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Molecular dysregulation of renal development: Congenital anomalies of the kidney and urinary tract

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ABSTRACT

Congenital anomalies of the kidney and urinary tract (CAKUT) occur in approximately 1 in 500 foetal ultrasound examinations. The CAKUT phenotype can involve varying degrees of renal dysplasia, renal hypoplasia, urinary tract obstruction, ureteropelvic anomalies such as megaureter, ureteral atresia, ectopic ureteral orifice, and duplex collecting system The nephrogenic (mesenchymal) and the ductogenic (ureteric) events are regulated by transcription factors, proto-oncogenes and growth factors in a complex fashion. Dysregulation of specific molecular pathways has been implicated as a primary mechanism for CAKUT. This review will attempt to clarify the molecular basis of CAKUT by focusing on these key developmental pathways. First, however, an examination of normal metanephric kidney development is necessary. Furthermore, clinical aspects of CAKUT, including prenatal diagnosis and current treatments, will be introduced. Through the critical evaluation of a range of diverse scientific literature, it is hoped that an overview of the current status of this important area of developmental anatomy is achieved.

1. Development of the metanephros

The development of the mammalian metanephric kidney begins at gestational week 4-5 in humans and at E11 in mice[1]. Metanephros formation is initiated by the ureteric bud, which sprouts out of the posterior end of the Wolffian duct and invades the surrounding metanephric mesenchyme [2]. The subsequent interaction between the two tissues induces the ureteric bud to branch, thus initiating the morphogenesis of the collecting duct system[3]. The metanephric mesenchyme then condenses at the tips of the ureteric buds, and mesenchymal cells form aggregates which epithelialise and form, in succession, the vesicle stage, the comma body stage, and the S body stage. Each S-shaped body, after fusion with the ureteric bud-derived collecting duct, differentiates into a definitive nephron. The branching pattern is the result of sequential ureteric bud arborisation, which proceeds from the deep cortex to the periphery in a process of induction, morphogenesis, and differentiation[1]. The underlying process of developing the subsequent stages of comma and S-shape is not fully understood, although many growth factors and molecular regulators, including glial cell-derived neurotrophic factor (GDNF), paired box genes (PAX2), Six1, Eya1, and the bone morphogenetic proteins (BMPs), among others, are implicated[4]. These stages of morphogenesis represent the onset of nephron differentiation[5].

The nephrogenic (mesenchymal) and the ductogenic (ureteric) events are regulated by transcription factors, proto-oncogenes and growth factors. Cell adhesion molecules, or CAM complexes, and their associations with the cytoskeleton and extracellular matrix (ECM) glycoproteins facilitate normal development[6]. The proto-

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oncogenes that encode for receptor tyrosine kinases are involved in mesenchymal-epithelial interactions, in which the proto-oncogene encoded tyrosine or serine/threonine kinase is the ureteric receptor for signalling molecules secreted by the metanephric mesenchyme[7]. Figure 1 below illustrates the dynamic interaction between the metanephric mesenchyme and the Wolffian duct via GDNF, the c-ret/ GDNF complex and BMP4.

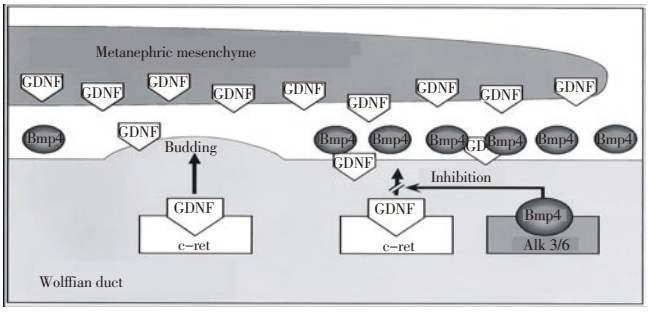

Figure 1. Navigation of the site of ureteral budding by Bmp4 through its inhibitory effect[8].

