Int. J. Dev. Biol. 43: 463-468 (1999)

Pax2 in development and renal disease

GREGORY R. DRESSLER[1]* and ADRIAN S. WOOLF[2]

[1]*Department of Pathology, The University of Michigan Medical Center, Ann Arbor, Michigan, USA and* [2]*Nephrourology Unit, Institute of Child Health, University College London Medical School, London, United Kingdom*

ABSTRACT ***Pax*** **genes are associated with a variety of developmental mutations in mouse and man that are gene dosage sensitive, or haploinsufficient. The** ***Pax2*** **gene encodes a DNA binding, transcription factor whose expression is essential for the development of the renal epithelium. Both gain and loss of function mutants in the mouse demonstrate a requirement for** ***Pax2*** **in the conversion of metanephric mesenchymal precursor cells to the fully differentiated tubular epithelium of the nephron. However,** ***Pax2*** **expression is down-regulated as cells leave the mitotic cycle. Humans carrying a single** ***Pax2*** **mutant allele exhibit renal hypoplasia, vesicoureteric reflux, and optic nerve colobomas. Conversely, persistent expression of** ***Pax2*** **has been demonstrated in a variety of cystic and dysplastic renal diseases and correlates with continued proliferation of renal epithelial cells. Thus,** ***Pax2*** **misexpresssion may be a key determinant in the initiation and progression of renal diseases marked by increased or deregulated cell proliferation.**

KEY WORDS: *Pax2, fetal kidney obstruction, multicystic dysplastic kidney, renal-coloboma syndrome, renal tumours, vesicoureteric reflux*

Introduction

Although many genes which code for transcription factors, growth/survival factors and adhesion molecules have been functionally implicated in the control of murine nephrogenesis, the relevance of most of these molecules to human disease is unclear. In this respect, the *PAX2* paradigm is perhaps the best example so far in which results from animal experiments appear relevant to the understanding the pathobiology of a variety of human diseases which are disorders of cell differentiation. Since most of these disorders are congenital anomalies of the kidney and lower urinary tract, we will focus on these malformations. However, we will also briefly allude to the relation between *PAX2* and human kidney malignancies.

Pax genes and normal development

The *Pax* gene family encodes DNA binding, transcription factors whose functions are required for the normal development of a variety of structures and cell types in *Drosophila*, mouse and man (for review see Stuart and Gruss, 1996). Vertebrate *Pax* genes were originally identified based on sequence homology to the *Drosophila* segmentation gene *paired*. These homologies are maximal within the DNA binding domain, or paired-box, of the proteins but also encompass other conserved domains such as the paired-type homeobox and the octapeptide sequence. In mouse and man, there are 9 known *Pax* genes, (Fig. 1) whose expression patterns are restricted to developing structures such as the CNS, the vertebral column, the eye, the neural crest, and the kidney. Both naturally occurring mutations in mice and humans, as well as gene targeting in mouse, have revealed essential functions for *Pax* genes during embryogenesis. For example, the mouse *small eye* mutant and human aniridia are due to mutations in the *Pax6* gene, whereas mouse *splotch* and human Wardenburg syndrome are caused by *Pax3* mutations (Read, 1995). In most of all cases studied, phenotypes are dominant and are due to a gene dosage effect, or haploinsufficieny. Thus, a reduction to one functional *Pax* gene allele already has deleterious effects on development. This effect has serious implications for human disease, since, unlike many disease genes, loss of heterozygosity is not required for phenotypic manifestations.

Pax2 in kidney development

The expression pattern of the *Pax2* gene has been characterised in many vertebrate species, including frog, chick, mouse and human, yet the essential character of this pattern remains constant. Both mRNA and Pax2 protein can be detected in the

***Address for reprints:** Department of Pathology, Howard Hughes Medical Institute, The University of Michigan Medical Center, MSRB I, 1150 W. Medical Center Drive, Ann Arbor, Michigan 48109-0650, USA. FAX: 734 763 6640. e-mail: dressler@umich.edu

0214-6282/99/$15.00

Printed in Spain
www.ehu.es/ijdb

pronephric duct, the earliest epithelial structure derived from the intermediate mesoderm, around the level of the 10th to 12th somite. As the duct extends into the mesonephric region, *Pax2* expression remains and becomes evident in mesonephric tubules, which are thought to derive from periductal mesenchyme. At the time of ureteric bud outgrowth and invasion into the metanephric mesenchyme, *Pax2* expression is activated in mesenchyme cells with particularly high levels in condensing mesenchyme at the ureteric bud tips. Thus, *Pax2* is one of the earliest markers for induced mesenchyme and may be activated by the primary inductive signals emanating from the bud. Such *Pax2* activating signals have not been identified, although a growing body of evidence points to members of the wnt family of secreted, matrix associated proteins. In the classical transfilter induction assay, the wnt-4 protein can substitute for much of the inducing activity previously associated with the dorsal spinal cord, as can other related wnt-proteins (Herzlinger *et al.*, 1994; Kispert *et al.*, 1998). Although kidneys from *wnt-4* mutant mice arrest at the mesenchymal aggregate stage (Stark *et al.*, 1994), they still express *Pax2*, consistent with the fact that *wnt-4* expression follows *Pax2* normally.

