Unravelling the genetics of vesicoureteric reflux: a common familial disorder

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Primary vesicoureteric reflux (VUR) is one of the more common genetic disorders. Little is yet known about the genetics of this potentially manageable childhood condition, which is characterised by regurgitation of urine from the bladder to the kidney. The VUR phenotype is associated with shortness of the submucosal segment of the ureter due to congenital lateral ectopia of the ureteric orifice. VUR is found in 30–50% of infants and young children with a urinary tract infection. A serious concern in families with an affected patient is that approximately one half of siblings or offspring will be affected, but up to a half of these affected siblings and offspring may be asymptomatic in childhood. If left untreated, these patients may present later in life with proteinuria, hypertension or renal failure. VUR is the commonest cause of end-stage renal failure in children, and an important cause in adults. As the kidney damage resulting from severe VUR is preventable, early detection is desirable. The techniques for clinical diagnosis are invasive and costly, reinforcing the importance of identification of a gene for VUR to facilitate genetic screening.

Although family studies suggest a major dominant gene, the inheritance pattern is still a matter of debate. In rare instances, VUR occurs in association with other diseases, such as the coloboma–ureteric–renal syndrome, which is caused by a PAX2 gene mutation. In this review, we present evidence that this common disorder may be caused by mutations in the developmental pathway of which the PAX2 gene forms a part.

INTRODUCTION

Primary vesicoureteric reflux (VUR) (OMIM # 193000) is a common disorder in children, and shows a strong familial association (1–9). VUR is the regurgitation of urine from the bladder into the ureter and kidney (10). The true incidence of VUR in an unselected population has not been established, although current estimates suggest it is between 1/100 and 1/1000 (11,12). This may be compared with autosomal dominant polycystic kidney disease (ADPKD) which, at a frequency of 1/1000 in the population, is considered to be one of the commonest dominant genetic diseases of man (13).

It is clear that in a large proportion of patients VUR is genetic in origin. Forty five percent of children with primary VUR are from families where at least one additional family member is affected, and often the disease occurs in two or more generations (10,14). In addition, there have been several documented instances in identical twins and triplets (reviewed in 15).

Approximately one-third of individuals with VUR have some degree of renal parenchymal damage, termed reflux nephropathy (16). Reflux nephropathy, like ADPKD, is an important cause of end-stage renal failure (17).

THE NATURE OF VUR

VUR is the regurgitation of urine through a vesicoureteric junction which is either incompetent, immature or abnormally placed (18). The defect, which is one of length, diameter, musculature and/or innervation of the submucosal segment of ureter, usually involves a shortening of the submucosal ureteric segment due to congenital lateral ectopia of the ureteric orifice (shown in Fig. 1) (19–24). It could be envisaged that this anomaly may be caused by mutations in one or several developmental genes.

With increasing age, the intravesical segment of the ureter elongates, which increases the ratio between the submucosal tube length and ureteric diameter (25). The end result is that VUR may resolve spontaneously in many children. Renal scarring, however, is permanent.

VUR varies in degree, and is best divided into five grades using the classification proposed by the International Reflux Study in children (26) (Fig. 2). Grades III–V VUR are all associated with increasing degrees of dilatation of the collecting system.

CLINICAL FEATURES OF REFLUX NEPHROPATHY

Reflux nephropathy, which may be complicated by hypertension, proteinuria or renal failure (27), usually occurs before 18 months of age (28,29), and frequently is not detected until investigation following a urinary tract infection. In Australia and New Zealand, 25% of children <6 years of age who entered renal replacement programmes had reflux nephropathy (17). In adult Caucasians <50 years of age, 5–15% of cases of end-stage renal disease were due to reflux nephropathy (30). Interestingly, VUR is much less
In terms of identifying which siblings or offspring of an affected patient to screen for VUR, there are no simple clinical or laboratory parameters available at present, and a current strategy is to screen all siblings or offspring. A major goal of antenatal and postnatal screening of siblings and offspring is to identify those children who may be at risk of reflux nephropathy, because with early detection the incidence of renal damage in siblings may be reduced compared with index patients (7).

