Published in final edited form as:
*Pediatr Nephrol*. 2010 June ; 25(6): 1005–1016. doi:10.1007/s00467-009-1392-6.

# Molecular anatomy of the kidney: what have we learned from gene expression and functional genomics?

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## Abstract

The discipline of paediatric nephrology encompasses the congenital nephritic syndromes, renal dysplasias, neonatal renal tumours, early onset cystic disease, tubulopathies and vesicoureteric reflux, all of which arise due to defects in normal kidney development. Indeed, congenital anomalies of the kidney and urinary tract (CAKUT) represent 20–30% of prenatal anomalies, occurring in 1 in 500 births. Developmental biologists have studied the anatomical and morphogenetic processes involved in kidney development for the last five decades. However, with the advent of transgenic mice, the sequencing of the genome, improvements in mutation detection and the advent of functional genomics, our understanding of the molecular basis of kidney development has grown significantly. Here we discuss how the advent of new genetic and genomics approaches has added to our understanding of kidney development and paediatric renal disease, as well as identifying areas in which we are still lacking knowledge.

### Keywords

Functional genomics; Gene expression; Kidney development; Metanephros; Mesonephros; Microarray; Pronephros

## An introduction to the embryology of the urinary tract

The vertebrate kidney is derived from the intermediate mesoderm (IM) located lateral to the somites. The metanephros is the final of three embryonic excretory organs that develop in a temporally and spatially distinct order, these being the pronephros, mesonephros and metanephros. The pronephros is the earliest to arise and the most primitive. Functional during development only in lower vertebrates, it consists of simple tubules that empty into the primary nephric duct. The mesonephros develops more caudally along the nephric duct as the pronephros degenerates. Mesonephric tubules are more complex, consisting of a glomerulus and proximal tubule-like structures. The mesonephros is functional in adult higher fishes and amphibians, but only during embryogenesis in mammals. The metanephros is the final of the three embryonic organs; in mammals, this forms the permanent kidney. The metanephros is derived from two key structures: the ureteric bud (UB), which branches from the nephric duct, and a region of spatially defined intermediate mesoderm termed the metanephric mesenchyme (MM). The nephric duct (ND), also referred to as the Wolffian duct, empties into the cloaca. Paired UB arise from the ND at embryonic day (E) 10.5 in the mouse (at approximately 5 weeks gestation in the human). The epithelial UB grows towards the MM in response to inductive signals from the MM. Reciprocal interactions between the



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UB and MM result in growth and branching of the UB to ultimately form the collecting system of the kidney. A mesenchyme-to-epithelial transition (MET) of the MM at each of the newly formed UB tips results in the development of the nephrons, the filtration units of the kidney (Fig. 1).

This level of understanding regarding the origin of the metanephros is the result of careful anatomical observations and organ culture experiments. Pioneers in this field, Clifford Grobstein and Lauri Saxén, performed classical experiments in which the UB was dissected from the MM to demonstrate that a primary inductive event from the UB is essential for initiating MET in the MM. Without the UB, the MM fails to form epithelial tubules and rapidly degenerates [1]. However, a number of other tissue types, including embryonic brain and spinal cord, can induce MET and tubulogenesis when placed in contact with the MM, indicating the factors responsible are not specific to UB [2, 3]. MET induction requires cell-cell contact. Hence, even when the inducer is separated from the MM by microporous filters, induction only occurs if cells from the inducing tissue are able to extend processes through the pores into the MM [4].

## Comprehensive profiling of gene expression during kidney development

Our molecular understanding of how these early morphogenetic events occur has grown slowly, based initially upon anatomical observations of renal defects in specific mutant mouse strains. However, the development over the last 20 years of a diverse array of functional genomics technologies has led to a far more comprehensive view of kidney development. This has included the development of high-throughput expression analysis techniques. Table 1 comprehensively lists all studies performed over the last decade in which genomics approaches have been applied to the analysis of kidney development. In the late 1990s, differential display PCR (ddPCR) was introduced as a means of defining mRNA expression differences in two different samples. This technique was applied to kidney morphogenesis by Leimeister et al. [5] and Plisov et al. [6]. However, ddPCR is difficult to automate. Consequently, techniques allowing a higher throughput analysis were established, such as comparative expressed sequence tag analysis (EST), serial analysis of gene expression (SAGE), high-throughput real time PCR (RT-PCR) and microarrays. cDNA or oligonucleotide microarrays have proven to be the most popular approach for global gene expression analysis as they are progressively more comprehensive [Affymetrix (Santa Clara, CA) ver. U133 Plus 2.0 Genechips now cover >47,000 human transcripts], sensitive, quantitative, rapid, and increasingly cost effective. Research on differential kidney gene expression in vivo initially provided a global and temporal overview of organ gene expression [7], identifying genes and hence pathways showing specific dynamic patterns of expression across kidney development. However, given the diversity of cell types within the developing kidney, such data were not particularly illuminating. To address spatially restricted gene expression, Valerius et al. [8] first analysed gene expression in immortalised cell lines representing either early metanephric mesenchyme (mK3), as determined by the expression of *Hoxa11/d11, collagen 1* and *vimentin*, or MM undergoing epithelialisation (mK4), as determined by the coexpression of *Pax2/8, Wnt4, collagen IV* and *LFB3* (mK4). While this study defined over 100 genes showing a greater than tenfold higher gene expression in mK4 versus mK3, these data were hard to relate back to the organ itself. Indeed, in 2002, in an editorial in *Genome Biology*, the relevance of microarray profiling to developmental biology was questioned [9]. However, spatial analysis of kidney development dramatically improved as research moved away from cell lines to direct isolation of specific kidney subcompartments, initially using manual microdissection and then via laser capture microdissection (LCM) and fluorescence-activated cell sorting (FACS)-based isolation from fluorescently tagged transgenic animals. Spatial experiments comparing UB and MM in vivo at various embryonic time points [10, 11] and in vitro [12]