Little has been known until recently for the mechanism of determining the specific site of the ureteral budding from the Wolffian duct. Although the tightly regulated expression pattern of c-ret and its ligand GDNF may be part of the mechanism to specify the site, both c-ret and GDNF are expressed surprisingly broadly throughout the branching ureter and metanephric mesoderm, respectively, at the time of budding. In this regard, Bmp4 antagonizes the function of GDNF from the metanephric blastema that would otherwise induce the budding from the Wolffian duct. Moreover, normally, Bmp4 is diffusely expressed in the mesenchymal cells surrounding the Wolffian duct except for the highly localized locus for the initial ureteral budding. Therefore, it is thought that Bmp4 serves as an inhibitory factor for GDNF-ret signaling along the stalk of the branching ureters, thereby limiting the site of ureteral bud formation. This inhibition of ureteral branching results from the antagonistic function of Bmp4 on GDNF signaling, as Bmp4 down-regulates the Wnt 11, a target molecule of GDNF-ret signaling[7,8].

2. Defining CAKUT: a new molecular paradigm

Congenital anomalies of the kidney and urinary tract are a family of diseases with a diverse range of phenotypes. The kidney is most frequently affected; however, the ureter, bladder and ureterovesical (UV) junction are also involved. Renal anomalies such as renal agenesis, multicystic dysplasia, and hypoplasticity result from growth failure of the metanephric cells[9]. Ureteropelvic anomalies such as megaureter, ureteric atresia, ectopic ureteral orifice, duplex collecting system, and anomalies of the bladder and urethra result from ureteral growth anomalies and abnormal ectopic budding[9,10]. The development of the common forms of human CAKUT is attributed to an accumulation of mutations in multiple genes, each of which has multiple ontogenic functions on the urinary system[11]. Figure 2 below illustrates the effect of genetic mutations on the structure of the renal and urinary systems.

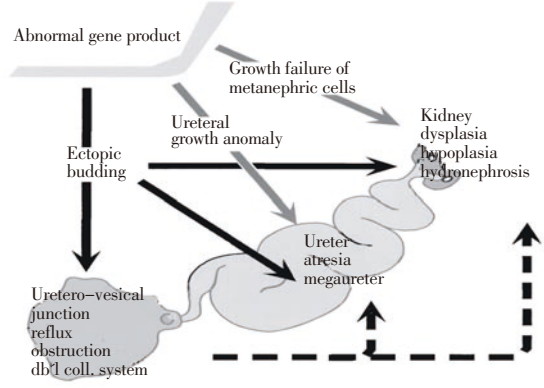

Figure 2. Overview of the ontogenic mechanism of CAKUT [8].

Pluripotentiality of the single gene mutation underlies the wide spectrum of clinical anomalies involving the ureterovesical (UV) junction, the ureter and the kidney. The loss-of-function mutation of the single gene can produce multiple anomalies due in part to its multiple biological actions on the morphogenesis of the three tissues of the excretory system, that is, the ureterovesical junction, the ureter and the kidney, at multiple developmental stages of these tissues. It is also due to the multipotentiality of the initial ectopic budding to produce three clinical entities, that is, ectopic ureteral orifice, anomalous ureter and hypo/ dysplastic kidney. Although urinary tract obstruction may cause anomalous ureter and dysplastic kidney, evidence is yet to be obtained to support the possibility that reflux can lead to those anomalies.

Glial cell-derived neurotrophic factor (GDNF) is a member of the TGF β superfamily. GDNF functions as a ligand secreted by the metanephric mesenchyme that binds to the Ret tyrosine kinase receptor and GFR 1 co-receptor, both of which are expressed within the ureteric epithelium [12]. Glial cell-derived neurotrophic factor is responsible for the initiation

of budding of the ureteric duct from the Wolffian duct, branching of the ureteric epithelium within the metanephric mesenchyme, and the development of new nephrons at the branch tips. At the tips of the branches of the ureteric duct, the mesenchyme is induced to form a mesenchymal condensate. Following this, the condensate forms an epithelial vesicle. Homozygous GDNF knockout mice die within the first 24 hours of postnatal life as the result of bilateral renal agenesis and the absence of an enteric nervous system[12].

Heterozygous GDNF mice show varying degrees of renal abnormalities. These alternate phenotypes led to the discovery of new GDNF family receptors designated GDNF family receptor- (GDNFR-) and GDNFR-[12]. GDNFR- is expressed in condensing mesenchyme, nephrons, and ureteric duct tips. Heterozygous GDNF knockout mice display an array of renal phenotypes, ranging from renal hypoplasia and cortical cysts, to unilateral renal agenesis. There is also a reduction in filtration surface area that leads to the development of glomerular hypertrophy and hypertension in order to maintain adequate renal function. These findings suggest that the GDNF heterozygous mice may prove to be useful in the elucidation of essential hypertension[13].

