Genetics of vesicoureteral reflux

Prem Puri, Jan-Hendrik Gosemann, John Darlow and David Barton

Abstract | Primary vesicoureteral reflux (VUR) is the most common urological anomaly in children, affecting 1–2% of the pediatric population and 30–40% of children presenting with urinary tract infections (UTIs). Reflux-associated nephropathy is a major cause of childhood hypertension and chronic renal failure. The hereditary and familial nature of VUR is well recognized and several studies have reported that siblings of children with VUR have a higher incidence of reflux than the general pediatric population. Familial clustering of VUR implies that genetic factors have an important role in its pathogenesis, but no single major locus or gene for VUR has yet been identified and most researchers now acknowledge that VUR is genetically heterogeneous. Improvements in genome-scan techniques and continuously increasing knowledge of the genetic basis of VUR should help us to further understand its pathogenesis.

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Introduction
Primary vesicoureteral reflux (VUR) (Online Mendelian Inheritance in Man [OMIM] 193000)—the retrograde flow of urine from the bladder into the upper urinary tract—is the most common urological anomaly in children. Primary VUR is a congenital condition caused by maldevelopment and malfunction of the ureterovesical junction. By contrast, secondary VUR is an acquired condition resulting from increased intravesical pressure, and is seen in conditions such as neurogenic bladder and bladder outlet obstruction.1

Primary VUR occurs in 1–2% of the pediatric population and in 30–40% of children presenting with urinary tract infections (UTIs).1–5 The association of VUR, UTI and renal parenchymal damage is well known.6,7 Renal parenchymal damage can be congenital or acquired. With the widespread use of prenatal ultrasound, it has become evident that a substantial number of infants who go on to be diagnosed with VUR have congenital renal damage.5–10 Congenital reflux nephropathy occurs as a result of abnormal embryological development with subsequent renal dysplasia, and is largely seen in male infants with high-grade VUR. Exposure to UTIs in patients with congenital renal dysplasia can lead to progression of renal parenchymal damage. Both experimental and clinical studies have shown that acquired renal scarring associated with VUR is the result of an acute inflammatory reaction caused by bacterial infection of the renal parenchyma.11 It is well recognized that the risk of renal scarring after an episode of pyelonephritis (proven by dimercaptosuccinic acid [DMSA] scan) is substantially increased in children with high-grade VUR, affecting up to 89% of children with grade IV–V VUR.12 Furthermore, despite improvements in diagnosis and treatment of VUR, reflux nephropathy is still recognized as an important cause of childhood hypertension and chronic renal failure.10,13

The familial nature of VUR is well established. Familial clustering of VUR has been described by several groups, with a prevalence of between 27% and 51% in siblings of index patients with VUR,14–17 and a 66% rate of VUR in children whose parents had reflux.18

VUR can resolve spontaneously with age,4,19 so it is difficult to accurately determine the exact prevalence in family members. It has become increasingly evident that the familial clustering of VUR must have a genetic basis; however, to date there has been no agreement on the mode of inheritance. A range of inheritance patterns have been suggested, including autosomal dominant with incomplete penetrance,20–24 autosomal recessive,25,26 X-linked27,28 and polygenic,29 and a substantial amount of work has evaluated candidate genes and conducted genome-wide scans. However, an understanding of the genetics of primary VUR remains elusive. This Review will summarize the research efforts that have led to the current understanding of normal and pathological gene signaling during urinary tract development, discussing VUR-associated syndromes, possible candidate genes and chromosomal candidate regions and loci.

VUR is a complex disease
Familial clustering of VUR and observations of sex-dependent differences in VUR prevalence support the hypothesis that VUR is a genetic disorder. Reports regarding gender distribution of VUR patients vary.10,14,16,21,30–32 However, other authors have observed that the male:female ratio in children with reflux is dynamic across different age groups, ranging from a male preponderance in infants to a clear female preponderance in older children.30,34 It is also important to recognize that the calculated ratio between the sexes is dependent upon the method of ascertainment. UTIs are more common in...
Key points

- Increasing amounts of evidence suggest that vesicoureteral reflux (VUR) is a genetically heterogenous disorder.
- Although VUR transgenic or knockout mouse models exist, the cognate genes in humans do not seem to be major contributors to primary VUR.
- Genome-wide linkage and association studies show little overlap of the major linkage peaks, although there is some intriguing evidence of overlaps in the minor peaks.
- Improvements in genome-scan techniques and increasing knowledge of the genetic basis of diseases should help us to search for VUR susceptibility genes.
- Improved knowledge of the genetic basis of VUR should help us to understand the reasons why some patients develop reflux nephropathy while others do not.

females and it is no surprise that when VUR is detected by screening children who present with symptoms of UTI, more girls than boys are diagnosed with VUR. However, 80% of cases detected by the appearance of hydrenephrosis on prenatal ultrasound are boys, and these patients often have high-grade VUR-associated renal damage.

The impact of sex on familial VUR was studied by Pirker et al. The authors hypothesized that the VUR phenotype is caused by more than one genetic disorder and analyzed 159 families with at least 2 children diagnosed with VUR. In this study, sisters of female index patients were at a significantly higher risk for VUR than brothers (P<0.01). Furthermore, they reported that brothers of index male patients have higher grade reflux and a higher rate of associated duplex systems than sisters of index patients. Boys in families in which only boys are affected might represent a genetically different subgroup, with a more severe type of reflux and an increased rate of associated urinary tract malformations. The findings led the authors to conclude that sex-related differences in VUR expression are likely to occur and that they could have implications for genetic counselling and modelling inheritance in genetic studies of VUR.

