Primary, Nonsyndromic Vesicoureteric Reflux and Nephropathy in Sibling Pairs: A United Kingdom Cohort for a DNA Bank

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Summary

Background and objectives Primary vesicoureteric reflux (VUR) can coexist with reflux nephropathy (RN) and impaired renal function. VUR appears to be an inherited condition and is reported in approximately one third of siblings of index cases. The objective was to establish a DNA collection and clinical database from U.K. families containing affected sibling pairs for future VUR genetics studies. The cohort’s clinical characteristics have been described.

Design, setting, participants, & measurements Most patients were identified from tertiary pediatric nephrology centers; each family had an index case with cystography-proven primary, nonsyndromic VUR. Affected siblings had radiologically proven VUR and/or radiographically proven RN.

Results One hundred eighty-nine index cases identified families with an additional 218 affected siblings. More than 90% were <20 years at the study’s end. Blood was collected and leukocyte DNA extracted from all 407 patients and from 189 mothers and 183 fathers. Clinical presentation was established in 122; 92 had urinary tract infections and 16 had abnormal antenatal renal scans. RN was radiologically proven in 223 patients. Four patients had been transplanted; none were on dialysis. In 174 others aged >1 year, estimated GFR (eGFR) was calculated. Five had eGFR 15 to 59 and 48 had eGFR 60 to 89 ml/min per 1.73 m². Values were lower in bilateral RN patients than in those with either unilateral or absent RN.

Conclusions The large DNA collection from families with VUR and associated RN constitutes a resource for researchers exploring the most likely complex, genetic components predisposing to VUR and RN.

Introduction

Vesicoureteric reflux (VUR) describes urine flowing into the upper urinary tract. It can be secondary to bladder outflow obstruction (1) and can occur in several multiorgan congenital disorders (2). Usually, VUR is primary and nonsyndromic, this article’s topic. VUR occurs in 25 to 40% of children presenting with urinary tract infection (UTI) and in 3 to 19% of infants with hydronephrosis on antenatal ultrasound scan (USS) screening (3). VUR’s true prevalence remains uncertain because early diagnosis requires invasive radiology and most VUR spontaneously resolves. It may be as low as 1 to 2% but may be higher (3). Although VUR can exist in isolation, Hodson and Edwards (4) associated VUR with renal segmental parenchymal thinning and calyceal clubbing. This “chronic pyelonephritis” was subsequently called “reflux nephropathy” (RN). In the United Kingdom (U.K.), RN respectively accounts for 8 and 12% of end-stage renal failure (ESRF) in children and adults (5), and RN exists in 5% of North American children receiving kidney transplants (6). These may be underestimations because some RN patients are probably hidden in diagnostic categories including “tubulo-interstitial disease” and “unknown” (7). RN occurs in 5% of children investigated after the first UTI, allowing estimation that around 0.5% of girls aged 0 to 16 years have RN (8). As addressed below, hypertension and proteinuria sometimes accompany RN.

Perceptions that UTI with VUR leads to RN via ascending infection stimulated an era of “active-treatment” of VUR (9). Certainly, RN can follow UTI, especially without prompt antibiotic treatment (10,11). Prospective studies comparing antireflux surgery with antibiotic prophylaxis fail to show significant differences in acquisition of kidney defects (12–14) or progression to ESRF (15). However, not all chronic radiologic kidney defects after UTIs are associated with VUR (16) and not all RN is pyelonephritic because some individuals born with VUR have malformed kidneys (17,18). Accordingly, the rationale for active treatment of VUR has been questioned (9) and
prospective trials have been initiated in children with VUR and UTI, including a “watchful waiting” arm (19–22). Brandstrom et al. (20,21) reported that either prophylaxis or endoscopic VUR correction reduced risks of febrile UTIs and acquisition of renal defects and Craig et al. (22) provided evidence for efficacy of antibiotic prophylaxis in reducing UTI.

