents of apical plaques [13, 14]. This includes four major Upk proteins: Upk1a, Upk1b, Upk2, Upk3a and one minor Upk protein: Upk3b [13, 15, 16]. Upk1a and Upk1b are members of the tetraspanin family of proteins and possess four transmembrane (TM) domains, whereas, Upk2 and Upk3 have a single TM domain. TM domains anchor each Upk to the plasma membrane and separate a large extracellular domain from a relatively small intracellular domain, resulting in the formation of an AUM [16].

Upks are assembled in the endoplasmic reticulum (ER; Figure 2.1), where they heterodimerize with their obligate binding partners prior to transport from the ER (Figure 2.2) (Tu et al., 2002) The tetraspanin uroplakins, Upk1a and Upk1b, form heterodimers with the single TM domain uroplakins, Upk2 and Upk3, respectively [17, 18]. Further processing of Upk2 in the Golgi results in a conformational change to the Upk1a/2 heterodimer leading to heterotetramer formation with Upk1b/3a (Figure 2.3) [19]. In the trans Golgi network,

Upk heterodimers form concentric hexameric rings (Figure 2.4). Six Upk1a/2 heterodimers form the inner ring and are connected to an outer ring composed of six Upk1b/3 heterodimers [20]. Highly organized Upk particles are then packaged into vesicles and trafficked to apical hinge regions at the surface of the cell where they become an integral part of the AUM.

Although the structural properties of Upks have been well characterized, their functional properties during urinary tract development and pathogenesis remain ambiguous. Recent studies have linked defects in urothelial plaque assembly to the development of hydronephrosis, VUR, structural anomalies and obstruction. *Upk3a*$^{-/-}$ mice showed diminished plaque formation and developed spontaneous hydronephrosis accompanied by abnormal ureterovesical orifices and VUR [9]. In a similar manner, Upk2 ablation led to absent plaque formation and the development of hydronephrosis, VUR and variable obstruction due to urothelial overgrowth in the distal ureter [10]. These studies indicate that Upks play a functional role in urothelial fidelity and when disrupted can result in urinary tract dysmorphogenesis and pathogenesis.

Evidence from lower vertebrates suggests that Upks may also have signaling roles. For example, *Xenopus* Upk3 (xUpk3) plays a functional role in oocyte activation by acting as a sperm receptor that becomes phosphorylated initiating limited proteolysis of xUpk3 and egg activation [21]. The potential importance of these signaling events is born out by the identification of heterozygous missense mutations in the *UPK3a* gene in children with renal adysplasia and multicystic dysplastic kidneys that correspond to the phosphorylation/protease domains identified in *Xenopus* [22]. In addition, bacterial binding to Upk1a is known to induce transcriptional changes in basal and intermediate cells before superficial cells are compromised [23, 24]. Finally, *Upk1b* is distinct from its other family members in that it can escape the ER without heterodimerization suggesting it may have a functional role beyond plaque formation [9, 25].

Urothelium and Pathogenesis

Studies from our lab and others have shown that urothelial remodeling is an early response to a variety of urinary tract pathologies including congenital obstructive nephropathy, ischemia, unilateral ureteral obstruction and infection [26–30]. Six of the top ten most differentially expressed genes in severely hydronephrotic *mgb*$^{-/-}$ kidneys were found in the renal urothelium, with four of the top five including the four major uroplakins - *Upk1a*, *Upk1b*, *Upk2* and *Upk3a* [26]. Based upon these observations, we hypothesized that the CKD-induced changes observed during renal adaptation in *mgb*$^{-/-}$ mice have the potential to alter urothelial function and increase susceptibility for disease progression. For example, altered apical plaque composition in severely hydronephrotic *mgb*$^{-/-}$ kidneys may increase susceptibility to infection, since specific Upk proteins have been postulated to function as bacterial binding sites [8, 20]. The subsequent development of pyelonephritis could serve as a second pathogenic ‘hit’ that tips the balance away from renal repair to permanent renal damage and altered renal function [31]. In fact, this precise scenario occurs in *mgb*$^{-/-}$ mice that develop pyelonephritis [32]. Such observations support our working hypothesis that the

urothelium and its corresponding Upk proteins play a critical role in modulating normal urinary tract development, homeostasis and disease.