A high concordance of VUR has been demonstrated in identical twin siblings, further supporting a genetic basis for the transmission of the disorder. In another study, 12 families in which twins were affected with VUR were investigated. The authors reported that 11 out of 12 index patients were females with high-grade VUR and a low incidence of renal scarring, and suggested that twins with VUR might represent a genetically different subgroup with female preponderance and severe reflux. Furthermore Menezes et al. reported that, although the total incidence of renal scarring in a population of siblings of index cases with VUR was similar in males and females, the incidence of mild scarring was higher in females, whereas the incidence of severe scarring was significantly higher in males (P<0.05).

**Embyrology**

Urinary tract development in the embryo normally begins with the formation of the ureteric bud, which is an outgrowth of the mesonephric duct. Growth of the ureteric bud is stimulated by reciprocal signaling between the bud and the metanephric mesenchyme, and results in the formation of the ureter and branching to form the collecting ducts. Furthermore, signaling between the bud and the metanephric mesenchyme stimulates the metanephric nephron to form the kidney. The part of the mesonephric duct between newly developed ureter and urogenital sinus is subsequently removed by apoptosis whereupon the free end of the developing ureter inserts into the bladder wall and the vesicoureteric valve is formed.

The mechanism of VUR and subsequent reflux nephropathy is based on the disruption of a proper vascular mechanism at the vesicoureteric junction, which leads to retrograde flow of urine into the ureter or kidney. This disruption is most likely the result of a shortened intravesical ureter, and an enlarged or malpositioned ureteric orifice. Furthermore, abnormalities during embryogenesis, especially a caudally shifted ureteric bud, predispose to ureteric reflux in animal models. The precise position at which the ureteric bud grows out from the mesonephric duct is critical. A range of abnormalities of the kidneys and urinary tract can result from aberrant or multiple budding. Many genes are involved in ureteric budding and subsequent urinary tract and kidney development—several of these genes could be responsible for isolated VUR, although the precise mutations might differ from those that cause other anomalies.

**GDNF/RET pathway**

The most important and thoroughly investigated genes and proteins involved in urinary tract morphogenesis are those in the GDNF/RET pathway. During embryonic kidney and urinary tract development, glial-derived neurotrophic factor (GDNF) is expressed in the metanephric mesenchyme along the length of the mesonephric duct. Formation of the ureteric bud is promoted by GDNF binding to the tyrosine kinase receptor, RET, and the co-receptor GDNF family receptor α-1 (GFRα1), both of which are expressed in the mesonephric duct.

GDNF expression is positively regulated by the transcription factors PAX2 (paired box gene 2), GATA3 (transacting T-cell-specific transcription factor GATA3), EYA1 (eyes absent homolog 1), SIX1 (sine oculis-related homeobox 1) homolog protein SIX1), SALL1 (Sal-like 1), and HOX11 (homeobox 11). Furthermore, expression of the protein WNT11 in the epithelial tip of the ureteric bud is required to propagate mesenchymal GDNF signaling (Figure 1).

By contrast, the FOXC1/FOXC2 (forkhead box C1 and C2) transcription factors and the SLIT2–ROBO2 (slit homolog 2–roundabout homolog 2) signaling complex restrict GDNF activity, and GDNF/RET signaling has been shown to be negatively regulated by the receptor tyrosine kinase antagonist Sprouty1 (SRY1). Negative regulation of the GDNF/RET pathway is required for a unique origin of a single ureteric bud—deletion of the negative regulators results in multiple buds and urogenital malformations in mouse models. Transforming growth factor β1 (TGFβ1) and bone morphogenic protein 4 (BMP4) are endogenous
inhibitors of the pathway and restrict the site of outgrowth of the ureteric bud to one location. By contrast, fibroblast growth factor receptor type 2 (FGFr2) signaling in the mesenchyme defines the site of ureteric bud outgrowth independently of GDNF/RET signaling and stimulates ureteric bud branching. The Wilms tumor suppressor gene WT1 has also been reported to be a GDNF-independent mediator of ureter induction. 43

The renin–angiotensin system has also been implicated in ureteric budding. 59 The angiotensin type II receptor (AGT2R) is expressed in the epithelium of the ureteric bud, metanephric mesenchyme and stroma, and is essential for early stages of ureteric bud morphogenesis. AGT2R-deficient mice exhibit abnormal ureteric budding, increased incidence of double ureters, and VUR. 59 Furthermore, antagonism of the AGT2R results in impaired branching of the ureteric bud and downregulation of GDNF, Ret, Wnt11, and Spry1. By contrast, antagonizing AGT2R has been shown to upregulate Bmp4 expression. 59

**Uroplakins**

Uroplakins are integral membrane proteins expressed at the luminal surface of the urothelium. The uroplakins UPK1A, UPK1B, UPK2 and UPK3A are major markers of urothelial terminal differentiation. 60,61 Their main function is to strengthen the membrane during bladder filling and emptying, prevent bacterial adherence and contribute to the permeability barrier function of the urinary tract. 61 By forming plaques, they strengthen the membrane during bladder filling and emptying, preventing the urothelium from rupturing. 60,62 UPK3A is the only uroplakin with a cytoplasmic domain and Upk3 deletion has been shown to disrupt UPK1B maturation, so that the UPK1A/UPK2 pair can only form abnormally small patches of urothelial plaques, leading to incompetent function of the urothelium and urine leakage. 60

**Genetics of VUR**

**Animal models**

To date, seven mouse models of VUR have been reported (Table 1). Hu et al. 60 reported that ablation of the Upk3 in mice resulted in enlarged, reflexing ureteral orifices, hydronephrosis, reduced renal function and rendered the epithelium permeable to methylene blue dye. Furthermore, the same group found that Upk2 knockout mice had a similar phenotype to Upk3−/− models, with additionally reduced thickness of the renal parenchyma.
They concluded that uroplakin defects could be involved in major urinary tract anomalies and associated renal failure.\textsuperscript{63} However, it is not clear how these defects lead to VUR.