Primary VUR can occur in the renal coloboma syndrome, caused by mutations of PAX2 (23) encoding a transcription factor expressed in developing renal tracts (24). Although PAX2 mutations have not been found in nonsyndromic VUR (25), defects in other genes might be relevant. Indeed, numerous genes control ureteric morphogenesis and functional maturation of the bladder and ureter (2,3,26). There is a high concordance of VUR in identical twins (27) and kindreds with multiple affected members with apparent dominant (28,29) or recessive (30) inheritance exist. When first-degree relatives of index cases are screened by cystography, around one third of siblings (31) and up to two thirds of offspring (32,33) have VUR. Thus, although yet-to-be defined environmental factors might predispose to primary VUR, genetics must be important. The definition of genetic factors that contribute to VUR and RN will illuminate mechanisms of normal human renal tract development. Information from such studies may also have clinical utility by giving families reasons why a malformation has occurred and may also allow the prediction of VUR status in relatives of index cases. As a step toward these ends, we established a DNA collection and clinical database from U.K. families containing affected sibling pairs. This bank, which we have called “The UK VUR DNA collection” (34), could then be used as a resource with which researchers can begin to unravel the perhaps complex genetics underlying VUR. We here present the detailed clinical description of the patient cohort.

Materials and Methods

After approval by the South West Research Ethics Committee, informed consent/assent was obtained. All U.K. tertiary pediatric nephrology centers were approached initially through the British Association for Pediatric Nephrology, and subsequently by individual contact, letters, and visits from the researchers to those and some regional pediatric centers. Information sheets and a Web site link (http://www.vur.org.uk/) were publicized to encourage family involvement and inform Study Group members. Families were recruited from January 2003 to July 2006. Databases in nephrology and radiology departments were searched for validating reports. Secondary or syndromic VUR was excluded. For this collection, no new patients were identified nor were additional tests undertaken, other than collection of blood for leukocyte DNA (35).

Each family comprised an “index case” with VUR of any grade diagnosed by micturating cystourethrography or radionuclide cystography. Some index cases also had RN. RN was defined as an abnormality on most recent imaging, predominantly using renography with 99mTc-dimercaptosuccinic acid (DMSA). In a minority, RN was diagnosed using USS or intravenous urography. A diagnosis of RN was accepted by finding focal or global defects and/or an abnormal split kidney function over 60/40%. A normal DMSA scan excluded RN but a normal USS was inconclusive because this modality is less sensitive at detecting RN (36). Each family also contained one or more “affected siblings” with VUR and/or RN. A minority of affected siblings had RN but not VUR. These tended to be older siblings and we believe that they would have had been shown to have VUR if investigated when younger because VUR usually spontaneously resolves by the second decade (37). For each index case and affected siblings, verification of VUR and RN status was essential and established from clinical records and review of original imaging pictures and reports.

For the index cases and affected siblings, medical records were interrogated for information about BP, plasma creatinine, and proteinuria. “Hypertension” was present either when an individual was on hypotensive medication and/or had a raised BP; when height data were unavailable, age-related BP percentiles for children of 50th percentile height were used (38). For some patients, plasma creatinine data existed, allowing calculation of estimated GFR (eGFRs). The most recently available creatinine result was used (providing there was no contemporaneous UTI) in the Schwartz formula (39) for those over 1 but under 18 years old and the Modification of Diet in Renal Disease (MDRD) formula (40) for the 13 older patients. We defined “proteinuria” as ≥ + + on dipstick testing. GFRs were not calculated in patients under 1 year old because of the known maturation of filtration rate in infancy, which can occur even with structurally abnormal kidneys (41) and the limitations of available formulae.

Normality of the data for age and eGFR was tested using the Kolmogorov-Smirnov test. Comparisons between groups for age were undertaken using Kruskal-Wallis and Mann-Whitney tests. Comparisons between groups for eGFR were undertaken using ANOVA and pairwise comparison.

Clinical details of parents and other family members were sought but only 21 mothers and 10 fathers had undergone traceable renal investigations.

