Nephrol Dial Transplant (1996) 11: 976–978

Nephrology
Dialysis
Transplantation

The Molecular Basis of Renal Disease

(Section Editor: L. Fine)

Genetics of human kidney malformations

P. Winyard and S. Feather

Developmental Biology and Nephro-Urology Units, Institute of Child Health, London, UK.

The spectrum of human renal malformations

Renal malformations are anatomic defects of the kidney caused by aberrations of normal kidney development. The range of malformations includes *aplasia*, where there is anatomic absence of renal tissue, *hypoplasia*, in which the kidneys are small and have reduced numbers of nephrons, and *dysplasia*, where the metanephros fails to differentiate normally. Dysplastic kidneys contain primitive ducts surrounded by sheaths of fibromuscular and undifferentiated cells, often with islands of metaplastic cartilage [1,2] and/or cysts in the multicystic dysplastic kidneys. Vesicoureteric reflux (V-UR) and horseshoe kidneys are also included in some classifications of renal malformations.

From a developmental standpoint, these heterogeneous abnormalities can be considered to be a spectrum of 'adysplasia' rather than distinct conditions. Support for this hypothesis comes from (a) families containing individuals with aplasia, dysplasia and V-UR, (b) patients with coexistent renal aplasia and dysplasia, and (c) the natural history of some multicystic dysplastic kidneys which involute leading to renal aplasia [3].

In humans at birth the risk of a having renal or urinary tract malformations is reportedly as high as 10%. Bilateral renal aplasia complicated by the Potter sequence of oligohydramnios, face and limb deformities, and lung hypoplasia occurs in 1–3 per 10 000 births, bilateral renal dysplasia has an incidence of 1 in 10 000 and, unilateral aplasia has a widely variable reported incidence of 1–30 per 10 000 births. V-UR

Evidence for genetic causation of human renal malformations

Most human kidney malformations are sporadic, with no family history of renal disease. However, genetic causation is implied by studies which have demonstrated autosomal dominant inheritance of adysplasia and the increased risk of 3–5% of recurrence of kidney malformations in siblings of index children with bilateral disease [4,5]. In mice null mutations in nephrogenesis genes such as *ret, wt-1* and *wnt-4* lead to renal abnormalities and it is likely that these genes may be implicated in human malformations. At present, however, there are only a few proven human genetic defects which lead to renal malformations. These are detailed below.

Vesicoureteric reflux and optic nerve colobomas

A New Zealand family with renal hypoplasia, proteinuria, V-UR and optic nerve colobomas have recently been described with mutations of *PAX-2* [6]. *PAX-2* is part of the family of nine *PAX* genes which contain paired box domains and mutations of *PAX-3* cause Waardenberg syndrome and *PAX-6* cause aniridia. *PAX-2* is located on human chromosome 10q24-q25 and contains an octapeptide domain in addition to the paired box region. These domains allow it to bind DNA, hence it is thought to act as a transcriptional regulator. The newly described mutation leads to a frame shift which eliminates the octapeptide DNA binding domain and the whole C-terminal portion of the PAX-2 protein. *Pax-2* has been implicated in murine nephrogenesis by several experiments: (a) it is normally expressed in the ureteric bud and induced renal mesenchyme; (b) *Krd* mice with a transgene-induced deletion of *PAX-2* have a high incidence of kidney defects including aplasia, hypoplasia, and cystic kidneys; (c) antisense oligonucleotides which specifically block PAX-2 protein production prevent renal mesenchyme to epithelium conversion in metanephric organ culture; and (d) constitutive overexpression of PAX-2 in transgenic mice leads to congenital nephrotic syndrome and polycystic kidneys.

V-UR is a common congenital abnormality of the urinary tract which is often associated with renal malformations, urinary infection and kidney scarring, sometimes leading to hypertension and chronic renal

Correspondence and offprint requests to: Paul Winyard, Developmental Biology Unit, Institute of Child Health, 30 Guilford St., London WC1E 1EH, UK.

failure. Mutation of a single major locus inherited in a dominant fashion is the likely cause of V-UR [5]. *PAX-2* mutations may be involved in some cases but it is unlikely to be the only gene involved since V-UR is usually an isolated finding, with no evidence of renal or optic abnormalities. *PAX-8*, may be a better candidate, since it has a similar expression in the kidney but not in the eye.