Inactivation of AGT2R function causes ectopic ureteric budding, double budding and a phenotype of congenital abnormalities of the kidney and urinary tract (CAKUT), which includes VUR and ureter duplications.\textsuperscript{64} The incidence of ectopic budding seen during organogenesis exceeds the actual development of CAKUT phenotypes in Agt2r\textsuperscript{−/−} mice, suggesting that other inhibitory mechanisms—for example BMP4 signaling—inhibit AGT2R-related ectopic budding.\textsuperscript{64,65}

Overexpression of RET in the Hoxb7/Ret overexpressed mouse model has been shown to result in short intravesical ureteric tunnels, caudally malpositioned ureteric buds, small cystic kidneys and VUR. The phenotype of this model is thought to corroborate the hypothesis that abnormal ureteric bud position disrupts ureterovesical junction formation.\textsuperscript{66} Similarly, reduced PAX2 signaling caused by a heterozygous Pax2 mutation has been shown to result in shortened intravesical ureters, caudally shifted ureteric buds, small hypoplastic kidneys, reduced nephron number and particularly VUR.\textsuperscript{51,67}

The transcription factor Lim1 (also known as homeobox protein Lhx1) is a homeobox gene expressed in the organizer region of mouse embryos. Conditional knockout of Lim1 in the ureteric bud and mesonephric duct has been shown to delay bud formation, slow mesonephric duct migration to the bladder, and cause the kidneys to be small and malformed, with dilated refluxing ureters.\textsuperscript{68}

The fibroblast growth factor receptors are tyrosine kinase receptors with important roles in embryonic development. Several different conditional knockout models of various fibroblast growth factor receptors have been described. Conditional knock out of Fgfr2 in the metanephric mesenchyme led to renal and urinary tract anomalies as well as VUR.\textsuperscript{69} Transgenic and gene knockout studies of FGFR2 and other FGFrs (such as FGFR1, FGFR2IIIb, FGFR11) have shown that fibroblast growth factor receptor signaling is required for patterning of all renal lineages, including the ureteric bud, nephrogenic mesenchyme, and renal stromal mesenchyme. These results led him to conclude that FGFR signaling is critical for patterning of virtually all renal lineages at both early and late stages of development.\textsuperscript{69}

Other knockout models resulting in CAKUT have been established to investigate the effect of candidate genes on kidney and urinary tract malformations (Table 2), and have provided new insights into the role of genes during urinary tract morphogenesis.

Syndromes associated with VUR in humans

VUR is a feature of numerous congenital syndromes, and mutations in genes essential for urinary tract morphogenesis are linked to the development of the majority of such disorders.

Genetic studies of syndromes with associated VUR have revealed several possible candidate genes involved in the pathogenesis of VUR and related urinary tract malformations (Table 3).

Renal coloboma syndrome

Renal coloboma syndrome (RCS) is also known as the papillorenal syndrome or isolated renal hypoplasia, and is characterized by hypoplasia of kidneys and optic nerve abnormalities.\textsuperscript{70} VUR has been reported in 26% of patients with RCS.\textsuperscript{51} To date, PAX2, the gene encoding the transcription factor PAX2 (10q24.31), is the only gene known to be associated with RCS.\textsuperscript{70} Studies have reported that 50% of individuals with RCS have mutations in PAX2,\textsuperscript{71} and three genomic rearrangements of PAX2 have been shown to be linked with RCS—a de novo 10;13 chromosome translocation, and two deletions of the entire PAX2 locus.\textsuperscript{72} As RCS is inherited in an autosomal dominant manner, individuals from families with an identified mutation or probands can be tested by sequence analysis.

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<thead>
<tr>
<th>Table 1</th>
<th>Animal models resulting in VUR phenotype and urinary tract anomalies</th>
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<tr>
<td>Animal model</td>
<td>Modified gene function</td>
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<tr>
<td>Upk2\textsuperscript{−/−} knockout mouse</td>
<td>Upk2 knockout</td>
</tr>
<tr>
<td>Upk3\textsuperscript{−/−} knockout mouse</td>
<td>Upk3 knockout</td>
</tr>
<tr>
<td>Agtr2\textsuperscript{−/−} knockout mouse</td>
<td>Agtr2 knockout</td>
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<tr>
<td>Hoxb7/Ret\textsuperscript{−/−} overexpressed mouse</td>
<td>Overexpression of Ret</td>
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<tr>
<td>Pax2\textsuperscript{−/+} mouse</td>
<td>Heterozygous Pax2 mutation</td>
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<tr>
<td>Lim1 conditional knockout mouse</td>
<td>Lim1 conditionally removed in the nephric epithelium after beginning of nephric duct formation</td>
</tr>
<tr>
<td>Fgfr2 conditional knockout mouse</td>
<td>Fgfr2 conditionally removed in the metanephric mesenchyme</td>
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in order to initiate treatment to prevent ophthalmological or renal complications. 50% of individuals diagnosed with RCS are found to have a de novo mutation and the other 50% have an affected parent.70

