De Novo Uroplakin IIIa Heterozygous Mutations Cause Human Renal Adysplasia Leading to Severe Kidney Failure

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Human renal adysplasia usually occurs sporadically, and bilateral disease is the most common cause of childhood end-stage renal failure, a condition that is lethal without intervention using dialysis or transplantation. De novo heterozygous mutations in Uroplakin IIIa (UPIIIa) are reported in four of 17 children with kidney failure caused by renal adysplasia in the absence of an overt urinary tract obstruction. One girl and one boy in unrelated kindreds had a missense mutation at a CpG dinucleotide in the cytoplasmic domain of UPIIIa (Pro273Leu), both of whom had severe vesico ureteric reflux, and the girl had persistent cloaca; two other patients had de novo mutations in the 3' UTR (963 T→G; 1003 T→C), and they had renal adysplasia in the absence of any other anomaly. The mutations were absent in all sets of parents and in siblings, none of whom had radiologic evidence of renal adysplasia, and mutations were absent in two panels of 192 ethnically matched control chromosomes. UPIIIa was expressed in nascent urothelia in ureter and renal pelvis of human embryos, and it is suggested that perturbed urothelial differentiation may generate human kidney malformations, perhaps by altering differentiation of adjacent smooth muscle cells such that the metanephros is exposed to a functional obstruction of urine flow. With advances in renal replacement therapy, children with renal failure, who would otherwise have died, are surviving to adulthood. Therefore, although the mechanisms of action of the UPIIIa mutations have yet to be determined, these findings have important implications regarding genetic counseling of affected individuals who reach reproductive age.


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anomaly: Indeed, Nishimura et al. (19) found an association with a common polymorphism of the angiotensin II receptor type 2 (AT2) gene with diverse urinary tract malformations; however, this could not be replicated (20). In rare families with more than one affected individual, renal adysplasia might have a dominant inheritance with reduced penetrance and variable expressivity (6,7,21). Our hypothesis to explain the sporadic occurrence of bilateral renal adysplasia is that such individuals have de novo dominant mutations of genes expressed in renal tract differentiation; because such individuals would often have severe renal failure, they would not generally have lived to reproduce in the era before the advent of modern dialysis and transplantation.

Uroplakins (UP) are key components of the urothelium. They are transmembrane proteins that pass the lipid bilayer once (UPII, UPIIIa, and UPIIIb) or four times (UPIa and UPIb; both belonging to the “tetraspanin” family) (22–24). All have large luminal/extracellular domains, but only UPIIIa and UPIIIb have significant cytoplasmic portions in their C-termini (22). UP heterodimers (UPIb/UPII and UPIa/UPII) are major components of plaques, also known as the “asymmetric unit membrane,” covering almost the entire urothelial apical cell surface (25). Indeed, these specific heterodimers must form as a prerequisite for their transport to the apical membrane of urothelial cells, and in the absence of the appropriate partner, UPIa/UPII/UPIIIa cannot escape the endoplasmic reticulum, whereas UPIb becomes mistargeted to the basolateral membrane (23,26). UPIIIa null mutant mice have high-grade VUR leading to hydrenephrosis, without anatomic bladder obstruction (26); their plaques are structurally abnormal and their urothelia are excessively water permeable, and some mutants die soon after birth with renal failure. Given the emerging cell biology and murine genetic data that UPIIIa is a critical molecule for the integrity of urothelium and also urinary tract development, we hypothesized that UPIIIa (or its partner, UPIb) might be mutated in humans with congenital kidney and urinary tract malformations; moreover, we reasoned that such mutations might occur de novo.

Materials and Methods

Patients

The research project was approved by the Local Ethical Committees, and venous blood was collected from patients and first-degree relatives after informed consent; leukocyte DNA was extracted by the salt-precipitation method. We studied three groups of patients: Group 1 was 17 children with renal failure associated with bilateral renal adysplasia without overt urinary tract obstruction; group 2 was six children with posterior urethral valves leading to bilateral dysplastic kidneys and renal failure secondary to urinary tract obstruction; group 3 was 19 patients with unilateral multicystic renal dysplasia without renal failure (4). The first two groups were ascertained at the nephrology clinic at Great Ormond Street Hospital, London; the third group was ascertained at St. James’ University Hospital, Leeds. None of these patients had defined clinical evidence of genetic multiorgan syndromes. The clinical histories of the four index cases in whom UPIIIa mutations were subsequently found (Table 1), all from group 1, are as follows (imaging comprised ultrasonography, isotope renography, and micturating cystography).