Results

Overview

Index cases were indentified from 189 overtly unrelated families and there were 218 affected siblings, giving a total of 407 patients (223 female and 184 male), of which 96% were white. Over 90% of them were under 20 years old at the end of the study period (Figure 1). VUR was proven in 363 (89%) of the 407 patients, the others (i.e., 44 of the affected siblings) having radiologic evidence of RN only. In 161 families there was 1 affected sibling, 25 families had 2 affected siblings, and 2 families had 3 affected siblings. Thus, most families contained one affected sibling pair. In total, there were 250 affected sibling pairs: 55 male/male pairs; 111 male/female pairs; 84 female/female pairs. Leukocyte DNA samples were collected from all 407 patients and from 189 mothers and 183 fathers. Clinical presentation was established in 122 (65%) of the 189 families; 92 (75%) had UTI and 16 (13%) had abnormal antenatal renal scans. The other 12% comprised a variety of presentations including renal impairment and hypertension. Duplex kid-
Nephropathy

Of the 407 patients, radiologically proven RN was present in 223 (55%), being bilateral in 61 (15% of the 407). In 122 (30%) of the 407 patients, appropriate imaging had been undertaken, permitting the conclusion that they did not have RN, whereas for the other 62 (15% of the 407) patients, definitive imaging to assign a RN status did not exist. Of the 189 index cases (all, by definition, with proven VUR), 120 (63%) had RN, 60 (32%) did not have RN, and in 9 the RN status could not be established. Of the 218 affected siblings, 174 had evidence of VUR; 74 of these also had RN, 62 had no RN (normal DMSA), and 38 had insufficient information to assign RN status. The remaining 44 siblings only had evidence of RN; 8 had a negative micturating cysto-urethrogram (MCUG) and 36 had not had a test for VUR (Table 1).

In 40% of all (250) sibling pairs in the cohort, both individuals had RN. After a UTI, some DMSA defects represent acute pyelonephritis and these may resolve over 6 months (42). Accordingly, we noted that 165 patients had RN assigned on the basis of an abnormal first DMSA scan. Eight other patients had BP recordings including the four post-transplant patients, there was information on urine analysis on 302 individuals. Eight had proteinuria of ++ or more on dipstick testing. Six of these had RN, one had a normal DMSA scan, and one lacked appropriate imaging to assess RN status.

Renal Excretory Function
eGFR was calculated in 174 patients over a year old and these, together with the four transplanted cases, are shown in Table 2. eGFR was normally distributed and data were expressed as mean ± SD. eGFR was normal in 121 patients. Even after excluding the four patients with ESRF who had been transplanted (who inevitably would have had bilateral RN in their native kidneys), there was a significant difference in eGFR (ANOVA; \( P < 0.001 \)) between the three subsets: with no RN, with unilateral RN, and with bilateral RN (Figure 2). Pairwise analysis using Fisher 95% confidence intervals showed that eGFR in the bilateral RN group (\( n = 48; \text{mean} ± \text{SD}, 92.2 ± 27.8 \text{ml/min per 1.73 m}^2 \)) was significantly lower than those in unilateral RN (\( n = 88; 104.8 ± 22.1 \)) and the group without RN (\( n = 38; 112.8 ± 21.5 \)). There was no significant difference, however, between the last two groups. Notably, there was also a significant difference (Kruskal-Wallis test; \( P < 0.001 \)) between the ages of the three groups (Figure 3). Age was not normally distributed and was therefore expressed as median (range). Mann-Whitney test showed that the bilateral RN group (median 12.3 years, range 1 to 33) was significantly older than those in unilateral RN (median 6.4 years, range 1 to 23) and the group without RN (median 3.7, range 1 to 20 years) (\( P < 0.001 \) for both comparisons). There was also a significant difference (\( P < 0.05 \)) in age between the last two groups.