Branchio-otorenal syndrome

Branchio-otorenal (BOR) syndrome is characterized by malformations of the outer, middle and inner ear which are associated with conductive, sensorineural, or mixed hearing impairment, branchial fistulae and cysts, as well as renal malformations. 5% of patients with the disorder have VUR.73 BOR syndrome is inherited in an autosomal dominant manner,73 and mutations or deletions of the gene *EYA1* (the human homolog of the *Drosophila* eyes absent gene) have been identified as a cause of BOR syndrome in ~40% of affected individuals.74 Furthermore, BOR-associated mutations of the homeodomain-containing transcription factor SIX5 have been described,75 and seven different mutations of homeobox protein SIX1 have been identified in kindreds with BOR syndrome.75 In contrast to RCS, ~90% of patients with BOR syndrome have an affected parent, whereas only 10% are result of de novo mutations.73

<table>
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<tr>
<th>Animal model</th>
<th>Resulting urinary tract phenotype</th>
<th>Study</th>
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<tr>
<td>Bmp4&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Ectopic ureteric buds, double ureteric buds, hydroureter, double collecting systems, less smooth muscle actin in ureter, hydrenephrosis, dysplastic kidneys, reduced renal parenchyma</td>
<td>Miyazaki et al.141,142</td>
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<td>Foxc1&lt;sup&gt;−/−&lt;/sup&gt; and Foxc2&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>More cranial ectopic ureteric buds that are broader in size, duplex ureters, hydrenephrosis, hydroureter, ectopic mesonephric tubules, GDNF expression expanded more anteriorly, duplex kidneys</td>
<td>Kume et al.57</td>
</tr>
<tr>
<td>Eya1&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Duplex collecting systems, renal agenesis, dysplastic kidneys</td>
<td>Xu et al.143</td>
</tr>
<tr>
<td>Slt2–/– and Robo2–/– mouse</td>
<td>Multiple ureteric buds and ureters, dilated ureters, GDNF expression expanded more anteriorly, abnormal nephrogenic zone, multiple kidneys</td>
<td>Grieshammer et al.36</td>
</tr>
<tr>
<td>Spry1&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Multiple ureteric buds, multiple ureters, dilated ureters, no difference in GDNF expression, mesonephric duct is more sensitive to GDNF, disorganized kidneys</td>
<td>Basson et al.58</td>
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<tr>
<td>RaraB2&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Impaired distal ureter maturation, impaired kidney branching, renal hypoplasia</td>
<td>Batourina et al.46</td>
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<tr>
<td>RET&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Impaired distal ureter maturation, impaired kidney branching, renal agenesis</td>
<td>Batourina et al.46 Schuchardt et al.146</td>
</tr>
<tr>
<td>Aldh1a2&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Defective ureter insertion, hydroureter, hydrenephrosis, bladder hypoplasia, bladder agenesis</td>
<td>Batourina et al.45</td>
</tr>
<tr>
<td>Agtr1AB&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Obstructive nephropathy, no pelvis development</td>
<td>Yerkes et al.145</td>
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<tr>
<td>Shh&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Hydroureter, dilated pelvis, less smooth muscle actin in ureter, kidney hypoplasia</td>
<td>Yu et al.146</td>
</tr>
<tr>
<td>Cnb1&lt;sup&gt;−/−&lt;/sup&gt; mouse</td>
<td>Ureteropelvic junction obstruction, defective ureter peristalsis, underdeveloped pelvic and ureter smooth muscle, hydrenephrosis</td>
<td>Chang et al.447</td>
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HD dysplasia syndrome

Hypoparathyroidism–deafness–renal dysplasia (HDR) syndrome includes the association of hypoparathyroidism, sensorineural deafness and renal disease, including VUR.76,77 The majority of cases of HDR syndrome have been mapped to chromosome 10p, particularly mutations of the GATA3 gene, which encodes the transcription factor GATA-3 protein, and is found at this locus.78–80 It is now widely recognized that HDR is an autosomal dominant disorder caused by mutations of GATA3.81 Despite an association, the only data regarding HDR syndrome are case reports, so the exact incidence of VUR in HDR syndrome remains unknown.

Kallmann syndrome

Kallmann syndrome is characterized by the association of anosmia and isolated gonadotropin-releasing hormone (GnRH) deficiency and resulting hypothalamic–hypogonadal hypogonadism, with autosomal dominant, autosomal recessive or X-linked inheritance. The *KAL1* gene encodes the protein anosmin-1 (an extracellular–matrix-associated glycoprotein), and is located on the human X-chromosome. *KAL1* is mutated in ~10% of patients with Kallmann syndrome.82 The frequency of de novo mutations is unknown. Reports suggest that the X-linked recessive form of the disorder is linked with VUR, duplex ureters, duplex kidneys and renal agenesis.83 *FGFR1, PROKR2, PROK2, CHD7 and FGF8* are also associated with Kallmann syndrome and, together with *KAL1*, account for about 25–35% of cases.82 The exact incidence of VUR in Kallman syndrome is unknown.