BMP4 is also a member of the TGF- β superfamily. BMP4 is expressed in the metanephric mesenchyme, the ureteric epithelium, the collecting ducts, and the ureteric duct leading up to the nephrogenic zone[14]. In the cultured metanephros deprived of sulfated glycosaminoglycans (S-GAGs), BMP4-loaded beads promote growth and elongation of the ureter. When S-GAGs synthesis is not inhibited, however, BMP4 beads inhibit ureteric branching and expression of Wnt 11, a downstream target of GDNF signaling. BMP4 therefore has two main roles in kidney organogenesis: the inhibition of ectopic budding from the Wolffian duct and ureteric duct by antagonising inductive signals from the metanephric mesenchyme, and the maintenance of elongation of the branching ureter within the metanephros itself[14,15]. Homozygous BMP4 knockout mice die between E6.5 and E9.5[7]. The homozygous null genotype is therefore termed "embryonic lethal".

Homozygous BMP4 knockout is not compatible with life. Heterozygous BMP4 knockout mice display abnormalities that resemble human CAKUT, including hypoplastic and dysplastic kidneys, ectopic ureterovesical (UV) junction, and a duplicate collecting system[7,8]. Cases of hypoplasia and dysplasia result from reduced branching of the ureter, while the ectopic UV junction and duplicate collecting system are predominantly initiated by ectopic ureteral budding from the Wolffian duct[8]. Figure 3 below demonstrates the role of BMP4 in both heterozygous and homozygous states at E11 in a murine model (whole mount in situ hybridization). The position of the initial ureter budding from the Wolffian duct is shown by arrows, and the 24th, 25th and 26th somite pairs are also labeled. The position of the initial budding in the wild type corresponds to the 26th somite, whereas that in the mutant corresponds to the 25th somite.

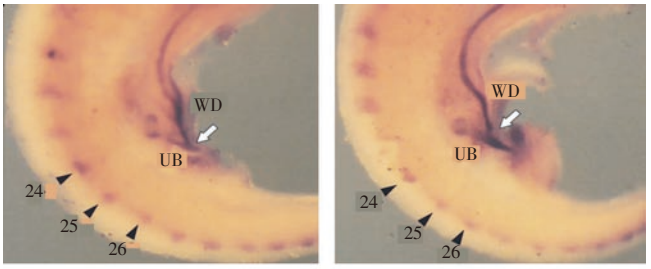

Figure 3. Ectopic budding in a Bmp4 mutant embryo[8].

In the metanephros, induction of nephrogenesis by the ureter is accompanied by an increase in expression levels of the PAX2 gene[3]. PAX2 is normally expressed in cells of the mesenchyme, as well as in the ureteric bud. PAX2 activates GDNF in metanephric mesenchyme[3]. High concentrations of PAX2 have been found in early nephrogenic epithelial cells, derived from metanephric mesenchyme, and in developing ureteric bud cells[16]. PAX2 is also involved with the expression or regulation of other renal growth factors or signals, including proper function of the c-ret pathway, and WNT4 factor expression in nephrogenesis [5]. Renal hypoplasia, a common form of CAKUT, is caused by inadequate ureteric bud arborisation as a result of increased ureteric bud apoptosis[7]. Although nephrons appear normal histologically, their numbers are reduced, leading to an increased risk of developing hypertension in adult life[13]. PAX2 haploinsufficiency occurs when there is only a single functional copy of the gene, as the other copy is inactivated by a mutation; this confers a heterozygous genotype for the condition. In humans, PAX2 haploinsufficiency causes renal-coloboma syndrome (RCS) involving eye abnormalities, renal hypoplasia, and renal failure in childhood. Overexpression of PAX2 can also cause problems. One group used immunochemistry to examine the extent of glomerulosclerosis seen in Denys-Drash syndrome (characterised by congenital kidney disease, Wilms tumour, malformation of gonads) caused by WT1 and persistent expression of PAX2 by podocytes[17].

In the developing kidney, Six1 is expressed in the uninduced metanephric mesenchyme at E10.5 and in the induced mesenchyme around the ureteric bud at E11.5 [18]. At E17.5 to P0, Six1 expression is restricted to a subpopulation of collecting tubule epithelial cells. Indeed, this group generated Six1 mutant mice to elucidate the mechanisms implicated in this process. They found that loss of Six1 leads to a failure of ureteric bud invasion into the mesenchyme and subsequent apoptosis of the mesenchyme. These results indicate that Six1 plays an essential role in early kidney development, especially in relation to mesenchymal and ureteric interaction[18]. Another gene, Eya1, is implicated in branchio-oto-renal (BOR) syndrome, an autosomal dominant disorder characterised by varying combinations of branchial, otic and renal anomalies[19]. Individiduals with BOR may have hypoplastic or absent kidneys, with resultant renal insufficiency or renal failure.