highlighted large numbers of differentially regulated genes in each murine cell population. This work was advanced with the microdissection of the ureteric tip from the ureteric bud at E11.5 [13] and E12.5 [14]. The microarray analysis of cells isolated from *Sall1*-GFP knock-in [15] and transgenic *Hoxb7*-GFP mice [11] went a step further in defining the compartments of MM-derived populations and the ureteric tree/tip, respectively. Challen et al. [16] also compared the expression of the E10.5 MM to that of the adjacent intermediate mesoderm and identified a number of early MM-specific markers, including *CD24*, which have subsequently formed the basis for studies looking for renal progenitors. Yano et al. [17] and Higgins et al. [18] profiled macroscopic compartments of the human kidney but concluded that greater spatial specificity was required to elucidate pathways involved in specific patterning events. Brunskill et al. [19] were the first to present a global atlas of gene expression of subcompartments of the developing kidney. They achieved this by combining the techniques of laser capture microdissection and FACS of compartment-specific transgenic mice to isolate 14 major subcompartments for microarray profiling. From this analysis, they were able to identify the top 223 genes showing the most compartment-enriched gene expression patterns. Validation by in situ hybridisation further defined the distribution of mRNA expression within a restricted component of the compartment, allowing the further subdissection of segmentation during the formation of the uriniferous tubule as it unwinds [19]. This was the first step in identifying anchor genes absolutely restricted to each anatomical structure and time point in the process of kidney development. This work was performed within the Genitourinary Development Molecular Atlas Project (GUDMAP), with the objective of identifying molecular markers that would enable the development of reporter mouse strains for further subdivision of kidney development [20]. Such mice will allow further subfractionation across time and space for subsequent expression analyses as well as provide tools for genetic fate mapping through the generation of GFP:Cre:ERT2 lines driven by kidney anchor gene promoters.

## Expression profiling of mutant models

As well as being used for expression profiling of normal kidney development, such genomic tools have also been applied to a growing number of mouse mutant strains to increase our understanding of the pathways interrupted to cause specific phenotypes and, concommitently, to infer normal function for many genes. Table 2 lists such studies, describing the mutant model examined, the tissue isolated for analysis and the overall conclusions of the studies. In some cases, these mouse models were of known human conditions, such as the *Gata3*$^{-/-}$ mice, where defects in this gene are implicated in the autosomal dominant HDR syndrome (hyperparathyroidism, sensorineural deafness and renal anomaly) [21], and the *Hnf-1 beta* knockout mice, which mimic human MODY5 (maturity-onset diabetes mellitus and congenital malformations of the kidney) [22]. The latter highlighted a role for Hnf-1 beta in the suppression of SOCS3, thereby promoting tubulogenesis. As for normal development, the application of microdissection, FACS and LCM to mutant tissue has focused such analyses to the affected subcompartment. Cui et al. [23] profiled isolated glomeruli from *Pod1* knockout mice, showing that the loss of this gene resulted in a reduction of alpha 8 integrin expression. LCM of individual renal vesicles was used to analyse changes in RV-gene expression in *Lim1* mutant mice, showing significant down-regulation of *Dkk1* and placing this gene downstream of *Lim1* [24]. One of the most interesting developments of late has been the investigation of the role of specific micro-RNAs (miRNAs) in kidney development. These short non-coding RNAs bind to the 3′ untranslated region of mRNAs, thereby regulating levels of gene expression at the posttranscriptional level. An association had been noted between the expression of specific miRNAs and a number of renal disease states, including miR-17–92 in Wilms' tumour and miR-15a in cystic kidney disease [25]. Three independent groups have now shown that a knockout of the miRNA processing enzyme, Dicer, in the podocytes results in dramatic

cytoskeletal rearrangement and dedifferentiation [26–28]. This resulted in proteinuria, podocyte effacement and apoptosis. Profiling of these mice compared to littermates enabled the in silico identification of miRNA target sequences in podocyte-specific genes.