As the induced mesenchyme undergoes epithelial conversion, forming first the renal vesicle and then the S-shaped body, *Pax2* expression is down regulated. This is first apparent in the podocyte precursors at the proximal end of the S-shaped body where *Pax2* repression may be mediated by increasing levels of the Wilms' tumour suppressor gene, *WT1* (Ryan *et al.*, 1995). In mature proximal and distal tubule cells, *Pax2* expression is undetectable but still persists in the major collecting ducts. How *Pax2* expression is suppressed in proximal and distal tubules remains unclear, but most probably works through a *WT1* independent mechanism.

Both *in vitro* loss of function experiments (Rothenpieler and Dressler, 1993) and gene targeting (Torres *et al.*, 1995) demonstrate a clear requirement for *Pax2* to generate tubules from the mesenchyme. Homozygous *Pax2* null mice initiate pronephric duct development and express at least some of the known markers, such as the receptor tyrosine kinase *c-ret*, in the ductal epithelium as it extends caudally. However, at the level of the mesonephros, there is no evidence of tubule formation in the *Pax2* null mice. Furthermore, the mesonephric duct fails to extend completely caudal to the level of the metanephric mesenchyme and is unable to produce a ureteric bud. *In vitro* tissue recombination experiments suggest that *Pax2* mutant mesenchyme is unable to respond to wild-type inductive signals (G. Dressler, personal observation), just as the mesonephric mesenchyme is unable to form tubules despite the apparently normal nephric duct more anterior. The phenotype of the *Pax2* null mice is supported by previous organ culture experiments which indicated that *Pax2* was required for the early aggregation stage of the induced mesenchyme. It remains to be determined whether the block in nephric duct extension and maintenance is due to lack of signals emanating from mesonephric tubules or is a cell autonomous effect due to lack of *Pax2* expression in the ductal epithelium.

Biochemistry of Pax proteins

As outlined in Figure 1, *Pax* genes fall into three main subgroups based on conserved domains and expression patterns. The subdivision of the *Pax* gene family was based on the presence or absence of the paired-type homeodomain and the octapeptide sequence, as well as common elements of the expression patterns. Pax proteins interact with DNA through the characteristic 128 amino acid paired-domain at the amino-terminal end of the protein and/or the homeodomain located at the amino-terminus. Experiments in the fly demonstrated that the paired-domain and the homeo-domain could bind DNA independently (Treisman *et al.*, 1991). Although most *Pax* genes were cloned by sequence homology, the *Pax5* gene was identified because it encodes the B-cell specific transcription factor BSAP which binds to an immunoglobulin enhancer sequence (Adams *et al.*, 1992). Thus, *Pax5* is probably the best model for paired domain/DNA interactions because of the known recognition sequences. Much of the available evidence now indicates that the 128 amino acid paired-domain binds to a bipartite DNA recognition sequence, allowing for a high degree of sequence divergence (Czerny *et al.*, 1993; Epstein *et al.*, 1994b; Phelps and Dressler, 1996). The bipartite nature of the paired-domain was confirmed by the crystal structure of the *Drosophila* paired protein bound to its recognition oligonucleotide (Xu *et al.*, 1995). The paired-domain consists of three amino-terminal α-helices that resemble a homeo-domain, followed by a carboxyl terminal region with three smaller α-helices. The amino terminal α-helices contact the 3' part of the bipartite target sequence, whereas the carboxyl terminal tail recognizes the 5' end of the DNA sequence. Furthermore, interactions between the paired-domain and the DNA recognition sequences can change the conformation of the Pax protein (Epstein *et al.*, 1994a) and the target DNAs (Chalepakis *et al.*, 1994). The importance of DNA binding is underscored by specific mutation in the DNA binding domains of *Pax2* (Sanyanusin *et al.*, 1995a) and *Pax6* (Hanson and van Heyningen, 1995) in human disease.

The *Pax2, 5,* and *8* constitute a subfamily that was probably derived from a single ancestral gene. Each gene contains a paired domain and an octapeptide, but only the first helix of the homeo domain. Although DNA binding activities map to the amino terminal paired domain, transcription activation and repression is mediated by more C-terminal domains of the Pax2 family of proteins (Lechner and Dressler, 1996). For example, amino acid residues (aa) 197-415 of murine Pax2 are required for maximal transcription transactivation in cultured mammalian cells (Lechner and Dressler, 1996). The activation domain is generally rich in proline (P), serine (S), threonine (T) and tyrosine (Y) and is phosphorylated at serine residues. Missense mutations have rarely been identified in the C-terminal coding region of *Pax* genes, most likely because multiple regions can contribute to full activity. Surprisingly, *Pax2* also contains a repressor motif, the octapeptide sequence, that is similar to the *engrailed* and *goosecoid* repression domains (Smith and Jaynes, 1996; Mailhos *et al.*, 1998). In all these proteins, the octapeptide is found N-terminal to the HD suggesting a potential interaction which has survived evolution. The presence of both activation and repression domains in Pax proteins suggests multiple functions that may ultimately depend on the context of target sequences within a given locus.