Furthermore, in Six1-/- kidney development, it has been found that PAX2 and Six2 expression was markedly reduced in the metanephric mesenchyme at E10.5, indicating that Six1 is required for the expression of these genes in the metanephric mesenchyme[18]. Again, these findings suggest that Six1 plays a significant role in early (i.e. pre-E10.5) kidney development. In contrast, Eya1 expression was unaffected in Six1-/- metanephric mesenchyme at E10.5, indicating that Eya1 may function upstream of Six1. Moreover, it has been suggested that both Eya1 and Six1 expression in the metanephric mesenchyme is preserved in PAX2-/- embryos at E10.5, further indicating that PAX2 functions downstream of both Eya1 and Six1 in the metanephric mesenchyme[19]. The epistatic relationship between PAX2, Eya1 and Six1/2 in the metanephric mesenchyme during early kidney development is essential for the initiation and maintenance of mesenchymal and ureteric interaction.

3. Clinical considerations

Congenital anomalies of the kidney and urinary tract involve renal dysplasia, renal hypoplasia, urinary tract obstruction, ureteropelvic anomalies, including megaureter, ureteral atresia, ectopic ureteral orifice, and duplex collecting system[20]. CAKUT can occur bilaterally or unilaterally. Children with CAKUT often have varying degrees of a reduced number of nephrons at birth (low nephron endowment), leaving them susceptible to adult-onset diseases including hypertension. CAKUT are now the leading cause of renal failure in children[21]. Renal dysplasia and obstructive conditions lead to loss of water and sodium in urine because of abnormal tubulogenesis. Children with severe ureteric reflux often develop urinary tract infections and renal fibrosis. Renal fibrosis can further increase the risk of renal failure in children who already have other CAKUT, leading to a vicious cycle. Furthermore, hypertension and proteinuria may develop in children with renal dysplasia and further exacerbate renal function [19-21]. The majority of renal malformations are detected antenatally because of the widespread use and sensitivity of foetal ultrasound[22]. The optimal timing is between 16 to 20 weeks' gestation because this period facilitates excellent visualisation of anatomy with a high sensitivity in detecting anomalies[23]. Furthermore, it is early enough in the pregnancy to allow completion of prenatal diagnostic procedures, including foetal karyotyping and additional imaging studies[10]. CAKUT are problems that often require surgical intervention, including ureter resections, debridement of fibrotic tissue, and, in some cases, kidney transplantation[21]. In addition to surgery, and often prior to it, dialysis can be an effective temporising measure until a donor organ becomes available or corrective surgery becomes a viable therapeutic alternative.

4. Conclusion

Glial cell-derived neurotrophic factor is responsible for the initiation of budding of the ureteric duct from the Wolffian duct and branching of the ureteric epithelium within the metanephric mesenchyme. BMP4 is expressed in the metanephric mesenchyme, the ureteric epithelium, and the collecting ducts. BMP4 plays a significant role in the inhibition of ectopic budding from the Wolffian duct and ureteric duct and the maintenance of elongation of the branching ureter within the metanephros itself. PAX2 is expressed in cells of the mesenchyme, as well as in the ureteric bud. PAX2 activates GDNF in the metanephric mesenchyme. Six1 also plays a significant role in early kidney development. The dynamic interplay between PAX2, Eya1 and Six1 in the metanephric mesenchyme during early kidney development is essential for the induction and maintenance of mesenchymal and ureteric interaction.

An appreciation of the molecular basis of CAKUT facilitates an understanding of the pathogenesis of these myriad conditions and may assist the design of genetic screening tests for early diagnosis and management. Furthermore, an insight into the relationship between abnormal genes and their products in the pathogenesis of CAKUT will provide an aetiological classification of CAKUT.

These insights will allow the coupling of molecular biology and classical epidemiologic methods, thus expanding our knowledge of the pathogenesis of CAKUT and deliver improved treatments for patients.

Conflict of interest statement

I declare that this article is entirely my own work, and any material from other sources is correctly acknowledged.

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