Cell Biology of Ureter Development

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Cell biology of ureter development

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Conflict of interest
None declared

Abstract
The mammalian ureter contains two main cell types: a multilayered water-tight epithelium called the urothelium, surrounded by smooth muscle layers which, by generating proximal to distal peristaltic waves, pump urine from the renal pelvis toward the urinary bladder. Here, we review the cellular mechanisms involved in the development of these tissues, and the molecules which control these processes. We consider the relevance of these biological findings for understanding the pathogenesis of human ureter malformations.

Key words
epithelium, growth factor, kidney, malformation, metanephric mesenchyme, mutation, nephric duct, signaling, ureteric bud

Molecule Abbreviation Box

ALK Activin receptor-like kinase (growth factor receptor)
AngII Angiotensin II (growth factor)
BMP Bone morphogenetic protein (growth factor)
DLGH Discs-large homolog (intracellular scaffolding protein)
ERK Extracellular signal-regulated kinase (intracellular signaling molecule)
ETV ETS transcription factor (transcription factor)
FGFR Fibroblast growth factor receptor (growth factor receptor)
FOX Forkhead box (transcription factor)
FRAS Fraser syndrome (basement membrane molecule)
FREM FRAS1-related extracellular matrix (basement membrane molecule)
GDNF Glial cell line-derived neurotrophic factor (growth factor)
GATA GATA-binding factor (transcription factor)
GFR GDNF family receptor (growth factor receptor)
HCN3 Potassium/sodium hyperpolarization-activated cyclic nucleotide-gated channel 3 (ion channel)
HNF1B Hepatocyte nuclear factor 1B (transcription factor)
KIT v-kit Hardy-Zuckerman 4 feline sarcoma viral oncogene homolog (growth factor receptor)
MYOCD (transcription factor associated protein)
PAX Paired box (transcription factor)
PI3K Phosphatidylinositol 3-kinase (intracellular signaling molecule)
PLC Phospholipase C (intracellular signaling molecule)
PTCH Patched (growth factor receptor)
RET Rearranged during transfection (growth factor receptor)
ROBO Roundabout (growth factor receptor)
ROCK Rho-associated protein kinase (intracellular signaling molecule)
SMAD Homologs of drosophila protein, mothers against decapentaplegic and *Caenorhabditis elegans* protein SMA (intracellular signaling molecule)
SHH Sonic hedgehog (growth factor)
SLIT Slit homolog (growth factor)
SOX SRY-related HMG-box (transcription factor)
TBX T-box (transcription factor)
TGF Transforming growth factor (growth factor)
TSHZ Teashirt (transcription factor)
UPK Uroplakin (urothelial membrane protein)
VANGL Van gogh-like (planar cell polarity protein)

Initiation of the ureteric epithelium

(see *Molecule Abbreviation Box*)

The nephric (or Wolffian) ducts (NDs) are a pair of epithelial tubes, each of which runs along the intermediate mesoderm. Each ND gives rise to a ureteric precursor, the ureteric bud (UB), which grows into metanephric mesenchyme (MM) cells condensing out of intermediate mesoderm. Normally, a single bud emerges from each ND near its distal (caudal) end, a precision facilitating optimal interactions between UB and MM which are required to generate a single ureter-kidney functional unit, of normal shape and internal structure (Mackie and Stephens 1975; Kume et al 2000; Ichikawa et al 2002). In principle, normal budding could be controlled either by pre-patterning within the duct itself or by external signals. Experiments with explanted NDs provide no evidence for a strong intrinsic pre-pattern. Instead, any part of the duct, even the more proximal (cranial) section lying alongside the mesonephric kidney, can be stimulated to emit ectopic UBs by applying certain molecules (Sainio et al 1997; Davies et al 1999; Maeshima et al 2006) the actions of which can be understood by considering intracellular pathways under their control (Davies 2002) (Figure 1).