ASSOCIATION OF VUR WITH OTHER CONDITIONS

VUR may occur in association with other conditions, such as a bifid or duplex pelvicalyceal collecting system or ureter (34), pelvicureteric junction obstruction (35), nocturnal enuresis (36), detrusor instability or detrusor–sphincter dysssnergia (37), hypospadias and undescended testicles (38). VUR has also been observed in association with several syndromes, notably Hirschsprung’s disease (R.R.B., unpublished), prune-belly syndrome (39,40), a syndrome of ectrodactyly, ectodermal dysplasia and cleft lip/palate (41), coloboma–ureteral–renal syndrome (42) and Robinow dwarfism (43). In addition, chromosomal abnormalities of the short arms of chromosomes 8 or 10 have been reported in two studies where VUR was associated with developmental disabilities, skeletal anomalies, hypotonia, rectal atresia, horseshoe kidney and malrotation of the intestine (44), and multicystic kidney, hearing loss, heart defects, growth retardation and psychomotor delay (45), respectively.

Figure 1. Some anatomical features of the non-refluxing and refluxing ureter. The ureter traverses the bladder wall (intramural portion), and then tunnels obliquely through the submucosa (submucosal tunnel) before exiting at the ureteric orifice. The non-refluxing ureteric orifice has a longer submucosal tunnel compared with the refluxing orifice, which is more laterally placed on the bladder wall. [Adapted from Glenn, J. (ed.) (1975) Urologic Surgery, 2nd edn. Harper and Row, New York.]

Figure 2. Classification of grades of VUR used by the International Reflux Study Committee. Grade I reflux involves only the ureter. Grade II involves the ureter, pelvis and calyces with no dilatation and normal calyceal fornices. Grade III involves moderate dilatation and/or tortuosity of the ureter, moderate dilatation of the pelvis, no or slight blunting of the fornices. Grade IV involves moderate dilatation and/or tortosity of the ureter, moderate dilatation of the pelvices, blunting of the sharp angles of the fornices and maintenance of papillary impressions in most of the calyces. Grade V involves gross dilatation and tortuosity of the ureter, pelvis and calyces. The papillary impressions are no longer visible in the majority of the calyces. Reproduced with permission from the Report of the International Reflux Study Committee (26).

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prevalent in Afro-American populations (31). Reflux nephropathy and its associated complications should be preventable by identifying and treating all children with VUR (17).

VUR IN SIBLINGS AND OFFSPRING

Thirty four to forty five percent of an affected patient’s siblings will have reflux between birth and 18 months of age (14,32,33). The severity of VUR in siblings does not correlate with the grade of reflux in the index patient (32). VUR also appears to be more frequent in female siblings (5), although the reason for this is not known.

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Figure 3. Pedigrees of eight families with VUR examined for mutation of PAX2. Filled symbols represent individuals with VUR and/or reflux nephropathy. Note that in family A there are two disease-free individuals who may be inferred to be carriers because they have affected siblings and affected offspring.

cystourethrography, showed that 24/36 offspring (66%) exhibited VUR (9). The same authors reviewed the literature, revealing a 65% rate of reflux in the offspring of known affected patients (9). Although these results were obtained from a pre-selected group of patients, and the degree of genetic heterogeneity, if any, could not be estimated, the high rate of transmission favours autosomal dominant inheritance with 100% penetrance. The alternative, that VUR is a multifactorial trait, cannot be excluded, but at least one study, described below, suggests that VUR is not likely to be a polygenic disease.

That VUR is polygenic is rendered unlikely by the finding that in one family, where VUR occurred as part of a syndrome, the disease was associated with mutation of the PAX2 gene (56). We have identified frameshift mutations in exons 2 and 5 of PAX2 in several patients with coloboma–ureteric–renal syndrome, which involved VUR as part of the phenotype (56,57, M.R.E., unpublished). These results suggest that mutation of a single gene, such as PAX2 or similar genes, could be sufficient to cause primary VUR.