Table 1. Radiologically proven VUR and RN status of the 407 individuals who together comprise summary of cases and phenotypes “index cases” and “affected siblings”

<table>
<thead>
<tr>
<th>Vesioureteric Reflux and Reflux Nephropathy Status of Index Cases and Affected Siblings</th>
<th>VUR, +</th>
<th>VUR, -</th>
<th>RN, +</th>
<th>RN, -</th>
<th>RN, ?</th>
<th>VUR, +</th>
<th>VUR, ?</th>
<th>RN, +</th>
<th>RN, -</th>
<th>RN, ?</th>
</tr>
</thead>
<tbody>
<tr>
<td>Index cases</td>
<td>189</td>
<td>120</td>
<td>60</td>
<td>9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Affected siblings Individuals</td>
<td>218</td>
<td>74</td>
<td>62</td>
<td>38</td>
<td>8</td>
<td>36</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: +, present; -, absent; ?, uncertain.
Researchers have begun to undertake linkage analyses using both families with numerous members affected by VUR and/or RN (29,30,43) and kindreds with two to three affected individuals (35,44). Studies using genetic association (35,45–47) and candidate gene sequencing (45,48) strategies are also being undertaken. The DNA Bank from the current cohort represents one of the largest such collections, nearly rivaled by Irish (44) and Slovenian (35) collections. Cordell et al. (35) undertook an initial genetic study using both the U.K. and Slovenian collections. In studies of white subsets, to control for potentially confounding racial variations in genetic background, modest evidence of linkage was found to several loci but there was no clear overlap with previous linkage studies (29,30,43,44). Indeed, major loci may not exist for this common renal tract malformation within some European populations. Furthermore, Cordell et al. (35) found no association with polymorphisms in candidate genes \textit{AGTR2}, \textit{HNF1B}, \textit{PAX2}, \textit{RET}, \textit{ROBO2}, and \textit{UPK3A}.

Of the 407 patients in the U.K. cohort, 96% were white. In the 2001 census (http://www.statistics.gov.uk/focuson/ethnicity/), 8% (i.e., 4.6 of 57.5 million) of the U.K. population were non-white, of which approximately 1 million were black and 2 million were from either India, Pakistan, or Bangladesh. Thus, non-whites are apparently underrepresented (\( \chi^2 \) test, \( P = 0.002 \)) in the UK VUR DNA collection versus the whole population. Perhaps this represents a putative inequality of health care access but could also be explained by a different incidence of VUR between racial groups. Indeed, when Chand et al. (49) analyzed 15,504 UTI patients, black children were less likely as white children (\( P < 0.0001 \)) to have VUR. Duplex kidney was noted in nine patients in the U.K. cohort. The incidence of duplication in the general population is approximately 2\% (50) and VUR and duplex kidney can coexist in an individual (51). Families have also been described in which some members can have either VUR or duplex kidney (51).

The incidence of RN in the U.K. cohort is similar to some other reports (14) whereas in some series of children with VUR the incidence of RN was lower (52). Williams et al. (3) estimated that for each 6000 individuals who are born with VUR, 1600 (27\%) will have their clinical course complicated by UTI and 2000 (33\%) will have RN, with just one (0.02\%) having ESRF. Compared with this, the current patient cohort therefore appears enriched for RN, which was present in 55\% of all patients; furthermore, four (1\%) had ESRF. The cohort may not be representative of all cases of VUR as most referrals for entry into the collection were from specialist pediatric nephrology centers likely to have seen the more severe end of the VUR/RN spectrum of disease. Centers differ in their clinical policies on use of MCUG for early diagnosis of VUR after UTI and in sib-

**Table 2.** Chronic kidney disease status of patients over 1 year of age

<table>
<thead>
<tr>
<th></th>
<th>Bilateral RN (( n = 52 ))</th>
<th>Unilateral RN (( n = 88 ))</th>
<th>No RN (( n = 38 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR ( \geq 90 \text{ ml/min per 1.73 m}^2 ) (stage 1 CKD)</td>
<td>23</td>
<td>65</td>
<td>33</td>
</tr>
<tr>
<td>GFR 60–89 \text{ ml/min per 1.73 m}^2 ) (stage 2 CKD)</td>
<td>21</td>
<td>22</td>
<td>5</td>
</tr>
<tr>
<td>GFR 15–59 \text{ ml/min per 1.73 m}^2 ) (stage 3/4 CKD)</td>
<td>4</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>ESRF (transplanted; stage 5 CKD)</td>
<td>4</td>
<td></td>
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</tbody>
</table>

CKD, chronic kidney disease.