Upk1b$^{RFP/RFP}$ Ablation Mice and Urothelial Dysmorphogenesis

To begin to investigate the complex interactions associated with CKD initiation and progression, we sought a genetic model of urothelial dysfunction. A survey of the published literature and website resources identified the uroplakin 1b-RFP knock-in mouse (*Upk1b*$^{RFP/RFP}$) model generated by the GenitoUrinary Development Molecular Anatomy Project (GUDMAP) consortium [33]. *Upk1b*$^{RFP/RFP}$ mice possess a red fluorescent protein and polyadenylation sequence (RFP-pA) inserted just after the first coding exon in *Upk1b* that disrupts translation resulting in the production of an inactive Upk1b protein and the generation of a functional Upk1b knockout.

As discussed above, Upk1b is a tetraspanin protein that heterodimerizes with Upk3a or Upk3b [15, 17, 18]. Loss of Upk1b expression in *Upk1b*$^{RFP/RFP}$ mice is predicted to block Upk1b/3a heterodimer production completely abrogating apical plaque/AUM formation. Our studies confirmed that *Upk1b*$^{RFP/RFP}$ mice lack *Upk1b* expression and do not develop apical plaques/AUMs throughout their urinary tract [12]. Interestingly, even though *Upk1b* is more widely expressed than its other family members (see below), we did not observe any other gross morphological or functional deficits in *Upk1b*$^{RFP/RFP}$ mice besides those associated with the urinary tract.

The murine *Upk1b* gene is located on chromosome 16 and studies implicate a retinoic acid-mediated GATA4/6 mechanism in the regulation of *Upk1b* expression and Bach1, αMEF-2, MEF-2A, and the AP-1, c-Fos, c-Jun transcription factors are predicted to regulate the expression of *Upk1b* [34]. Principally associated with epithelia, *Upk1b* is most highly expressed in the developing and mature urinary and reproductive tracts with significantly lower levels of expression observed in other tissues [35]. *Upk1b* mRNA expression has been detected in the placenta, urogenital sinus, genital tubercle, metanephros, urothelial lining of the developing urinary tract, gonad, oviduct, ovary, testes, epididymis, kidney, ureter, bladder, breast, skin, as well as the corneal, pharyngeal and pharyngo-tympanic epithelia. *Upk1b* mRNA expression has also been detected in the mesothelial lining of the pericardial, pleural and peritoneal cavities as well as the renal capsule. Finally, although Upk1b is most often associated with epithelia, *Upk1b* mRNA expression has been detected in embryonic metanephric mesenchyme and pelvic ganglia, postnatal cap mesenchyme, and adult ovarium mesenchymal stroma and testicular interstitium [33, 36, 37].

Upk1b's Role in Urinary Tract Development

The novel identification of a duplicated collecting system in approximately 16% of *Upk1b*$^{RFP/RFP}$ mice indicates that Upk1b plays a fundamental role in normal urinary tract development [12]. The duplicated collecting system was composed of an orthotopic and ectopic ureter that drained the caudal and cranial poles of the kidney, respectively. The orthotopic ureter was patent and inserted into the bladder in a normal anatomic position, while the ectopic ureter ended blindly near the base of the bladder and was obstructed,

resulting in mild hydronephrosis in the cranial pole of the kidney. These observations suggest that the duplicated *Upk1b*$^{RFP/RFP}$ collecting system results from the induction of two independent ureteric buds from the Wolffian duct, and provide the first suggestive evidence that Upk1b is involved in these processes.

Although expression of Upk1b, or any other Upk protein for that matter, has never been reported during the early stages kidney development, recent data from our lab shows that *Upk1b*$^{RFP/RFP}$ mice do indeed possess duplicated ureteric buds and possible spurious branching events later in development (Figure 3). Clearly, a more thorough evaluation of Upk1b expression during normal and abnormal urinary tract development is needed to determine its precise temporal and spatial patterns of expression.

These observations suggest that Upk1b plays a critical role in early metanephric kidney development and represents a potential novel gene target for the development of CAKUT. Precisely how Upk1b modulates early kidney development remains to be determined. Even so, its association with structural defects is consistent with the prior genetic data presented above for other Upk knockout mice. In addition, Upk1b expression independent of apical plaque formation may provide an alternative mechanism for its action during early kidney development.