Pax protein function must be mediated by interactions with other nuclear proteins that complex at the DNA binding site and mediate activation or repression. For example, Pax5 can recruit members of the Ets family of proteins to a B-cell specific promoter sequence (Fitzsimmons *et al.*, 1996). This recruitment implies Pax5 binds first

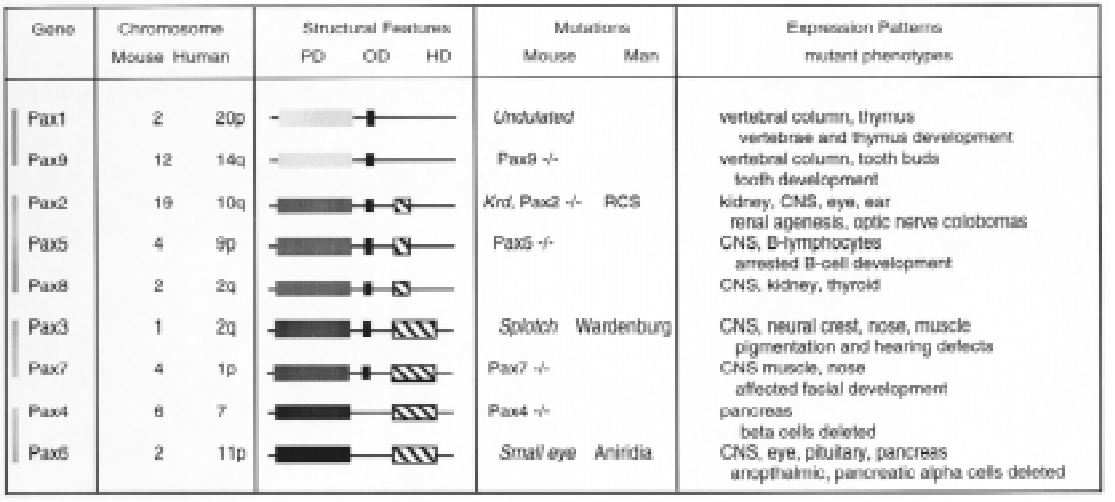

Fig. 1. The mammalian *Pax* gene family. *Nine Pax genes have been identified to date that fall into 4 subgroups based on sequence and expression patterns. The positions of the conserved domains are noted: the paired domains are indicated by shaded gray boxes, the octapeptide is a solid black box, and the homeodomain is represented by a striped box. Naturally occurring mutations are indicated by their name or syndrome in mouse and man respectively. Engineered mutants in mouse are indicated by -/-.*

and requires both Pax5 binding sites and an Ets consensus sequence. However, if the Pax5 binding site is sub-optimal, the Ets protein binds first and can recruit, or stabilise, Pax5 binding, although it is not clear if Ets proteins interact with unbound Pax5. A recent Pax3 binding protein was identified mapping to the DiGeorge /velocardiofacial syndrome locus (DGCR) (Magnaghi *et al.*, 1998). This protein, called HIRA, shares homology with yeast transcriptional co-repressors. However, it remains to be determined how these protein interactions affect Pax mediated function.

PAX2 in human kidney disease

The human metanephros appears at five weeks of gestation and the first glomeruli form by nine weeks (Risdon and Woolf, 1998). Nephrons are generated until 34 weeks with tubule maturation continuing postnatally. Malformations of the kidney and urinary tract are often diagnosed by fetal ultrasound examination, a routine procedure performed in many countries from midgestation. Such malformations contribute to 30% of all prenatally diagnosed anomalies and the most severe cases are associated with a significant decrease in fetal urine formation and hence reduced amounts of amniotic fluid: this is often associated with lung hypoplasia, the so-called 'Potter sequence' (Noia *et al.*, 1996). The human fetus can survive without kidney function since the placenta removes circulating waste but, after birth, severely affected infants may require dialysis and subsequent renal transplantation. In fact, kidney malformations are the commonest causes of chronic renal failure in young children (McEnery *et al.*, 1992). Renal malformations can occur in isolation or as part of a multiorgan syndrome and, additionally, they can be sporadic or inherited (Woolf and Winyard, 1998). The following are some varieties recognised in clinical practice: in 'renal agenesis' the kidney is absent; in 'multicystic renal dysplasia' poorly branched ducts terminate in cysts and are surrounded by undifferentiated cells and metaplastic cartilage; in 'renal hypoplasia' the organ is small with fewer nephrons than normal; in 'vesicoureteric reflux' urine passes retrogradely from the urinary bladder into the ureter and into a kidney which may itself be malformed. Finally, kidney malformations are sometimes associated with urinary tracts which are physically obstructed.

The primary causes of human renal malformations most likely include mutations, the effects of physical impairment of urine flow and teratorgens: currently *PAX2* can be implicated in the first two of these categories. The expression patterns of *PAX2* have been documented during human nephrogenesis with protein first localised to the mesonephric (Wolffian) and paramesonephric (Mullerian) ducts and then appearing in the primitive ureter epithelium and condensing renal mesenchyme in the early metanephros (Winyard *et al.*, 1997). Subsequently, *PAX2* is highly expressed in condensing mesenchyme and fetal collecting ducts, especially at the branching tips of the ureteric bud (Eccles *et al.*, 1995; Winyard *et al.*, 1996b) but is downregulated during maturation with only very low levels of protein appearing in postnatal collecting ducts (Winyard *et al.*, 1996b). This temporal and spatial expression pattern within the renal mesenchymal and ureteric bud lineages correlates with cell proliferation as assessed by nuclear staining for proliferating cell nuclear antigen (Winyard *et al.*, 1996b).