Hand–foot–genital syndrome

Limb malformations and urogenital defects such as VUR are the main characteristics of the hand–foot–genital syndrome (HFGS), which is inherited in an autosomal dominant manner.84 Mutations in the *HOXA13* gene, which encodes the transcription factor homeobox protein HOXA13, are responsible for the development of HFGS.85 and 14 mutations in *HOXA13* have been reported to date, of which 40% are point mutations and 60% are expansions in one of the three polyalanine tracts of the gene.84 Like HDR syndrome, the only data
regarding HFGs are case reports, so the exact incidence of VUR in the syndrome remains unknown.

**Townes–Brocks Syndrome**
The Townes–Brocks syndrome (TBS) is a rare complex of malformations, mainly characterized by the triad of imperforate anus, dysplastic ears and thumb malformations. Approximately one-third of affected individuals display renal impairment, including VUR and end-stage renal disease; congenital heart disease and foot and genitourinary malformations are frequently found in TBS patients. To date SALL1—a positive regulator of GDNF signaling sururing ureteric bud formation—is the only gene shown to be associated with TBS, and can be identified in ~70% of patients with TBS using DNA sequencing and deletion analysis. Together with the characteristic clinical findings, the detection of a SALL1 mutation confirms the diagnosis of TBS. Approximately 50% of TBS cases are due to de novo mutations.

**Urofacial syndrome**
Urofacial syndrome (UFS), also known as Ochoa syndrome, is characterized by facial abnormalities and dysfunctional urinary voiding, which is often accompanied by VUR. Mutations affecting the gene HPSE2, which encodes the heparanase-2 enzyme, provide the genetic basis of UFS.

**de Lange syndrome**
Patients with de Lange syndrome have distinctive facial features, growth retardation, hirsutism, and upper limb reduction defects. VUR has been reported in 12% of patients with the disorder. Although de Lange syndrome is inherited in an autosomal dominant or X-linked manner, the vast majority of affected patients have de novo mutations. Three studies have described 179 affected individuals with the syndrome, and a mutation of the nipped-B homolog (Drosophila) gene (NIPBL) has been identified in around 50% of these known cases. Once again, the exact mechanisms by which NIPBL contributes to VUR is not known.

**FGFR-related craniosynostosis syndromes**
The FGFR-related craniosynostosis syndrome complex includes a number of disorders caused by mutations of the FGFR family. Several syndromes associated with FGFR2-activating mutations have been reported to lead to urogenital anomalies such as hydronephrosis and solitary kidney. VUR has been reported in Pfeiffer syndrome, an acrocephalosyndactyly syndrome characterized by bicornal craniosynostosis, midface hypoplasia, broad thumbs, broad big toes, and partial/variable soft-tissue syndactyly of hands and feet. Most of the FGFR-related craniosynostosis syndromes are inherited in an autosomal dominant manner. De novo mutation rate
is high and is further elevated with advancing parental age. 82

Epstein syndrome
Epstein syndrome (ES) is a MYH9-related disorder (MYH9RD), mainly characterized by thrombocytopenia, nerve deafness, cataracts and renal disease. 85 It has been associated with VUR in one case report. 42 The gene encoding MYH9 protein is located at chromosome 22q12.3 and is the only gene in which mutations are known to cause the MYH9RDs. 85

VACTERL association
The association of vertebral, anal, tracheoesophageal, renal and limb defects is called VACTERL association when at least three organ systems are involved. VUR has been reported in 17 patients with VACTERL in a study of 44 patients (39%). 94 The genetic background of VACTERL is not fully understood, although deletions of the distal 13q chromosome have been described in more than 140 patients with the VACTERL-association phenotype. 95 Furthermore, other syndromes caused by single gene mutations display VACTERL-related malformation patterns including renal malformations. Such syndromes include Feingold syndrome (also known as oculodigitoesophageal duodenal syndrome), which is caused by mutations in N-MYC, CHARGE syndrome (CHD7), anophthalmia-oesophageal-genital syndrome (SOX2), Pallister-Hall syndrome (GLIS), and VACTERL-hydrocephalus (FANCB), which is the only X-linked syndrome—all the others are inherited as autosomal dominant traits. 89 In addition, a microdeletion within the FOX transcription factor gene cluster at 16q24.1 (FOXF1, MTHFSD, FOXC2 and FOXL1) has been described in patients with VACTERL-related malformations. 89

Hirschsprung disease and CAKUT
Hirschsprung disease and related disorders such as Bardet–Biedl syndrome, have been reported to be associated with congenital anomalies of the kidney and urinary tract (CAKUT), including VUR. Whereas the reports of CAKUT implicate various loci, 100 nonsyndromic Hirschsprung disease is mainly associated with mutations of the RET proto-oncogene, which encodes the protein receptor tyrosine kinase for GDNF and is located on chromosome 10q, although mutations have been found in at least seven other genes: 101 However, a study of 21 patients with Hirschsprung disease and CAKUT investigated by Pini-Prato et al. found a mutation in RET in only one patient. 102 Sampson et al. suggested an alternative locus associated with the CAKUT-HSCR phenotype, describing three patients with CAKUT–Hirschsprung disease who had microdeletions at 16p11.2, a region that contains eight genes including the SH2-domain-containing binding protein 1 (SH2B1), which is involved in RET signaling. 103

Human genetic studies
Broadly speaking, two approaches have been taken to identify genes involved in primary VUR: targeted investigation of genes that cause VUR when mutated in animal models or those known to be involved in urogenital development, and genome-wide scans for linkage or association to VUR in families or cohorts of affected patients.