Upk1b's Role in Urinary Tract Integrity

Upk1b ablation leads to cell-autonomous, age-dependent, progressive hydronephrosis in *Upk1b*$^{RFP/RFP}$ mice in the absence of anatomic obstruction (Figure 4) [12]. The AUM composed of apical plaques is associated with intracellular cytoskeletal elements and the tensile strength of these interactions is hypothesized to stabilize the apical membrane. We hypothesize that AUM loss in *Upk1b*$^{RFP/RFP}$ mice results in a structurally weakened urothelium. As urine production and storage pressure increase following postnatal nephrogenesis, the weakened renal urothelium begins to distend resulting in the development of age-dependent, progressive hydronephrosis in *Upk1b*$^{RFP/RFP}$ mice. The renal specificity of this defect is most likely a reflection of the fact that the bladder and ureters possesses a well-defined muscular coat that has intrinsic tone capable of compensating for their weakened urothelium that is lacking in the kidney. This hypothesis is consistent with studies suggesting that the functional integrity of the urothelium is intimately associated with Upk expression and proper development of the AUM and provides a unique pathogenic basis for the sequential progression of CKD in these animals.

Upk1b's Role in Urinary Tract Infections

Upk1b$^{RFP/RFP}$ mice that are challenged with uropathogenic E. coli (UPEC) show significantly less bacterial burden in their kidneys than controls (Figure 5). Similar reductions in bacterial burden were also observed in the *Upk1b*$^{RFP/RFP}$ bladder and ureter. This data supports prior studies showing that the assembled uroplakin plaque is a receptor for type I fimbriae of UPEC, and that Upk3a signaling is necessary for UPEC invasion and urothelial cell apoptosis [8, 38]. The lower bacterial burden observed throughout the urinary tract of *Upk1b*$^{RFP/RFP}$ mice demonstrates that alterations in Upk expression have the

potential to modify bacterial binding, invasion and burden and hence affect the development and progression of urinary tract infections. This observation provides a mechanistic model for increased susceptibility to urinary tract infections during obstruction where *de novo* Upk expression occurs providing a clear pathogenic basis for potential CKD progression and the development of ESRD [26].

Upk1b's Role in Urinary Tract Cancer

The absence of *Upk1b* expression and plaque/AUM formation in $Upk1b^{RFP/RFP}$ mice resulted in the lack of terminally differentiated umbrella cells, an increase in cellular proliferation and cell layers, and a shift towards a less mature cellular composition within the urothelium [12]. These changes caused dramatic alterations in urothelial morphology in $Upk1b^{RFP/RFP}$ mice that resulted in the appearance of dysplastic features in both the bladder and kidney (Figure 6) [12].

Prior studies have suggested an association of UPK1B with the development of ovarian Brenner tumors, which represent spurious urothelial differentiation within the female genital tract [39] In addition, dysplastic urothelium is postulated to be a putative precursor of carcinoma *in-situ* of the urinary tract as well as invasive bladder cancer [40, 41]. Urothelial cancers are considered multifocal and can be found throughout the urinary tract, with the presence of upper urinary tract tumors significantly increasing the chance of developing bladder cancer [42].

Whether $Upk1b^{RFP/RFP}$ mice eventually develop urothelial cancers as they age remains to be determined. Alternatively, it is plausible that urothelial dysplasia predisposes $Upk1b^{RFP/RFP}$ mice to the development of urothelial cancers when exposed to secondary pathogenic insults. For example, chronic inflammation and stones have both been identified as substantial risks factors for the development of urothelial cancers suggesting that chronic urothelial injury may lead to urinary tract tumor development in certain cases [43].

Summary

The development and progression of CKD in children is known to be highly variable and its clinical management is often challenging [44]. Studies with $Upk1b^{RFP/RFP}$ mice show how a single genetic locus can produce CAKUT followed by the development of spontaneous, age-dependent hydronephrosis and dysplastic features within the urothelium. Some of the urothelial changes observed in $Upk1b^{RFP/RFP}$ mice have the potential to generate pathogenic susceptibilities within the urinary tract that may only become apparent upon further challenge (ie: infection, congenital obstruction). To test this hypothesis, we generated $Upk1b^{RFP/RFP};mgb^{-/-}$ mice to determine what effect alterations in urothelial morphology and function would have on the development of congenital hydronephrosis and subsequent CKD progression. Compound homozygotic $Upk1b^{RFP/RFP};mgb^{-/-}$ mice develop more severe bilateral hydronephrosis at an earlier age than $mgb^{-/-}$ mice alone suggesting that the presence of a structurally weakened urothelium exacerbates the development and progression of bilateral hydronephrosis in these animals (Figure 7).

