

The Genetics of Primary Vesico-Ureteric Reflux

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Abstract

Introduction: Vesico-ureteric reflux (VUR), reflux of urine from the bladder into the ureter and towards the kidney, is an important cause of end-stage renal failure in both children and adults. Primary VUR is considered to be a result of a disruption of the normal anti-reflux mechanism of the ureterovesical junction (UVJ). VUR is common, occurring in approximately 1% to 2% of newborns in Caucasian populations. The aetiology of VUR is thought to involve a substantial genetic component, supported by the observation that VUR frequently occurs in multiple members of the same family. The purpose of this article is to review the literature supporting a genetic cause of VUR, and to draw together observations and make suggestions regarding differential diagnosis of VUR, which might help in future studies on the genetic aetiology of VUR. **Results:** A common theme arising was the notion that VUR may be caused by multiple genes in the population. However, any one individual with VUR may carry a single dominant mutant allele. Overall, progress has been made in mapping putative VUR loci in both humans and mice, although the mode(s) of inheritance and the exact nature of the underlying defect are still poorly understood. **Conclusions:** It is likely that over the next few years VUR genes will be mapped and, once identified, the challenge will be to understand how changes in the expression of these genes lead to the underlying defect in VUR.

Ann Acad Med Singapore 2000; 29:337-45

Key words: Kidney damage, Pyelonephritis, Reflux nephropathy, Ureter development, Urinary tract infection

Introduction

Vesico-ureteric reflux (VUR, OMIM#193000), the name given to reflux of urine from the bladder into the ureter and towards the kidney, is believed to be due to a developmental abnormality of the ureteric bud during embryogenesis.¹ Ureteral reflux has been known for centuries; in the 15th century Leonardo da Vinci depicted refluxing ureters in his anatomical drawings.² VUR is an important cause of end-stage renal failure in adults, and is responsible for approximately 15% of end-stage renal failure in children.^{3,4} Many studies have shown that VUR occurs frequently in families, suggesting that it is inherited.⁵ The disease may be transmitted from parents to approximately half of offspring, but the actual mode of inheritance is unknown. It is clear that VUR is very common, occurring in an estimated 1% to 2% of Caucasian children in early post-natal life.^{6,7}

The Physical Abnormality

The defect in patients with primary VUR is thought to involve malformation of the ureterovesical junction, which normally prevents urine from leaving the bladder via the ureter.^{1,4} The ureterovesical junction acts as a valve and closes during micturition or when the bladder contracts. The length of the submucosal ureter,⁸ the width of the

ureteric opening,⁹ the musculature of the trigone and distal ureter,¹⁰ and ureteric peristalsis (including innervation of the ureter¹¹) may all contribute to an anti-reflux mechanism. The defect is thought to be, at least in part, due to a shortening of the submucosal ureteric segment due to congenital lateral ectopia of the ureteric orifice (Fig. 1). Depending on the length of the submucosal segment of the ureter, several different types of ureteric orifices may form in the bladder wall.¹² These openings are associated with progressively less competency at preventing reflux the more laterally displaced the ureter opening is from its normal position. The underlying abnormality could be due to mutations in one or more developmental genes, or alternatively, to abnormal levels of exogenously derived or endogenous diffusible factors (e.g. TGF- β 1¹³).

Histochemical studies¹⁴⁻¹⁶ have raised the possibility that an additional partial ring of muscle is present in the ureterovesical junctions of VUR patients. There may exist a continuum where some normal patients do not have this additional muscle tissue, those with VUR have a partial ring of muscle and those with primary obstructive megaureter have a complete ring. The muscle tissue was reported to be like that from the mesonephric duct, from which both the vas deferens and ureteric bud originate.^{14,17}