Renal-coloboma syndrome

The renal-coloboma syndrome is a rare collection of anomalies including blindness due to an optic nerve malformation called a 'coloboma' associated with congenitally small kidneys and vesicoureteric reflux (Rieger, 1977; Schimmenti *et al.*, 1995; OMIM #120330). The renal lesion has sometimes been called 'hypoplasia', although the absolute nephron numbers have not been formally counted. The syndrome has a striking phenotypic similarity to *Pax2* heterozygous mutant mice (Keller *et al.*, 1994; Torres *et al.*, 1995; Favor *et al.*, 1996) and, following this clinical observation, Sanyanusin and colleagues (1995b) defined a heterozygous mutation of *PAX2* in a human kindred where the mutation arose *de novo* and was transmitted from father to sons in an autosomal dominant manner consistent with the gene location on 10q24.3-q25.1. The mutated gene was predicted to code for a protein with an intact DNA-binding paired box, an interrupted octapepide domain and a novel, truncated, carboxyterminal: it was postulated that this would perturb the transactivation of target genes. Subsequent reports of further patients confirmed other *PAX2* mutations including a *de novo* t(10;13) translocation (Narahara *et al.*, 1997) and a mutation in the paired box which would affect DNA binding

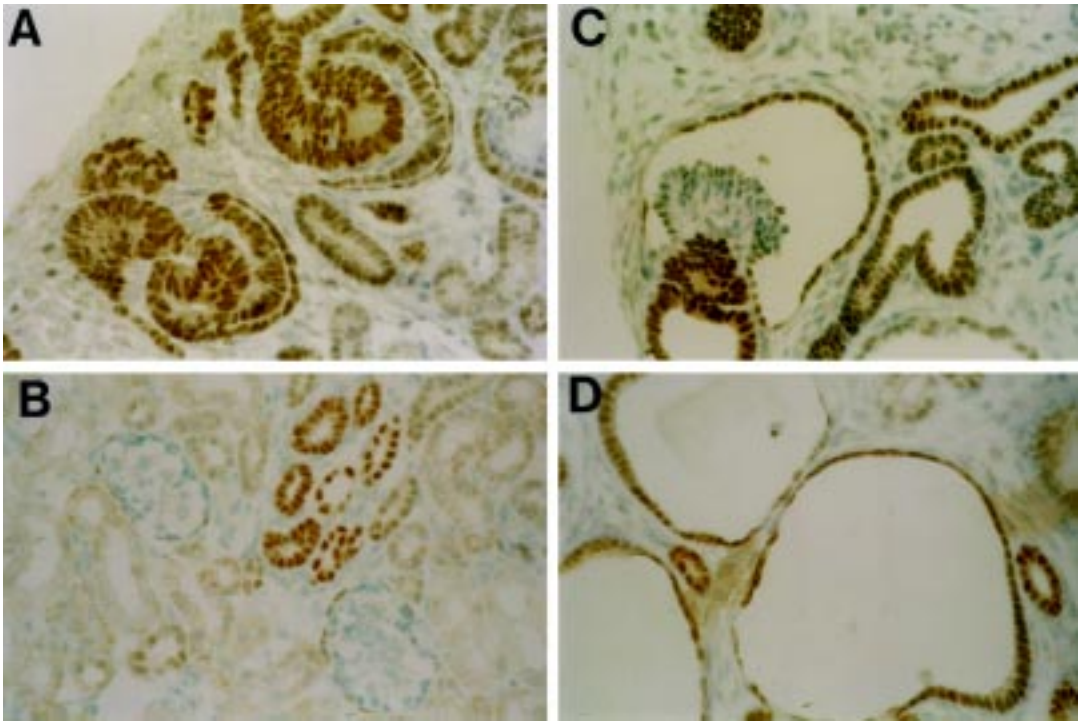

Fig. 2. Deregulation of PAX2 in experimental fetal kidney obstruction. (A) *PAX2 protein expression in the nephrogenic cortex of the normal fetal sheep metanephros.* **(B)** *In the inner cortex, expression is downregulated in glomerular parietal epithelia and proximal tubules with low levels maintained in collecting ducts.* **(C)** *In the nephrogenic cortex of the obstructed organ, PAX2 is expressed in early cysts, seen to be expansions of glomerular parietal epithelia and adjoining proximal tubule.* **(D)** *Expression is maintained in all cells lining cysts in the deeper cortex.*

(Sanyanusin *et al.*, 1995a). The phenotype of this disease would be consistent with a haploinsufficiency state causing a lack of growth of both the kidney and the ureter. The ocular coloboma, which can cause severe visual impairment, is explained by *PAX2* expression during eye development and the high frequency hearing loss, which is a more variable feature of the syndrome (Schimmenti *et al.*, 1995, 1997) is consistent with gene expression in the inner ear. Homozygous *PAX2* mutations have yet to be described in humans, although kindreds with kidney and Mullerian malformations (Battin *et al.*, 1993) superficially resemble mouse null-mutants (Torres *et al.*, 1995).

