**Kif3a Controls Murine Nephron Number Via GLI3 Repressor, Cell Survival, and Gene Expression in a Lineage-Specific Manner**

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**Abstract**

The primary cilium is required during early embryo patterning, epithelial tubulogenesis, and growth factor-dependent signal transduction. The requirement for primary cilia during renal epithelial-mesenchymal tissue interactions that give rise to nephrons is undefined. Here, we used Cre-mediated recombination to generate mice with Kif3a deficiency targeted to the ureteric and/or metanephric mesenchyme cell lineages in the embryonic kidney. Gradual loss of primary cilia in either lineage leads to a phenotype of reduced nephron number. Remarkably, in addition to cyst formation, loss of primary cilia in the ureteric epithelial cell lineage leads to decreased expression of Wnt11 and Ret and reduced ureteric branching. Constitutive expression of GLI3 repressor (GLI3<sup>D</sup>) rescues these abnormalities. In embryonic metanephric mesenchyme cells, Kif3a deficiency limits survival of nephrogenic progenitor cells and expression of genes required for nephron formation. Together, our data demonstrate that Kif3a controls nephron number via distinct cell lineage-specific mechanisms.

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**Introduction**

Primary cilia are microtubule-based organelles that function as signaling centers during development and cell differentiation [1]. The primary cilium arises in a quiescent cell from the basal body as a microtubule-based plasma membrane-invested cytoskeletal structure termed the axoneme. Cilia assembly and maintenance and growth of the axoneme is mediated by a kinesin motor protein-based transport process termed intraflagellar transport (IFT), by which particles are transported in a bidirectional manner along the axoneme [2]. Deficiency of KIF3A, a component of the kinesin II motor complex, disables anterograde IFT, and causes both failure of formation and maintenance of the primary cilium [3]. A critical role for the primary cilium during embryogenesis was initially demonstrated by the finding that mice with Kif3a deficiency lack nodal cilia and exhibit defects in left-right asymmetry [3]. Many human congenital malformation syndromes are caused by mutations in proteins that are localized to cilia and ciliary basal bodies [1]. Some of the mutated proteins are downstream effectors of the Hedgehog (Hh), WNT and FGF signaling pathways. Hh ligands signal by binding the cell surface protein Patched (PTC), which functions as a constitutive inhibitor of Smoothened (SMO). In the absence of Hh ligand, inactive SMO promotes the processing of full length GLI3 to a C-terminally truncated transcriptional repressor, GLI3 repressor (GLI3R). Hh activates SMO, leading to the blockage of GLI3 processing and the nuclear translocation of full-length GLI proteins to induce transcription. Several lines of evidence implicate the primary cilium in mammalian Hh signaling. First, disruption of Hh signaling generates a phenotype very similar to that described in embryos with deficiency of IFT proteins [4]. Second, PTC, SMO, and GLI are localized to the primary cilium [5–7]. Third, IFT proteins act downstream of PTC1 and SMO and upstream of GLI proteins [4,8]. Cilia defects alter the ratio of GLI activator to GLI3R resulting in aberrant Hh signaling [1]. The primary cilium is also implicated in WNT signaling since NPHP2 (inversin), NPHP3, and GLIS2, each of which promotes non-canonical WNT signaling, are localized to the cilium. Inactivation of any of these noncanonical WNT effectors increases canonical WNT activity [9,10]. In contrast to Hh and WNT signaling, the role of the primary cilium in regulating FGF signaling is largely unknown. FGFs have been shown to regulate cilia length [11] but a role for the cilium in regulating FGF signaling has not been demonstrated previously. However, the localization of FGF receptors to cilia in murine airway cells suggests a possible role for the cilium in regulating FGF signaling [12].

The discovery that proteins mutated in polycystic kidney diseases are localized to the primary cilium identified the primary cilium as critical to renal epithelial cell differentiation [13]. In direct support of this cilia-dependent function, kidney-specific inactivation of Kif3a in the ureteric epithelial cell lineage inhibits...
ciliogenesis and induces epithelial cysts [14]. The observation that nephron formation may be impaired in mice with deficiency of NPHP2 (inversin), a cilia-localized protein and polycystic kidney disease gene [9] suggests that primary cilia may function during stages of renal development that control nephron formation and which precede epithelial differentiation.

Nephron formation is dependent on inductive mesenchymal-epithelial tissue interactions between the ureteric bud (an epithelial tubule) and the metanephric blastema (a mesenchymal tissue). Metanephric mesenchyme cells adjacent to ureteric branch tips are induced to form nephrogenic precursors that constitute the mature nephron (glomerulus, proximal tubule, loop of Henle, and distal tubule), a process that has been termed nephrogenesis. In turn, the ureteric bud and its branches are stimulated to undergo successive branching events in response to signals by adjacent mesenchyme cells, resulting in formation of the collecting ducts, calyces and pelvis, a process termed renal branching morphogenesis [15]. Investigation of the molecular mechanisms that control nephrogenesis and branching morphogenesis has elucidated critical roles for signaling by Hh, WNT and FGF proteins. Sonic Hh (Shh) controls inductive tissue interactions during murine kidney development by inhibiting formation of GLI3 repressor [16]. During branching morphogenesis, GLI3R plays a critical role in distal ureteric branch tips by promoting the expression of Ret and Wnt11, both of which are required for ureteric branching [17]. Canonical WNT signaling is required for renal branching morphogenesis [18] and formation of nephrogenic precursors in response to WNT9b and WNT4 [19–21]. Nephron formation is also dependent on expression of FGF8 by metanephric mesenchyme cells. Deficiency of Fgβ abrogates expression of Wnt4 and limits nephron formation to stages prior to the formation of the glomerulus [22].

Here, we tested our hypothesis that the primary cilium is required during growth factor-mediated renal mesenchymal-epithelial interactions. We investigated our hypothesis by generating mouse strains with deficiency in Kif3a in all kidney cells or in the ureteric or metanephric mesenchyme cell lineage. Our results demonstrate that Kif3a deficiency and subsequent loss of primary cilia is accompanied by a decrease in the number of nephrons. Analysis of mice with lineage-specific Kif3a deficiency showed that Kif3a performs distinct functions in ureteric and metanephric mesenchyme cells. In ureteric cells, Kif3a deficiency disrupts ureteric branching and expression of Ret and Wnt11, which act in concert to promote ureteric branching. Remarkably, constitutive expression of GLI3R in Kif3a-deficient ureteric cells rescues each of these abnormalities. Analysis of mice with Kif3a deficiency in metanephric mesenchyme cells revealed two further mechanisms by which Kif3a controls nephron number. First, Kif3a-deficient cells exhibit reduced survival, negatively impacting the mass of mesenchyme cells that can contribute to nephrons. Second, expression of FGF8 and its downstream effectors by Kif3a-deficient cells is markedly reduced. Yet, expression of Hh signaling effectors is unaffected. Together, these results demonstrate a fundamental role for Kif3a and the primary cilium in controlling nephron number during murine kidney development.