This observation supports our overall hypothesis that complex, overlapping genetic traits may contribute, at least partially, for the variation in disease progression observed in children with CKD. Determining how these various susceptibility loci interact is critical in gaining a better understanding of how diverse genetic backgrounds contribute to pathogenic initiation and progression within the urinary tract. Future studies designed to clearly identify these overlapping genetic susceptibilities as potential prognostic markers and therapeutic targets may permit the personalized management of each child in the clinical setting.

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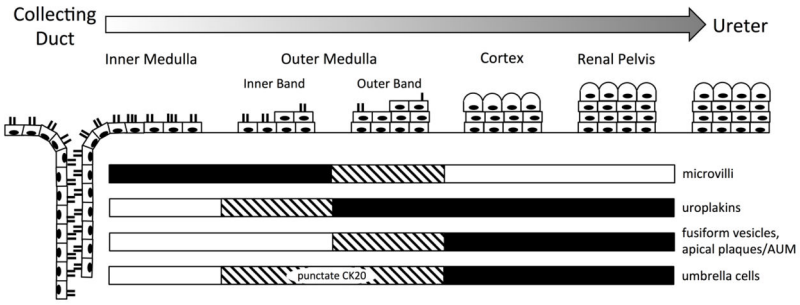

Figure 1.

Schematic model of renal urothelium morphology from the collecting duct to ureter showing an increase in morphologic complexity and expression patterns of key components.

Abbreviations: CK – cytokeratin, AUM – asymmetric membrane unit.