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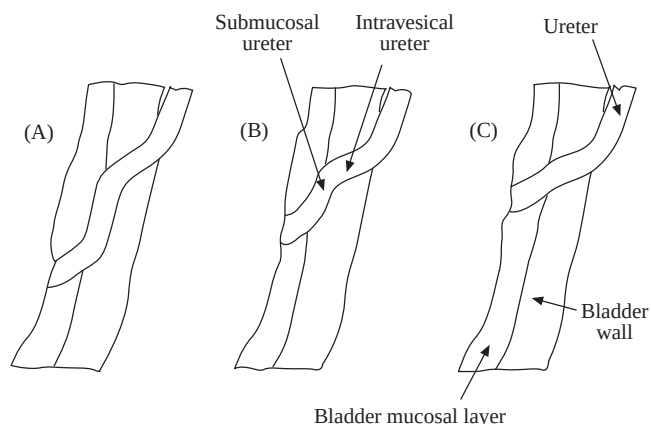


Fig. 1. Features of non-refluxing and refluxing ureters. The ureter traverses the bladder wall (intravesical portion, panel A, B or C), and then tunnels obliquely through the submucosa (submucosal portion, panel A, B or C) before exiting at the ureteric orifice. The non-refluxing ureteric orifice has a longer submucosal tunnel (shown in panel A) compared with the refluxing orifice (shown in panels B or C), and in some patients the ureteric orifice is extremely laterally placed on the bladder wall (panel C).

However, the type of musculature of the ureterovesical junction was indistinguishable in post-mortem specimens obtained from male and female Afro-American and Caucasian children.¹⁶ In addition, it was shown that innervation of the ureterovesical junction in reflux patients^{14,18} was indistinguishable from that in control patients.¹⁹ In the latter study though, immunostaining for PGP (protein gene product), a general nerve marker, was found to be reduced in parts of the ureterovesical junction in VUR patients.¹⁴ Moreover, studies in normal volunteers suggest that the vesicoureteral junction excitatory reflex is important in preventing reflux of urine into the ureter.¹¹

Since VUR primarily involves abnormalities of the ureter and the ureteric orifice, it has been suggested that the timing and position of branching of the ureteric bud from the Wolffian duct may be related to VUR.¹² Glial-derived neurotrophic factor (GDNF) plays an important role in initiating ureteric bud branching from the Wolffian duct,²⁰ and many other factors are involved.²¹ The ureteric bud also induces differentiation of the nephrogenic mesenchyme,²¹ suggesting that abnormalities in the ureteric bud could also be responsible for abnormal development, or dysgenesis of the collecting ducts or renal papillae, features which are commonly associated with VUR.²²

Clinical Presentation

Reflux is generally asymptomatic, although patients may present with a urinary tract infection, following which reflux is discovered.⁵ Only a relatively small proportion of patients with reflux present with end-stage renal failure, having never had a urinary tract infection. Progression to end-stage renal failure is correlated with severity of VUR, which is graded from I to V.²³ While VUR grade has an

influence on the outcome, the grade of VUR or the outcome in the index patient does not bear any relationship to the grade of VUR in other family members.²⁴ Many key clinical questions still need to be resolved, of which several are re-stated in a recent review.²⁵

VUR is diagnosed by voiding cystourethrogram (VCUG) both in index patients and in asymptomatic relatives. VUR has a tendency to spontaneously resolve with age, with yearly resolution rates exceeding 28%.^{26,27} It has been suggested that spontaneous resolution occurs because the length of the intravesical segment of the ureter increases with age as does muscular support for the ureter,²⁸ lending itself to the notion that in some individuals, VUR may be the outcome of delayed maturation.

Dimethylsuccinic acid (DMSA) scan is used to detect renal scarring caused by VUR. This can potentially be used to identify family members in whom reflux has already resolved.²⁹ However, unless scarring has occurred, not all spontaneously resolved reflux will be detected by DMSA scan.