Results

**Kif3a Deficiency Decreases Nephron Formation in the Murine Kidney**

We initiated investigation of primary cilium function during renal morphogenesis by examining the cellular distribution of primary cilia in distinct lineages that give rise to the kidney. Acetylated alpha-tubulin (α-AcT) is expressed in the ciliary axoneme and the basal body from which the axoneme emerges; expression on the apical cell surface marks the primary cilium. Examination of α-AcT expression at E11.5, the stage at which the ureteric bud invades the metanephric blastema, demonstrated expression in virtually all ureteric and mesenchymal cells (Figure S1A and S1A'). By E13.5 and E15.5, α-AcT could be clearly localized to the apical surface of ureteric cells (Figure S1B, S1B', S1C, and S1C'), as well as the mesenchyme-derived structures, condensing mesenchyme, vesicles and S-shaped bodies (Figure S1D and S1D'), that precede formation of the mature nephron. Together, these data indicate that primary cilia are formed during early stages of ureteric branching and nephron formation.

We investigated the functional contribution of the primary cilium to renal development by generating mice with loss of primary cilia in both ureteric and metanephric mesenchyme cells. Kif3a is a component of the microtubule heterotrimeric kinesin II motor complex, which mediates anterograde IFT. kif3a deficiency disables anterograde IFT and leads to failure in formation and maintenance of cilia [3]. Since germline deficiency in Kif3a is embryonic lethal prior to the onset of kidney development [3], we used a conditional Kif3a<sup>tm</sup>-<sup>WT</sup> allele [3] and a Tamoxifen-inducible Cre mouse strain [23] to generate Cre<sup>ER<sup>TM</sup></sup>;<kif3a<sup>tm</sup>-<sup>WT</sup> tm</kif3a<sup>tm</sup>-<sup>WT</sup> tm> mice. Administration of Tamoxifen (3mg/40g body weight) prior to E10.5 induced embryonic demise prior to kidney formation. In contrast, embryos of pregnant dams treated with Tamoxifen at E10.5 survived until shortly after E13.5 (Figure 1A), thus providing a means to analyze primary cilium function.

Loss of Kif3a in Tamoxifen-treated mice was confirmed using anti-KIF3A antibody (Figure 1B, 1C'). In control mice (WT mice injected with Tamoxifen at E10.5), KIF3A and α-AcT co-localized in ureteric and metanephric mesenchyme cells (Figure 1B, B'). In ureteric branches, characterized by a lumen, co-localization of KIF3A and α-AcT was restricted to the apical cell surface (Figure 1B, arrow located in UB apical domain). In contrast, expression of KIF3A was markedly diminished in kif3a<sup>tm</sup>-<sup>WT</sup> mice (Figure 1C'). Further, co-localization of KIF3A with α-AT in the apical domain of epithelial cells was not detected (Figure 1C'). We assessed the impact of KIF3A deficiency on primary cilia by counting the fraction of ureteric cells and nephrogenic metanephric mesenchyme (SIX2-positive) cells with primary cilia by imaging these respective cells in three randomly selected optical fields in a 5 micrometer sagittal tissue section generated from the mid-point of each kidney (n = 6 mice/group) (Figure 1D and 1E). The number of cells with primary cilia was decreased by 54% in each of these cell populations in Kif3a-deficient mice compared with controls (Figure 1H). Next, we determined the effect of KIF3A and primary cilium loss on kidney development. Ureteric branches and nephrogenic precursors were identified in tissue sections with cytokeratin (CK) and NCAM, respectively (Figure 1F and 1G). Both ureteric branches and nephron precursors formed normally in mutant mice. The number of NCAM-positive nephrogenic precursors was determined for each kidney in five tissue sections - a mid-sagittal section and two sections generated 40 micrometer in mutant mice. The number of NCAM-positive nephrogenic precursors in Tamoxifen-treated mice was confirmed using anti-KIF3A antibody (Figure 1B, 1C'). In control mice (WT mice injected with Tamoxifen at E10.5), KIF3A and α-AcT co-localized in ureteric and metanephric mesenchyme cells (Figure 1B, B'). In ureteric branches, characterized by a lumen, co-localization of KIF3A and α-AcT was restricted to the apical cell surface (Figure 1B, arrow located in UB apical domain). In contrast, expression of KIF3A was markedly diminished in kif3a<sup>tm</sup>-<sup>WT</sup> mice (Figure 1C'). Further, co-localization of KIF3A with α-AT in the apical domain of epithelial cells was not detected (Figure 1C'). We assessed the impact of KIF3A deficiency on primary cilia by counting the fraction of ureteric cells and nephrogenic metanephric mesenchyme (SIX2-positive) cells with primary cilia by imaging these respective cells in three randomly selected optical fields in a 5 micrometer sagittal tissue section generated from the mid-point of each kidney (n = 6 mice/group) (Figure 1D and 1E). The number of cells with primary cilia was decreased by 54% in each of these cell populations in Kif3a-deficient mice compared with controls (Figure 1H). Next, we determined the effect of KIF3A and primary cilium loss on kidney development. Ureteric branches and nephrogenic precursors were identified in tissue sections with cytokeratin (CK) and NCAM, respectively (Figure 1F and 1G). Both ureteric branches and nephron precursors formed normally in mutant mice. The number of NCAM-positive nephrogenic precursors was determined for each kidney in five tissue sections - a mid-sagittal section and two sections generated 40 micrometer in both directions from the mid-sagittal section, resulting in a total of five sections. Quantitation of NCAM-positive nephrogenic precursors demonstrated a 34% decrease in mutant mice (n. NCAM-positive structures/section, WT versus kif3a<sup>tm</sup>: 15.2±2.96 versus 10±1.5, p = 0.001, n = 4 mice/group) (Figure 1I). These data demonstrate that Kif3a controls the number of nephrons formed during renal embryogenesis.
Kif3a Functions in a Cell-lineage Specific Manner to Control Nephron Number

