Am. J. Hum. Genet. 66:1420–1425, 2000

Report

Primary, Nonsyndromic Vesicoureteric Reflux and Its Nephropathy Is Genetically Heterogeneous, with a Locus on Chromosome 1

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Primary vesicoureteric reflux (VUR) affects 1%–2% of whites, and reflux nephropathy (RN) causes up to 15% of end-stage renal failure in children and adults. There is a 30–50-fold increased incidence of VUR in first-degree relatives of probands, compared with the general population. We report the results of the first genomewide search of VUR and RN; we studied seven European families whose members exhibit apparently dominant inheritance. We initially typed 387 polymorphic markers spaced, on average, at 10 cM throughout the genome; we used the GENEHUNTER program to provide parametric and nonparametric linkage analyses of affected individuals. The most positive locus spanned 20 cM on 1p13 between *GATA176C01* and *D1S1653* and had a nonparametric LOD score (NPL) of 5.76 ($P = .0002$) and a parametric LOD score of 3.16. Saturation with markers at 1-cM intervals increased the NPL to 5.94 ($P = .00009$). Hence, VUR maps to a locus on chromosome 1. There was evidence of genetic heterogeneity at the chromosome 1 locus, and 12 additional loci were identified genomewide, with $P < .05$. No significant linkage was found to 6p, where a renal and ureteric malformation locus has been reported, or to PAX2, mutations of which cause VUR in renal-coloboma syndrome. Our results support the hypothesis that VUR is a genetic disorder.

Vesicoureteric reflux (VUR [MIM 193000]) is the retrograde passage of urine from the bladder into the upper urinary tract. VUR may occur as a secondary effect of anatomical or neurological bladder outflow obstruction. However, the primary form of the disorder occurs in the absence of these features, with an incidence in infants of 1%–2% (Peters et al. 1967; Bailey 1975). Almost 30% of children presenting with urinary tract infection (UTI) have VUR, and the reflux of infected urine into the kidney in the presence of compound papillae can cause acute pyelonephritis and subsequent renal parenchymal scarring (Smellie and Prescod 1986). In addition, some infants born with VUR have associated dysplastic or hypoplastic renal malformations, which themselves impair renal function (Hinchliffe et al. 1992; Risdon et al. 1993). Reflux nephropathy (RN), the renal disease associated with VUR, accounts for up to 15% of end-stage renal failure in children and adults (Kincaid-Smith et al. 1984).

Previous studies demonstrated that VUR and RN can occur in families, and systematic studies of first-degree relatives of individuals affected with VUR show a 30%–50% incidence of VUR, which implies that genetic factors play an important role in the pathogenesis (Noe 1992; Noe et al. 1992). Different modes of inheritance of VUR have been suggested, including a dominant single gene (Chapman et al. 1985) and polygenic inheritance (DeVargas et al. 1978). Previous studies have suggested a urinary tract malformation locus on chromosome 6p on the basis of two lines of evidence: a balanced translocation including 6p and association with HLA types (Macintosh et al. 1989; Izquierdo et al. 1992; Groenen et al. 1998). VUR can also occur as part of complex syndromes (Winter and Baraitser 1998) such as the re-

Received November 8, 1999; accepted for publication January 6, 2000; electronically published March 17, 2000.

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0002-9297/2000/6604-0026$02.00

nal-coloboma syndrome, which is caused by PAX2 mutations on chromosome 10q (Sanyanusin et al. 1995). Recent work has found an association between diverse renal tract malformations and a polymorphism of the angiotensin II receptor type 2 (AT2) on Xq (Nishimaura et al. 1999). Furthermore, studies in mice demonstrate that the function and interaction of numerous molecules, including transcription factors, growth factors, and cell-adhesion molecules, are critical for the normal development of the urinary tract (Woolf and Winyard 1998). This raises the possibility that primary, nonsyndromic VUR may be genetically heterogeneous.

We performed a genomewide search for susceptibility loci for primary VUR of seven European kindreds with three to seven affected individuals in each family and an apparently dominant pattern of inheritance. The seven families that we studied were assessed by pediatric and adult nephrologists. VUR was diagnosed by cystogram and RN by intravenous pyelogram or isotope renogram. There was no evidence of secondary causes of VUR or other clinical features that suggested a syndromic diagnosis. In each family, at least one individual presented with symptomatic renal disease and had been established by radiological investigations to have VUR, RN, or both, as indicated in figure 1. This prompted further inquiry into the rest of the family. Some family members had already been investigated and diagnosed as having VUR, RN, or both, but some affected relatives were subsequently diagnosed by radiological screening. We chose to analyze only affected individuals, because VUR can regress with age (Tamminen-Mobius et al. 1992), and, furthermore, not all individuals had a cystogram, an investigation that was not thought to be clinically relevant in asymptomatic adults. All individuals in whom VUR, RN, or both were not clinically proven were classified as having unknown status. Specifically, the presence of "soft" renal symptoms and signs such as UTI or hypertension were not regarded as precise enough for a positive diagnosis of VUR or RN, so these individuals were labeled as having unknown status. Informed consent was obtained from individuals before they were included in the study.