Family 1. The white female index case was born by emergency cesarean section after cord prolapse. By day 3 of life, her plasma creatinine was 378 μM (normal for age < 50 μM = 0.56 mg/dl). Imaging failed to detect a right kidney; the left kidney had abnormal cortical echogenicity, and its malformed pelvis was connected to a tortuous ureter with severe VUR and intrarenal reflux of urine. The urinary bladder had two diverticula, and there was a common passage-way between rectum and urethra. There was no vaginal orifice, and the right uterine horn was absent. The child was classified as having a persistent cloaca. Both gonads were ovarian. Initially, the patient required neonatal peritoneal dialysis to sustain life, but the plasma creatinine stabilized to 128 μM, allowing withdrawal of dialysis. Over several years, she was treated with cutaneous ureterostomy, ureteric reimplantation, bladder neck reconstruction, and bladder reconstruction (ileocystoplasty). At most recent follow-up, aged 12 yr, she has renal failure of moderate severity (plasma creatinine 267 μM) and requires treatment with vitamin D for secondary hyperparathyroidism and erythropoietin for anemia. She also required surgical correction of a large ventricular septal defect. Her karyotype is 46XX.

Family 2. The white male index case was born at 41 wk gestation. He presented at 2 yr of age, with a history of being “unwell” for >3 mo. He had severe anemia (hemoglobin 6.3 g/dl) and renal failure (plasma creatinine 285 μM). On imaging, both kidneys were present, but they had abnormally increased cortical echogenicity and thinned parenchyma. There was bilateral hydrenephrosis (dilated renal pelvis) with severe bilateral VUR into tortuous ureters. The urethra was normal. Now aged 15 yr, he has progressive chronic renal failure (creatinine 345 μM) and requires treatment with vitamin D for secondary hyperparathyroidism and erythropoietin for anemia. It is considered that he will require renal transplantation in the next few years.

Family 3. The Pakistani female index case presented with intrauterine growth retardation at 34 wk gestation, with induction of labor at 37 wk. She was admitted as a neonate with a history of vomiting and found to have renal failure (plasma creatinine 176 μM). Both kidneys were small (<2 SD below the mean for age) and had increased cortical

Table 1. Clinical features of index patients with heterozygous UPIIIa mutations

<table>
<thead>
<tr>
<th>Family</th>
<th>Renal Phenotype</th>
<th>VUR</th>
<th>Bladder</th>
<th>Other Malformations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Right aplasia</td>
<td>Severe</td>
<td>Diverticula</td>
<td>Cloaca</td>
</tr>
<tr>
<td></td>
<td>Left dysplasia</td>
<td></td>
<td></td>
<td>Ventricular septal defect</td>
</tr>
<tr>
<td>2</td>
<td>Bilateral dysplasia</td>
<td>Severe</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Bilateral dysplasia</td>
<td>Absent</td>
<td>Normal</td>
<td>No</td>
</tr>
<tr>
<td>4</td>
<td>Bilateral dysplasia and cortical cysts</td>
<td>Absent</td>
<td>Small volume</td>
<td>Simple external right ear</td>
</tr>
</tbody>
</table>

aUPIIIa, Uroplakin IIIa; VUR, vesicoureteric reflux.
echogenicity with no evidence of obstruction or VUR. Subsequently, her renal excretory function slowly deteriorated, and she received a cadaveric renal transplant at age 9 yr.

**Family 4.** The white male index case was delivered at 42 wk gestation by emergency cesarean section because of fetal distress. By day 4 of life, he was noted to have severe renal failure (plasma creatinine 411 μM). Subsequent assessment showed a “simple right ear” and evidence of short stature. Imaging showed two small highly echogenic kidneys 2 and 3 cm long, each with cortical cysts. Cystogram revealed a small-volume, “irritable” bladder with no VUR and a normal urethra. His karyotype is 46XY. He was initially treated with alkali for acidosis and erythropoietin for anemia, but renal excretory function deteriorated and he received a cadaveric renal transplant at age 3 yr.