Potential candidate genes originally emerged from studies focusing on the pathogenesis and anticipated genetic background of VUR, and subsequently mutations of selected genes were investigated in the humans using linkage and association studies.

Linkage studies investigate the segregation of diseases in large families with polymorphic markers for each candidate gene locus. Whereas classical linkage studies map genes with just a few large multigenerational families, the affected sib-pair linkage study design includes only pairs of affected siblings and their parents. This approach is favored in diseases such as VUR, where multigenerational affected families are difficult to find.

Association studies evaluate the relationship between alleles and phenotypes by comparing allele frequencies in affected and control populations. Thus, association studies can use related or unrelated individuals. However, in contrast to linkage studies, which exploit recent recombination using family data for markers, association studies evaluate recombinations that could have occurred sometime in the past. Association studies are, therefore, most useful in diseases caused by common ancestral mutations, whereas linkage studies are useful when samples from families are available. Linkage can identify both ancestral and more recent mutations. Both methods may be applied to the investigation of candidate genes or for genome-wide scans. Candidate genes can also be investigated directly, by sequencing the coding and regulatory regions in affected individuals.

The RET pathway
In 2008, a Gly691Ser mutation (SNP rs1799939) was detected in the RET gene. This mutation was present in 29% of the French-Canadian control population and in 70% of patients with primary VUR. 104 However, another study investigated the Gly691Ser mutation in 221 unrelated index cases, 190 of their affected siblings and 592 controls from the Irish population, and found no evidence of any influence of RET SNP rs1799939 on VUR phenotype, which is consistent with the absence of evidence for linkage of VUR to RET observed by other studies. 24,105,106

ROBO2/SLIT2
To date, two groups have reported ROBO2 gene missense mutations associated with VUR, 107,108 and a further study has supported the hypothesis that variations in ROBO2 and SLIT2 are rare causes of VUR in humans. 109 However, subsequent candidate gene linkage studies including the ROBO2 locus found no evidence for linkage, 24 and similar study has found no evidence of linkage at SLIT2. 24

PAX2
Although PAX2 knockout mice models and an association with renal coloboma syndrome suggest that PAX2
would be a promising candidate gene for VUR, linkage studies and mutation scanning have shown that the PAX2 gene is not mutated in primary VUR.\textsuperscript{113,114}

**SOX17**
Mutations in SOX17, an high mobility group box transcription factor and Wnt signaling antagonist, have been identified in 7 patients with VUR, 4 of these patients derived from 2 small families.\textsuperscript{111}

**TGF-β**
In 2004, Lee-Chen et al. reported that polymorphisms of the kallikrein 1 (KLK1) promoter and of the TGFβ1 gene contribute to progressive renal scarring in Taiwanese children with primary VUR.\textsuperscript{112} No association with VUR susceptibility was observed in this population, whereas two other groups investigated the effect of polymorphisms on renal scarring and demonstrated that a TGFβ1 polymorphism is a risk factor for primary VUR.\textsuperscript{113,114}

**The renin–angiotensin system**

**ACE polymorphism**
A 287 bp insertion/deletion polymorphism of the angiotensin-converting-enzyme gene (ACE I/D) has been investigated in 196 patients with congenital uropathies (67 of whom had VUR) compared to 163 individuals without urological malformations, finding no difference in ACE I/D distribution between children with urogenital malformations and those without.\textsuperscript{115} However, the study found a significant overrepresentation of the ACE DD genotype in the uropathy group with renal lesions compared to controls and children with uropathies but no renal lesions (P<0.005), a finding that was even more pronounced in the VUR cohort. These data led the authors to conclude that the DD polymorphism is a risk factor for renal parenchymal damage in patients with congenital urological abnormalities, which is particularly relevant in children with VUR. These findings have been supported by two further studies, one of which also reported an association between the D allele of ACE to small congenital kidneys with refluxing ureters in patients with primary VUR, and to the progression of reflux nephropathy.\textsuperscript{116,117} Contrastingly, these data could not be reproduced in studies by Park et al.\textsuperscript{118} and Yoneda and colleagues,\textsuperscript{119} who drew the opposite conclusions.

**AGTR2 mutation**
The angiotensin type II receptor (AGT2R) has also been thoroughly investigated with regard to VUR pathogenesis. Whereas Hohenfellner et al.\textsuperscript{120} and Yoneda et al.\textsuperscript{121} concluded from their data that AGT2R is not involved in the pathogenesis of primary VUR, Nishimura et al.\textsuperscript{122} reported AGT2R polymorphisms in CAKUT patients and concluded that the establishment of CAKUT is preceded by delayed apoptosis of undifferentiated mesenchymal cells surrounding the urinary tract during key ontogenic events, from the ureteral budding to the expansive growth of the kidney and ureter. Furthermore, Rigoli et al.\textsuperscript{123} found AGT2R polymorphisms in approximately 50% of VUR patients in an Italian cohort and concluded that the discrepancy between their and other studies could be explained by the differences in genetic and ethnic backgrounds.