The renal coloboma syndrome is a rare entity but the question arises whether *PAX2* mutations might account for some individuals with non-syndromic malformations of the kidney and ureter. Primary, non-syndromic vesicoureteric reflux occurs in 1% of children and can be associated with renal dysplasia and hypoplasia (Feather *et al.*, 1996). Inheritance in some kindreds appears dominant with variable penetrance and expression. So far, families with this disorder have failed to show linkage to the *PAX2* locus or mutations of the gene (Feather *et al.*, 1997; Cho *et al.*, 1998). Very recently, there is preliminary evidence that a subset of patients who have non-syndromic renal hypoplasia have *PAX2* mutations (Salomon *et al.*, 1998). Finally, although the closely-related *PAX8* gene is expressed during human kidney development (Poleev *et al.*, 1992; Eccles *et al.*, 1995), mutations have yet to be associated with renal malformations.

PAX2 expression in human cystic dysplastic kidneys

Human multicystic dysplastic kidneys represent a model of perturbed epithelial/mesenchymal interaction (Woolf and Winyard, 1998). Microdissection studies had revealed that dysplastic epithelia were malformed branching tubules terminating in cysts surrounded by poorly differentiated cells resembling mesenchymal cells. We found that human renal dysplastic epithelia had a high level of cell division and expressed an array of fetal proteins including PAX2, the cell survival molecule BCL2 (Winyard *et al.*, 1996b) and galectin-3, a ureteric bud lineage cell signal molecule (Winyard *et al.*, 1997). Strikingly, dysplastic organs harvested postnatally show persistent patterns of fetal gene expression whereas normal organs downregulated proliferation as well as these genes in mature epithelia (Winyard *et al.*, 1996b). Since transgenic *Pax2* expression causes renal cysts (Dressler *et al.*, 1993), persistent expression in human fetal kidney epithelia may drive proliferation and cause cyst growth. We are now aware that the biology of multicystic kidneys is complex, with cell death upregulated in undifferentiated cells located around proliferating epithelia (Winyard *et al.*, 1996a). Recently, Groenen *et al.* (1996, 1998) described a patient with bilateral multicystic renal dysplasia with a t(6:19) (p21;q13.1) translocation which interrupted the upstream stimulator factor 2 transcription factor gene on chromosome 19 and the *CDC5L* cell cycle gene on chromosome 6. However, the significance, if any, of these mutations to the abnormal biology the multicystic dysplastic kidney is currently unclear.

Multicystic dysplastic kidneys are usually associated with an atretic ureter, and obstruction early in gestation has been invoked as a cause of the malformation. Therefore, in order to follow up our observations on cell turnover and gene expression in the human disease, we decided to follow the biological changes in a reproducible animal model of fetal kidney obstruction (Attar *et al.*, 1998). The sheep metanephros appears at 27 days of gestation and this species constitutes a good experimental model because the urinary tract can be obstructed *in vivo* relatively early in gestation to generate pathologies resembling human malformations. We caused urinary flow impairment in fetal sheep by surgical unilateral ureteric anatomical obstruction at 90 days of gestation when a few layers of glomeruli had formed (Attar *et al.*, 1998). After ten days, the nephrogenic cortex was replaced by disorganised cells separated by oedema and prominent

vascular spaces. Cortical histology was dominated by cysts which expressed PAX2 (Fig. 2) and PCNA. In addition, there was an increase in apoptosis in tissues around obstructed nephrons. Hence proliferation, PAX2 expression and programmed cell death were upregulated, as described in human dysplastic organs (Winyard *et al.*, 1996a,b). The mechanisms by which urinary flow impairment might initiate these profound effects remain to be explained.

PAX2 and human renal tumours

Renal tumours are disorders of cell growth and differentiation and some varieties may even originate in the nephrogenic period (Grignon and Eble, 1998). Therefore, it was logical to examine these lesions for *PAX2* expression. The gene was found to be expressed in the condensed blastema and dysplastic epithelium of human Wilms' tumours (Dressler and Douglas, 1992; Eccles *et al.*, 1995), a relatively common childhood neoplasm, and in renal carcinoma (Gnarra and Dressler, 1995), which affects adults. Furthermore, the reduction of PAX2 expression by antisense oligonucleotides in human renal carcinoma cell lines was associated with reduction of growth (Gnarra and Dressler, 1995). Therefore, it can be speculated that the continued, postnatal expression of *PAX2* may drive kidney tumour growth *in vivo*. In support of this contention are other experiments demonstrating that the gene can transform murine cells (Maulbecker and Gruss, 1993) and inhibit the promoter of p53, a tumour suppresser (Stuart *et al.*, 1995). Germline and somatic mutations of diverse genes have been documented in some Wilms' tumours (e.g. *WT1*) and adult kidney cancers (e.g. *VHL* and *MET*), but further work is necessary to unravel the relation of these genetic lesions to deregulated *PAX2* expression. Finally, it is notable that a variety of tumours occasionally arise in multicystic dysplastic kidneys and these organs may contain nephrogenic blastema and perilobular rests, the latter being considered a Wilms' tumour precursor (Barrett and Winel, 1980; Cromie *et al.*, 1980): as discussed above, there is evidence for PAX2 overexpression in these organs.