The short-term viability of embryos in Tamoxifen-treated pregnant mice limited the availability to investigate mechanisms underlying the requirement for Kif3a during nephron formation. To address this limitation, we generated mice with loss of Kif3a targeted specifically to the ureteric (Kif3a<sup>−/−</sup>UB) mice or metanephric mesenchyme (Kif3a<sup>−/−</sup>MM) mouse strains, using Hoxb7-CreER<sup>+</sup> [24] and Rarb2-Cre [25] mouse strains, respectively. Kif3a<sup>−/−</sup>UB and Kif3a<sup>−/−</sup>MM mouse embryos were generated in the proportion predicted by Mendelian segregation and survived to birth. The efficiency and specificity of Kif3a deletion was demonstrated by analyzing Kif3a mRNA expression in ureteric bud and metanephric mesenchyme tissue fractions isolated at E11.5 using quantitative PCR. Kif3a mRNA was reduced by over 95% in ureteric and metanephric mesenchyme cells in Kif3a<sup>−/−</sup>UB and Kif3a<sup>−/−</sup>MM mouse, respectively, but was not significantly decreased in cells that were not targeted by the respective Cre alleles (Figure S2). KIF3A protein expression was examined by immunofluorescence in embryonic kidney tissue. Comparison of anti-KIF3A antibody-generated signals on the apical surface of control and mutant cells revealed specific identification of KIF3A. In control embryos, KIF3A (green color) co-localized with α-AcT (red color) on the apical surface of ureteric cells (Figure 2A, UB: arrow) and in mesenchyme cells (Figure 2A, arrow in box). In Kif3a<sup>−/−</sup>UB mice, KIF3A was lost in ureteric cells (Figure 2A<sup>′</sup>, box inset) but was expressed in metanephric mesenchyme cells (Figure 2A<sup>′</sup>, upper arrow). In Kif3a<sup>−/−</sup>MM mice, KIF3A was lost in metanephric mesenchyme cells (box inset, Figure 2A<sup>′</sup>) but was expressed in ureteric cells (Figure 2A<sup>′</sup>, arrow in UB).

Next, we determined the effect of KIF3A deficiency on cilia. Cilia number was quantitated in sagittal tissue sections generated from the mid-point of the kidney by counting the number of cells, identified by DAPI, associated with a primary cilium, identified with anti-α-AcT. Deletion of Kif3a in ureteric or metanephric mesenchyme cells resulted in a gradual loss of primary cilia during embryogenesis. Kif3a<sup>−/−</sup>UB mice demonstrated 64% fewer ureteric-derived cells with primary cilia at E13.5 (Figure 2A<sup>′</sup> versus 2A; % cells with primary cilia, Kif3a<sup>−/−</sup>UB versus WT: 64 ± 1.07, n = 5 mice/group and 59% fewer cells with primary cilia at E13.5 (Figure 2B<sup>′</sup> versus 2B; % cells with primary cilia, Kif3a<sup>−/−</sup>UB versus WT: 40 ± 2.95, n = 5 mice/group). By E15.5, primary cilia could be detected in collecting ducts only rarely (Figure 2D<sup>′</sup> versus 2D). Furthermore, scanning electron microscopy (SEM) of collecting duct cells revealed decreased cilia number at E15.5 and complete absence of cilia at P0 (Figure 2F<sup>′</sup> versus 2F, and 2G<sup>′</sup> versus 2G, boxes). In Kif3a<sup>−/−</sup>MM kidneys, the number of metanephric mesenchyme cells with primary cilia was decreased by 70% at E13.5 (Figure 2A<sup>′</sup> versus 2A, asterisk; % cells with primary cilia, Kif3a<sup>−/−</sup>MM versus WT: 29 ± 5.67 versus 94 ± 1.14, n = 5 mice/group) and by 67% in proximal tubule cells at E15.5 (Figures 2C<sup>′</sup> versus 2C; % cells with primary cilia, Kif3a<sup>−/−</sup>MM versus WT: 29 ± 5.67 versus 94 ± 1.14, n = 5 mice/group). By P0, cilia were virtually absent from proximal tubule cells (Figure 2E<sup>′</sup> versus 2E). These results were confirmed by SEM analysis of proximal tubules, identified by apical brush border villae, and stained with DAPI at E15.5 and P0.
Figure 2. Gradual loss of primary cilia after induction of Kif3a deficiency in ureteric or metanephric mesenchyme cell lineages. (A, A', A'') KIF3A expression in E13.5 kidney tissue. (A) KIF3A co-localizes with α-AcT (arrow) on the apical surface of ureteric cells and in metanephric mesenchyme cells (box) in WT mice. (A') In Kif3a−/−UB mice, KIF3A expression (arrow) is markedly decreased in ureteric cells (box) but is comparable to WT in metanephric mesenchyme cells. (A'') In Kif3a−/−MM mice, KIF3A expression (arrow) is lost in metanephric mesenchyme cells (box) but is retained in ureteric cells. (B, B', B'') Primary cilia (arrow) in ureteric cells at E15.5. The number of cilia is decreased in Kif3a−/−UB mice (B') but is comparable to WT (B) in Kif3a−/−MM mice (B''). (C, C', C'') Primary cilia (arrow) in proximal tubules at E15.5. The number of cilia is decreased in Kif3a−/−MM mice (C'') but is unaffected in Kif3a−/−UB mice (C') compared to WT (C). (D, D', D'') Primary cilia in collecting ducts at P0. Cilia (red) are absent from the collecting duct lumen and the cell body (asterisk) (D') but are unaffected in Kif3a−/−MM mice. (E, E', E'') Primary cilia (arrow) in proximal tubules at P0. Cilia (arrows) are absent from the tubule lumen in Kif3a−/−MM mice (E''). Expression of α-AcT (asterisk) is visible within the body of some proximal tubule cells. Cilia are unaffected in the proximal tubule of Kif3a−/−UB mice (E'). (F–G'') SEM of collecting ducts in WT (F, G), Kif3a−/−UB (F', G'), and Kif3a−/−MM (F'', G'′) mice.
and 2I versus 2I, boxes). Taken together, these results indicate that deletion of Kif3a results in a cell-specific gradual loss of primary ciliature during embryonic kidney development with complete absence of cilia by P0. Kif3a/−/−UB and Kif3a+/−MM heterozygote mice were viable and characterized by normal renal development (data not shown). In contrast, both Kif3a−/−UB and Kif3a−/−MM mice exhibited a remarkably similar histologic phenotype characterized by epithelial cysts (Figure S3H, I), a reduction in the number of NCAM-positive nephrogenic precursor structures (Figure 3A–3C), and glomeruli, which are characterized by expression of WT1 in podocytes (Figure 3D–I). As in previous analyses, quantitation of NCAM-positive and WT1-positive structures was performed in sagittal tissue sections generated starting at the mid-point of a kidney. The number of NCAM-positive structures in Kif3a−/−UB and Kif3a+/−MM mice was reduced by 25% and 32%, respectively, at E13.5 (Figure 3J, no. NCAM-positive structures/section, WT versus Kif3a−/−UB: 16.5±3.05 versus 12.33±2.36, p = 0.003; WT versus Kif3a−/−MM: 16.5±3.05 versus 11.16±2.27, p = 0.001, n = 4 mice/group). The number of WT1-positive structures in Kif3a−/−UB and Kif3a+/−MM mice was reduced by 24% and 34%, respectively, at E15.5 (Figures 3D–F and 3K, no. WT1-positive structures/section: WT versus Kif3a−/−UB: 25.5±4.35 versus 18.8±2.71, p = 0.002; WT versus Kif3a−/−MM: 25.5±4.35 versus 17.51±1.25, p = 0.001, n = 4 mice/group), and by 25% and 35%, respectively, at P0 (Figure 3G–I and Figure 3K, no. WT1-positive structures/section: WT versus Kif3a−/−UB: 34.25±3.52 versus 25.12±3.68, p = 0.002; WT versus Kif3a−/−MM: 34.25±3.52 versus 21.6±2.56, p = 0.0001, n = 4 mice/group). Nephron number was also quantitated by counting the number of glomeruli, identified by their characteristic morphology in sagittal tissue sections generated in both directions from the mid-point of a kidney at 36 micrometer intervals to the outer limit of the organ [17]. At E13.5, this analysis demonstrated a reduction in glomerular number of 24% in Kif3a−/−UB kidneys and 33% in Kif3a+/−MM kidneys (Figure S3B and S3C versus S3A and Figure S3J); no. glomeruli/kidney, WT versus Kif3a−/−UB: 78.14±7.81 versus 62.65±4.29, p = 0.002; WT versus Kif3a−/−MM: 78.14±7.81 versus 54.85±7.20, p = 0.756E-05, n = 7 mice/group). Glomerular number was similarly reduced at E15.5 (Figure 3D–F, Figure S3J); no. glomeruli/kidney, WT versus Kif3a−/−UB: 1153±151 versus 887±74, p = 0.01; WT versus Kif3a−/−MM: 1153±151 versus 827±169, p = 0.005, n = 6 mice/group) and at P0 (Figure S3G–I, Figure S3J); no. glomeruli/kidney, WT versus Kif3a−/−UB: 2025±211 versus 1513±290, p = 0.01; WT versus Kif3a−/−MM: 2025±211 versus 1205±255, p = 0.001, n = 4 mice/group). Cyst formation in glomeruli and tubules (Figure 3I and Figure S3H and S3I, arrowheads) was consistent with published analysis of KIF3A function in the kidney [26]. Together, these results demonstrate that Kif3a deficiency in either the ureteric or metanephric mesenchyme cell lineage causes nephron deficiency.