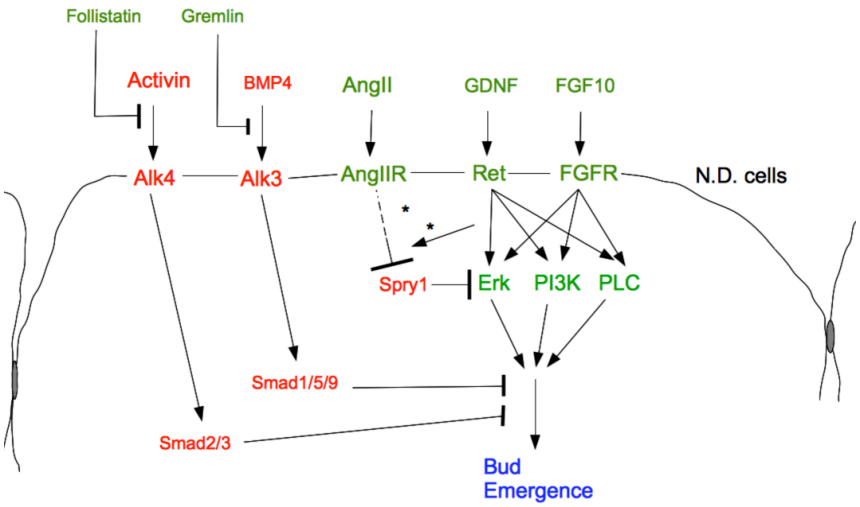

Figure 1. Intracellular pathways modulating UB emergence from the ND.
Pathways that encourage (green) and pathways that inhibit (red) bud emergence are depicted. (see also *Molecule Abbreviation Box*)

UB emergence is antagonized by SMAD signaling but favored by ERK, PI3K and PLC activation (Costantini 2010). NDs express activin A which acts in an autocrine manner to activate SMADs and prevent budding (Maeshima et al 2006). However, when an isolated ND is treated with both an activin antagonist and a growth factor that activates ERK, PI3K and PLC pathways, multiple buds emerge along its length (Maeshima et al 2006). In such experiments, numerous normal diameter buds rather than one large cyst are generated, implying a yet-be-be defined lateral inhibition mechanism whereby bud tip cells direct their immediately-neighbors to remain quiescent.

ND cells express various cell surface receptors, each of which binds pro- or anti-branching factors. RET and FGFR2 receptor tyrosine kinases, and their GFRα and sulphated glycosaminoglycan co-receptors, bind GDNF and FGFs, activating ERK, PI3K and PLC pathways and driving UB emergence (van Weering 1998; Eswarakumar et al 2005). Expression of such receptors depends on duct cells expressing the GATA3 transcription factor (Grote et al 2008), and β-catenin, a multifunctional intracellular protein, (Marose et al 2008; Michos 2009), and on nearby stromal cells synthesizing retinoic acid, an effector metabolite of vitamin A (Rosselot et al 2010). The extent of intracellular signaling triggered by receptor tyrosine kinases is limited by the cytoplasmic protein sprouty-1, without which the ND produces multiple ectopic buds (Basson et al 2005). Additionally, signaling between SLIT2 and ROBO2 (Grieshammer et al 2004; Lu et al 2007), members of molecular families first implicated in neural guidance, together with expression of FOXC1 transcription factor (Kume et al 2000), guard against UB ectopia by limiting the cranial extent of the GDNF expression domain within intermediate mesoderm. As alluded to above, bud emergence is also antagonized by TGFβ family members (e.g. activins and BMPs), autocrine and paracrine factors which bind ALK receptor threonine kinases, activating the SMAD pathway (Michos et al 2007). Normally, *in vivo*, SMAD activation is favored along most of the ND (Bush et al 2004). By contrast, near the duct's caudal end, MM secretes the BMP antagonist Gremlin-1 (Michos et al 2007) and the RET agonist GDNF and these, together with ANGII-mediated Sprouty-1 downregulation (Yosypiv et al 2006 and 2008), favor formation of a solitary, correctly-placed UB (Figure 2). An autocrine loop involving neuropeptide Y may enhance the commitment of these ND cells to budding (Choi et al 2009).