Conclusion

Normal renal development requires two copies of the *PAX2* gene, which is expressed in the early mesenchymal derived epithelium and in the branching ureteric bud. In both mice and humans, *PAX2* deficiency causes defective growth of the fetal kidney and ureter, while overexpression is associated with epithelial overgrowth with cyst or tumour formation.

Acknowledgement

This work was supported by Action Research project grant SP3178 and R01 DK51043.

References

ADAMS, B., DOERFLER, P., AGUZZI, A., KOZMIK, Z., URBANEK, P., MAURER-FOGY, I. and BUSSLINGER, M. (1992). *Pax-5* encodes the transcription factor BSAP and is expressed in b lymphocytes, the developing CNS, and the adult testis. *Genes Dev. 6:* 1589-1607

ATTAR, R., QUINN, F., WINYARD, P.J.D., MOURIQUAND, P.D.E., FOXALL, P., HANSON, M.A. and WOOLF, A.S. (1998). Short-term urinary flow impairment deregulates PAX2 and PCNA expression and cell survival in fetal sheep kidneys. *Am. J. Pathol. 152:* 1225-1235.

BARRETT, D.M. and WINEL, R.E. (1980). Renal cell carcinoma in multicystic dysplastic kidney. *Urology 15:* 152-154.

BATTIN, J., LACOMBE, D. and LENG, J.J. (1993). Familial occurrence of hereditary renal adysplasia with Mullerian anomalies. *Clin. Genet. 43:* 23-24.

CHALEPAKIS, G., JONES, F.S., EDELMAN, G.M. and GRUSS, P. (1994). Pax-3 contains domains for transcription activation and transcription inhibition. *Proc. Natl. Acad. Sci. USA 91*: 12745-12749.

CHO, K-L., MCNOE, L.A., FRENCH, M.C., GUILFORD, P.J. and ECCLES, M.R. (1998). Absence of PAX2 gene mutations in patients with primary familial vesicoureteric reflux. *J. Med. Genet. 35*: 338-339.

CROMIE, W.J., ENGELSTEIN, M.S. and DUCKETT, J.W. (1980). Nodular renal blastema, renal blastema and duplicated collecting systems. *J. Urol. 123:* 100-102.

CZERNY, T., SCHAFFNER, G. and BUSSLINGER, M. (1993). DNA sequence recognition by Pax proteins: bipartite structure of the paired domain and its binding site. *Genes Dev. 7:* 2048-2061.

DRESSLER, G.R. and DOUGLAS, E.C. (1992). Pax-2 is a DNA-binding protein expressed in embryonic kidney and Wilms tumor. *Proc. Natl. Acad. Sci. USA 89:* 1179-1183.

DRESSLER, G.R. WILKINSON, J.E., ROTHENPIELER, U.W., PATTERSON, L.T., WILLIAMS-SIMONS, L. and WESTPHAL, H. (1993). Deregulation of Pax-2 expression in transgenic mice generates severe kidney abnormalities. *Nature 362:* 65-67.

ECCLES, M.R., YUN, K., REEVE, A.E. and FIDLER, A.E. (1995). Comparative *in situ* hybridisation analysis of PAX2, PAX8 and WT1 gene transcription in human fetal kidney and Wilms' tumors. *Am. J. Pathol. 146:* 40-50.

EPSTEIN, J., CAI, J., GLASER, T., JEPEAL, L. and MAAS, R.L. (1994a). Identification of a Pax paired domain recognition sequence and evidence for DNA dependent conformational changes. *J. Biol. Chem. 269:* 8355-8361.

EPSTEIN, J., GLASER, T., CAI, J., JEPEAL, L., WALTON, D.S. and MAAS, R.L. (1994b).Two independent and interactive DNA-binding subdomains of the Pax6 paired domain are regulated by alternative splicing. *Genes Dev. 8:* 2022-2034..

FAVOR, J., SANDULACHE, R., NEUHAUSER-KLAUS, A., PRETSCH, W., CHATERJEE, B., SENFT, E., WURST, W., BLANQUET, V., GRIMES, P., SPORLE, R. and SCHUGHART, K. (1996). The mouse *Pax2*^{1Neu} mutation is identical to a human PAX2 mutation in a family with renal-coloboma syndrome and results in developmental defects of the brain, ear, eye and kidney. *Proc. Natl. Acad. Sci. USA 93:* 13870-13875.

FEATHER, S., WOOLF, A.S., ALLISON, J., BARRATT, T.M., BLAYDON, D.C. and MALCOLM, S. (1997). Exclusion of key nephrogenesis genes as candidates for familial vesicoureteric reflux. *J. Am. Soc. Nephrol. 8:* 388A.

FEATHER, S., WOOLF, A.S., GORDON, I., RISDON, R.A., and VERRIER-JONES, K. (1996). Vesicoureteric reflux - is it all in the genes? *Lancet 348:* 725-728.