Reflux Nephropathy

VUR can potentially damage the kidney in two ways. Infectious agents can be carried up to the kidneys during reflux, resulting in pyelonephritis and subsequent kidney scarring.³ Alternatively, it is thought that damage can occur directly by reflux of urine physically impacting on the kidney (e.g. in high-grade reflux, where most damage occurs at the poles of the kidney). The kidney damage caused by VUR is called reflux nephropathy, and is often associated with hypertension, or in more severe cases, proteinuria and renal failure.³ Kidney damage occurs in up to one-third of individuals with VUR³, although the outcome in patients with VUR is very variable.^{30,31} The renal scarring caused by VUR is permanent.

In addition to VUR, renal dysplasia, scarring and small kidneys have been observed in newborn babies.³² Moreover, hydronephrosis has been detected prenatally by ultrasound in babies who have later been shown to have reflux.³³

The Argument for VUR Being a Genetic Disease

There are several reasons for suspecting that VUR has a genetic contribution to its aetiology. Perhaps the strongest evidence is that VUR occurs in approximately 20% of patients (6/29 patients) who have renal-coloboma syndrome associated with *PAX2* gene mutations.^{34,35} *PAX2* is a member of the paired-box family of developmental genes, and plays a critical role in the development of the central nervous system (CNS), eyes, ears and urogenital tract (reviewed in reference 36).³⁶ *PAX2* is expressed during development of the Wolffian duct prior to branching of the ureteric bud, and also in the ureteric bud and the differentiating

nephrogenic mesenchyme during kidney development.^{37,38} The expression patterns of *PAX2* suggest that this gene is a good candidate for mutations in VUR patients. To date, 8 different *PAX2* mutations have been identified in 10 families with renal-coloboma syndrome. At least 4 of these mutations were associated with VUR (varying from grade I to grade IV) as part of the syndrome.^{34,35} Neither the type of *PAX2* mutation nor the position of the mutation was predictive for the presence VUR (Fig. 2). These studies may, however, be an underestimate of the frequency of VUR in patients with *PAX2* mutations, as not all the patients in these studies were evaluated for reflux. In addition, in some patients an early episode of reflux may have already resolved. While VUR occurs in patients who have *PAX2* mutations and renal-coloboma syndrome, there have been no patients or families with *PAX2* mutations in whom VUR was the only abnormality. Several other syndromes associated with VUR are mentioned below.

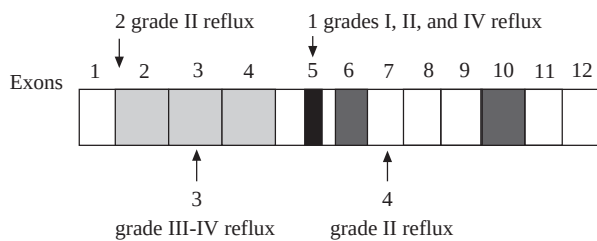


Fig. 2. Mutations identified in the *PAX2* gene associated with VUR.³⁴ Shown is the *PAX2* gene (black and grey bar), which is divided into 12 exons as denoted by the 12 segments. The paired box is shown in exons 2, 3 and 4, (light grey shading) while the octapeptide is shown in exon 5 (black box). Exons 6 and 10 (dark grey shading) are alternatively spliced. The mutations 1–4, associated with VUR, are shown above and below the bar. Mutations 1 and 2 are frameshift mutations in exons 5 and 2, respectively.³⁴ Mutation 3 is a missense mutation in exon 3,³⁴ while mutation 4 is a stop-codon mutation in exon 7.³⁵

Risk Estimates

Renal-coloboma syndrome is rare, and the majority of patients with primary VUR do not have any other associated abnormalities. Primary VUR is by far the most common form of VUR. In genetic studies, it is important to determine the frequency of the allele(s) of interest in the population and the rate at which it is passed on. Estimates of the rate of VUR in the population at large, parent to child transmission, and the chance of a sibling of an affected exhibiting VUR are useful clues to the genetics of the disorder. For monogenic disorders, the allele frequency can be estimated from population studies. In the case of multigenic disorders this is more difficult. (It is not clear whether there is one or several genes interacting to cause primary VUR. If there are several genes, these estimates will be lower than the individual allele frequencies.) Risk estimates for VUR from the recent literature are given in Table I.