**Sequencing of Genomic DNA**

The exonic structure and polymorphic variation of UPIIIa and UP Ib used as a reference for mutation analysis was that defined by Jiang et al. (27) (Ensembl accession numbers: UPIIIa, ENSG00000100373; UP Ib, ENSG00000114638). Nucleotide positions are numbered as the position after the start codon of full-length mRNA. All six exons of UP IIIa and seven exons of UP Ib were sequenced in all index cases, using previously published primers (27), except for exon 3 of UP IIIa, where we identified a novel SNP (A→G, intron 2, 78 bp before the splice donor site) causing allele dropout; here, we used the following forward and reverse primers, respectively, at an annealing temperature of 67.4°C: 5′-AAAGCAGGGAGGTGATCCTT-3′ and 5′-CTGTACTCCAGCCTGGAAA-3′.

PCR were performed using standard reactions. The products were treated with 10 units of Exonuclease (New England Biolabs, Beverly, MA), 2 units of Shrimp-Alkaline-Phosphatase (Roche, East Sussex, UK), and 1X SAP buffer in a 25-μl reaction to remove residual dNTP and primers from the PCR; the mix was incubated at 37°C for 30 min followed by 85°C for 15 min to deaminate the enzymes so that these products could be used directly in sequencing reactions. Sequencing reactions were performed using a BigDye Terminator kit (Applied Biosystems, Foster City, CA). Products were desalted by centrifugation through Sephadex-G50 plates (Sigma, St. Louis, MO) at 911 g for 5 min. Samples were run on a MegaBACE 500 (Amersham), and sequences were analyzed using Sequencher v4.1 software. Sequencing was repeated three times using both forward and reverse primers to ensure sequence fidelity.

**UP Immunohistochemistry**

Paraffin sections from 14 normal human embryos ranging from 5 to 13 wk gestational age were provided by the Medical Research Council and Wellcome Trust-funded Human Embryo Bank, Institute of Child Health, London. This time window spans the early organogenesis of the kidney and urinary bladder (12,28). Paraffin sections were prepared for immunohistochemistry as described (27) using primary antibodies specific for UP IIIa (mouse monoclonal; 1:50), UP Ib (raised in rabbit; 1:100), and α-smooth muscle actin (α-SMA; mouse monoclonal; 1:200) overnight at 4°C in a humidity chamber. Bound antibodies were detected with a peroxidase-based method, and slides were counterstained with hematoxylin. Primary antibodies were replaced with purified IgG or rabbit serum as negative controls, and no signal was obtained (data not shown).

**UP Transfection Experiments and UP IIIa Immunocytochemistry**

Bovine pcDNA3 expression constructs for wild-type UP IIIa and UP Ib were made as described previously (23). For the missense mutant UP IIIa construct, C was changed for T at position 818 of cDNA using a Site-directed Mutagenesis Kit (Stratagene, La Jolla, CA), resulting in a Pro273Leu change in the expressed protein. COS-1 adult monkey kidney cells that do not display endogenous UP IIIa or UP Ib expression (as shown by Western blot; data not shown) were cultured at 37°C in DMEM (Life Technologies BRL, Grand Island, NY) supplemented with 10% FCS and antibiotics to 30 to 40% confluence. FuGENE-6 Transfection Reagent (Roche) was used for lipofection (1 μg of each construct was transfected, and 4 μl of FuGENE-6 was used per μg of DNA), and cells were allowed to continue to grow to 80% confluence. Then they were fixed in 4% paraformaldehyde for 1 h; some were permeabilized by using 1× PBS with 0.5% Triton-X (Sigma). Cells then were blocked with FCS and incubated with UP IIIa mAb (1:5) overnight at 4°C. Cells were washed and incubated for 1 h at room temperature with FITC-conjugated anti-mouse secondary antibody (Dako, Glostrup, Denmark) that was seen as a green signal. For visualization, camera shutter speed was fixed such that nonpermeabilized cells that were transfected with only UP IIIa gave no signal, as expected (23). All immunofluorescence pictures were taken using the same shutter speed. Pictures were taken from representative fields.