FITZSIMMONS, D., HODSDON, W., WHEAT, W., MAIRA, S.-M., WASYLYK, B. and HAGMAN, J. (1996). Pax-5 (BSAP) recruits Ets proto-oncogene family proteins to form functional ternary complexes on a B-cell-specific promoter. *Genes Dev. 10:* 2198-2211.

GNARRA, J.R. and DRESSLER, G.R. (1995). Expression of Pax-2 in human renal cell carcinoma and growth inhibition by antisense oligonucleotides. *Cancer Res. 55:* 4092-4098.

GRIGNON, D.J. and EBLE, J.N. (1998). Renal neoplasms. In *Heptinstall's Pathology of the Kidney*. Chapter 34. 5th edition. (Eds. J.C. Jennette, J.L. Olson, M.M. Schwartz, F.G. Silva). Lippincott-Raven, Philadelphia-New York, USA. pp.1541-1573.

GROENEN, P.M.A., GARCIA, E., DEBEER, P., DEVRIEND, T.K., FRYNS, J.P. and VAN DE VEN, W.J.M. (1996). Structure, sequence, and chromosme 19 localisation of human USF2 and its rearrangement in a patient with multicystic renal dysplasia. *Genomics 38:* 141-148.

GROENEN, P.M., VANDERLINDEN, G., DEVRIENDT, K., FRYNS, M.P. and VAN DE VEN, W.J. (1998). Rearrangement of the human CDC5L gene by a t(6;19)(p21;q13.1) in a patient with multicystic renal dysplasia. *Genomics 49:* 218-229.

HANSON, I. and VAN HEYNINGEN, V. (1995). Pax6: more than meets the eye. *Trends Genet. 11:* 268-272.

HERZLINGER, D., QIAO, J., COHEN, D., RAMAKRISHNA, N. and BROWN, A.M.C. (1994). Induction of kidney epithelial morphogenesis by cell expressing Wnt-1. *Dev. Biol. 166:* 815-818.

KELLER, S.A., JONES, J.M., BOYLE, A. BARROW, L.L., KILLEN, P.D. GREEN, D.G., KAPOUSTA, N..V, HITCHCOCK, P.F., SWANK, R.T. and MEISLER, M. (1994). Kidney and retinal defects *(Krd),* a transgene-induced mutation with a deletion of mouse chromosome 19 that includes the *Pax2* locus. *Genomics 23:* 309-320.

KISPERT, A., VAINIO, S. and McMAHON, A.P. (1998). Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development 125:* 4225-4234.

LECHNER, M.S. and DRESSLER, G.R. (1996). Mapping of Pax-2 transcription activation domains. *J. Biol. Chem. 271:* 21088-21093.

MAGNAGHI, P., ROBERTS, C., LORAIN, S., LIPINSKI, M. and SCAMBLER, P.J. (1998). HIRA, a mammalian homologue of Saccharomyces cerevisiae transcriptional co-repressors, interacts with Pax3. *Nature Genet. 20:* 74-77.

MAILHOS, C., ANDRE, S., MOLLEREAU, B., GORIELY, A., HEMMATI-BRIVANLOU, A. and DESPLAN, C. (1998). *Drosophila* goosecoid requires a conserved heptapeptide for repression of paired-class homeoprotein activators. *Development 125:* 937-947.

MAULBECKER, C.C. and GRUSS, P. (1993). The oncogenic potential of Pax genes. *EMBO J. 12:* 2361-2367.

McENERY, P.T., STABLEIN, D.M., ARBUS, G. and TEJANI, A. (1992). Renal transplantation in children. *New Engl. J. Med. 326:* 1727-1732.

NARAHARA, K., BAKER, E., ITO, S., YOKOYAMA,Y., YU, S., HEWITT, D., SUTHERLAND, G.R., ECCLES, M.R. and RICHARDS, R.I. (1997). Localisation of a 10q breakpoint within the PAX2 gene in a patients with a de novo t(10;13) translocation and optic nerve coloboma-renal syndrome. *J. Med. Genet. 34:* 213-216.

NOIA, G., MASINI, L., DE SANTIS, M. and CARUSO, A. (1996). The impact of invasive procedures on prognostic, diagnostic and therapeutic aspects or urinary tract anomalies. In *Neonatal Nephrology in Progress*. (Eds. L. Cataldi, V. Fanos, U. Simeoni) Agora, Lecce, Italy. pp. 67-84.

PHELPS, D.E. and DRESSLER, G.R. (1996).Identification of novel pax-2 binding sites by chromatin precipitation. *J. Biol. Chem. 271*: 7978-7985.

POLEEV, A., FICKENSCHER, H., MUNDLOS, S., WINTERPACHT, A., ZABEL, B., FIDLER, A., GRUSS, P. and PLACHOV, D. (1992). PAX8, a human paired box gene: isolation and expression in developing thyroid, kidney and Wilms' tumors. *Development 116*: 611-623.

READ, A.P. (1995). Pax genes - *Paired* feet in three camps. *Nature Genet. 5:* 333-334.

RIEGER, G. (1977). On the clinical picture of Handmann's anomaly of the optic nerve Morning glory syndrome? *Klin. Monatsbl. Augenheilkd. 170:* 697-706. (German).