DNA was extracted from peripheral blood leukocytes by standard techniques. The primers for the markers were obtained from Research Genetics. Set 6.0 was used for chromosomes 2–7, 11, 12, 15, 17–20, and X in pedigrees 1–5. The remainder of the chromosomes in pedigrees 1–5 and all chromosomes in pedigrees 6 and 7 were analyzed with fluorescently labeled primers of set 8.0 (Research Genetics). A total of 387 markers was used in the analysis, and 10 additional markers were used to saturate the region on chromosome 1. Semiautomated fluorescent genotyping was undertaken with the ABI 377 Genescan/Genotyper system by comparison of the fragment sizes with an internal standard. The PCR products of the remainder of the primers were labeled with radioactive $\alpha[^{32}P]$-CTP and the products separated with a nondenaturing polyacrylamide gel system. The average heterozygosity of the markers was 0.76, and the average spacing was 10 cM.

The program GENEHUNTER (Kruglyak et al. 1996) was used to compute multipoint parametric and nonparametric linkage analysis by use of data from the seven pedigrees. An analysis of only affected family members was performed by use of clinical criteria as described. The genetic model for VUR used in the parametric analysis was the most likely model predicted by the segregation analysis of Chapman et al. (1985): gene frequency of 1% and a dominant disorder and phenocopy of 1%. Heterogeneity (α) at each locus was also estimated by GENEHUNTER. Stringent criteria for statistical significance of genomewide scans in sibling pairs were used: $P < .0007$ was suggestive of linkage, and $P < .00002$ was significant linkage (Lander and Kruglyak 1995), although it was recognized that the families we studied might correspond to a dominant disorder with $P < .0017$, suggestive of linkage, and $P < .000049$, significant linkage. All areas with $P < .05$ are reported (Lander and Kruglyak 1995). Figure 1 shows the pedigrees of the seven families included in the study. Table 1 shows the most significant regions (with $P < .05$ on a nonparametric analysis) genomewide by generation of a multipoint analysis on the entire data set by GENEHUNTER. Results are presented for affected individuals with VUR, RN, or both.

The most positive susceptibility locus was a 20-cM interval on chromosome 1 between *GATA176C01* and *D1S1653* in cytogenetic band 1p13. The nonparametric LOD (NPL) score was 5.48 ($P = .0002$) on the initial genomewide set of markers. Saturation of the region with markers spaced at 1-cM intervals increased the NPL score to 5.94 ($P = .00009$). The parametric LOD score for this region, under a dominant genetic model with a gene frequency of 0.01 and a phenocopy of 0.01, was 3.12 ($\alpha = .78$) and was 3.17 ($\alpha = .78$) with saturation of the region.

Pedigree 6 had the highest parametric LOD score at this locus (2.16), and both pedigrees 1 and 2 had negative parametric LOD scores. Heterogeneity at this locus was 0.78. It was not possible to establish whether the additional pedigrees mapped to this region, since the maximum parametric LOD score was 0.85 in pedigree 4. Because pedigree 6 weights the chromosome 1 data, the genomewide analysis was repeated without this pedigree, to search for additional loci. The results for this analysis are presented in table 2 and show that the next most positive locus was on chromosome 20 between *D20S477* and *D20S481*, with a parametric LOD score of 2.90 and an NPL of 3.42 ($P = .003$). Pedigree 6 had a negative LOD score (-1.74) at this locus.