**Results**

At 7 wk gestation, the nascent tubelike bladder has recently formed from the cloaca, and surrounding mesenchyme has begun to differentiate, as evidenced by α-SMA expression (Figure 1A). At this time point, UP IIIa was immunolocalized in the luminal epithelial layer of the urogenital sinus (Figure 1B), and a similar pattern was noted for UP Ib (Figure 1C). At the same stage, the ureter and metanephric kidney has begun to form, but the UP were not detected in these structures (data not shown). At 13 wk gestation, when the bladder has become a muscular organ, expression of both UP was maintained in the bladder urothelium (data not shown). At 13 wk, unlike the bladder at this stage, mesenchyme surrounding the ureter has not yet formed muscle bundles (Figure 1D; the ureter was negative for α-SMA; data not shown).

At this stage, UP IIIa and UP Ib were expressed in urothelium of the ureters and also the renal pelvis (Figure 1, D through I). Expression of UP IIIa (and UP Ib) always appeared apical (Figure 1G), consistent with the formation of urothelial plaques. By contrast, mesenchymal/stromal cells adjacent to the ureteric and pelvic urothelium did not express UP.

Next, we sequenced UP IIIa and UP Ib in patient group 1, the 17 children with bilateral renal adysplasia and renal failure. No mutations were found in UP Ib. In three white patients and one Pakistani patient, with a spectrum of phenotypes including renal adysplasia, cloacal malformation, and VUR (Table 1), a heterozygous UP IIIa base change was found (Figure 2). In the two unrelated index cases from families 1 and 2, the same heterozygous mutation was found in exon 6 of UP IIIa, a change in nucleotide 818 (C/T), leading to a Pro273Leu missense change. In two other index cases, a heterozygous mutation was found in the 3′ untranslated region (UTR; family 3: 963T→G change, 67 nucleotides after the stop codon; family 4: a 1003T→C change, 107 nucleotides after the stop codon). No similar sequence changes were detected in 96 white and 96 Pakistani control individuals. In these four index cases, the mutation was absent in both parents (paternity was confirmed.
using seven unlinked autosomal microsatellites), and in the two families with siblings, the mutations were absent in two brothers and one sister. Furthermore, these parents and siblings had ultrasonographically normal urinary tracts. No family reported consanguineous marriage, and there was no history of renal disease in any of the extended families. These were heterozygous de novo mutations and correlated with the new occurrence of renal adysplasia within each of the four families.

The specificity of the above mutations for bilateral renal adysplasia was established when we genotyped two other groups of patients with different types of malformation; the first set comprised six cases with posterior urethral valves, most of whom had secondary VUR and renal failure, and the second comprised 19 patients with unilateral multicystic dysplastic kidney, none of whom had renal failure. In both conditions, impairment of fetal urine flow, at the level of obstructed urethras and ureters, respectively, is thought to cause renal dysplasia (3,8). No mutations in UPIIIa or UPIb were found, suggesting that UPIIIa and UPIb are not major causes of these varieties of renal dysplasia.

Residue 273 of UPIIIa is conserved as proline in human, mouse, rat, and cow (Figure 3), and the change would significantly alter the secondary structure of the UPIIIa cytoplasmic C-terminus. We tested the hypothesis that the Pro273Leu mutation would interfere with normal transport of UPIIIa to the cell surface by transfecting bovine UP pcDNA3 constructs into COS-1 cells. Using immunocytochemistry of permeabilized and nonpermeabilized cells, we were able to distinguish UPIIIa that was localized within cells (permeabilized) from UPIIIa that reached the cell surface (nonpermeabilized). When the wild-type UPIIIa was expressed alone, it was contained within the cytoplasm, but it reached the cell surface after co-transfection with its plaque partner, UPIb (Figure 4, A through D). When transfected alone, 273Leu UPIIIa failed to reach the cell surface (Figure 4E) and was localized within the cells in a pattern reminiscent of endoplasmic reticulum (Figure 4F). By transfecting 273Leu UPIIIa with UPIb, the mutant protein was detected at the cell surface of nonpermeabilized cells (Figure 4G), whereas the pattern in permeabilized cells was consistent with a Golgi apparatus localization (Figure 4H). These results suggested that 273Leu UPIIIa was able to escape the endoplasmic reticulum and reach the cell surface by forming a heterodimer with UPIb, i.e., it behaved exactly like its wild-type counterpart.

