nature portfolio

https://doi.org/10.1038/s41581-022-00598-5

Supplementary information

Principles of human and mouse nephron development

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Supplementary Table 1 | Genes and pathways associated with nephron patterning phenotypes

Gene	**Species**	**Genetics**	**Phenotype**	**Reference(s)**
Notch2	Mouse	*Pax3*$^{CreTg/+}$; *Notch2*$^{f/f}$	1. No LTL$^+$ tubules, but tubules do form 2. No RC 3. Abnormal SSB 4. Postnatal lethal	1
Notch2	Mouse	*Six2*$^{+/Cre}$; *Notch2*$^{f/f}$	1. *Tfap2b*$^+$, *Krt8/18*$^+$ distal cell types do form, but LTL$^+$ PTs do not 2. Postnatally lethal (all die by P2) 3. PT cyst formation at P1	2
Rbpj	Mouse	*Pax2*$^{CreTg/+}$; *Rbp-J*$^{f/f}$	1.No LTL$^+$ tubules 2. No RC 3. E12.5 lethal	1
N1-ICD	Mouse	*Six2*^{TGC}; *Rosa26*$^{N1ICD/+}$	1. Rescue of LTL$^+$ tubules 2. RCs form (abnormal morphologies) 3. Postnatal lethal	1
Jag1	Mouse	*Six2*^{Cre}; *Jag1*$^{f/f}$ *Cdh16*^{Cre}; *Jag1*$^{f/f}$ *Atp6v1b1*^{Cre}; *Jag1*$^{f/f}$	*Six2*^{Cre}: 1. Reduced number of nephrons 2. Disrupted podocyte (*Wt1*$^+$) and PT (LTL$^+$) formation *Cdh16*^{Cre}: 1. Greater number cells expressing *both* principal and intercalated markers 2. [Those] tubules enlarged 3. Adult hydronephrosis *Atp6v1b1*^{Cre}: 1. Greater number of cells expressing *both* principal and intercalated markers 2. Decreased number of principal cells	3,4
Dll1	Mouse	*Six2*^{Cre}; *Dll1*$^{f/f}$	1. Mildly disrupted LTL$^+$ tubules	3
Bmp7	Mouse	*Bmp7*$^{-/-}$ (exon deletion)	1. Reduced *Wt1*, *Pax2*, *Wnt4* expression 2. High nephron cell death 3. Reduced kidney size 4. Reduced *Wt1*, *Pax2*, *Wnt4* expression 5. Accumulation of interstitial mesenchyme	5
		Bmp7$^{+/-}$		6
Bmp4	Mouse	*Bmp4*$^{+/-}$	1. Embryonic hypoplastic kidney 2. Urinary tract anomalies	7
Fgf8	Mouse	*Pax3*$^{CreTg/+}$; *Fgf8*$^{fl/-}$ (two variants)	1. Loss of *Wnt4*$^+$ & *Lhx1*$^+$ early nephrons 2. Early block of nephrogenesis	8
Fgf8	Mouse	*Fgf8*$^{neo/-}$ (hypomorph)	1. Loss of tubular components of nephron 2. RCs form but are less frequent 3. Smaller kidneys	8
Fgf9; Fgf20	Mouse/*In vitro* NPs	*HoxB7*$^{Cre+/Tg}$*; Fgf9*$^{-/-}$*; Fgf20*$^{\beta Gal/\beta Gal}$	1. Decreased NP self-renewal 2. Perturbed UB outgrowth and branching	9,10
Wnt4	Mouse	*Wnt4*$^{-/-}$	1. Complete loss of nephrogenesis 2. Postnatal lethal	11
Wnt9b	Mouse	*Wnt9b*$^{-/-}$ *Wnt9b*$^{neo/neo}$	1. Loss of mesonephric tubules 2. Disruption of secondary branching after T-stage 3. Cystic kidneys 4. Increased cell number per PT wall	12,13
Wnt7b	Mouse	*Wnt7b*$^{c3/-}$*; Sox2Cre*	1. Absence of tubular network that demarcates the renal medulla 2. Disrupted distal CD epithelium morphogenesis 3. Arrest of LOH elongation (proliferation)	14
Cdh4 (R-cadherin)	Mouse	*Cdh4*$^{-/-}$	1. Mild defects to nephrogenesis 2. Mild defects to PT formation	15
Cdh6 (K-cadherin)	Mouse	*Cdh6*$^{-/-}$	1. Mild defects to nephrogenesis 2. Mild defects to PT formation	16
Ctnnb1 (β-catenin)	Mouse	*Ctnnb1*$^{c/c}$*; Osr2*$^{IresCre/+}$ (loss of function)	1. Aberrant arrangement of *Wt1*$^+$ podocytes 2. Loss of *Pecam1*$^+$ endothelial cells in the RC 3. Loss of *Akap12* expression in parietal epithelial cells	17