## Development databases and *in silico* modeling

The databasing of information on the molecular basis of kidney development began in the mid-1990s with the creation of the Kidney Development Database (KDD) [29, 30]. This began as a spreadsheet of gene expression in different cellular compartments or structure in the developing kidney as well as a list of mutant mice displaying defects in renal development. The database still exists (http://golgi.ana.ed.ac.uk/kidhome.html), but for the most part it is no longer comprehensive as the flow of data from expression profiling has become too large. The National Institutes for Digestion, Diabetes and Kidney Disease have more recently supported the creation of a large public database of gene expression in the developing urogenital tract as a whole which supercedes the KDD. This Genitourinary Development Molecular Anatomy Project database (http://www.GUDMAP.org) [20] houses >255 microarrays and >7200 wholemount and section in situ hybridisation entries covering >2900 genes analysed for expression in the developing genitourinary tract (UGT) from TS17 (E10.5) to adult tissue (as of August 2009). Access to such a rich source of expression data that have been annotated to the level of detail as this will prove to be a valuable resource in the prioritisation or even identification of genes responsible for specific phenotypes. An example of this was reported recently by Berkovic et al. [31], who used single-nucleotide polymorphism chips to localise a region responsible for the action of myoclonus-renal failure syndrome (AMRF) to chromosome 4q13–21 and then used microarray-expression analysis to prioritise candidate genes. As a result, SCARB2/Limp2, a lysosomal-membrane protein, was shown to contain mutations in all three affected pedigrees. Re-analysis of a *Limp2* knockout mouse verified the presence of cerebral and renal changes. There is also an extensive gene expression database documenting the expression of genes in the developing pronephros of *Xenopus laevis* established by the Euregene consortium (XGEbase http://www.euregene.org/xgebase).

## Flies, fish, frogs and worms

While the mouse has been a key tool in our understanding of kidney development, the most powerful model organisms for functional genomics are the fly *Drosophila melanogaster*, the zebrafish *Danio rerio*, the African clawed toad *Xenopus laevis* and the worm *Caenorhabditis elegans*. Whilst only zebrafish and *Xenopus* are vertebrates, all of these model systems have made valuable contributions to our understanding of kidney development and disease.

Arguably, the animal that has had the most influence on the kidney field is *C. elegans*, where the analysis of the role of large numbers of genes involved in cilia function have significantly advanced the field of cystic kidney disease. Identification of the *C. elegans* genes *lov-1* and *pkd-2* revealed the association between cilia and autosomal dominant polycystic kidney disease (ADPKD). Other mammalian renal diseases, such as autosomal recessive PKD (ARPKD), nephronophthisis (NPHP) and Bardet–Biedl syndrome (BBS), which also have orthologues in *C. elegans*, share the common elements of cystic kidneys and ciliary localised gene products [32]. This link came with the realisation that the human PKD1 and PKD2 proteins, mutations in which cause PKD, localise to the primary cilia of renal epithelial cells as do the worm homologues. Cilia in the worm are critical for appropriate male mating behaviour, and a large number of genes identified as being involved in cilia morphogenesis or function have subsequently been shown to result in renal cysts when mutated in mice [33].

Teleosts, such as zebrafish, do not form a metanephros. Instead, they have a functional pronephros during embryonic and larval stages and a mesonephros acting as their excretory organ in the adults. Nevertheless, there is considerable similarity in gene expression and cell type between the paired pronephric tubules and ducts and the single midline glomerulus of the developing zebrafish pronephros and the nephron of the mammalian metanephros (Fig. 2b). Zebrafish mutant embryo models and gene knockdown using morpholino antisense oligonucleotides have proven useful for studying defects of glomerular formation and slit diaphragm morphology [34] and for identifying several genes with roles in cyst formation and tubular defects [35]. Indeed, such studies have identified more than a dozen gene disruptions, leading to cystic phenotypes affecting either the glomerulus or the pronephric tubules [36]. Some of the defects have been due to mutations in intraflagellar transport genes associated with ciliogenesis, again highlighting the link between cilia defects and PKD.

In developing *Xenopus*, there is one pronephric glomus, a pronephric tubule, and a connecting tubule leading to the cloaca where the nephron joins its bilateral counterpart (Fig. 2c). Large-scale gene expression studies of the pronephros in *Xenopus* have also been performed, and after an analysis of the expression of over 240 genes, eight distinct cellular regions have been identified within the pronephric tubule and pronephric duct [37]. The pronephric nephrons can be segmented into proximal tubule, intermediate tubule, distal tubule and connecting tubule. These data are available via XGEbase (http://www.euregene.org/xgebase), which houses over 1200 in situ hybridisation (ISH) images. A large number of the expression patterns delineating different regions are represented by distinct solute transporters, and there is a direct correlation between the expression of these genes in *Xenopus* and that in mouse/human, suggesting that the more tractable *Xenopus* model system is pertinent to the understanding of development in humans. Nephron segmentation has also been investigated in *Xenopus* via microinjection of mRNA into *Xenopus* blastocysts [38]. This has allowed the investigation of the role of key transcription factors, including *Lhx1*, *Wt1*, *Pax8* and *Hnf1b*, in nephron patterning and tubulogenesis. Gain- and loss-of-function experiments have also shown that the *Irx* genes are required early for maintenance of the kidney anlage, and later for segmentation of the nephron [39, 40]. *Irx3* was found to be both necessary and sufficient for the formation of the *Xenopus* intermediate segments that are equivalent to the loop of Henle segments of the mammalian nephron [39]. Patterns of *Irx1*, *Irx2* and *Irx3* expression in both the embryonic and adult mouse nephron suggest that these genes also have roles in loop of Henle patterning in mammals [39].