Next, we interrogated the possible effect of the genetic changes found in the 3’ UTR using the program Pfold (29) (http://www.daimi.au.dk/~compbio/rnafold/). This program aligns RNA sequences on the basis of their combined probabilities of forming conserved secondary structures such as hairpin loops that might be important for mRNA stability and/or interaction with proteins involved in transcription or translation. Using Pfold, we aligned the mouse and human 3’ UTR (which happen to share no significant similarity in sequence), and the analysis (data not shown) suggested that an evolutionary conserved secondary structure exists in the do-
main of the *UPIIIa* mutation in family 3 (963T → G change, 67 nucleotides after the stop codon); inclusion of this mutant RNA sequence in the alignment reduced the probability of forming a secondary structure. Similarly, inclusion of the *UPIIIa* mutation in family 4 (1003T → C change, 107 nucleotides after the stop codon) increased the probability that an additional secondary structure in the 3′ UTR is formed, as predicted by this program.

**Discussion**

Here, we show for the first time using specific antibodies that *UPIIIa* and *UPIb* proteins are expressed in the developing human ureter and renal pelvis and report *de novo* *UPIIIa* mutations in children with severe renal adysplasia with kidney failure. In fact, this is the first report of *UP* mutations in a human renal disease, and we believe that this report also constitutes the first *de novo* genetic changes associated with non-syndromic urinary tract malformations. The genetic findings also have potentially considerable clinical implications. The occurrence of *de novo* *UP* mutations in children with adysplasia and with severe renal failure leads to the possibility that, because it is now possible to save the lives of such children with medications, dialysis, and transplantation (1), a new generation inheriting dominant mutations may arise; furthermore, this next generation may be at risk of experiencing severe congenital renal disease. In this manner, advances in medical care may generate new problems for future generations. Thus, our findings of *UPIIIa* mutations not only begin to illuminate the pathogenesis of human sporadic urinary tract malformations but also have important implications regarding the genetic counseling of these individuals as they reach reproductive age.

We identified mutations in *UPIIIa* in a subset of patients with renal adysplasia with no overt urinary tract obstruction. The mutations were absent in all four sets of parents and siblings, none of whom had radiologic evidence of renal adysplasia. Indeed, others (30,31) have cogently reasoned that establishing that a genetic change has arisen *de novo* in an individual who has a congenital disease that was not present in the rest of the family can be taken as definite evidence that the mutation is causative. The Pro273Leu missense mutation arose at a CpG dinucleotide, consistent with the occurrence in two unrelated index cases, and this residue is strictly conserved in all mammalian species whose *UPIIIa* sequence is known. Collectively, these observations alone make it highly likely that the *de novo* missense change is involved in the pathogenesis of the renal malformation. Recurrent mutations, which arise in CpG dinucleotides and which can occur *de novo*, have been reported in other diseases such as craniofrontonasal syndrome (31).

We also found two cases of mutations in the 3′ UTR of a gene, *UPIIIa*. Using the (purely theoretical) Pfold analyses, we noted
that the de novo changes found in the UPIIIa 3' UTR would be predicted to alter the secondary structure of the mRNA, giving some credence to possible functional effects of the 3' UTR nucleotide changes. Although 3' UTR mutations have rarely been recognized, they have been reported to cause human disease, as described for α-globin in thalassemia (32). There is

Figure 3. Alignment of UPIIIa amino acid sequence in four different species. Using Boxshade (http://bioweb.pasteur.fr/seqanal/interfaces/boxshade.html) human (Homo sapiens, OR75631), mouse (Mus musculus, Q9JKX8), bovine (Bos taurus, P38574), and rat (Rattus norvegicus, gi:34867511), UPIIIa amino acid sequences were aligned showing conserved residues with a black background (Swiss-Prot accession numbers in brackets). The cytoplasmic domain is underlined in red according to the human entry in Swiss-Prot (http://us.expasy.org/sprot/), and the residue at which the missense change was identified is conserved as proline in all four species (as indicated by the arrow). No complete amino acid sequences for other species are available.