Kif3a Controls Branching Morphogenesis in a GLI3R-dependent Manner

Formation of nephrons is initiated by signals released from ureteric bud-derived cells adjacent to mesenchymal nephrogenic progenitor cells [15]. Since nephron number is directly related to the number of ureteric branches elaborated during branching morphogenesis, we analyzed the effect of Kif3a deficiency on formation of ureteric branches by quantitating the number of ureteric bud tips, marked by expression of either GFP or Glut1. In Kif3a−/−UB mice, formation of the initial ‘T’ shape ureteric bud at E11.5 and the first two branch generations at E12.5 was normal compared with WT (Figure 4A, 4A’, 4B and 4B’). However, at E13.0 and E14.0, the number of ureteric bud tips was significantly reduced in Kif3a−/−UB mice (Figures 4C versus 4C, 4D versus 4D and Figure 4R; no. UB tips/kidney, WT versus Kif3a−/−UB at E13.0:19.2±2.28 versus 16.2±1.41, p = 0.03; WT versus Kif3a−/−UB at E14.0:39±2.59 versus 34±1.15, p = 0.03, n = 6 mice/group). Since ureteric branching is dependent, in part, on ureteric tip cell proliferation, we next identified ureteric tip cells undergoing mitosis using antibody specific for phospho-histone H3. Consistent with the decrease in ureteric branching, mitotic ureteric tip cell was decreased in Kif3a−/−UB mice (Figure 4K, 4L, and 4S; no. mitotic tip cells/kidney, WT versus Kif3a−/−UB: 7.72±1.81 versus 3.23±0.54, p = 0.001, n = 3 mice/group). These results are consistent with decreased nephron number observed in Kif3a−/−UB mice (Figure 3B versus 3A, and Figure 3F).

Ureteric branching is controlled by a Wnt11/Ret/Gdf6 signaling axis [27,28]. GDNF, an extracellular ligand expressed by metanephric mesenchyme cells, binds to RET on the surface of ureteric tip cells. In turn, GDNF/RET signaling controls ureteric tip cell expression of Wnt11, a positive regulator of ureteric branching (Figure 4Q). In Kif3a+/−UB mice, overall expression of Wnt11 and Ret was markedly reduced (Figures 4F and 4I, inserts). Higher resolution imaging of ureteric tips revealed weak expression of Wnt11 and Ret in ureteric cells (Figure 4F and 4I, arrowheads).

Previously, we demonstrated that expression of Wnt11 and Ret in ureteric tip cells is controlled by GLI3R [17]. Suppression of GLI3R formation in Patched1 deficient mice decreases Wnt11 and Ret expression and lowers ureteric branch and nephron number. Obligate expression of GLI3R via the Gli3D/0 allele, which expresses GLI3R in a constitutive manner [29], rescues these abnormalities [17]. Since defects in the primary cilium alter the ratio of GLI3 activator to GLI3R in favor of GLI3 activator [1], we hypothesized that GLI3R deficiency in Kif3a−/−UB mice could cause reduced branching morphogenesis. Thus, we determined whether constitutive expression of GLI3R in Kif3a−/−UB mice rescues ureteric branching. Analysis of Kif3a−/−UB,Gli3D/0/+ mice at E13.0 and E14.0 revealed that the number of ureteric tips was significantly increased compared to Kif3a−/−UB mice (Figure 4G, 4D’ and 4R; no. UB tips/kidney, Kif3a−/−UB,Gli3D/0/+ mice at E13.0:19.2±2.28 versus 13.33±0.82, p = 0.01; at E14.0:40.0±2.33 versus 33.33±2.5, p = 0.001, n = 6 mice/group). Further, expression of Wnt11 and Ret was markedly increased compared to that observed in Kif3a−/−UB mice (Figure 4G and 4J) versus Figure 4F and 4I). Concomitant with a rescue of ureteric branching, the number of NCAM-positive nephrogenic precursors in Kif3a−/−UB,Gli3D/0/+ mice was comparable to that observed in WT mice (Figure 4P versus 4O, and 4T; NCAM-positive structures/section - Kif3a−/−UB,Gli3D/0/+ versus WT: 15.3±3.40 versus 14.7±2.42, p = 0.27, n = 4 mice/group). Taken
Kif3a Controls Metanephric Mesenchyme Cell Survival