Kallmann's syndrome

Unilateral renal aplasia occurs in 40% of patients with X-linked Kallmann's syndrome, and bilateral disease has been reported in some kindreds. The syndrome is caused by mutations of the *KAL* gene on Xp22.3 which encodes a 680 amino-acid protein containing a signal peptide, but lacking membrane insertion or anchorage sequences, suggesting that the protein is secreted. The putative protein has homologies to both antiproteases and to cell–matrix and cell–cell adhesion molecules. Kallmann's syndrome is characterized by the association of anosmia and isolated hypogonadotrophic hypogonadism. These symptoms result from defective migration of olfactory and gonadotrophin releasing hormone axons from the nose into the olfactory bulb and forebrain during fetal development.

The link between the *KAL* gene and renal aplasia was unclear until we recently demonstrated the expression pattern of *KAL* in the kidney during the first trimester of human gestation [7]. Low levels of mRNA were detected in the mesonephros and metanephros at 45 days after fertilization by reverse transcriptase polymerase chain reaction. The mesonephric kidneys later degenerate and remnants of the mesonephric duct give rise to the *vas deferens* in males which is often absent in Kallmann's syndrome. The metanephroi develop into the adult kidneys and later in gestation the nephrogenic cortex of the fetal kidney continues to express low levels of KAL mRNA whilst high levels are located in the olfactory bulb. These observations implicate *KAL* as a critical gene in nephrogenesis when it may play a role in the mesenchymal to epithelial transition similar to either uvomorulin or the laminin A chain since the KAL protein contains areas with structural homology to cell–cell and cell–matrix adhesion molecules.

Branchio-oto-renal syndrome

The Branchio-oto-renal syndrome is an autosomal dominant disorder of high penetrance and variable expression [8]. Significant renal disorders occur in two-thirds of affected individuals, often varying within the same family from renal aplasia and dysplasia to abnormalities of the renal pelvis and ureters. Branchial defects consist of preauricular pits, cervical fistulas or cysts, and ear defects include structural defects of the outer, middle, or inner ear, and sensorineural, conductive, or mixed deafness. The gene has not been cloned but linkage analysis has isolated it to a 500-kb interval around 8q12.2–q21.2.

Di George syndrome

The Di George syndrome is part of the spectrum of phenotypes associated with deletions around chromosome 22q 11.2 [28] and over half of the patients have a broad spectrum of kidney abnormalities. Microdeletions within this region can cause: **C**ardiac defects, **A**bnormal facies, **T**hymic hypoplasia, **C**left palate and **H**ypocalcaemia, leading to the acronym CATCH 22. Candidate genes for the Di George syndrome include DGDR2, which encodes a membrane bound adhesion receptor protein, and DGDR3, which shares some homologous regions with a murine androgen receptor gene. Interestingly, DGDR3 also contains a leucine zipper motif which confers DNA binding activity and it may act as a transcriptional regulator in a similar manner to *PAX-2*.

Modifying factors in human renal malformations

There are some paradoxical observations regarding the genetics of human renal malformations. For example, mutations of the *KAL* gene in Kallmann's syndrome usually lead to unilateral kidney disease even though all cells have the mutation. Similarly, the severity of V-UR and renal adysplasia can vary within individual members of the same kindred. Multiple gene defects and/or modifying environmental factors may explain these findings.

Multiple genes

Defects in several genes may be required to cause some renal malformations. For example, mice with single null mutations of the paralogous *hoxa-11* and *hoxd-11* transcription factors have minor defects in the axial skeleton but normal kidneys. In contrast, mice with double knockouts have lethal renal malformations, along with severe skeletal malformations. Genetic background may also be important in generating variable phenotypes since the human population is relatively heterogeneous. This hypothesis is supported by a recent article where transgenic mice were generated with homologous null mutations of the gene coding for the epidermal growth factor receptor. Three genetic backgrounds were examined and transgenic offspring died at the time of implantation, midway through gestation or after 3 weeks, depending on their genetic background.