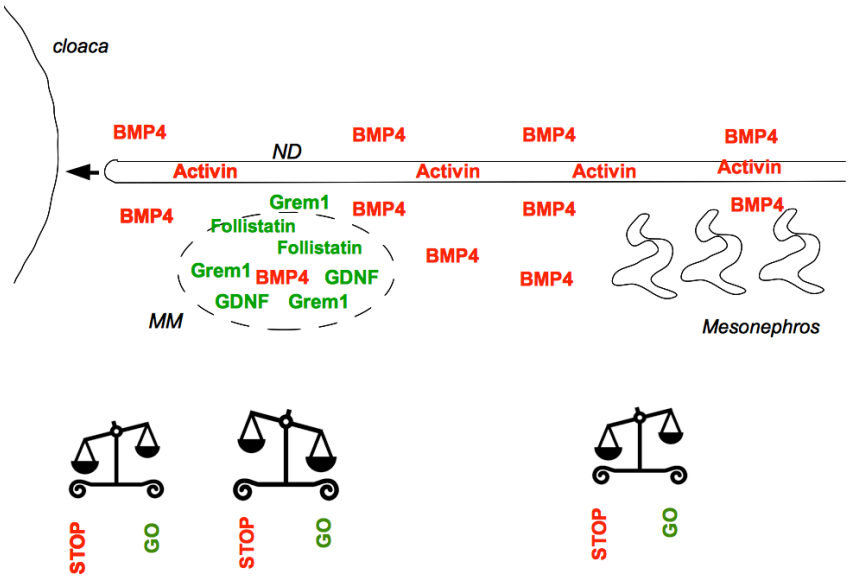

Figure 2. Growth factors controlling UB emergence from the ND.

The caudal part of the embryo, with the cloaca/urogenital sinus, is on the left of the diagram. Except near the MM, inhibitory signals such as BMP4 and activin dominate the molecular landscape. MM produces activators such as GDNF in addition to gremlin-1 (Grem1) and follistatin which respectively antagonize the anti-branching factors BMP4 and activin. At this precise point, the balance between activation and inhibition favors emergence of a single UB. (see also *Molecule Abbreviation Box*)

ND budding is preceded by increased epithelial proliferation (Michael and Davies 2004) and thickening to a pseudostratified epithelium (Chi et al 2009). RET signaling leads to rearrangement of ND cells such that those with the greatest ERK/PI3K/PLC activation move together and produce the bud (Kuure et al 2010a). This movement is also modulated by ETV4 and ETV5, transcription factors upregulated by GDNF/RET signaling (Kuure et al 2010a). During UB branching, epithelial cells become wedge-shaped, implicating cytoskeletal changes involving actin microfilaments. Indeed, mutation of genes coding for the actin depolymerizing factors cofilin 1 and destrin affect branching (Kuure et al 2010b), as does inhibition of ROCK, a molecule driving actin rearrangements (Michael et al 2005; Meyer et al 2006). ROCK is itself modulated by the planar cell polarity protein VANGL2 (Yates et al 2010). *In vitro*, UB epithelia undergo apoptotic death if physically separated from MM, and mesenchymal-derived factors such as GDNF may facilitate UB survival as well as emergence (Towers et al 1998). The PAX2 transcription factor is normally expressed in the ND and the emerging bud and is anti-apoptotic in the UB/collecting duct lineage (Torban et al 2000; Dziarmaga et al 2006). Prominent ND/UB apoptosis and impaired UB formation occurs in embryos lacking HNF1B (also known as vHNF) (Lokmane et al 2010). This transcription factor is normally expressed in the ND/UB where it may directly upregulate PAX2 (Lokmane et al 2010).

What happens to the top and bottom of the bud?

Once the UB's enters the MM, it begins to branch to produce kidney collecting ducts. Consideration of these events is beyond the remit of the current review and has been covered elsewhere (Davies & Fisher 2002; Michos 2009). It is unclear how similar are the mechanisms of UB emergence to its subsequent arborisation. Interestingly, the UB's proximal-distal axis does not initially restrict the branching ability of its cells because, experimentally, a collecting duct tree can be generated from either end of the nascent ureter (Sweeney et al 2008).

The just-formed ureter is separated from the urogenital sinus, the bladder precursor, by a length of ND extending beyond the point of UB emergence (Chia et al 2011). When development is complete, however, the ureter connects directly to the bladder, an anatomical change requiring substantial remodeling. Previous teaching postulated that the caudal-most ND cells migrated into the base of the bladder where they formed the urothelium of the trigone, the triangular zone

between the ureteric orifices and the urethral outlet of the bladder; as this occurred, the ureter/ND junction would approximate to the bladder wall. Lineage tracing of genetically-labeled ND cells shows that the first part of this model is incorrect (Mendelsohn 2009). In fact, the caudal-most part of the ND involutes by apoptosis induced by signals from the forming bladder (Batourina et al 2005). The vesicoureteric junction then becomes physically separated from the opening of the ND, maintained in males as the ejaculatory duct, as they are pushed apart by growth of the bladder wall.