**Uroplakins**
UPK3A and other members of the uroplakin family have been investigated in various studies. Mutations in UPK3A have recently been found in some patients with renal aplasia, hypoplasia and dysplasia, including some who had VUR.\textsuperscript{121,122} By contrast, other investigators found no evidence for major involvement of UPK3A in VUR,\textsuperscript{123–126} but concede that their results do not rule out mutations in regulatory elements affecting gene expression or function.\textsuperscript{123,126}

**The X chromosome**
Some strong candidate genes on the X chromosome (KAL1 and AGT2R) as well as reports of X-linked transmission and evidence suggesting linkage of primary VUR to the pseudoautosomal region\textsuperscript{22} led Kelly et al.\textsuperscript{125} to investigate the possibility of linkage in these areas in a study in 2009. However, microsatellite linkage analysis of the X-linked gene KAL1 (which is associated with Kallmann syndrome) and the pseudoautosomal regions PAR1 and PAR2 did not reveal significant linkage. The authors concluded that discrepancy between their and other studies could be explained by the different ascertainment criteria, genetic approaches, ethnic backgrounds and environmental factors.

**Summary**
Overall, candidate gene studies have been largely disappointing, with just a small percentage of VUR cases found to be caused by mutations in many candidate genes that have been studied to date. It was previously suspected that VUR is an autosomal dominantly inherited disease.\textsuperscript{20} However, so far candidate gene studies have failed to determine a single major dominantly inherited allele. It has now become clear that VUR is a genetically heterogeneous disorder, and the high frequency of VUR is likely to be due to the cumulative effect of numerous rare mutations in many different genes.

**Genome-wide scans**
When candidate gene studies do not yield positive results, a scan of the whole genome for shared alleles at polymorphic markers might identify regions containing causative genes. The approach could be a case-control or family-based association study, a family-based linkage study or a combination of both, depending on the genetic model and samples available.

The first genome-wide scan for VUR was reported by Feather and co-workers,\textsuperscript{22} whose data suggested linkage to chromosome 1p13 and identified 12 other possible loci in seven European families. Two attempts to replicate their findings were unsuccessful.\textsuperscript{23,24} This may be explained by the fact that the map positions of some of the markers linked to VUR by Feather et al.\textsuperscript{22} have since been revised—the marker defining one side of their
linkage region was found to be on 2q11 and the marker defining the other side is now known to be on 1q23.24

In 2007, Kelly et al.67 carried out a genome-wide scan investigating 609 individuals from 129 Irish families with more than one affected member with VUR and reported that nonparametric linkage (NPL) analysis revealed a region on chromosome 2q37, reaching genome-wide statistical significance, when families with duplex kidneys were removed from the analysis. Data regarding linkage with regions identified in this study on chromosomes 2, 3, 4, 10, 13, 16 and 20 were supported by smaller linkage studies or studies of chromosomal rearrangements associated with VUR, usually in association with other urinary tract malformations.22,127-131 For example, the second highest linkage peak in this study was in chromosome 10q26, with the linkage peak clearly distal to potential candidate genes FGFR2 and PAX2. A study of 10 patients with monosomy of 10q26 and urinary anomalies (such as VUR and hypoplastic kidney) with or without genital anomalies provided evidence for a gene or genes involved in kidney development in the same region. The results specifically demonstrated that PAX2, GFRα1 and EMX2 were present in two copies in 8 cases suggesting that one or more novel genes for urinary tract development and for genital development reside in this region.131

Special attention should be drawn to the region on chromosome 13q32-33, which was not the highest peak in the study by Kelly et al.67 but received support from two other linkage studies22,127 and a study of chromosomal rearrangements.132 It is particularly noteworthy that a genome-wide linkage scan for end-stage renal disease (ESRD)128 and a candidate gene association study for chronic kidney disease (CKD)129 provide further data to support a role for this region in renal disease or CAKUT. The ESRD study also revealed evidence for linkage at 10q26, 1q25, 6q24 and 4p15.32,130 all of which are close to or overlapping with regions identified by Kelly et al. The CKD candidate gene study provided support for loci identified by Kelly et al. at 6q24-25, 16q24.3, and 20p11.2.133 These results indicate that the study of the genetics of VUR has wider implications than the understanding of VUR alone, and is likely to be important to the understanding of chronic renal disease and renal failure.

A genome-wide study conducted by Conte et al. included 72 patients with primary VUR from 24 families and provided evidence further supporting the heterogeneity of VUR, finding several loci mapping to chromosomes 1, 3, 4, and 22,129 although only the locus on chromosome 3q22.2-23 was fully overlapping with a locus reported by Feather et al.22

These three genome-wide scans reported by Feather, Kelly and Conte were performed using an autosomal dominant model of inheritance.22,47,129 However, dominant as well as recessive models were used to analyze 72 VUR-affected individuals from 12 large families in a study by Weng et al.26 Whereas the dominant model yielded no signals across the entire genome, a unique linkage peak on chromosome 12p11-q13 was found in the recessive model and was confirmed by fine mapping.26 Although the clinically observed increased risk of VUR in siblings of index cases and the high transmission from parents to children suggest that VUR is not generally inherited in a recessive fashion, the data suggest that there may be a recessive form of the disorder. Interestingly, two large families in this study failed to yield a significant logarithm (base 10) of odds (LOD) score across the entire genome under either a recessive or a dominant model, possibly suggesting that VUR in these particular families might be caused by multiple risk alleles segregating along different lines of descent.