Decreased nephron number in Kif3a-/-MM mice [Figure 3J, 3K and Figure S3J] demonstrated that Kif3a functions in a cell autonomous manner within metanephric mesenchyme. We investigated mechanisms underlying Kif3a-dependent functions by first analyzing the effect of Kif3a deficiency on metanephric mesenchyme cells that are progressively committed to a nephrogenic fate. Kif3a(+/-)/Fgf8(-/-) mice were intercrossed with R26R-lacZ mice, which were used as a reporter for Rarb2-Cre activity and to label Kif3a-deficient cells [31]. Analysis of lacZ expression at E11.0 demonstrated that the metanephric blastema was smaller in Kif3a-/-MM mice compared to controls (Figure 5A and 5B, area of lacZ-positive tissue [µm²]: control –53806.86±662; Rarb2-Cre;R26R;Kif3a(+/-) – 33486±2563; p = 3.27×10^-5, n = 6 mice/group). Consistent with this finding, the number of SIX2-positive nephrogenic progenitor cells was decreased in Kif3a-/-MM mice (Figure S4D versus S4E, S3F; no. SIX2-positive cells/tissue section, WT versus Kif3a-/-MM: 48.5±2.65 versus 43.25±3.5, p = 0.008, n = 6 mice/group). This finding is consistent with the decreased number of NCAM-positive structures in Kif3a-/-MM mice (Figure 3A, 3C and 3J). Next, we determined whether these Kif3a-dependent effects on nephron number were associated with altered cell proliferation and/or apoptosis. We analyzed apoptosis at the stages when the process of nephron formation is well established (E12.5). The number of apoptotic nuclei, identified by the TUNEL assay, was significantly increased in metanephric mesenchyme cells in Kif3a-/-MM kidney tissue (Figure 5F versus 5E and 5H; no. TUNEL-positive cells/tissue section, WT versus Kif3a-/-MM: 3.25±0.96 versus 7.2±1.29, p = 0.0006, n = 4 mice/group). In contrast, the proportion of proliferating (BrdU-positive) cap mesenchyme cells did not differ in mutant mice (Figure S4A, S4B and S4C) (% BrdU-positive cap mesenchyme cells, WT versus Kif3a-/-MM: 89.6±2.38 versus 88.2±3.56, p = 0.51, n = 5 mice/group). Previously, we demonstrated that deficiency of nephrogenic progenitors during early stages of murine kidney development can limit the number of nephrogenic cells available to participate in more advanced stages of nephron formation [31]. We investigated this possibility in Kif3a-/-MM mice using the R26R allele and examining cortical sections for lacZ expression in nephrogenic structures. In control mice, lacZ-marked cells comprised 95% of the total number of cells (4547 of 4613 cells) resident within nephrogenic structures. In contrast, in mutant mice, lacZ-marked cells constituted 82% of the total number of cells in nephrogenic structures (2413 of 3251 cells) (p = 0.02; n = 6 cortical section counted in each of 6 mice/group, Figure 5C, 5D and 5G). These data suggest that depletion of Kif3a deficient nephrogenic progenitors may provide a selective advantage for Rarb2-Cre-negative cells with WT levels of KIF3A to participate in nephron formation. Together, these analyses of cell fate and cell survival indicate that Kif3a deficiency interferes with survival of nephrogenic progenitor cells and decreases the size of the cellular pool available to participate in nephron formation.

Kif3a Deficiency Leads to Fgf8 Deficiency in Nephrogenic Mesenchyme

Nephron formation is dependent on a signaling axis in which Fgf8 functions upstream of Wnt4 and Lim1 (Figure 6A). In the absence of Fgf8, neither Wnt4 nor Lim1 is expressed and nephron formation largely fails to progress to the stage of the S-shaped body.
Figure 4. Kif3a controls renal branching morphogenesis in a Gli3R-dependent manner. (A–C'). Cytokeratin immunofluorescence demonstrating ureteric branching at E11.5 and E12.5. The branch pattern is similar among WT (A,B), Kif3a+/−/UB (A',B') and Kif3a−/−/UB;Gli3+/−/UB, Kif3a−/−/UB;Gli3−/−/UB mice at E13.0 and E14.0. In Hoxb7-GFP-Cre mice, GFP expression in the kidney is restricted to the ureteric cell lineage. Ureteric branches expressing GFP are visualized in whole mount preparations of kidney explants generated from E13.0 (C, C' and C'') and E14.0 (D, D' and D''). Imaging suggests that branch number in Kif3a−/−/UB mice (C, D') is less than that in WT (C, D) and Kif3a+/−/UB;Gli3+/−/UB mice (C', D'), (E–J) Wnt11 and Ret expression. In WT kidney, Wnt11 (E) and Ret (H) are strongly expressed in ureteric tip cells (arrows). Expression of Wnt11 (F) and Ret (I) is markedly decreased in Kif3a−/−/UB mice. In Kif3a−/−/UB;Gli3−/−/UB mice, Wnt11 (G) and Ret (J) expression are rescued to levels similar to that observed in WT mice. (K–M) Phospho-Histone H3 (pHH3) is stained in mitotic cell of ureteric tip (red color) that is marked by cytokeratin (green color) at E13.5. (N–P) NCAM stain-demonstrating ureteric branching at E11.5 and E12.5. The branch pattern is similar among positive and −negative cells (G) and TUNEL-positive cells (H). (R) Quantitation of ureteric branch tip number reveals a significant decrease in Kif3a−/−/UB mice compared to WT at E13.0 and E14.0 but increased branching in Kif3a−/−/UB;Gli3−/−/UB mice compared to Kif3a−/−/UB mice. (S) Quantitation of mitotic tip cells in K, L and M. pH3 marked cells are decreased in Kif3a−/−/UB mice (L) compared to WT (K). pH3-positive cells is remarkably increased in in Kif3a−/−/UB;Gli3−/−/UB mice compared to Kif3a−/−/UB mice (M versus L). (T) Quantitation of NCAM-positive structures in N, O and P reveals a significant decrease in Kif3a−/−/UB mice compared to WT and Kif3a−/−/UB;Gli3−/−/UB mice. (*, P<0.05, **, P<0.01, *** P<0.001). Scale bars: 50 micrometer.

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[22]. Since FGF receptors have been localized to the cilium in nonrenal tissues [12], we investigated the possibility that Kif3a deficiency and loss of primary cilia interfere with FGF8-mediated signaling during nephron formation. In support of this possibility, analysis of kidney tissue derived from Kif3a−/−/MM mice at P0 revealed a 28% reduction in the number of S-shaped bodies (Figure 6H and 6I, Kif3a−/−/MM versus control – no, S-shape bodies/kidney: 6.16±0.5 versus 3.83±1.73; P<0.0003, n=5 mice/group). While expression of Fgfb mRNA was only mildly decreased at E13.5 (Figure S5), by E15.5, Fgfb expression was

Figure 5. Kif3a controls survival of nephrogenic precursor cells. (A–D) LacZ expression in kidney tissue. (A, B) LacZ-marked metanephric blastema at E11.0 is demarcated by red dotted lines. The position of the ureteric bud is marked by white dotted lines. The metanephric blastema is smaller in Kif3a−/−/MM mice (B) compared to control (A). (C, D) Incorporation of pink (lacZ-negative; Kif3a+/−) cells into nephrogenic structures (arrows) is greater in Kif3a−/−/MM mice (D) compared to controls (C). (E,F) TUNEL assay identify the apoptotic cell (arrows) of the metanephric mesenchyme at E12.5. The number of TUNEL-positive cells is greater in Kif3a−/−/MM mice (F, arrows) compared to control (E, arrows). (G, H) Quantitation of lacZ-positive and −negative cells (G) and TUNEL-positive cells (H). Kif3a−/−/MM mice exhibit a significantly larger contribution of lacZ-negative cells to nephrogenic structures (D), and increased apoptosis in metanephric mesenchymal cells (F). (***, P<0.001; **, P<0.01; *, P<0.05). Scale bars: 50 micrometer.