Further growth and differentiation of ureteric epithelia

The shaft of the UB, between the kidney and the ND, grows and differentiates to become the mature ureter. In contrast to UB initiation, less is known about the cell biology of ureteric growth. Once emerged, the bud runs straight to the MM but the guidance mechanisms are not understood. When extra UBs are induced with beads soaked in stimulatory growth factors, they do not always grow towards the beads (Davies et al 1999), arguing against simple chemotaxis. Initial extension of the emerging UB depends on its epithelia expressing FRAS1 (McGregor et al 2003). This basement membrane protein acts in a complex with two related molecules, FREM1 and FREM2, probably optimizing presentation of MM-derived growth factors to the bud (Pitera et al 2008) and also physically stabilizing UB/MM interactions by binding integrin $\alpha 8$ (Kiyozumi et al 2005). A similar lack of UB progression occurs in mutant mice lacking this matrix receptor which is normally expressed on the surfaces of MM cells (Muller et al 1997).

As it extends, the bud becomes thinner than the zone of ND that produced it, suggesting cell rearrangements involving convergent extension, which is known to drive the remarkable longitudinal growth of Malpighian kidney tubules in fly embryos (Jung et al 2005). Ureters are shorter than normal in TBX18 null mutant mice (Airik et al 2006). This transcription factor is normally expressed in mesenchymal cells surrounding the urothelial stalk and its absence is associated with decreased epithelial proliferation (Airik et al 2006). Once initiated, further longitudinal growth occurs in isolated wild-type embryonic ureters maintained in organ culture (Caubit et al 2008) and in ureters of certain mutant embryos lacking kidneys (Bush et al 2006). Both observations show that exposure to fetal urine is not needed for longitudinal growth, although these experiments do not rule out a more subtle, differentiation-optimizing influence conferred by urine flow which, in mice, probably begins several days after UB initiation when the metanephros has formed its first layers of vascularized glomeruli (Figure 3).

Urothelia in both the ureter and bladder have evolved to stop movement of urine back into the body. Prevention of movement of water and solutes through the apical-most epithelial layer is mediated by plaques made of UPK protein heterodimers (Jenkins and Woolf 2007; Wu et al

2009). UPK expression occurs early in urinary tract development, being present in epithelia lining the urogenital sinus (Jenkins 2005 and 2007). In mutant mice lacking either UPK3A or UPK2 proteins, plaques are disorganized and urothelia are leaky. These animals also have malformed urinary tracts with gaping (instead of normal slit-like) vesicoureteric junctions, and dilated ureters associated with either reflux of urine from the bladder or occlusion by exuberant urothelial growth (Hi et al 2000; Kong et al 2004). These structural anomalies might simply be secondary disruptions following on from loss of the urothelial physical barrier. It has, however, been postulated (Jenkins and Woolf 2007) that they may also result from perturbed intracellular signaling by analogy with the proven role for uroplakin proteins in triggering embryogenesis in frog eggs. UPK expression is compromised in ureters of mouse embryos engineered to have downregulated BMP4 (Brenner-Anantharam et al 2007) or TBX18 (Airik et al 2006), both proteins being normally expressed in adjacent SM precursor cells. Furthermore, application of BMP4 to explanted metanephroi induces UPK expression in ureteric bud branch tips within the organ (Brenner-Anantharam et al 2007), suggesting that these UB descendants can be reprogrammed into a urothelial fate.

Ureteric muscle formation and function

The shaft of the embryonic ureter initially comprises an epithelial tube extending through loose mesenchyme. This epithelium acts as a paracrine signaling centre, driving surrounding cells to differentiate into SM (Lye et al 2010). The urothelium secretes SHH, a growth factor that binds to the PTCH1 receptor in immediately adjacent mesenchymal cells, stimulating them to proliferate (Yu et al 2002). Peri-urothelial mesenchymal cells are also stimulated to express BMP4 which itself effects their own differentiation into SM (Yu et al 2002; Brenner-Anantharam et al 2007). Here, BMP4 enhances intracellular levels of phospho-SMADs (Caubit et al 2008; Wang et al 2009) and upregulates TSHZ3, a transcription factor-like protein. TSHZ3 is needed for MYOCD expression within nascent ureteric SM cells. MYOCD is transcriptional co-activator then upregulates genes coding for muscle contractile proteins, such as a smooth muscle actin and myosin heavy chains (Caubit el al 2008; Lye et al 2010). Lack of another transcription factor, SOX9, which is, like TSHZ3, is normally expressed by mesenchyme aggregating around the urothelial ureteric tube, also leads to failed SM differentiation (Airik et al 2010). The aggregation of SM precursor cells around urothelia depends on mesenchymal expression of TBX18; in mice engineered to lack this transcription factor prospective SM precursors become mislocalized to the surface of the metanephric kidney (Airik et al 2006). Correct orientation of ureteric SM cells depends on DLGH1, an intracellular scaffolding protein highly expressed in urothelia and more weakly in nascent SM cells (Mahoney et al 2006). When DLGH1 is inactivated, circular muscle bundles misalign in a longitudinal orientation. In mutant embryos lacking this protein, the differentiation of stromal cells between the urothelium and SM layer is