Finally, even the fly has played a role in our understanding of kidney development. The secretory and absorptive Malpighian tubules in *Drosophila melanogaster* have provided insights into tubular morphogenesis [41]. The filtration barrier of the fly is called the nephrocyte. Like podocytes, these cells are mesodermal in origin and contain specialised slit diaphragms analogous to those in the mammalian renal podocyte [42] (Fig. 2a). Of note, the fly orthologues of *Nphs1* (sticks and stones; *Sns*), *Kirrel* (kin of irre; *kirre*), *Cd2ap* (*CG31012*), *Tjp1* (polychaetoid; *pyd*) and *Nphs2* (*Mec2*) are genes critically important for podocyte structure and function in humans and mice, and all are expressed in nephrocytes in analogous intracellular locations. These cells form a size-selective barrier and remove products from the haemolymph by filtration and endocytosis [42]. However, the similarities in gene expression and the ready ability to perform mutation screens in flies may prove a rich source of understanding of renal development and disease. In addition, Liu et al. [43] have created a large-scale network diagram based on known gene function in the fly and identified roles for the JNK pathway in renal cancer based on these network predictions. This same pathway appears to be critical for the self-renewal of a population of cells within the Malpighian tubules in the fly [44]. This observation of self-renewing cells in the adult

Malpighian tubules may translate to a greater understanding of the tubular regenerative capacity of the human kidney.

## Lineage and selective gene deletion

While such comparative models have shown parallels to the mammalian situation, the primary animal model of kidney development has remained the mouse, and advancements in gene targeting technologies to allow for cell-specific gene deletion has revolutionised this field. Previous reviews have summarized available Cre transgenic lines targeting specific cell types/regions of the developing kidney [45, 46]. To these lists can be added three distinct alleles of Six2-cre transgenic mice [47], which allow gene inactivation in the cap mesenchyme, and the recently reported Wnt4EGFPCre, which inactivates genes from the renal vesicle stage of nephron development [48]. Another line that has been widely used to examine the role of genes in the metanephric mesenchyme of the kidney is the Pax3-Cre transgenic line originally described by Li et al. [49] and shown to result in recombination in the metanephric mesenchyme by Chang et al. [50].

These tools have contributed significantly to our understanding of kidney development. The definition of podocyte-specific expression of *Nphs1*, for example, has facilitated the inactivation of a large number of genes in this cell type alone. This involved the expression of the enzyme Cre-recombinase under the control of the *Nphs1* promoter crossed to a strain in which sequences for the gene of interest are flanked by loxP sites recognised by Cre-recombinase. Such cell-specific gene deletion versus cell-specific promoter-driven overexpression has facilitated the development of an allelic series in which the level of vascular endothelial growth factor-A (VEGF-A) in the developing glomerulus was varied from too high to too low [51]. This revealed a requirement for exquisite control of VEGF-A expression from the podocytes in order to establish and maintain normal glomerular function. The same technology has been adapted to investigate the lineage fate of cells during development. More recently, the same podocin-Cre (Nphs2-Cre) mouse, which also expresses Cre recombinase in the podocytes, has revealed a critical role for microRNAs in the maintenance of glomerular function when crossed with a Dicer (flx/flx) strain, Dicer being the enzyme required for miRNA processing [27]. By incorporating the requirement for an inducible agent, such as tamoxifen or doxycycline, to initiate Cre-recombinase expression, specific cells can be permanently marked to visualise what they give rise to. In this way, it has been possible to prove that all segments of the nephron, other than the collecting duct, do indeed arise from the metanephric mesenchyme [47], but that only a subset of the MM, the *Six2*-expressing cap mesenchyme, condenses around the ureteric tips. Lineage tracing has also been applied to the intermediate mesodermal *Osr1*-expressing population to show that these cells can give rise to all parts of the kidney, other than the resident macrophages, but only at specific time points [52]. This has not eliminated the possibility that at least some of the *Foxd1*-positive interstitial cells do migrate into the kidney from another location. As well as defining fate during development, lineage tracing approaches are now being used to establish what cells give rise to what in response to damage. Humphreys et al. [53], using Six2 lineage tracing, showed that tubular repair after induced damage does not involve any cell population that was not derived from the *Six2*-positive cap mesenchyme. None of this would have been possible without the identification of cell types of compartment-specific gene expression patterns, many of which have and will come from gene expression analyses.