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almost undetectable (Figure 6C versus 6B, insert and Figure 7G, lane 3). Further, expression of Wnt4 and Lim1 was markedly reduced with only a focal pattern of expression within some nephrogenic structures (Figure 6E versus 6D and 6G versus 6F, insert). Together, these results indicate that Kif3a deficiency decreases Fgf8-dependent signaling during renal development.

Kif3a Controls Expression of Fgf8 by Metanephric Mesenchyme Cells

Previous studies have demonstrated that Fgf8 signalling regulates cilia length via the IFT pathway in diverse epithelia [11]. Yet, a role for Kif3a in controlling Fgf8 expression has not been previously elucidated. Our results demonstrating that Kif3a deficiency precedes Fgf8 deficiency in metanephric mesenchyme suggested the possibility that Kif3a controls Fgf8 expression in metanephric mesenchyme cells. We tested our hypothesis in cultured metanephric mesenchyme cells isolated from WT and Kif3a<sup>2/2</sup> MM mice and transfected with a DNA construct encoding a Kif3a-GFP fusion protein (Figure 7). Analysis of primary cilia length, identified by α-ACT expression and imaged by confocal microscopy in cultured primary metanephric mesenchyme cells, revealed that cilia were markedly shorter in cells isolated from Kif3a<sup>2/2-MM</sup> mice (Figure 7B versus 7A and Figure 7E, cilium length, WT versus Kif3a<sup>2/2-MM</sup> MM cells: 4.585±1.523 micrometer versus 1.576±0.449 micrometer; p = 0.03, n = 10 culture wells/group). Transfection of Kif3a fused with GFP in metanephric mesenchyme cell cultures caused localization of KIF3A and GFP to primary cilia and a significant increase in cilia length in metanephric mesenchyme cells isolated from Kif3a<sup>2/2-MM</sup> mice (Figure 7C and 7D and Figure 7E, cilium length, untransfected versus transfected Kif3a<sup>2/2-MM</sup> metanephric mesenchyme cells: 1.576±0.449 micrometer versus 1.962±0.360 micrometer; p = 0.03, n = 10 culture wells/group). Next, we analyzed the effect of Kif3a on expression of Hh signaling effectors, the expression of which has been shown to be dependent on Kif3a in nonrenal tissues. Surprisingly, neither loss of Kif3a expression in Kif3a<sup>2/2-MM</sup> metanephric mesenchyme tissue, cultured metanephric mesenchyme cells nor transfection-mediated Kif3a expression in these cells was associated with a detectable change in Ptc1 or Gli1 mRNA levels assayed by quantitative PCR (Figure 7G and 7F). In contrast, Fgf8 mRNA expression was significantly lower in cultured Kif3a<sup>2/2-MM</sup> metanephric mesenchyme cells compared to controls (Figure 7G and 7H). Moreover, Kif3a transfection significantly increased expression of Fgf8 mRNA in both WT and Kif3a-deficient metanephric mesenchyme cells (Figure 7G and 7H). Together, these data indicate that Kif3a controls Fgf8 expression in metanephric mesenchyme cells.

Discussion

Cilia proteins KIF3A, IFT88 and IFT20, which are involved in IFT [2,32,33], are required for renal ciliogenesis; inactivation of each is known to cause cystic kidney disease [3,14,34,35]. To our knowledge this is the first study demonstrating a role for the primary cilium in the regulation of nephron number. Our data show that Kif3a expression and primary cilia are found in both
ureteric cells and metanephric mesenchyme cells from the onset of murine kidney development. CRE-mediated recombination using a Kif3aloxP allele results in near total loss of Kif3a in CRE-expressing cells. Loss of cilia occurs with slower kinetics. Yet, Kif3a deficiency and a decrease in the number of primary cilia reduce the number of nephron precursor structures formed and the final number of mature nephrons. Experiments that investigated the mechanisms underlying this phenotype support a model of Kif3a function

Figure 7. Kif3a acts upstream of Fgf8. (A–D) Analysis of primary cilia (arrows) in metanephric mesenchyme cells derived from WT and Kif3a1/1-MM metanephiroi dissected free of ureteric bud. Cilia are identified by expression of α-Act. (A′–D′) Higher magnification of images in A–D, respectively. WT and Kif3a-deficient cells were transfected with a plasmid encoding Kif3a fused to GFP. Cilia in Kif3a-deficient mesenchyme cells (B, B′) are vestigial in comparison to cilia on WT cells (A, A′). Transfection with Kif3a results in localization of GFP to the cilium in each treatment group (C, D) and lengthening of the cilium in Kif3a1/1-MM cells (D versus B). (E) Quantitation of cilia length in untransfected and transfected WT and Kif3a1/1-MM cells. Expression of Kif3a partially rescues cilia length in Kif3a-deficient cells. (F) Quantitation of Ptc1 and Gli1 mRNA expression, measured by quantitative RT-PCR in untransfected and transfected WT and Kif3a1/1-MM cells. Ptc1 and Gli1 mRNA expression is not affected by Kif3a deficiency or transfection with Kif3a. (G) Expression of Fgf8, Ptc1, and Gli1 mRNA, measured by real time RT-PCR using RNA isolated from kidney explants and from untransfected and transfected cultured metanephric mesenchyme cells. (H) Quantitation of Fgf8 mRNA levels measured by quantitative RT-PCR as in panel G. MM, metanephric mesenchyme; UB, ureteric bud; WT, wild-type. (**, P < 0.01; *, P < 0.05), Scale bars: (A–D) 25 micrometer, (I–L) 50 micrometer.
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during murine renal development (Figure 8). Our model suggests that the functions of Kif3a in controlling nephron formation are specific to the ureteric and metanephric mesenchyme cell lineages. In the ureteric lineage, Kif3a controls the number of ureteric branches formed in a GLI3R-dependent manner. Control of branch number is a critical determinant of nephron number. In metanephric mesenchyme cells, Kif3a exerts two major distinct effects. First, Kif3a controls cell survival such that the metanephric blastema that gives rise to nephrons is smaller in Kif3a deficient mice and that surviving Kif3a deficient metanephric mesenchyme cells are less able to take part in forming nephrogenic structures compared to their wild type counterparts. Second, Kif3a controls expression of genes required during nephron formation in metanephric mesenchyme cells.