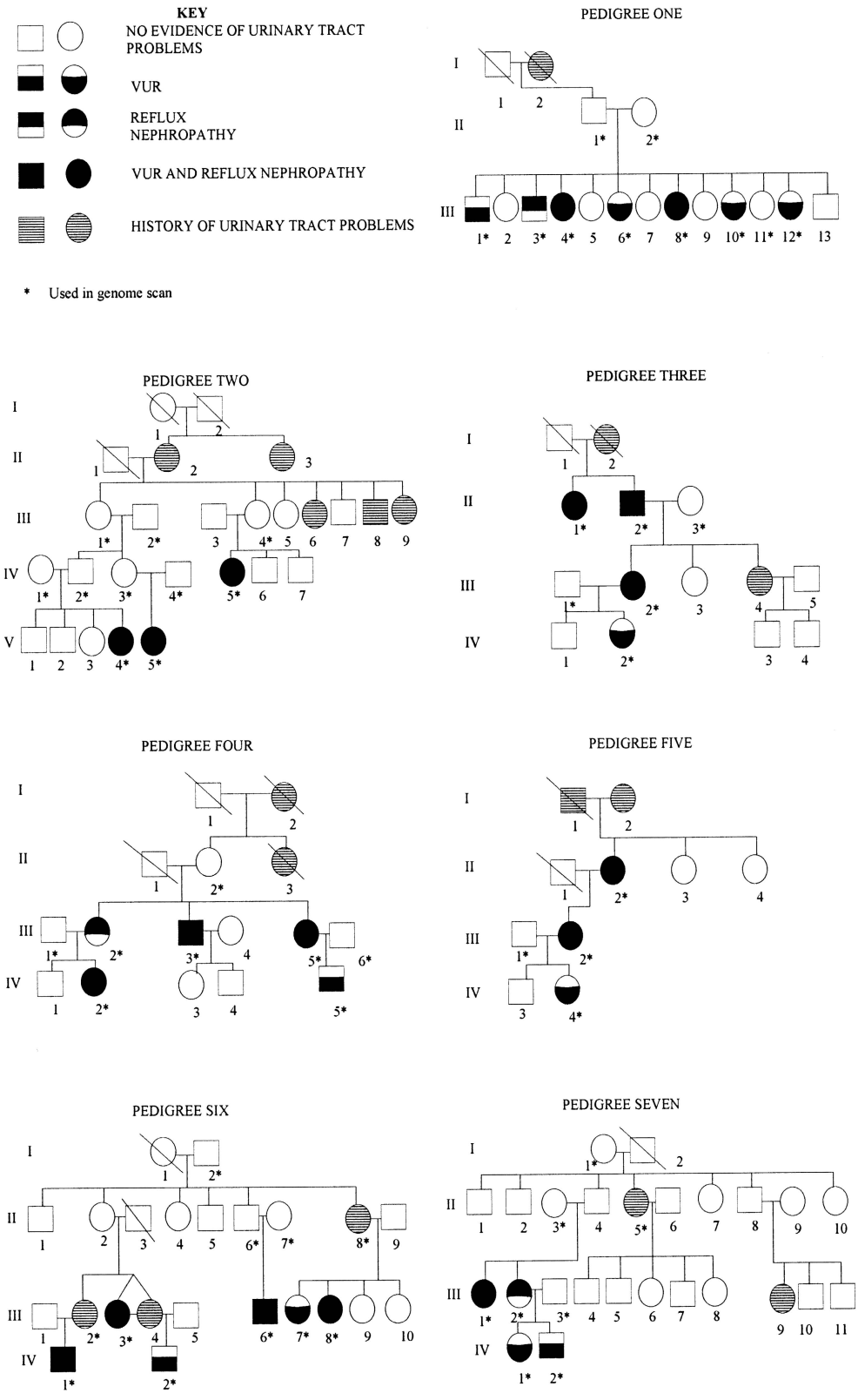

Figure 1 Pedigrees used in the genomewide analysis. Affected individuals have either VUR, as established by cystogram, or RN, as established by intravenous pyelogram or isotope renogram. Individuals who have a history of urinary tract problems such as UTI or hypertension but have not had the appropriate radiological investigations to clarify whether they have VUR or RN were labeled as having unknown status in the statistical analysis.