			4. Weak LTL staining compared to wildtype 5. Thin *Slc12a1*$^{+}$ tubules	
		Ctnnb1$^{ex3/+}$*; Wnt4GFP*$^{cre/+}$ (gain of function)	1. Loss of *Wt1* expression 2. Significantly fewer cortical *Hnf4a*$^{+}$ PT cells 3. Expansion of the *Jag1* expression domain into the proximal domain of the SSB	
Erbb4	Mouse	*R26ERBB4*$^{+/-}$*; Pax8*$^{Cre/+}$ (gain of function)	1. Defective PT development and polarization, cyst formation	[18]
		Erbb4$^{fl/fl}$*/Pax8*$^{Cre/+}$ (loss of function)	1. Reduced medullary cell density, increased CD lumen size	
Transcription Factors	**Species**	**Genetics**	**Phenotype**	**Reference(s)**
Pou3f3	Mouse	*Pou3f3*$^{-/-}$	1. Arrested development of LOH and distal nephron segments 2. RCs form 3. PCT form 4. Postnatally lethal	[19]
Sim1	Zebrafish	*sim1a* MO	1. Loss of PST and CS, PST elongation	[20]
		sim1a MO + RA	1. RA dose-dependent PST elongation, loss of PCT, CS	
Mecom	Zebrafish	*mecom* MO	1. *emx1* downregulation. 2. Anterior reduction of *slc12a3*$^{+}$ DL domain 3. *tbx2a/b* downregulation 4. posterior PST expansion 5. MCC domain expansion	[21,22]
Emx1	Zebrafish	*emx1* MO	1. Posterior expansion of *slc12a1*$^{+}$ DE domain 2. *irx1a, sim1a* upregulation	[21]
Kctd15	Zebrafish	*kctd15a, b* MO	1. Expansion of *tfap2a*$^{+}$ boundary 2. Expansion of *slc12a1*$^{+}$ DE domain 3. Loss of PST/DE and DE/DL boundaries	[23]
Irx3	*Xenopus*	*irx3* MO	1. Loss of intermediate tubule	[24]
	Zebrafish	*irx3b* MO	1. Loss of *slc12a1* expression in DE 2. PCT, PST domain expansion	[25]
Tfap2a	Fish	*trm*$^{-/-}$ (point mutation altering *tfap2a* exon 1 donor splice site)	1. Significant reduction of *slc12a1*$^{+}$ domain 2. Loss of *irx1a* expression in DE segment	[26]
Tfap2b	Mouse	*Six2*^{Cre}*; Tfap2b*$^{f/f}$	1. Loss of *Pvalb* expression (P0)	[27]
Hnf1b	Mouse	*Six2*^{Cre}*; Hnf1b*$^{f/f}$	1. RV and CSB form, few to no SSB	[28]
		Wnt4$^{+/EGFPCre}$*; Hnf1b*$^{lacZ/f}$	2. Loss of epithelial bulge in medial SSB due to high apoptosis 3. Loss of tubules (PT, LOH, DT) 4. Glomeruli form 5. Postnatal lethal (*Six2*^{Cre}*; Hnf1b*$^{f/f}$)	[29]
Hnf4a	Mouse	2018: *Six2*$^{GFP/Cre}$ (leaky) *Hnf4a*^{tm1Sad}*, Rosa26*^{EYFP} 2020: *Osr2*^{IresCre}*, Hnf4a*^{tm1Sad}*,* *Cdh6*^{CreER}*, Rosa26*^{Ai3}	1. Loss of mature PTs without affecting other nephron segments 2018: 1. Mice phenocopied Fanconi Syndrome 2. Decreased expression of PT maturation genes 2020: 1. Postnatally lethal – none survived to 4 weeks 2. P0: Visually similar to control 3. P7: Visually smaller, thinner cortex 4. P14: Severely damaged medullary region, cortical cysts, hydronephrosis	[30,31]
Wt1	Mouse	*Nes*^{Cre} *Pax8*^{Cre} *Wnt4*^{Cre}	1. All postnatally lethal, with specific effects depending on the Cre-driver used: *Nes*^{Cre}: 1. Mesenchymal cell expansion at the cost of severely diminished cellular condensation and epithelialization *Pax8*^{Cre}: 1. Blocks nephrogenesis and further maturation immediately after the MET 2. Loss of any proximal-distal patterning	[32]

			3. Normal condensation and epithelialization *Wnt4*^{Cre}: 1. Loss of tubule maturation and glomerulogenesis after the MET 2. Expanded mesenchyme with slightly reduced epithelialization & condensation	

CD, collecting duct; CSB, Comma-shaped body; DE, distal early; DT, distal tubule; DL, distal late; LOH, loop of Henle; MCC, multiciliated cells; MET; mesenchymal-to-epithelial transition; MO, morpholino; NP, nephron progenitor; PCT, proximal convoluted tubule; PST, proximal straight tubule; PT, proximal tubule; RA, retinoic acid; RC, renal corpuscle; RV, renal vesicle; SSB,S-shaped body; UB, ureteric bud.

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