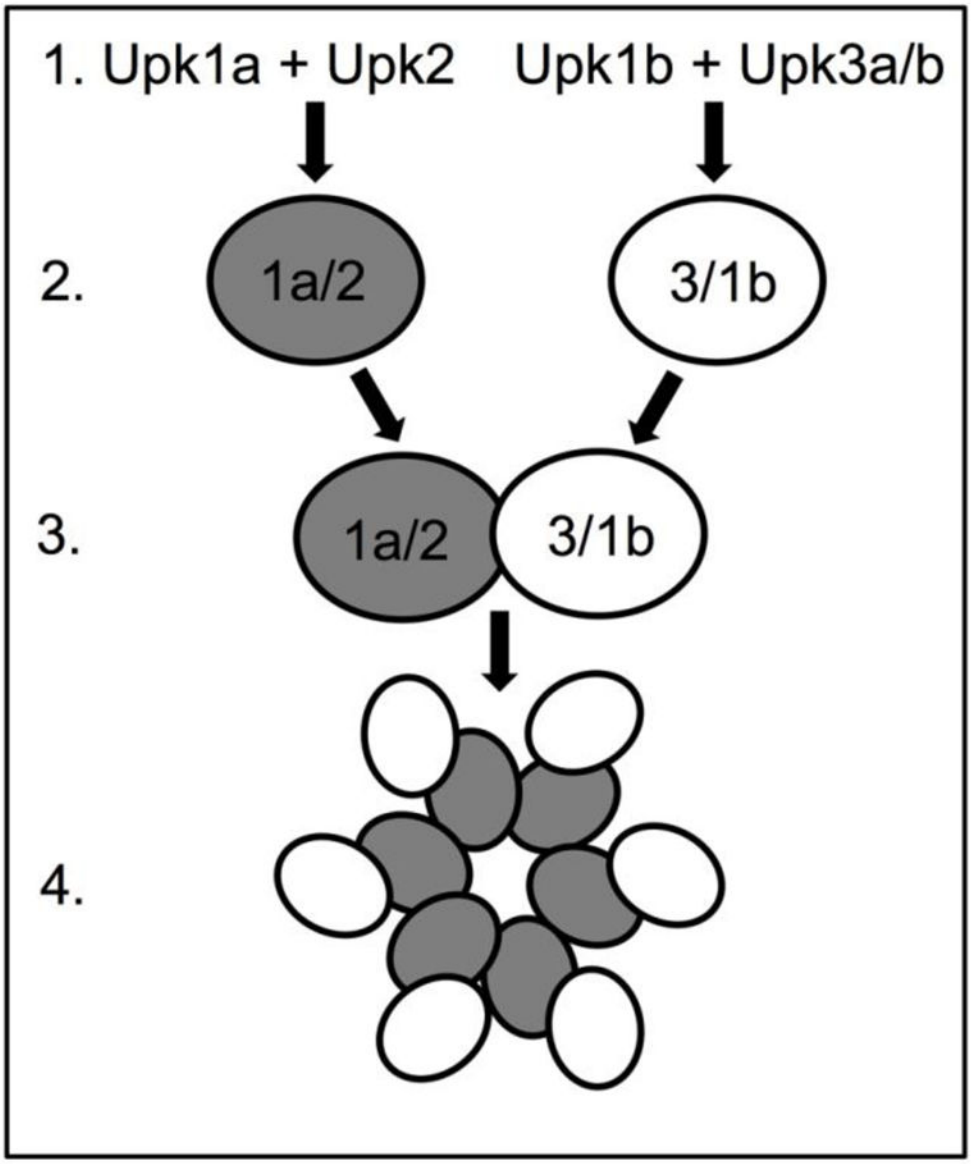

Figure 2.
Uroplakin Plaque Assembly. Abbreviations: Upk – uroplakin.

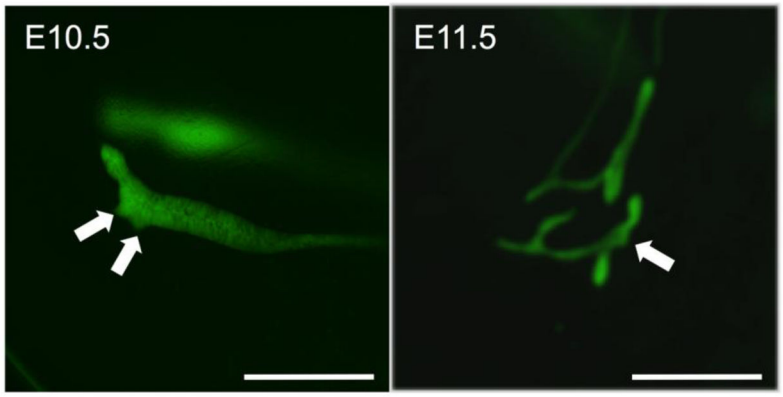

Figure 3.

Upk1b$^{RFP/RFP}$ mice show duplicated ureteric buds at E10.5 (white arrows) and spurious branching defects at E11.5 (white arrow). Abbreviations: Upk – uroplakin, E – embryonic day. Scale bar represents 1mm.

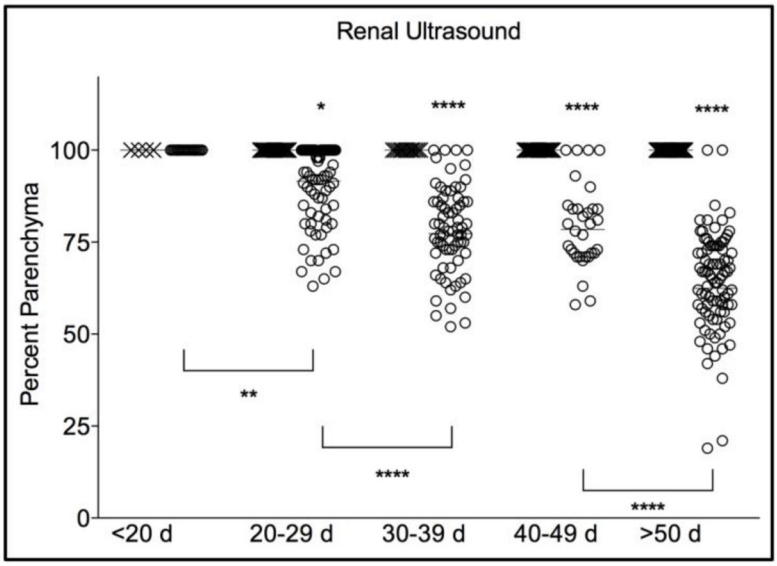

Figure 4.

Development of age-dependent, spontaneous hydronephrosis in *Upk1b*$^{RFP/RFP}$ mice showing percent of total parenchyma present in control *Upk1b*$^{+/+}$ (**X**) and *Upk1b*$^{RFP/RFP}$ (**O**) mice at the specified number of days (**d**) from birth. *P < 0.05, **P < 0.01, ****P < 0.0001.