## What questions remain?

As a result of these diverse functional genomics approaches, we now have a much more global and comprehensive view of which genes, gene networks and pathways are expressed

across time and space in kidney development, and we have identified many more critical players in podocyte maturation and function, nephron patterning and segmentation, ureteric bud outgrowth and branching as well as in the commitment of the MM and the various fates of these cells. Studies in worms, frogs, fish and mice have revealed a key role for cilia in the onset and progression of cystic disease and the advent of lineage tracing, and Cre-mediated selective gene ablation has particularly impacted on our understanding of glomerular disease. However, there are many key questions in kidney molecular development that remain unanswered. These include the regulation of cessation of nephrogenesis, the process governing the perinatal remodeling of the medulla and a clearer understanding of the normal repair response of the kidney to different types of damage. In mammals, nephron endowment is complete before or around birth. Hartman et al. [54] examined this process in mouse and proposed that it results from the exhaustion of the nephron progenitor cells, the cap mesenchyme. In humans, cessation of nephrogenesis occurs by 36 weeks of life. Consequently, premature babies or those suffering from intrauterine growth retardation (IUGR) are known to have a lower number of nephrons. Unlike mammals, teleosts and cartilaginous fish, including the skate, retain a progenitor blastema such that if the kidney is damaged, this blastema undergoes nephron induction to replace lost nephrons. While it is accepted that the remaining cap mesenchyme is exhausted by a burst of nephron differentiation at the end of mammalian development, what triggers this loss of self renewal and stimulates terminal differentiation is not understood. Intervention to prolong nephron endowment may play an important role in the treatment of IUGR or premature babies.

At birth, the medulla of the mammalian kidney still contains a large amount of interstitial tissue between the descending loops of Henle. Hence, an efficient counter-current mechanism is not yet in place, and considerable remodeling is required in the perinatal period for the newborn to be able to concentrate urine. Nothing is known about the regulation of this maturation, although a small number of genes showing medullary defects evident in newborn mice have been identified, including a mutation of Adamts-1 [55]. Of interest, a number of genes expressed during nephron formation in the cortex are also expressed in the developing and perinatal papilla, but their roles there are unknown. This includes *Wt1* and *Wnt4*, which are important for mesenchymal-to-epithelial transitions in nephron formation. Given the proposal of a papillary-located postnatal progenitor population in the adult kidney [56], it is possible that such cells are also involved in the normal remodeling of the papilla during medullary maturation and are used again after obstructive damage in the kidney.

Many of these key developmental questions are likely to involve epigenetic as well as genetic regulation. There is a growing appreciation of the role of non-coding RNAs, chromatin modification and DNA methylation and acetylation. A link between a key transcription factor in kidney development, Pax2, and the site-specific methylation of chromatin by histone methyltransferases has already been described [57]. Here, Pax2 can modify chromatin at different gene loci depending upon the proteins it is interacting with. Investigating these processes will represent the next phase of functional genomics and will be substantially enhanced by the advent of Next Generation (NextGen) sequencing. While this technology will complement current expression profiling by more comprehensively cataloging the gene expression of all alternate isoforms of coding as well as all non-coding transcripts, it will allow the assessment of DNA methylation and acetylation to globally determine why a given set of genes are active. The advent of genome wide lentiviral gene and shRNA libraries will also enable large-scale analysis of the overexpression or silencing of individual or combinations of genes either in cell lines or in embryo morphology screens.

## Conclusion

In conclusion, based on results from two decades of functional genetics and genomics, our understanding of the molecular basis of kidney development and disease has expanded considerably. We now have a detailed and comprehensive atlas of the developing kidney which has subdivided territories into smaller domains based upon gene expression and activity. Continued complimentary use of functional genomics in all model organisms will continue to reveal the molecular markers and regulatory pathways that underpin the development of the mammalian kidney. While we have not yet reached the level of the complete temporo-spatial wiring diagram, there is no doubt that the continued application of these technologies, and new additions to the functional genomics arsenal as they come on line, will bring us to the point where we can link cause and effect in each of the morphogenetic processes required to form a normal functional kidney. By extension, our understanding of what can and does go wrong will also improve. The final link is turning that understanding into diagnosis and treatment.

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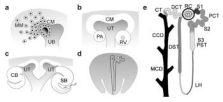

**Fig. 1.**

Overview of mammalian metanephric development. **a** The kidney is initiated via reciprocal induction events between the epithelial ureteric bud (*UB*), which arises from the mesonephric duct, and the metanephric mesenchyme (*MM*), which condenses to form a cap mesenchyme (*CM*). **b** Around the advancing ureteric tips (*UT*), the CM is induced to form a pre-tubular aggregate (*PA*) which then undergoes a mesenchyme-to-epithelial transition to form a renal vesicle (*RV*) surrounded by a basement membrane. The formation of each RV represents the initiation of a single nephron. **c** The RV proliferates and elongates to form first a comma-shaped body (*CB*) and then an S-shaped body (*SB*). Vascular endothelial cells are drawn into the cleft at the proximal end of this structure to form the glomerular capillaries. The other end of the forming nephron fuses with the UT at the late RV stage [73]. **d** A single nephron in the mammalian kidney. **e** Continued patterning of the elongating uriniferous tubule results in segmentation into the various functional regions of the nephron, including the proximal tubule and the distal tubule with an intervening loop of Henle (*LH*). The recruitment of vasculature and mesangial cells into the proximal end of this tubule forms the renal corpuscle (*RC*). *PCT* Proximal convoluted tubule, *PST* proximal straight tubule, *S1–3* segment 1–3, *DCT* distal convoluted tubule, *DST* distal straight tubule, *CT* connecting tubule, *CCD* cortical collecting duct, *MCD* medullary collecting duct