perturbed, suggesting that stroma may somehow control SM bundle alignment. Cell lineage experiments have shown that ureteric SM is distinct from muscle layers in the wall of the urinary bladder (Viana et al 2007). After the shaft of the ureter has become enveloped with SM, there appears to be a secondary wave of muscle differentiation at the proximal end (top) of the ureter where it merges into the renal pelvis. These events are mediated by the protein phophatase calcineurin (Chang et al 2004) and by ANGII signaling (Miyazaki et al 1998). Mice that are genetically engineered to lack key molecules in the ureteric SM-differentiation pathway have the common phenotype of hyroureter/hydronephrosis. The dilation arises not from anatomical obstruction but because of a back-up of urine in a functionally-obstructed tube lacking normal peristaltic waves.

Forming a network within the SM layers are neural-like, KIT receptor tyrosine kinase-expressing cells that are required for generation of contraction waves beginning before birth (David et al 2005). Notably, the explanted fetal ureter, even when physically disconnected from the kidney and bladder, undergoes regular peristalsis in a proximal to distal direction (Caubit et al 2008). *In vivo*, peristalsis is triggered by HCN3, a hyperpolarization-activated cation channel expressed in the renal pelvis/kidney junction (Hurtado et al 2010). When hedgehog signaling is experimentally downregulated in this region, expression of KIT and HCN3 are compromised and contractions are perturbed, even though SM cells themselves appear intact (Cain et al 2011). The mature ureter also contains adrenergic, cholinergic, nitrergic, and sensory nerves (Rolle et al 2008), the activities of which modify its contractility (Canda et al 2007).

A theoretical scheme, in which the onset of fetal urine production by the kidney enhances ureteric SM differentiation and function, is depicted in Figure 3.

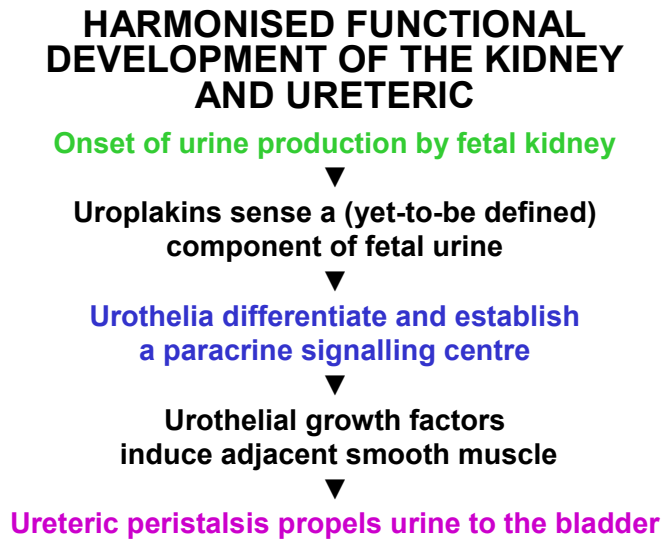

Figure 3. Harmonised kidney and ureteric functional development

Implications for understanding human congenital ureter malformations

The human ureter can be affected by several types of malformation (Williams et al 2008; Kerecuk et al 2008; Lye et al 2010), the. The most severe, and rarest (about 0.01-0.1% births), is its unilateral or bilateral absence, characteristically accompanied by kidney agenesis (Welch 1958). Ureteric dilation associated with ureteropelvic junction obstruction or primary megaureter affects up to 0.2% births (reviewed in Lye et al 2010). Even more common is ureteric duplication (2% of the population); in its most severe form the kidney is also "duplex", with the top part connected to an obstructed ureter with an ectopic termination in the urethra or vas deferens, and the bottom part connected to a refluxing ureter which inserts too laterally in the bladder wall (Mackie and Stephens 1975). Vesicoureteric reflux affects at least 0.5% of births, with some estimates of incidence an order of magnitude higher (Williams et al 2008). Sometimes these malformations occur as part of a syndrome affecting other parts of the body (see Online Mendelian Inheritance in Man), while at other times, the renal lesions occur in isolation.