RISDON, R.A. and WOOLF, A.S. (1998). Development of the kidney. *In Heptinstall's Pathology of the Kidney*. Chapter 2. 5th edition. (Eds. J.C. Jennette, J.L. Olson, M.M. Schwartz, F.G. Silva). Lippincott-Raven, Philadelphia-New York, USA. pp. 67-84.

ROTHENPIELER, U.W. and DRESSLER, G.R. (1993). *Pax-2* is required for mesenchyme-to-epithelium conversion during kidney development. *Development 119:* 711-720.

RYAN, G., STEELE-PERKINS, V., MORRIS, J., RAUSCHER, F.J.,III. and DRESSLER, G.R. (1995). Repression of Pax-2 by WT1 during normal kidney development. *Development 121:* 867-875.

SALOMON, R., TELLIER, A.L., ATTIE, T., ANTIGNAC, C., MUNNICH, A., NIAUDET, P., GUBLER, M.C. and BROYER, M. (1998). Pax-2 mutations in oligomeganephronic renal hypoplasia. *7th International Workshop on Developmental Nephrology*, Stockholm, Sweden.

SANYANUSIN, P., MCNOE, L.A., SULLIVAN, M.J., WEAVER, R.G. and ECCLES, M.R. (1995a). Mutation of PAX2 in two siblings with renal-coloboma syndrome. *Hum. Mol. Genet. 4:* 2183-2184.

SANYANUSIN, P., SCHIMMENTL, L.A., MCNOE, L.A., WARD, T.A., PIERPOINT, M.E.M., SULLIVAN, M.J., DOBYNS, W.B. and ECCLES, M.R. (1995b). Mutations of the *PAX2* gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nature Genet. 9:* 358-364.

SCHIMMENTI, L.A., CUNLIFFE, H.E., MCNOE, L.A., WARD, T.A., FRENCH, M.C., SHIM, H.H., ZHANG, Y-H., PROESMANS, W., LEYS, A., BYERLY, K.A., BRADDOCK, S.R., MASUNO, M., IMAIZUMI, K., DEVRIEND,T. K. and ECCLES, M.R. (1997). Further delineation of renal-coloboma syndrome in patients with extreme variability of phenotype and identical PAX2 mutations. *Am. J. Hum. Genet. 60:* 968-978.

SCHIMMENTI, L.A. PIERPONT, M.E., CARPENTER, B.L., KASHTAN, C.E., JOHNSON, M.R. and DOBYNS, W.B. (1995). Autosomal dominant optic nerve colobomas, vesicouretric reflux and renal anomalies. *Am. J. Med. Genet. 59:* 204-208.

SMITH, S.T. and JAYNES, J.B. (1996). A conserved region of engrailed, shared among all en-, gsc-, Nk1-, Nk2- and msh-class homeoproteins, mediates active transcriptional repression *in vivo*. *Development 122:* 3141-3150.

STARK, K., VAINIO, S., VASSILEVA, G. and McMAHON, A.P. (1994). Epithelial transformation of metanephric mesenchyme in the developing kidney regulated by Wnt-4. *Nature 372:* 679-683.

STUART, E.T. and GRUSS, P. (1996). PAX: developmental control genes in cell growth and differentiation. *Cell Growth Differ. 7:* 405-412.

STUART, E.T. HAFFNER, R., OREN, M. and GRUSS, P. (1995). Loss of p53 function through PAX-mediated transcriptional repression. *EMBO J. 14:* 5638-5645.

TORRES, M., GOMEX-PARDO, E., DRESSLER, G.R. and GRUSS, P. (1995). Pax-2 controls multiple steps of urogenital development. *Development 121:* 4057-4065.

TREISMAN, J., HARRIS, E. and DESPLAN, C. (1991). The paired box encodes a second DNA-binding domain in the paired homeo-domain protein. *Genes Dev. 5:* 594-604.

WINYARD, P.J.D., BAO, Q., HUGHES, R.C. and WOOLF, A.S. (1997). Epithelial galectin-3 during human nephrogenesis and childhood cystic diseases. *J. Am. Soc. Nephrol. 8*: 1647-1657.

WINYARD, P.J.D., NAUTA, J., LIRENMAN, D.S., HARDMAN, P., SAMS, V.R., RISDON, A.R. and WOOLF, A.S. (1996a). Deregulation of cell survival in cystic and dysplastic renal development. *Kidney Int. 49:* 135-146.

WINYARD, P.J.D., RISDON, R.A., SAMS, V.R., DRESSLER, G.R. and WOOLF, A.S. (1996b). The PAX2 transcription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. *J. Clin. Invest. 98:* 451-459.

WOOLF, A.S. and WINYARD, P.J.D. (1998). Advances in the cell biology and genetics of human kidney malformations. *J. Am. Soc. Nephrol. 9:* 1114-1125.

XU, W., ROULD, M.A., JUN, S., DESPLAN, C. and PABO, C.O. (1995). Crystal structure of a paired domain-DNA complex at 2.5 A resolution reveals structural basis for Pax developmental mutations. *Cell 80:* 639-650.