Our results demonstrate a central role for KIF3A in controlling ureteric and mesenchyme cell function. Lineage-specific deletion of Kif3a is efficient with little residual Kif3a mRNA expression in either the ureteric or metanephric mesenchyme cell populations by E11.5, the stage at which these cell populations were separated and analyzed in Kif3a<sup>−/−</sup>UB and Kif3a<sup>−/−MM</sup> mice (Figure S2). Our results also strongly suggest that the functions of KIF3A protein are related to its specific expression in the primary cilium. Analysis of KIF3A protein expression in Kif3a-deficient mouse strains demonstrated a specific KIF3A signal in the primary cilium (Figure 2A, 2A', 2A''). Interestingly, primary cilium exhibits a comparatively slower turnover rate, compared to KIF3A protein, in ureteric and mesenchyme cells. While we could detect very little KIF3A protein by E13.5, in the ureteric or metanephric mesenchyme cells of Kif3a<sup>+/−</sup>UB and Kif3a<sup>−/−MM</sup> mice, respectively, primary cilium could be detected at E15.5, albeit in reduced numbers. These observations suggest that primary cilium structure can be maintained in non-dividing cells in the face of KIF3A depletion. However, our studies do not provide information as to whether the function of KIF3A-deficient cilia is normal. Our data are also consistent with the rather modest effect of KIF3A deficiency on nephron number. Given the slow kinetics of KIF3A depletion and loss of cilia, it is likely that remaining number of cilia during early critical stages of nephron formation are sufficient to support this process.

Our results demonstrate a critical role for GLI3R in primary cilium function in ureteric cells and are consistent with our previous work related to GLI3R and ureteric branching [17]. The initial stage of ureteric bud invasion into the mesenchyme appears normal in Kif3a<sup>−/−UB</sup> kidneys. However, the expression of Wnt11 and Ret, both of which are required for ureteric branching [29,36], is decreased in mutant kidneys at E13.5 (Figure 4F,G and 4H, I). Primary cilia are present on only a subset of UB cells at this time point as primary cilia are gradually lost from E13.5 to P0. Concomitantly, the expression of the ureteric tip markers, and the number of ureteric tips is significantly reduced at E14.0.

In our previous published work, analysis of Hh signaling activity, using a Ptc1-lacZ reporter, demonstrated that ureteric tips are characterized by low Hh activity [17]. Activation of Hh signaling activity in ureteric cells in mice with Ptc1 deficiency causes decreased Ret and Wnt11 expression, decreased ureteric branching and low nephron number. But, constitutive expression of GLI3R (via the Gli3<sup>−/−</sup> allele) rescues these abnormalities [17] and suggests that GLI3R, rather than GLI activators, is the regulatory target of SHH signaling during formation of nephrons. Our prior analyses in Shh deficient mice also support the concept that regulation of GLI3R is the critical event during kidney development. Mice with homozygous deficiency of Shh are characterized by disruption of initial ureteric-metanephric mesenchyme tissue interactions and an elevated ratio of GLI3R to GLI activator proteins in Shh deficient renal tissue. Remarkably, these abnormalities are rescued by homozygous deficiency of Gli3 in Shh deficient mice, thus implicating regulation of GLI3R formation as a critical event during renal development [16]. Results here suggest that the primary cilium plays a critical role in GLI3R expression in ureteric cells.

In contrast to ureteric cells, our results do not invoke Hh signaling and GLI3R in regulating metanephric mesenchyme cell survival and nephron formation. Our data, are consistent with our published analysis of kidney development in mice with conditional inactivation of Smo in metanephric mesenchyme cells [37]. In these mice (Rarb2-Cre;Smoo<sup>ΔP/T</sup>), genetic inactivation of Smo was mediated by CRE recombinase, the expression of which was driven by a Rarb2 promoter element which directs expression in the intermediate and metanephric mesenchyme [31]. In Rarb2-Cre;Smo<sup>ΔP/T</sup> mice, renal development is normal until E15.5 when pelvic dilatation arises due to ureteric dyskinesia and abnormal pacemaker cell function, demonstrating that loss of Hh signaling in intermediate and metanephric does not disrupt the mass of cells available to take part in nephron formation. Our results in cultured metanephric mesenchyme cells (Figure 7) are consistent with these findings in Rarb2-Cre;Smo<sup>ΔP/T</sup> mice since Ptc1 and Gli1 are expressed in Kif3a deficient metanephric mesenchyme cells isolated at E11.5. Moreover, transfection of Kif3a in Kif3a-deficient cells has no significant effect on Ptc1 and Gli1 expression.

Our analyses in Kif3a<sup>−/−MM</sup> mice and in cultured metanephric mesenchyme cells suggest a role for KIF3A upstream of FGF8. Kif3a<sup>−/−MM</sup> mice are characterized by cilia in developing nephron structures and intact nephron formation before E13.5. However, the total pool of Kif3a-negative mesenchymal precursor cells is decreased in the kidney blastema at E11.0. Surviving Kif3a-

![Figure 8. Model of Kif3a-mediated regulation of nephron number. A Kif3a control is required in both ureteric and metanephric mesenchyme cells for processes that control nephron number. In ureteric cells Kif3a acts in a GLI3R-dependent manner to control expression of Wnt11/Ret and ureteric branching. In metanephric mesenchyme cells, Kif3a functions to control cell survival and expression of Fgf8, which is required for maturation of nephrogenic progenitors. The lineage-specific functions of Kif3a in ureteric and metanephric mesenchyme cells converge to control nephron number. doi:10.1371/journal.pone.0065448.g008](http://www.plosone.org/doi/10.1371/journal.pone.0065448.g008)
negative mesenchyme cells exhibit the ability to undergo a mesenchymal to epithelial transition probably because a sufficient level of Fgβ, Wnt4 and Lm1 mRNA is present to support this process (Figure S5). However by E15.5, Fgβ expression is lost – the same stage at which the number of cells is significantly reduced in mesenchyme-derived cells. By E15.5, Wnt4 and Lm1 expression is markedly reduced consistent with loss of Fgβ. Yet, our results related to Fgβ are distinct from previous published analyses of Fgβ activity during renal development. Mice with total loss of related to FGF8 are distinct from previous published analyses of Fgf8 S1 in File S1.

mice [30] were maintained on a B6 x129/SV mixed genetic background. R26R reporter and Rarb2-Cre;Kif3a PAX2 (1:500, PRB-276P, Covance), antibodies directed against: KIF3A (1:100, K3513, Sigma), was performed [45]. Isolated cells were cultured overnight then passaged to a new dish and cultured for 16 hours after which cells were transfected with a pEGFP-N1 vector containing full length Kif3a cDNA (0.8 micrograms/well) using Lipofectamine™ 2000 (Invitrogen) according to the manufacturers’ instructions. Cells were harvested 36 hours after transfection.