It should be borne in mind that all of the rates cited are under-estimates due to various forms of false negatives that occur in the different diagnostic methods used. Factors associated with under-estimation of the frequency of VUR include ascertainment bias and the fact that relative risk of VUR decreases with age (Table II). The age-dependency of VUR risk is a reflection of the fact that VUR spontaneously resolves over time. The exact time course of resolution and whether it is strictly related to age or infection still needs to be resolved. A recent study of the rate of resolution of VUR²⁶ is shown in Table III. This age-dependent resolution needs to be considered in genetic studies such as linkage analysis.

Estimates of the frequency of VUR in the population have been done in 1 of 3 ways:

1. An initial screen by VCUG: VCUG is the most direct method of identifying VUR and so should be considered

TABLE I: RISK ESTIMATES OF VUR IN THE GENERAL POPULATION REPORTED IN THE RECENT LITERATURE

Source (citation)	Bias	Parent → child	Siblings of affected	Population rate
32	Self-reporting survey			1–2%
32	New-born with <i>relative</i> with VUR	20.4% (38/186)		
83	Family history of VUR; ages tested up to 10 years	41.7% (5/12)	63.6% (7/11)	
7	Ultrasound then VCUG, DMSA; no prenatal VUR / abnormal development cases			1.26% (30/2384)
39	Siblings of affected; ages 2 weeks to 12.8 years		36.5% (176/482)	
82	?		32%	

VUR: vesico-ureteric reflux; VCUG: voiding cystourethrogram; DMSA: dimethylsuccinic acid

TABLE II: FREQUENCY OF VUR VERSUS AGE IN RECENT SURVEYS

Source (citation)	Bias	Total	Age (years)										
			0-1	1-2	2-3	3-4	4-5	5-6	6-7	7-8	8-9	9-10	>10
39	Incidence in siblings of affected patients		45.7% (113/247)		33.1% (59/178)			7% (4/57)					
56	UTIs	50	64% (32)	20% (10)	14% (7)			2% (1)					
29	Male, severe VUR, DMSA	70	61.4% (43)		21.4% (15)			17.1% (12)					
29	Female, severe VUR, DMSA	217	19.8% (43)		48.4% (105)			31.8% (69)					
41	At entry all have VUR	14	100% (14)	78.6% (11)	57.1% (8)	not applicable							

VUR: vesico-ureteric reflux; UTI: urinary tract infection; DMSA: dimethylsuccinic acid

TABLE III: REMAINING NUMBER OF CHILDREN WITH REFLUX AT GIVEN AGES FOR DIFFERENT GRADES OF REFLUX²⁶ (TIME IS GIVEN FROM FIRST VCUG)

Grade	0 years	4 years	8 years	10 years
I	20	7 (35%)	1 (5%)	0 (0%)
II	107	37 (34.6%)	18 (16.8%)	10 (9.3%)
III-V	37	20 (54.0%)	13 (35.1%)	5 (13.5%)

VCUG: voiding cystourethrogram

the more definitive in terms of determining the frequency of the underlying genetic disorder. These surveys^{26,39} tend to have ratios for the sexes close to 1:1, especially in younger children, suggesting the underlying disorder is autosomal, being present in approximately equal numbers in males and females at birth. This technique detects the highest number of children with low grade VUR.

2. An initial screen by renal ultrasound: Renal ultrasound surveys,^{7,40,41} when further investigated by VCUG, show more high-grade cases than with VCUG as the initial screen, and more males are detected (especially in the very young). Renal ultrasound detects dilation (hydronephrosis), a feature of high-grade reflux. The observed higher frequency in males in studies based on ultrasound screening implies that the expression of the autosomal defect is more severe in males. This implies that either anatomical differences or particular genes in males may modify the outcome of the underlying defect. It is possible that male and female VUR are genetically distinct disorders, but if this were the case, it would be difficult to explain the equal distribution of sexes in the VCUG-based studies.^{26,39} It may be more reasonable to think that low-grade VUR results from background genetic differences that are distinct from those causing high-grade VUR, but that there may be

common underlying genetics providing the original defect.