**Discussion**

Researchers have begun to undertake linkage analyses using both families with numerous members affected by VUR and/or RN (29,30,43) and kindreds with two to three affected individuals (35,44). Studies using genetic association (35,45–47) and candidate gene sequencing (45,48) strategies are also being undertaken. The DNA Bank from the current cohort represents one of the largest such collections, nearly rivaled by Irish (44) and Slovenian (35) collections. Cordell et al. (35) undertook an initial genetic study using both the U.K. and Slovenian collections. In studies of white subsets, to control for potentially confounding racial variations in genetic background, modest evidence of linkage was found to several loci but there was no clear overlap with previous linkage studies (29,30,43,44). Indeed, major loci may not exist for this common renal tract malformation within some European populations. Furthermore, Cordell et al. (35) found no association with polymorphisms in candidate genes \textit{AGTR2}, \textit{HNF1B}, \textit{PAX2}, \textit{RET}, \textit{ROBO2}, and \textit{UPK3A}.

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lings. Furthermore, those families with severely affected members may have been more likely to agree to be included in the collection.

Of interest, the fact that each member of 40% of all sibling pairs had RN raises the possibility of there being a genetic influence on development of nephropathy as well as VUR itself. We are unable to unravel what proportion of RN in the current U.K. cohort represents congenital anomalies versus defects acquired from pyelonephritis; indeed, it is debated what (nonhistologic) criteria can be used to distinguish these possibilities (10). There may be roles played by environmental factors such as variable access to health care and/or delay in treatment of UTIs common to members of the same family and which would result in RN.

Our data confirm that bilateral RN is a risk factor for decreased eGFR. However, the bilateral RN group was older than those with either unilateral or no RN. GFR normally begins to decline after 20 years of age (53), so we cannot exclude that the tendency to lower eGFR is related to aging. Having said this, most patients with bilateral RN are older than those with either unilateral or no RN. GFR decreased eGFR. However, the bilateral RN group was included in the collection.

Previous studies found that children with RN entering studies with a GFR of ≥70 ml/min per 1.73 m² show barely any risk of functional deterioration up to 10 years (14,15), although one would remain cautious about the long-term prognosis of these individuals (54). The number of younger patients with VUR but no RN in this cohort may have been influenced by changes in clinical practice such as the recognition of the importance of prompt treatment of UTI in babies and increased testing for VUR in newborn siblings of index cases.

In this U.K. cohort, predominantly under 20 years old, the incidence of hypertension (excluding four patients who had received a renal transplant) was only 4%. Accelerated arterial hypertension may be a presenting feature when RN coexists with impaired renal function (55), and prospective studies (13,56) show that some individuals with RN develop hypertension (56–58). Wennenstrom et al. (59), however, reported that individuals with RN not in renal failure had no increased risk of hypertension versus controls without RN. Indeed, the fact that, in this study, several of the patients with VUR but without radiologic evidence of RN had hypertension shows that hypertension is multifactorial in this reflux population. Perhaps longer term follow-up will show an increased incidence of hypertension in this cohort and indeed several had borderline high BPs. Proteinuria is common when RN and renal insufficiency coexist (60) and, in a retrospective adult study, progressive loss of kidney excretory function positively correlated with proteinuria (54). In our cohort, only 8 of 302 individuals had proteinuria of ≥++; of these, 6 had bilateral RN. Quantification of urine albumin/creatinine ratios may have revealed a higher incidence of abnormal proteinuria.

In conclusion, the current patient cohort represents a resource which investigators can use to unravel the genetic bases of VUR and/or RN. Studies for which the UK VUR DNA Bank could in future be utilized might exploit several complementary genetic strategies including the following: combining the current bank with others for larger and thus more highly powered linkage and association studies; targeted sequencing of the several tens of candidate VUR genes which already exist; seeking copy number variants (e.g., duplications and deletions) by comparative genomic hybridization by microarray. Potential investigators are now directed to “The United Kingdom Vesicoureteric Reflux (VUR) DNA Collection” Web site (http://www.vur.org.uk) in which instructions on how to access, and also terms and conditions of using, the collection are detailed (see also Supplemental Data).

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Disclosures
None.

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