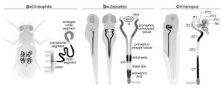

**Fig. 2.**

Comparison of the segmental patterning of the developing excretory systems in the fly, zebrafish and Xenopus. **a** In the fly, there are two pairs of excretory tubules, the Malpighian tubules, which empty into the hindgut. These consist of blind-ended tubules containing nephrocytes. *Hir* Hindgut imaginal ring. **b** The zebrafish has a pair of nephrons that fuse to form a single central glomerulus. The pronephric tubules lead to pronephric ducts which empty into the cloaca. *G* Glomerulus, *coS* corpuscle of Stannius. **c** *Xenopus* contain a bilateral pair of nephrons where blood filtration occurs through a single glomus into the coelom, and filtrate enters the segmented nephron tubules via nephrostomes. Connecting tubules empty into the cloaca. *G* Glomus, *c* coelom, *Ns* nephrostome, *PT1* proximal tubule 1, *PT2* proximal tubule 2, *PT3* proximal tubule 3, *IT1* intermediate tubule 1, *IT2* intermediate tubule 2, *DT1* distal tubule 1, *DT2* distal tubule 2, *CT* connecting tubule. Figure adapted from [74, 75] and [37] (used with permission)

*Pediatr Nephrol*. Author manuscript; available in PMC 2011 October 9.

**Table 1**

Gene expression profiling of human and murine kidney development

| Article title | Technology[a] | Experiment | Source/species | Outcome | Reference |
|---|---|---|---|---|---|
| Screen for genes regulated during early kidney morphogenesis | Transfilter organ culture and ddPCR | Spatial | E11 cultured mesenchymal cells from wildtype outbred CD1 mice | Compared uninduced state to that of explants cultured with spinal cord; 5/13 known genes and 4/8 novel gene tags verified by Northern blot and ISH | [5] |
| Mesenchymal-epithelial transition in the developing metanephric kidney: gene expression study by differential display | ddPCR, ISH validation | Spatial | E13 cultured MM, F344 rat | Induced metanephric explants vs. tubular induced explants; 72 differentially regulated mRNAs detected in a MET event | [6] |
| Changes in global gene expression patterns during development and maturation of the rat kidney | Affymetrix Rat Genome U34A GeneChip Array; custom data analysis with “The Equaliser” & “eBlot” | Temporal | Isolated RNA from E13, 15, 17, 19, P0, P7 and NP adult rats | 873/8740 genes were found to vary significantly ($P = 0.0025$) during rat kidney development. These genes clustered into five clear patterns of gene expression, each defined by a unique cluster-member gene function, tissue distribution, and embryonic expression | [7] |
| Microarray analysis of novel cell lines representing two stages of metanephric mesenchyme differentiation | Affymetrix Mu6500, Mu11K and U74A chip sets, ISH validation | Spatial | Clonal kidney cell lines of representative stages of uninduced and induced MM isolated from Hoxa11/SV40 4–12 week old transgenic mice | Microarray experiments were performed on representative stages of MM (mK3 early = prior to induction) and late mK4 (post-induction on way to MET); 121 differential genes showed >10fold difference in expression; identification of genes not previously implicated in MM differentiation. (*Hoxa7, Notch1, Fst, Igf2, sFRP1, Cdh16, shrom, Prx1, EST AA123934, NfiB*) | [8] |
| A catalogue of gene expression in the developing kidney | Affymetrix U74Av2, ISH validation | Temporal & Spatial | Microdissected wildtype murine UB & MM (E11.5); total kidneys E11.5, E12.5 (pooled), E13.5, E16.5 & adult | Identification of 428 genes with significantly elevated expression levels at E12.5 vs. adult kidney; 78 genes differentially expressed between E11.5UB vs. MM; 53 in UB, 25 in MM | [10] |
| Changes in gene expression patterns in the ureteric bud and metanephric mesenchyme in models of kidney development | High-density DNA arrays together with dIVT; Affymetrix Rat Genome U34A Gene chips | Temporal & Spatial | In vitro models of isolated E13 Sprague-Dawley rat UB and MM cultures | in vitro models for morphogenesis of the rat UB and MM examined and data compared to whole embryonic kidney at different stages of development; 740/8471 genes | [12] |