A knowledge of how specific molecules controling ureter development help us understand why mutations of certain genes cause human disease. Fraser syndrome often features bilateral ureter and renal agenesis and can be caused by biallelic mutations of either *FRAS1* or *FREM2*, each encoding a UB basement membrane protein (McGregor et al 2003; Jadeja et al 2005). Furthermore, mutations of *RET* have been implicated in humans with similarly severe renal tract malformations (Skinner et al 2008). Mutations in ROBO2 have been reported in individuals born with refluxing and/or duplicated ureters (Lu et al 2007). Congenitally-dilated ureters can occur in humans who have mutations of *SOX9* (in Campomelic dysplasia) or *GLI3* (Pallister-Hall syndrome), encoding a transcription factor involved in SHH signaling. By analogy with the mouse models described earlier, one may postulate that such ureters may be functionally-obstructed because of poorly formed an/or poorly functioning MS bundles. In the uro-facial syndrome, dilated ureters occur together with vesicoureteric reflux and dysfunctional urinary tract contractions (Daly et al 2010). These individuals have mutations of *HPSE2*, the gene coding for heparanase-2, an endogenous inhibitor of classical heparanse (Levy-Adam et al 2010). Both molecules are expressed in the developing ureter (Daly et al 2010) where they may mediate neuro-muscular functional differentiation. *UPK3A* mutations have been reported in humans born with ureteric malformations (Jenkins et al 2005) resembling those described in mice genetically engineered to lack the encoded urothelial plaque protein. Genes coding for several of other proteins (e.g. PAX2, GATA3, HNF1B) implicated in ureter development have been found to be mutated in humans renal tract malformations (Bilous et al 1992; Sanyanusin et al 1995; Adalat et al 2009). In some instances, the gene in question is also expressed in, and has intrinsic roles in, the kidney itself. Accordingly, the manifest renal malformation may reflect

multiple primary aberrations of upper and lower renal tract development. Good examples are *HNF1B*, where human mutations can cause ureteric atresia and cystic dysplastic kidneys (Adalat et al 2009), and PAX2, where human mutations are associated with vesicoureteric relfux and kidney hypoplasia (Sanyanusin et al 1995).

Ongoing discovery of novel or unsuspected ureter development genes

The genetic search for new human ureteric malformation genes continues, with numerous loci suggested by genome-wise analyses (e.g. Kelly et al 2007; Weng et al 2009; Cordell et al 2010). Fortunately for human geneticists, and also those researching the basic mechanisms of renal tract development, there is open access to a resource that makes high-throughput analyses of gene expression freely available to all. The GenitoUrinary Development Molecular Anatomy Project (GUDMAP) database holds information on RNA array analyses from microdissected tissues in the developing murine urogenital system (Harding et al 2011). At the time of writing, there are also over 1450 *in situ* hybridization entries showing gene expression in the developing ureter. Examples of transcripts which have a with particularly strong and specific ureter expression are shown in Table 1. Cross-referencing with OMIM, to ascertain whether any have been associated with human disease and/or might fit into what is already known about the biology of ureter development, revealed the following points. *HOXA1* mutation is associated with a brainstem dysgenesis syndrome, although the state of the renal tract was not reported; ISL1 is a known activator of BMP4 expression; *MNX1/HLXB9* mutations are implicated in Currarino syndrome, characterised by anorectal and sacral malformations and which can sometimes feature duplex ureter, hydronephrosis, vesicoureteric reflux; and *Nrap* encodes a protein implicated in achoring of myofibrillar proteins.

Table 1.
Transcripts with strong and specific ureteric expression in developing mice, as reported in the GUDMAP database.

Esrrb (estrogen-related receptor-β)
Hnf4g hepatocyte nuclear factor 4γ
Hoxa1 (Homeobox1α)
Isl1 (Isl LIM homeobox-1)
Lhx6 (Lim Homeobox gene 6)
Lix1 (Limb expression 1)
Mbd1 (Methyl-CpG-binding domain protein 1)
Neurod4 (Neuorgenic differentiation 4)
Nrap (Nebulin related anchoring protein)
Spdef (SAM pointed domain-containing ETS transcription factor)
Tox3 (Tox high mobility group box family member 3)
Zfhx4 (Zinc finger homeobox 4)

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