3. Surveys based initially on a screen for urinary tract infections. These surveys, as would be expected, identify females with VUR at a higher frequency because VUR leads to urinary tract infection in more females than males.

Risk estimates indicate that VUR is a common disorder, affecting at least 1% to 2% of the population.^{6,7} One can obtain an approximate estimate of the frequency of VUR in the general population by multiplying the rate of VUR in patients presenting with UTIs (~35%) by the rate of rate of patients presenting with UTIs in the population (~5%), giving an estimated population rate of 1.75%. In contrast, 13% to 45% of an affected patient's siblings have reflux, especially when analysed in siblings less than 24 months of age.^{39,42-44} These figures strongly suggest that there is an increased risk in siblings of an index patient over the general population. Moreover, transmission of VUR was found in 35% to 65% of newborns when a relative of an index patient, such as a parent or other relative in the family, was known to have either VUR or urinary tract disease.^{43,32} The estimated transmission rates and risks to siblings would tentatively suggest a dominant disorder. At present, a working model for the inheritance of VUR might be dominant, with high penetrance, but an age- and sex-dependent expression (i.e. high-resolution rates), perhaps with an environmental influence also (especially with respect to bilateral/unilateral frequencies of reflux and resolution). It has been calculated that alleles responsible for causing primary dominant familial VUR are present in the population at a frequency of 0.16%.⁴⁵ The evidence for concordant inheritance of VUR in twins and triplets is conflicting, which again is possibly because of the high rate of spontaneous resolution, and false negatives in diagnosis.⁴⁶

Sex Differences in VUR

Several sex differences have been noted in VUR patients. Young boys with VUR have on average a higher grade of VUR than girls,³³ and it is thought that this may in part be due to transient bladder outlet obstruction *in utero*.⁴⁷ Assessment of bladder function also suggests that there is a difference between males and females in voiding dynamics.⁴⁸

There has been suggested to be a difference in the frequency of VUR in males and females. However, in recent surveys based on VCUG diagnosis,^{26,39} the ratio of male to female with VUR is close to 1:1, especially in younger patients (Table IV). This suggests that the differences detected in earlier studies may be due to ascertainment biases that occur later in life. The ascertainment bias may be due to either (a) how patients present (e.g. females are more likely to present with urinary tract infections than males and hence, are more likely to be screened for VUR), or b) the rate of resolution of VUR (it is possible that the rate of resolution of the defect differs in males and females).

Several studies showed that in neonates there is a higher incidence of VUR in male infants, particularly high-grade VUR. This argument results from cases which were initially screened with ultrasound. The most recent study that favoured the preponderance of males to females with VUR was that of Tsai et al.⁷ This observation can be reconciled by considering that the rate of VUR at birth is essentially equal for both sexes, but differs in the grade of VUR

observed, as discussed above. A comparison of grade of VUR detected in males and females is given in Table V.

Ethnic Variation in VUR

Many studies have compared the frequency of VUR in Caucasians and either African or Afro-American populations, and all observed a distinctly lower rate of VUR or no VUR in black patients.⁴⁹⁻⁵³ Melhem and Harpen⁵⁴ found that the rate of VUR is low in Afro-American individuals compared to Caucasians ($P < 10^{-6}$), when analysed in 0 to 10-year-old patients with urinary tract infections. This suggests that rather than a gradual resolution of VUR, the rate of VUR in black patients is low from the onset, which might reflect a delayed maturation of the anti-reflux mechanism in Caucasians. Eke and Eke⁵² reported that of 66 children with UTIs from a large prospective study in Nigeria, none tested positive for VUR. By contrast, one would expect approximately 30% of these individuals in Caucasians to exhibit reflux. Other populations of African-origin also have low VUR rates. For example, West and Venugopal⁵⁵ reported that only 10% of Jamaican children undergoing VCUGs following diagnosis of UTIs exhibited VUR. Melhem and Harpen⁵⁴ sampled Black and White patients from the same geographical region, where one would expect similar environments and so the differences would be more likely to be attributed to genetics rather than environmental effects. Thus, a comparison of genetic differences between Caucasian and African-origin populations may provide a useful strategy for further genetic studies of VUR.