Figure 4. Missense UPIIIa expression in vitro. UPIIIa immunocytochemistry in nonpermeabilized (A, C, E, and G) and permeabilized (B, D, F, and H) COS-1 cells transfected with wild-type UPIIIa alone (A and B), wild-type UPIIIa and wild-type UPIb (C and D), Pro273Leu UPIIIa alone (E and F), and Pro273Leu UPIIIa and wild-type UPIb (G and H). Neither wild-type nor mutant UPIIIa reached the cell surface when transfected alone (A and E), but both were immunodetected (green) in a pattern reminiscent of endoplasmic reticulum in permeabilized cells (B and F). When co-transfected with UPIb, both wild-type and mutant UPIIIa reached the cell surface (C and G), and, in permeabilized cells, UP were detected in a Golgi-like pattern (D and H). Note that untransfected COS-1 cells do not express UP.
considerable evidence that the 3' UTR of many genes are critical in controlling the amount of proteins finally made (32–34), and it is conceivable that the UPIIIa 3' UTR mutations operate in this manner. It is known that in UPIIIa null-mutant mice, UPIb is upregulated and incompletely glycosylated, suggesting that the expression of UPIIIa and UPIb is interactive (26), and so altered UPIIIa expression levels may influence the expression of UPIb as well. Alternatively, the effect of a 3' UTR mutation may be more complex. For example, microinjection of wild-type Prohibitin 3' UTR RNA can suppress cellular growth and proliferation, whereas an allelic variant associated with an increased risk for breast cancer and differing, like our mutations, by only one nucleotide, cannot (35). Thus, it is also possible that the 3' UTR RNA of UPIIIa has independent effects on the biology of the cell. Clearly, additional studies are needed to test hypotheses about how these de novo mutations may lead to disease.

Urothelial cells undergo endo/exocytosis to modulate urothelial surface area in response to changes in bladder pressure (36), and binding of the Escherichia coli FimH receptor to UPla mediates urothelial invasion of this bacterium (37); unlike UPla, UPIb, or UPII, UPIIIa has a significant cytoplasmic tail, and it has been suggested that this region interacts with cytoplasmic proteins to mediate membrane/cytoplasmic interactions (25). We showed in COS-1 cells that wild-type UPIIIa protein can escape the endoplasmic reticulum and reach the cell surface only in the presence of UPIb, as was shown previously in 293T cells (23). Although we hypothesized that the Pro273Leu mutation in the cytoplasmic domain of UPIIIa might prevent UPIIIa from reaching the cell surface, we showed that it was possible for the missense mutant protein to reach the cell surface in COS-1 cells when co-transfected with UPIb. However, we must readily acknowledge that COS-1 cells might not be the ideal system with which to study targeting of an “epithelial-cell protein,” such as UP, because polarized monolayer formation is not a characteristic of these cells. Mindful of this, we have performed preliminary experiments co-transfecting UPIIIa (either the wild-type or missense mutant) with wild-type UPIb into MDCK cells, a prototypical renal epithelial line. The results (D.J., personal observations) first demonstrated that these cells have no endogenous expression of UPIIIa, at least as assessed by antibodies that detect human and bovine UPIIIa. Second, we observed that neither the wild-type nor the missense protein seems to reach the cell membrane, i.e., the UPIIIa protein is immunodetected in only permeabilized but not non-permeabilized cells. Difficulties with both the COS-1 and MDCK models may reflect lack of appropriate polarity and/or molecular machinery used for cellular transport of UP. Moreover, even if a mutant UPIIIa protein were targeted to the apical surface, following on from the hypothesis that the missense mutation may act in a “dominant-negative” manner, one would need to measure relatively subtle biologic readouts of plaque structure and epithelial function. We consider that the ideal cell to study will be the human urothelial cell itself. These cells not only are polarized but also are known to have specific machinery to deliver UP to the apical membrane, for example Rab27b (38). In addition, urothelial cells have the theoretical capacity to assemble plaques at the asymmetric unit membrane, and they also have specific physiologic properties such as high transepithelial resistance (39). In the future, we aim to express mutant UP proteins in “normal human urothelial” cells that can be propagated and also induced to differentiate in culture (40). These cells are susceptible to high-efficiency retroviral transduction when proliferative (J. Southgate, University of York, UK, personal observation) and, when differentiated, display physiologic and biochemical characteristics of human urothelium found in vivo.