| Article title | Technology[a] | Experiment | Source/species | Outcome | Reference |
|---|---|---|---|---|---|
| | | | | were highly expressed in UB; 624/8471 genes highly expressed in MM | |
| Gene expression in the normal adult human kidney assessed by complementary DNA microarray | 41,859 unique cDNA array (Stanford Functional Genomics Facility), IHC validation | Spatial | Human nephrectomy specimens removed for renal neoplasia, lobes separated and dissected into 5 fractions | RNA isolated from inner and outer cortex, inner and outer medulla, papillary tips, renal pelvis and glomeruli. cDNA microarray & IHC validation; 1548/41859 genes with variable expression; distinct expression of >250 genes in cortex, 139 in glomerulus, 130 genes in renal tubules, 58 genes in medullary vs. papillary tips, 102 genes in papillary tips/pelvis | [18] |
| Identification of kidney mesenchymal genes by a combination of microarray analysis and *Sall1*-GFP knock-in mice | Affymetrix Murine Genome U74v2 GeneChips, ISH validation | Spatial | Isolated murine *Sall1*-GFP-positive population of E17.5 kidneys | Compared mesenchymal vs. UB derivatives; reported 78% of top 50 mRNAs were in the mesenchyme; Identification of 17 novel mesenchymal genes | [15] |
| Temporal and spatial transcriptional programs in murine kidney development | Compugen long-oligonucleotide microarrays, ISH validation | Temporal & Spatial | Wildtype CD1 murine kidneys from 24-h intervals from E10.5 to P0; E10.5 MM vs. IM; E15.5 Hoxb7-GFP positive ureteric tree vs. Hoxb7–GFP-negative MM | Temporal: Profiling through developmental time course & boxed comparison of development at E10.5 vs. 11.5 vs. 13.5 kidneys; functional annotation clustered into 5 classes and pathway/network analysis to identify critical signaling pathways; Spatial: MM E10.5 compared to adjacent IM; E15.5 UB vs. GFP negative mesenchymal derivatives from the Hoxb7 mouse; 3600 dynamically expressed genes identified | [11] |
| Novel regulators of kidney development from the tips of the ureteric bud | Affymetrix Mouse Genome 430 2.0 Array, RT-PCR and ISH validation | Spatial | 13.5 rats, E12.5 mice isolated UB & MM | UB tips vs. tree have distinct gene expression profile, identified 20 predicted secreted protein transcripts upregulated in UB vs MM for both rat and mouse, e.g. Clf-1 | [14] |
| Spatial gene expression in the T-stage mouse metanephros | Compugen mouse long microarrays, ISH validation | Spatial | Dissected murine E11.5 ureteric tip, ureteric tree and MM | Identified genes spatially expressed in regions of the developing kidney e.g. Ureteric tip epithelium: sim-1, Arg2, Tacstd1, Crlf-1, BMP7; ureteric tree epithelium: Innp1, Itm2b, Mkm1, SPARC, Emu2 and Gsta3; MM genes: CSPG2 and CV-2 | [13] |

| Article title | Technology[a] | Experiment | Source/species | Outcome | Reference |
|---|---|---|---|---|---|
| Microarray interrogation of human metanephric mesenchymal cells highlights potentially important molecules in vivo | Affymetrix human U133 plus 2.0 Gene Chips, RT-PCR and IHC validation | Temporal | 10-week gestation human fetus used to generate a human MM cell line | Generated mesenchymal cell lines from first trimester human metanephros and stimulated with growth factors; 36 genes differentially regulated; changes in components of LIF signaling pathway reported | [58] |
| Atlas of gene expression in the developing kidney at microanatomic resolution | Affymetrix Mouse Genome 430 2.0 Array; ISH validation | Spatial | LCM-or FACS-sorted subpopulations from E12.5 and E15.5 kidneys of CD1, Six2-GFP and Meis1-GFP mice. | Microarray analysis of 14 subcompartments of the developing kidney; 223 genes identified as component-specific; identification of gene expression states of intermediate states of nephrogenesis; regulatory networks explored | [19] |

ddPCR, Differential display PCR; MET, mesenchyme to epithelial transition; MM, metanephric mesenchyme; IM, intermediate mesoderm; UB, ureteric bud; dIVT, double in vitro transcription; ISH, in situ hybridisation; IHC, immunohistochemistry; LCM, laser capture microdissection; FACS, fluorescence-activated cell sorting; E, embryonic day; GFP, green fluorescent protein