TABLE IV: SEX DISTRIBUTION OF VUR REPORTED IN RECENT STUDIES

Source (citation)	Ascertainment bias	Male	Female
39	Siblings of affected, VCUG	32.1%	39.3%
26	UTIs, VCUG	31% (57/183) of patients presented with UTIs	34% (173/505) of patients presented with UTIs
32	New-born with relatives with VUR	1.5	1
32	UTIs, US, cystography	4.3	1
40	Pre-natal ultrasound	80% (109)	20% (27)
7	Pre-natal ultrasound?	80% (24)	20% (6)
41	US first few days postnatal, then VCUG	84% (21)	16% (4)

US: ultrasound; UTI: urinary tract infection; VCUG: voiding cystourethrogram; VUR: vesico-ureteric reflux

TABLE V: GRADE OF REFLUX VERSUS SEX IN RECENT STUDIES

Source	Bias	Sex	Grade I	Grade II	Grade III	Grade IV	Grade V	Total
26	UTIs	Male	14.0% (8)	54.4% (31)	24.6% (14)	3.5% (2)	3.5% (2)	57
		Female	20.8% (36)	58.4% (101)	19.1% (33)	1.2% (2)	1.0% (1)	173
56	Referral centre; UTIs	Both	13.6% (11)	17.3% (14)	23.5% (19)	38.3% (31)	7.4% (6)	81
7	US	Both	0.0% (0)	25.8% (8)	48.4% (15)	19.4% (6)	6.5% (2)	31
41	US, VCUG	Both	7.3% (3)	12.2% (5)	24.4% (10)	31.7% (13)	24.4% (10)	41 ureters

US: ultrasound; UTI: urinary tract infection; VCUG: voiding cystourethrogram

While the differences in incidence of VUR between Caucasians and African-origin populations are distinct, the variation of reported frequencies of VUR in surveys from other regions of the world^{56, 57} (e.g. Singapore, Australia) are more subtle and clouded by issues of ascertainment bias and diagnosis.

Association with Chromosomal Defects

Genetic loci on the short arm of chromosome 6 have been implicated in ureteral abnormality syndromes such as hydronephrosis, multicystic kidney and VUR.⁵⁸ In contrast, a different study excluded a large region of chromosome 6 in familial ureteral anomaly syndrome using linkage analysis.⁵⁹ VUR has also been reported in association with chromosomal abnormalities on the short arm of chromosome 10 in patients with multiple congenital anomalies.⁶⁰

Congenital Abnormalities and Syndromes Associated with VUR

Several developmental anomalies of the urogenital tract are frequently associated with VUR. These include bifid or duplex pelvicalyceal collecting system or ureter,⁶¹ pelviureteric junction obstruction,⁶² detrusor instability or detrusor-sphincter dyssynergia,⁶³ hypospadias, and cryptorchidism.⁶⁴ VUR has also been observed in association with several syndromes, notably prune-belly syndrome,⁶⁵ a syndrome of ectrodactyly, ectodermal dysplasia and cleft lip/palate,⁶⁶ renal-coloboma syndrome,³⁴ Robinow dwarfism,⁶⁷ Kallman syndrome,⁶⁸ and the Currarino triad.⁶⁹

Points to Consider when Analysing the Genetics of VUR

There are several variables that need to be taken into consideration when studying the genetics of VUR:

1. VUR may be a heterogeneous disorder, i.e. VUR may be a common marker or symptom of several genetically distinct disorders. In selecting individuals and pedigrees, care must be taken to identify those with the same putative sub-grouping of VUR, for example, dominantly inherited primary VUR. It is possible that an X-linked gene is associated with a proportion of VUR.⁷⁰ Similarly, individuals with VUR that are recruited predominantly by identification of urinary tract infections may have a different genetic predisposition to those that are recruited predominantly by ultrasound and/or VCUG. It will be useful to identify sub-categories of VUR based on an association with various conditions, such as urinary tract infection alone, intra-renal high-grade reflux with scarring, duplex ureters, hypospadias and so on. Families that have these characteristics should be regarded as potentially having separate sub-types of VUR. Some families may have a combination of these anomalies

and so discretion will be required. Due to the commonness of VUR, it is possible that in a large pedigree, 2 or more VUR genes segregated. This may arise especially if an individual with asymptomatic VUR married into the family. While causing difficulties in the analysis, many linkage programmes are able to take into account phenocopy rates, allowing for occasional sporadic unrelated occurrences of the disease in an affected family.

2. The diagnosis of VUR is only partially reliable. It is important to be aware of the high rate of false negative data, especially as VUR is only detected if reflux occurs at the time of testing. In a large recent survey, Greenfield⁷¹ found that 27% of cases with a negative VCUG were positive in a follow-up VCUG. Taking into the account the frequency of spontaneously resolution, there are undoubtedly some asymptomatic affecteds in the population. In general, therefore, one can only have confidence in the diagnosis of those who have tested positive for VUR and anticipate that a significant proportion of those who were typed as unaffected are in reality affected.
3. VUR has a high rate of spontaneous resolution,²⁶ especially in patients with mild reflux. This is an age-dependent effect, so testing is best done at a very early age. In adults, VUR is inferred for the purpose of genetic studies from the presence of reflux nephropathy. Not all patients with reflux will go on to exhibit gross markers of reflux nephropathy in later life. Once again, it is only possible to have confidence in the diagnosis of those who have tested positive for VUR.

Analysis of Candidate Genes for Linkage or Mutations in VUR Patients

Although a model for the inheritance of VUR is lacking, the strong familial segregation of VUR has prompted a number of linkage studies. The first linkage studies produced weak evidence for association of the VUR gene with several histocompatibility (HLA) antigens or haplotypes (reviewed in reference 5).⁵ It is perhaps relevant at this stage to point out that these early studies relied heavily on either single individuals or small families. The patients selected for these studies were probably subject to ascertainment bias, as most were identified in the first instance with a urinary tract infection. If the HLA locus itself has an influence on susceptibility of individuals to urinary tract infections, then it is quite possible that these studies inadvertently scored an association with urinary tract infection and segregation of certain HLA types associated with a higher predisposition to urinary tract infection in the population. Future genetic studies of VUR should take ascertainment bias into consideration for the same reasons.

Several genes that are expressed either in the ureteric bud or the kidney during embryogenesis may be candidates for VUR. One such gene is *PAX2*, as discussed above. However, *PAX2* does not show linkage in families with primary VUR, and no mutations were identified in the *PAX2* gene in 8 families with primary familial VUR.⁷² Other candidate genes which have been analysed in families with primary VUR include *RET*, *GDNF* (encoding the ligand for RET receptor) and *GDNFR α* but each showed an absence of segregation with VUR.⁷³ Another candidate gene, the Kallman gene (*KAL*), has been shown to be mutated in patients with Kallman syndrome, which is associated with VUR and mental illness. In families carrying mutations in the *KAL* gene, VUR has not been commonly found.⁶⁸ Recently, mice with a null mutation of the angiotensin II receptor type 2 gene (*AT2*), located on chromosome X, were shown to have congenital anomalies of the kidney and urogenital tract (CAKUT).⁷⁴ A splice site mutation in the *AT2* gene was also found in some humans with CAKUT.^{74,75} Abnormalities in both mice and humans with CAKUT included ureteric abnormalities, suggesting that the *AT2* gene may be a candidate gene for VUR. Specific DNA sequence variants in the *AT1* and *AT2* genes have been analysed in 23 patients with congenital primary VUR showing that the sequence variants were present with the same frequency in these patients as those present in the normal population.⁷⁶ It is interesting to note that a possible association of VUR with an X-linked recessive pattern of inheritance has been described.⁶⁹