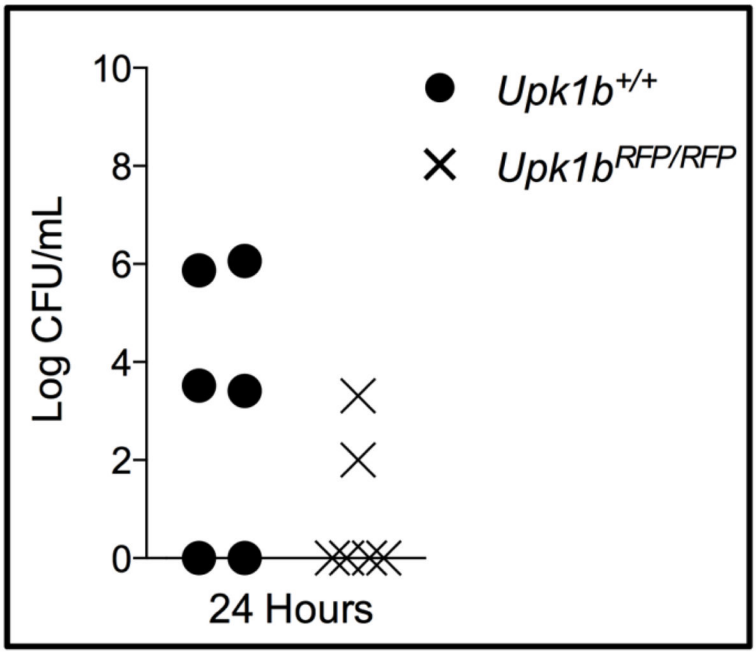

Figure 5.
Bacterial burden is less in *Upk1b*$^{RFP/RFP}$ kidneys versus *Upk1b*$^{+/+}$ control kidneys at 24 hours post-infection.

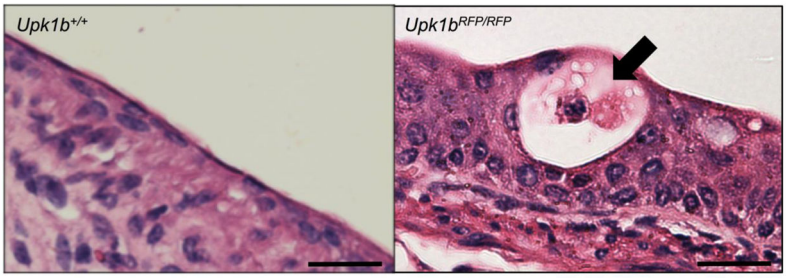

Figure 6.

Upk1b$^{RFP/RFP}$ renal urothelium displays several dysplastic features versus *Upk1b*$^{+/+}$ control including increased cellularity and nuclear disorganization, anisokaryosis, and vacuoles consistent with cell retraction associated with apoptosis (**white arrow**).

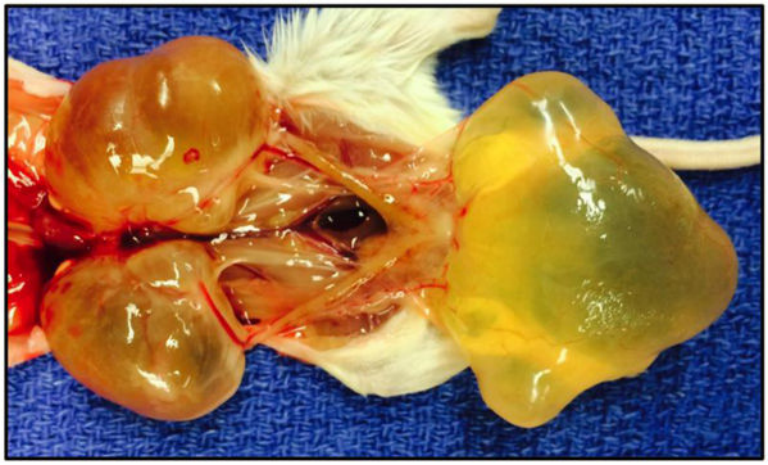

Figure 7.

Adult female *Upk1b*$^{RFP/RFP}$;*mgb*$^{-/-}$ mouse showing development of severe bilateral hydronephrosis, a condition that is not observed in adult female *mgb*$^{-/-}$ mice. This observation suggests that the weakened renal urothelium created by the knockout of Upk1b exacerbates the development and severity of hydronephrosis in the presence of a congenital obstruction. Scale bar represents 25μm.