In 2010, Cordell et al. published a study analyzing 661 individuals with VUR from 320 families from Slovenia and the UK for genome-wide linkage and family-based association. In addition, they tested for, but did not detect, associations with the major candidate genes AGTR2, HNF1B, PAX2, RET, ROBO2, and UPK3A.105 Modest evidence for linkage to several regions (Table 4) was found, particularly when the nonparametric analysis was restricted to cases with confirmed VUR (rather than reflux nephropathy or other proxy phenotypes) and when parametric analysis allowed for the existence of multiple loci. The results provided some support for the regions identified on chromosomes 3, 6 and 21 by Kelly et al. and on chromosome 1 by Conte et al. In the family-based analysis, associations were found with one single nucleotide polymorphism (SNP) in the UK families, three SNPs in the Slovenian families and three SNPs in combined families. Case-control analysis detected associations with three additional SNPs. However, data quality control procedures reduced the coverage of the genome for association studies to about 30% in this study and little concordance was seen in the results from the two study groups or between linkage and association study results, leading the authors to conclude that major loci may not exist for VUR, at least within European populations.105

Conclusions

Despite a large amount of work, the genetic basis of VUR is still unclear. Results of genome-wide linkage and association studies show little overlap of the most significant linkage peaks between studies, although there is some intriguing evidence of overlaps in the minor peaks. No linkage or association study in VUR has yet identified a specific causative gene, and although VUR can be demonstrated in transgenic or specific gene knockout mouse models, the cognate genes in humans do not seem to be major contributors to primary VUR. Examination of functional candidate genes known to be involved in urinary tract development, using linkage analysis and DNA sequencing, has likewise failed to identify any gene accounting for more than a small fraction of VUR cases. This could be due to different ascertainment criteria (for example, multigenerational families versus affected sibling pairs), different genetic approaches (microsatellites versus SNPs), population differences in frequencies of mutations in different genes, multiple contributing loci, modifying genes or environmental factors.125
Table 4 | Regions identified using SNP genome-wide linkage analysis (NPL>2)*

<table>
<thead>
<tr>
<th>Chromosome</th>
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<th>Families</th>
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</table>

*All regions yielding an NPL score (or LOD score) of 2 or greater in genome scans are listed, as well as supporting evidence from other studies, even if their NPL does not reach 2. NPL unless otherwise indicated as LOD. NPL>2 but supported by at least one study with NPL>2. Position later revised (see text).

‡Autosomal recessive model.
The majority of the genes investigated for VUR and urinary tract development are also involved in the development of other organs or tissues. To date, the study population investigated consists of deliberately selected patients with VUR with or without urinary tract anomalies. Linkage studies investigating VUR candidate genes did not implicate specific genes, probably because these genes are not exclusively responsible for urinary tract development and mutations normally cause multiple anomalies. It seems likely that rare, highly-penetrant mutations account for most cases of VUR, and that these mutations can occur in any one of a large number of genes. As VUR is thought to result from a subtle anatomical defect, it seems unlikely that highly deleterious mutations in genes involved in urinary tract development will cause isolated VUR: such mutations are much more likely to cause severe syndromes affecting multiple systems, and this is reflected in the data so far reported. More subtle changes in the same or other genes or changes in their regulation might cause VUR, but these changes can be difficult to distinguish from normal genetic variation.

Increasing the numbers of study participants, collaborative studies and leveraging high-density genomic data using techniques such as imputation and pathway SNP analysis will help to accelerate data acquisition to find precise candidate regions or VUR-specific genes. Genomic copy-number variation (CNV) has been shown to be an important pathogenic mechanism in many other disorders in recent years, but no CNV study in VUR has been reported to date.

Research groups investigating the genetics of VUR should be aware of the various phenotypic expressions of the disorder. The majority of the studies reported to date have neglected to consider the dynamic range of disease characteristics and, in many patients, VUR remains undetected because of the absence of symptoms. Thus, current study cohorts consist mainly of patients with symptomatic VUR or siblings of those patients.

To increase the statistical power of genetic studies, group distributions should be designed according to clinical features of VUR and severity (for example, VUR with risk for renal scarring, reflux nephropathy or end stage renal disease) in future family studies. Furthermore, subgroup characteristics such as sex, race, and parent of origin should be taken in account when designing future study groups. All such stratification requires larger numbers of patients than have previously been included, which in turn will drive collaboration between research groups.

The existing and continuously growing body of work indicates that numerous genes or loci contribute to the pathogenesis of VUR. Concordance between minor, if not major, linkage peaks of different genome scans has been found and is the basis for ongoing research efforts to improve the level of knowledge of the genetics of VUR and to identify specific genes involved in its pathogenesis. Other abnormalities of the urinary tract are more common in families with VUR and linkage studies for chronic kidney disease identify some of the same genomic regions as those for primary VUR, indicating that understanding the biology of VUR will provide further insights into the development of the urinary tract and kidney and its susceptibility to disease.

**Review criteria**

The references used in this manuscript were obtained by searching the PubMed database using the keywords “vesicoureteric reflux”, “vesicoureteral reflux”, “genetics”, “children”, “association”, “linkage”, “whole genome scan”, and “genome wide scan”. Full-text papers in English were retrieved and their bibliographies reviewed for further relevant articles. Papers were selected on the basis of the authors’ expertise and research interests.

from classic anatomic theories to contemporary cell biological views of CACUT. Kidney Int. 61, 889–898 (2002).
80. Zahirieh, A. et al. Functional analysis of a novel GATA3 mutation in a family with the hypoparathyroidism, deafness, and renal


**Author contributions**

P. Puri and J. H. Gosemann researched data for the article and reviewed and edited the article before submission. P. Puri, J. H. Gosemann and D. Barton wrote the article. All authors contributed significantly to discussions of content.