Antibodies

Immunofluorescence analyses of kidney tissue and cultured cells was performed using published methods [44,46,47] using antibodies directed against: KIF3A (1:100, K3513, Sigma), PAX2 (1:500, PRB-276P, Covance), α-Act (1:1000, T6793, Sigma), Cytokeratin (1:200, C2562, Sigma), NCAM (Neural Cell Adhesion Molecule) (1:50, C9672, Sigma), WT1 (1:500, sc-192, SANTA CRUZ), OTL (Lotus Tetragonolobus Lectin) (1:100, FITC, FL-1321, Vector Laboratories, Inc.), SIX2 (1:200, ab66908, Abcam), Phospho-Histone H3 (Ser28) (1:200, Cell Signaling) and GFP (1:1000, Ab16901, Millipore). Secondary antibodies used were Alexa 488 anti-rabbit IgG, anti-rat IgG and anti-chicken IgG, as well as Alexa 546 anti-rat IgG and anti-rabbit IgG, (1:1000, Molecular Probes, Invitrogen Detection Technologies). DAPI (1:1000, D9564, Sigma) was used for nuclear staining.

RNA Isolation and Real-Time PCR

Total RNA was purified from isolated E11.5 ureteric bud and metanephric mesenchyme or whole embryonic kidneys using the RNeasy Mini Kit (QIAGEN). cDNA was synthesized using a first strand cDNA synthesis kit (Invitrogen). Real-time RT-PCR [46] was performed to determine the expression of Kif3a, Ptc1, Gli1 and
Fgf8, Gaptb served as an endogenous control. The primers used, the fragments amplified, and the annealing temperatures are detailed in Table S2 in File S1. Quantitative RT-PCR (qRT-PCR) was performed using an Applied Biosystems 7900 HT fast RT-PCR system with TaqMan® Universal PCR Master Mix and TaqMan® probes for Kif3a (Mm00438922_m1) or Fgf8 (Mm00439287_m1). Mouse Gaptb was used as an endogenous control (Mm0330249_g1, Applied Biosystems). Primers for Ptc1 and Gld were designed using Primer 3 software. Relative mRNA expression was determined using the standard curve method. Samples were analysed in triplicates.

Image Capture and Data Analysis

Kidneys for SEM were perfused with 4% PFA and 2% glutaraldehyde in PBS, prepared as described previously [14], and visualized with a FEI XL30 Scanning Electron Microscope at the Advanced Bioimaging Center of Mount Sinai Hospital, University of Toronto. Microscopy was also performed using a spinning disk confocal laser scanning microscope or Zeiss Axiovision® light microscope. A minimum of four mice (derived from different litters) were analysed for each developmental stage, gene, antigen and genotype. Student's t-test (two-tailed) was used to analyze the mean differences between groups. The statistical significance was taken at a value of P<0.05. Images were combined using Adobe Photoshop CS2 and CorelDRAW 14 software.

Ethics Statement

All experiments using animals have been conducted according to the guidelines adopted by the Toronto Centre for Phenogenomics and which are in accord with national and international guidelines. The experiments, the results of which are reported here, were approved by the Institutional Animal Care and Use Committee (IACUC) of the Toronto Centre for Phenogenomics. Animals were sacrificed via inhalation of CO2.

Supporting Information

Figure S1 Primary cilia are present in both ureteric epithelial and metanephric mesenchyme cells in the developing murine kidney. (A–D) Schematic of ureteric bud epithelial cells (black), metanephric mesenchyme cells and metanephric-derived nephrogenic stuctures during progressive stages of kidney development (green). (A’–D’). Primary cilia (acetylated α-tubulin, red, arrows) are present in both ureteric (arrows), metanephric mesenchyme cells and their derivatives (Pax2, green, arrow heads) in E11.5 (A’), E13.5 (B’), and E15.5 (C’) kidneys. (A”–D”) Single color shows primary cilia in the developing kidney. CM, Condensate Mesenchyme; CSB, Comma-Shape Body; UB, Ureteric Bud; RV, Renal vesical; WT, wild type. Scale bar: C’–F”, 25 micrometer. (TIF)

Figure S2 Expression of Kif3a in kidney tissue. Ureteric bud was dissected free of metanephric mesenchyme in E11.5 kidney tissue of WT, Kif3a−/−UB, and Kif3a−/−MM mice. Kif3a mRNA expression was analyzed by quantitative RT-PCR and quantified. Kif3a is not expressed in the ureteric bud of Kif3a−/−UB mice but is expressed in metanephric mesenchyme. Kif3a is not expressed in the metanephric mesenchyme of Kif3a−/−MM mice but is expressed in ureteric bud. (**, P<0.001). (TIF)

Figure S3 Decreased nephron number in both Kif3a−/−UB and Kif3a−/−MM kidneys. (A–F) Histological sections, stained with hematoxylin and eosin demonstrate a qualitative decrease in the number of glomeruli (arrows) at E15.5 (A,B,C) and E18.5 (D,E,F) in both Kif3a−/−UB (B, E) and Kif3a−/−MM (C, F) mice compared to WT (A, D). (G–I) The decrease in mature glomeruli (arrows) in both Kif3a−/−UB (H) and Kif3a−/−MM (I) mice is greater at P0. Cysts are present in collecting duct (H, arrowheads) and tubules (I, arrowheads) in both mutant mouse strains. (J) Quantification of the number of mature glomeruli demonstrates a decrease in Kif3a−/−UB and Kif3a−/−MM mice at E15.5, E18.5 and P0 compared to controls. (**, P<0.001; *, P<0.01; *, P<0.05). Scale bars: 50 micrometer. (TIF)

Figure S4 Cell proliferation and SIX2-positive nephrogenic progenitor cells in Kif3a−/−MM kidney tissue. (A, B) In situ BrdU incorporation assay in E13.5 kidney tissue. Ureteric bud tip is demarcated by the yellow dotted line. (C) Quantification of BrdU-positive cap mesenchyme cells reveals no significant difference between Kif3a−/−UB and WT mice. (D, D’) SIX2-positive cells (nephrogenic precursors) are organized in a tightly packed layer around the ureteric bud tip at E13.5 in WT mice. (E, E’). The SIX2-positive cells are disorganized surrounding the ureteric tip in Kif3a−/−MM mice. (F) Quantification of the SIX2-positive cells demonstrates a significant decrease in Kif3a−/−MM mice versus WT control mice. (**, P<0.01). Scale bars: 50 micrometer. (TIF)

Figure S5 Expression of Fgf8, Wnt4, Lim1 and Wnt9b mRNAs in E13.5 Kif3a−/−MM mice. Expression was determined by in situ hybridization. Expression of Fgf8 is mildly decreased in Kif3a−/−MM mice (A’) compared to WT (A) but expression of Wnt4, (B, B’), Lim1 (C, C’) and Wnt9b (B, D’) is unchanged. Scale bars: 50 micrometer. (TIF)

File S1 Table S1, Primers used to genotype the various mutant mouse lines. Table S2, The primers and their RT-PCR products used to estimate the mRNA expression in kidneys and in MM cells. (DOC)

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Author Contributions

Conceived and designed the experiments: L. Chi L. Chen NDR AG. Performed the experiments: L. Chi L. Chen RM AG. Analyzed the data: L. Chi NDR CCH. Wrote the paper: L. Chi NDR.

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