It is notable that the two patients with the Pro273Leu mutation also had severe primary VUR in combination with renal adysplasia; in contrast, the individuals with a 3' UTR mutation had no bladder/ureteric phenotype, and the effect of these mutations may be a loss of function. With regard to primary VUR, it is interesting that the missense mutation was not detected in two studies that screened UPIIIa in a total of 101 individuals with the condition (27,41); perhaps this can be explained by the facts that only a minor subset of primary VUR patients have bilateral renal adysplasia and the Pro273Leu mutation is probably lethal without intensive therapy in early life. Furthermore, it has already been established, using a whole-genome search based on families with several affected members, that the condition called “primary VUR” is in fact genetically heterogeneous (42).

To study the cellular basis of renal adysplasia, we studied the expression of UPIIIa and its partner, UPIb, during human development. The metanephros forms 28 d after fertilization, when the ureteric bud penetrates the renal mesenchyme (12). Ultimately, the ureteric bud lineage forms urothelium, from the renal pelvis to bladder trigone, and kidney collecting ducts. The first six to 10 bud branch generations remodel, generating the renal pelvis and calyces, whereas the subsequent generations form collecting ducts; nephron tubules form as branch tips induce adjacent renal mesenchyme. Using microdissection studies of dysplastic kidneys, Potter (12) reasoned that the primitive tubules found in some human dysplastic kidneys represent early ureteric bud branches that fail to remodel into a normal renal pelvis and that also fail to produce further, nephron-inducing branches. As kidneys and ureters form, the urogenital sinus demarcates from the cloaca to form the bladder, and stroma around primitive urothelia forms smooth muscle; clearly, these developmental events have gone wrong in congenital malformations such as persistent cloaca. UP are expressed in postnatal human urothelia (43), and UPIb has been detected in human bladder urothelia at 10 wk of gestation (27). Our current data, using for the first time a UPIIIa-specific antibody, show that expression of both UPIIIa and UPIb plaque-partner proteins also occurs in the early stages of human organogenesis. UPIIIa and UPIb are expressed at 13 wk in the urothelium of the bladder, ureters, and renal pelvis and at 7 wk in the urogenital sinus, evagination of which is a crucial event in distal female genital tract formation (44) and which is relevant to the cloacal malformation seen in the proband in family 1.

Current concepts propose that human renal malformations arise from either a primary failure of metanephric mesenchy-
mal induction or a physical obstruction of urine flow (3). We have not found UPIIIa protein to be expressed within the metanephros itself, probably ruling out any direct role in nephrogenesis. In addition, although Kong et al. (45) recently reported that mice with null mutations of another member of the UP family, namely UPII, die from obstructive congenital nephropathy (urothelial hyperproliferation within the ureter), none of our patients with UPIIIa mutations had overt urinary obstruction; indeed, we excluded UPIIIa mutations in a separate group of patients with atretic ureters associated with unilateral multicystic kidneys. We suggest that another, more likely, possibility is that UPIIIa mutations cause a “functional obstruction” to fetal urine flow that perturbs kidney development. Several recent observations in genetically engineered mice are relevant here. First, Yu et al. (46) demonstrated that developing ureteric epithelia act as a signaling center by expressing sonic hedgehog; this secreted protein regulates proliferation and differentiation of nearby mesenchymal cells that are destined to become smooth muscle cells, and null mutation of sonic hedgehog in the Hoxb7/Cre line resulted in renal hypoplasia and hydrouroueter. Second, Chang et al. (47) demonstrated that urinary tract–specific deletion of calceineurin subunit b1, a gene expressed in mesenchyme near differentiating urothelia, reduced proliferation in nascent smooth muscle cells accompanied by defective pyloureteral peristalsis, progressive renal obstruction, and renal failure. As reviewed by Mendelsohn (48), functional obstruction of the developing kidney also occurs in mice mutant for AT1, an angiotensin I receptor. We speculate that UPIIIa mutations would disrupt the nurturing relationship between urothelia of the ureter/renal pelvis and the surrounding mesenchyme. This might result from a loss of structural integrity within the epithelium or from a more subtle effect, namely altered epithelial/mesenchymal signaling.

Acknowledgments

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