Environmental factors

Animal experiments suggest that renal adysplasia can be generated by teratogens. Examples in humans are

maternal alcohol abuse and exposure to high levels of blood sugar in maternal diabetes, but such factors can only be implicated in a small minority of cases. Mechanical obstruction of the urinary tract *in utero* causes renal dysplasia in some animals, and there is a strong correlation between posterior urethral valves and renal dysplasia in humans. This could be considered to be an environmental cause for adysplasia but this hypothesis ignores the underlying cause of the obstruction. Since twins, other siblings, and families have been described with posterior urethral valves it could be that mutation of the same genes may cause both obstruction and dysplasia.

Cell and developmental biology of human renal malformations

Very little is known about the cell biology of human renal adysplasias in contrast to polycystic kidney diseases where aberrations including enhanced epithelial proliferation and altered polarity occur. Histologically, dysplastic kidneys contain primitive ducts, which are considered to be 'frustrated' branches of the ureteric bud, surrounded by sheaths of undifferentiated cells that superficially resemble mesenchymal cells in the developing metanephros [1,2]. Therefore, a common theme in adysplasia and polycystic kidney disease is a lack of differentiation. Programmed cell death, or apoptosis, occurs in nephrogenesis where it has been implicated in morphogenesis, and fulminant apoptosis occurs in the hypoplastic, cystic kidneys which develop in mice with null mutations of *bcl-2*. Therefore we recently examined the location and extent of apoptosis in human multicystic dysplastic kidneys [10]. The incidence of apoptosis was greater in dysplastic kidneys compared to normal controls, particularly in cells located around the dysplastic tubules and thus apoptosis may contribute to the spontaneous involution reported in multicystic dysplastic kidneys. In addition our laboratory has preliminary data which suggest that *bcl-2* expression is reduced in the same disorder but it is currently unknown whether this is a primary genetic or a secondary event.

Conclusion

We are still in the early stages of trying to define the role of genetic factors in human renal diseases. A few defined genetic defects have been implicated in conditions such as Kallmann's syndrome (*KAL*) and reflux with optic nerve colobomas (*PAX-2*), but even in these cases the spectrum of renal malformations is variable, suggesting that other factors are involved in the generation of the renal disease. It is therefore likely that genetic background, environmental factors, and multiple gene defects interact in many cases of human renal malformations, which makes the search for further genetic causes significantly more arduous. There is, however, compelling evidence from transgenic animal work that genes such as *wt-1, ret, wnt-4* and *bcl-2* are essential in normal nephrogenesis and further study of these genes is warranted to define their role in human renal abnormalities.

Acknowledgements. P W and S F are supported by Training Fellowships from Action Research and the Kidney Research Aid Fund.

References

1. Potter EL. *Normal and Abnormal Development of the Kidney.* Year Book Medical Publishers, Chicago USA, 1972
2. Risdon RA. Renal dysplasia. Part 1A clinico-pathological study of 76 cases. *J Clin Pathol* 1971; 24: 57–71
3. Woolf AS. Clinical impact and biological basis of renal malformations. Semin Nephrol 1995; 15: 361–372
4. Roodhooft AM, Birnholz JC, Holmes LB. Familial nature of congenital absence a severe dysgenesis of both kidneys. *N Engl J Med* 1984; 310: 1341–1345
5. Bailey RR. Vesico-ureteric reflux and reflux nephropathy. *Kidney Int* 1993; [Supp]: 80–85
6. Sanyannusin P, Schimmentil LA, McNoe LA *et al.* Mutations of the *PAX2* gene in a family with optic ner colobomas, renal anomalies and vesicoureteral reflux. *Nature Genetics* 1995; 9: 35?–364
7. Duke VM, Winyard PJD, Thorogood P, Soothill P, Bouloux PMG, Woolf AS. *KAL*, gene mutated in Kallmann's syndrome, is expressed in the first trimester of human development. *Mol Cell Endocrinol* 1995; 110: 73–79
8. Heimler A, Lieber E. Branchio-oto-renal syndrome: reduced penetrance and variable expressivity in four generations of a large kindred. *Am J Med Genet* 1986; 25: 15–27
9. Glover T. Catching a break on 22. *Nature Genetics* 1995; 10: 257–258
10. Winyard PJD, Nauta J, Lirenman DS *et al.* Deregulation of cell survival in cystic and dysplastic renal development. *Kidney Int.* (in press)