ANALYSIS OF PAX2 AS A CANDIDATE GENE FOR VUR

The primary defect in VUR is thought to arise during embryogenesis of the urinary tract. It has been suggested that the timing and position of branching of the ureteric bud from the Wolffian duct is related linearly to where the ureteric orifice opens in the bladder wall; the sooner the branching occurs, the more lateral the orifice will be in the bladder (58). Abnormalities in the ureteric bud, which induces differentiation of the nephrogenic mesenchyme, could also be responsible for abnormal development, or dysgenesis of the collecting ducts or renal papillae, associated with intrarenal reflux (59,60). PAX2 is a good candidate gene for mutations associated with developmental abnormalities of the ureteric bud and kidney. During development of the urinary tract, PAX2 is expressed in the ureteric bud and in the differentiating nephrogenic mesenchyme (61,62). PAX2 is a member of the paired-box family of genes (63), and plays a critical role in the development of both the kidney and ureteric bud (56,64). The Krd mutant mouse, in which an allele of the PAX2 gene is deleted, has ureteric dilatation as part of its phenotype (64).

To test the possibility that a PAX2 mutation may cause primary VUR, we analysed the PAX2 gene in eight unrelated pedigrees with VUR, shown in Figure 3. The only clinical problem presenting in the families was VUR and/or reflux nephropathy. Exons 2–12 of the PAX2 gene were examined by single-stranded conformational polymorphism (SSCP) analysis in 23 clinically affected and 34 disease-free individuals from the eight families. No SSCP polymorphisms were detected in PAX2, suggesting that PAX2 was not mutated in any of these patients. Three markers near the PAX2 gene, including a dinucleotide repeat in intron 8 of PAX2, were also used for linkage analysis, but linkage could not be established. In a separate study, linkage to PAX2 was excluded in a three generation pedigree involving four individuals with VUR, and one with hypospadias (R.F. Gagel, pers. comm.).

While PAX2 is a promising candidate as a major gene for VUR, these studies have suggested that PAX2 may not be involved in the aetiology of primary VUR. The association of PAX2 mutation in coloboma–ureteric–renal syndrome (56,57) suggested that the expression of PAX2 may be part of a developmental pathway disrupted in VUR. Other candidate genes, such as c-ret and N-myc, are being tested for involvement in VUR using strategies similar to the above. Linkage studies are also under way to localise the VUR gene.

SUMMARY AND FUTURE DIRECTIONS

We have tried to convey in this review that VUR is a common, clearly genetic, but relatively poorly understood disorder genetically. While several studies (12,15,56) refute the suggestion that VUR is a polygenic disease, a gene for familial primary VUR remains elusive. Meanwhile, there is a real need to
identify children who have VUR prior to the development of reflux nephropathy. It is a tragedy that VUR causes as much end-stage renal failure as the commonest dominant genetic disease known to man, i.e. ADPKD, but all patients with VUR could quite effectively be treated if the affected individuals could all be identified. Identification of individuals with VUR may well be able to prevent all VUR-induced renal failure. Unfortunately, many individuals with VUR are asymptomatic until it is too late. Ideally the means of identification should be simple, inexpensive and non-invasive. Early diagnosis of VUR is important, as much of the damage to the kidney occurs in the first 2–3 years of life and probably even in utero.

VUR is diagnosed in children by micturating cystourethrogram or radionuclide micturating cystography (65). These methods involve urethral or suprapubic catheterisation, radiographic contrast or radionuclide injection and exposure to radiation. The decision to investigate children using these invasive tests underlies the importance and seriousness of the condition. Ultrasound techniques, on the other hand, are becoming much more sophisticated, but not yet reliable enough to diagnose VUR. The ideal would be to identify infants prior to their developing a urinary tract infection or reflux nephropathy using a genetic screening method. Once identified, infants could then be monitored and treated. Perhaps the greatest immediate application of a genetic test would be the identification of siblings and offspring of an affected patient who are not gene carriers, and who could be spared the anxiety and morbidity associated with medical investigations.

If a gene for VUR was identified, then patients with sporadic VUR could be analysed to determine if their condition was caused by mutations in the same gene as familial VUR. In addition, those at risk of developing renal failure may benefit from an analysis of genotype-phenotype correlations. Such studies would examine the association of certain mutations with reflux grade. Finally, identification of a VUR gene may allow a biochemical understanding of VUR, and possibly the development of new approaches to treatment.

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