[a]Affymetrix, Santa Clara, CA; Compugen, Toronto, ONT, Canada

## Table 2

Gene expression profiling of murine models of kidney dysgenesis

| Mutant strain (gene) | Source/species | Technology[a] | Outcome | Reference |
|---|---|---|---|---|
| *Megalin* $^{-/-}$ | 12- to 16-week-old mice kidneys | Affymetrix MullK chip B | Megalin regulates vitamin D homeostasis | [59] |
| *Meprin beta* $^{-/-}$ | Adult male mouse kidneys | Affymetrix MG-U74Av2 oligonucleotide array | Changes in genes associated with immune response and cell cycle, increase in TRAF6, Tlr6 | [60] |
| *Aqp1* $^{-/-}$ | 10-week-old mouse inner medulla | NIA mouse 15 K clone set GRL array | Downregulation of many medullary genes involved in heatshock/stress, mitochondrial pathways, vasopressin receptors | [61] |
| *Gata3* $^{-/-}$ | E9.5 mouse embryos | NIA mouse 15 K cDNA array | Downregulated Dad2 | [21] |
| *Pod1* $^{-/-}$ | E18.5 mouse isolated glomeruli | Affymetrix GeneChip Mouse Expression Array 430 2.0 | Almost 4000 differentially expressed genes. Fourfold reduction in alpha 8 integrin | [23] |
| *KininB1* and *kininB2* $^{-/-}$ | 12- to 16-week-old male mouse kidneys | Agilent oligonucleotide arrays | Large number of downregulated genes that are already implicated in renal physiology or pathology | [62] |
| *Pax 2* $^{lacZ/+}$ *Pax 2* $^{GFP}$ | GFP Pro/mesonephric cells | cDNA microarrays | Gata3 in the nephric duct is regulated by Pax2 and Pax8 | [63] |
| *Sprague Dawley Cy* $^{-/-}$ | 3-week-old rat total kidney | Agilent 60-mer Rat Array G4130A | Upregulation of extracellular matrix metabolism genes in PKD model | [64] |
| *Hoxd 11* $^{-/-}$ *Hoxd 11* $^{-/-}$ | E11.5, E12.5, E13.5 and E16.5 mouse kidneys | Affymetrix GeneChip Mouse Expression Array 430A&B | Upregulation of Irx3, downstream target of Hox; changes in, for example, Hoxa11s, GATA6, Slc40a1, Cxcl12; multiple downstream pathways | [65] |
| *c-maf* $^{-/-}$ | Mouse kidneys | Affymetrix GeneChip U74A array | Down-regulation of GPx3 in knockout mice, possibly regulated by c-maf | [66] |
| Pygo1 $^{-/-}$/ Pygo2 $^{-/-}$ | El8.5 mouse kidneys | Illumina Sentrix Mouse-6 expression Beadchip | Expression changes in candidate Wnt signaling targets but Pygol/Pygo2 not essential for canonical Wnt signaling. | [67] |
| *Rarb2Cr* $^{tg/+}$; *Lim1* $^{lz/flox}$ | LCM isolated renal vesicles | Affymetrix GeneChip Mouse Expression Array 430 2.0 | Differential expression of Chrdl2, Bmf, myob5, pdgfrl and 9× downregulation of Dkk1 | [24] |
| *Aqp 1* $^{-/-}$ | 8-week-old mouse renal medulla ± infusion of vasopressin | cDNA microarrays | Increased cyclin Dl and Egr accompanied by increased cell proliferation | [68] |
| *Hnf1 beta* $^{-/-}$ | mIMCD3 cells | CHIP-chip & Affymetrix GeneChip Mouse Expression Array 430A&B | Hnf-1 beta binds to and regulates Socs3 to control tubulogenesis | [22] |
| *Nephrin* $^{-/-}$ | E18.5 glomeruli mouse | Affymetrix GeneChip Mouse Expression Array 430 2.0 | Nephrin not essential for cell survival but plays a role in glomerular differentiation and development. | [69] |

| Mutant strain (gene) | Source/species | Technology[a] | Outcome | Reference |
|---|---|---|---|---|
| *Glis2*$^{mut}$ | PND25 & PND60 total kidney | Agilent whole-mouse genome microarrays | Upregulation of genes involved in ECM homeostasis and immune/inflammatory response | [70] |
| *Wnt4 n/+* | E14.5 mouse kidney | Affymetrix GeneChip MG-U74Av2 arrays | >50% genes expressed in renal vesicle or later stage | [71] |
| *Aqp 11*$^{-/-}$ and *Pkd1*$^{-/-}$ | PND1 mouse kidneys | Affymetrix GeneChip Mouse Expression Array 430 2.0 | Comparison suggests strong similarities between the two strains (same changes in Myc, Egfr, Egf, Mmp12, Timp1, Tgfb1) | [72] |
| *NPHS2-cre; Dicer*$^{Flx/Flx}$ | 3-week-old mouse isolated glomeruli | Exiqon's miRCURY LNA array | mmu-miR-23b, mmu-miR-24, and mmu-miR-26a identified in glomeruli | [27] |
| *NPHS2-cre; Dicer*$^{Flx/Flx}$ | 4- to 5-week old mouse glomeruli | Affymetrix GeneChip Mouse Expression Array 430 2.0 | Upregulation of podocyte specific members of mir-30 miRNA family | [28] |

PND, Post-natal day; GFP, green fluorescent protein; PKD model, polycystic kidney disease model; ECM, extracellular matrix

[a]Exiqon, Vedbaek, Denmark; Agilent Technologies, Santa Clara, CA; Illumina, San Diego, CA