Mouse Models of VUR

The CAKUT mouse model is discussed in the section above. At least one other mouse model of hydronephrosis appears to arise from VUR. Hereditary male-linked hydronephrosis is considered to be the hallmark of the DDD inbred strain of mice,⁷⁷ which has since been shown to be caused by VUR in DDD and ddY mice.⁷⁸ DDD mice also have altered numbers of lumbar vertebrae, usually 6, but the number of lumbar vertebrae did not correlate with severity of hydronephrosis.⁷⁷ Scanning electron microscopy revealed the cause of the hydronephrosis to be a grossly enlarged ureteric orifice into the bladder (100 to 300 μ M in DDD mice versus 30 μ M in C57BL/6 mice),⁷⁸ a defect one might expect to associate with VUR. Ureteric orifices in DDD mice did not remain closed, therefore providing poor protection against VUR. A larger ureteric orifice was found on the right than on the left for both sexes in all DDD mice.

Gonadectomised DDD mice treated with testosterone exhibited increased reflux in proportion to the level of testosterone administered in both males and females,⁷⁹ implying male hormones may play a significant role in development of the VUR in these mice. In the light of this,

it is interesting to recall the high rate of severe VUR in neonatal human males and the histochemical studies of Dixon et al,¹⁸ described elsewhere in this review. In contrast to DDD mice, ddY mice exhibit less severe VUR⁷⁸ and may be a more appropriate model for human VUR. These mouse strains and their relatives may provide a fruitful ground for genetic studies of VUR in the future.

In contrast to DDD mice, the inbred strain C57BL/KsJ has been shown to be a model for a defect in the ureteropelvic junction (the junction where the ureter joins the kidney⁸⁰), rather than the ureterovesical junction in the case of vesico-ureteric reflux.

Genome-Wide Linkage Analysis to Find a VUR Locus

Very recently, a genome-wide linkage analysis has produced evidence of linkage of a dominant VUR gene on chromosome 1 in 7 European families with primary VUR.⁸¹ The region identified was a 20 cM genetic interval between markers GATA176C01 and D1S1653. It will be of interest to determine in future studies whether VUR shows linkage to this region in the majority of families analysed, or whether there are several genetic loci that cause VUR in the general population.

Summary and Future Directions

There is considerable evidence that predisposition to VUR is heritable. However, it is difficult to determine whether VUR is a monogenic disorder, or whether it is a heterogeneous collection of diseases. Heterogeneity would imply that there is more than one genes involved, and that VUR is multifactorial in origin. On the other hand, several studies^{34, 81} suggest that VUR is not necessarily polygenic in any given family. That a high frequency of VUR occurs in siblings and offspring of an affected individual favours an autosomal dominant model of inheritance. It is possible that there are different sub-types of dominant VUR. To make progress in the genetics of VUR, there is a need to include new parameters in the differential diagnosis of VUR, perhaps using new criteria with which to sub-classify patients into different subgroups based on physiological characteristics. One suggestion to start with is to differentiate between patients (and families) ascertained primarily because of urinary tract infection, and patients ascertained by the presence of high-grade reflux. Ideally for this purpose, the high-grade reflux may have been identified either following pre-natal ultrasound screening, or following presentation of the patient with renal failure.

Acknowledgements

We thank Dr P Tomlinson and Professor G D Abbot for stimulating discussions. This work was supported by the Cancer Society of New Zealand, the New Zealand Lotteries Grants Board, and a University Otago Research Grant.

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