Table 1

Summary of GENEHUNTER Analysis

Chromosome (Distance [cM])	Markers	Parametric LOD Score (α)	NPL Score	P	LODs (Pedigrees) at Maximum P
1 (149–166)	*GATA176CO1–D1S1653*	3.12 (.78)	5.48	.0002	−.46 (1), −1.08 (2), .56 (3), .85 (4), .27 (5), 2.16 (6), .56 (7)
2 (55–68)	*D2S1788–D2S1352*	1.17 (.31)	3.35	.005	−.13 (1), −.86 (2), −.65 (3), −.54 (4), −.01 (5), 1.02 (6), .11 (7)
3 (37–66)	*GATA164B08–D3S1768*	2.43 (.87)	2.95	.009	−.45 (1), 1.25 (2), .16 (3), −.09 (4), .19 (5), 1.06 (6), −.19 (7)
3 (117–184)	*GATA128C02–D3S1763*	1.61 (.98)	3.00	.008	1.21 (1), −.13 (2), −.15 (3), .11 (4), .27 (5), −.24 (6), .51 (7)
8 (25–29)	*D8S1106–D8S1145*	1.05 (.45)	2.38	.02	−.46 (1), 1.32 (2), −.20 (3), .84 (4), .27 (5), −.85 (6), −.91 (7)
9 (64–69)	*D9S1122–D9S922*	.60 (.34)	2.16	.03	1.19 (1), −1.08 (2), .52 (3), −.95 (4), .27 (5), −.74 (6), −.36 (7)
9 (143–145)	*ATA59H06–D9S158*	.93 (.48)	1.87	.04	1.14 (1), −.85 (2), .44 (3), −1.70 (4), .07 (5), .68 (6), −.50 (7)
13 (90–117)	*D13S793–D13S285*	2.03 (.99)	2.34	.02	−.03 (1), .81 (2), .31 (3), .72 (4), .04 (5), .09 (6), .55 (7)
20 (3–35)	*D20S103–D20S470*	2.79 (.99)	2.75	.01	−.37 (1), .95 (2), .50 (3), .84 (4), .27 (5), .53 (6), .38 (7)
20 (45–53)	*D20S477–D20S481*	1.77 (.74)	2.92	.009	1.20 (1), .85 (2), .55 (3), −.61 (4), .27 (5), −1.74 (6), .54 (7)
22 (24–25)	*GCT10C10–D22S689*	.91 (.48)	2.19	.02	1.11 (1), −.74 (2), .55 (3), −1.17 (4), .24 (5), .23 (6), −.28 (7)
22 (40–44)	*D22S685–D22S445*	1.20 (.71)	2.04	.03	−.28 (1), .39 (2), .56 (3), −1.4 (4), .26 (5), .78 (6), .30 (7)
X (70–84)	*GATA144D04–DXS6800*	.58 (.75)	1.9	.04	.01 (1), .43 (2), .50 (3), −.40 (4), −.05 (5), −.36 (6), .39 (7)

NOTE.—Areas with $P < .05$ are described. The most positive region is on chromosome 1, with genetic heterogeneity at this locus, since pedigrees 1 and 2 have negative LOD scores.

Prior to the genome scan, a number of regions were considered to be candidates for linkage to VUR and RN, including chromosome 6p (the HLA locus), chromosome 10q (where the genes PAX2, RET, and FGFR2 are located), and the X chromosome (where AT2 is located). There were no areas on chromosome 6p and chromosome 10q with $P < .05$. However, there was a locus on the X chromosome that was worthy of report ($P = .04$).

In this study, we present the results of the first genomewide search in primary, nonsyndromic VUR and its nephropathy. We have investigated seven large pedigrees in which inheritance of VUR appears to be dominant. Our analysis identifies a 20-cM locus on chromosome 1 that is highly suggestive of linkage ($P = .00009$). The criteria of Lander and Kruglyak (1995) assume no prior genetic model, and, when we use what is considered to be the most likely model for VUR on the basis of segregation analysis (Chapman et al. 1985), a significant parametric LOD score of 3.17 is reached. The order and level of significance of the nonparametric and parametric scores correspond closely, which suggests that the dominant model previously suggested for VUR (Chapman et al. 1985) is likely to be correct.

Table 2

Summary of GENEHUNTER Analysis Excluding Pedigree 6

Chromosome (Distance [cM])	Markers	Parametric LOD Score	NPL Score	P
1 (152–166)	*GATA176CO1–D1S1653*	.7	2.65	.01
3 (61–65)	*GATA164B08–D3S1768*	1.01	1.90	.04
3 (114–177)	*GATA128C02–D3S1763*	1.89	3.79	.004
8 (24–30)	*D8S1106–D8S1145*	.86	2.57	.01
13 (96–113)	*D13S793–D13S285*	2.08	2.37	.02
20 (2–36)	*D20S103–D20S470*	1.22	1.57	.02
20 (42–54)	*D20S477–D20S481*	2.90	3.42	.003
22 (24–28)	*GCT10C10–D22S689*	−.3	1.9	.04
X (74–84)	*GATA175DO3–DXS7132*	.89	2.04	.03

NOTE.—The most positive area is now on chromosone 20, with a parametric LOD score that approaches 3.0. Areas with $P < .05$ are described.

A number of biological processes, including those involved in development, neoplasia, and inflammation, could be involved in the pathogenesis of VUR and its nephropathy, and genes implicated in these processes are potential candidates for VUR. The region on chromosome 1 contains several interesting candidate genes. The ras-related protein 1A (RAP1A or KREV1) contains GTP-binding motifs and shares 50% amino acid homology with RAS proteins. The expression of KREV1 has been noted to be reduced in some tumors (Pizon et al. 1988; Kitayama et al. 1989). Another potential candidate is the Ras homolog gene family member C gene. This gene is a member of the Ras superfamily of small GTP-binding proteins, which are a large group of proteins that are involved in signal transduction, proliferation, vesicle trafficking, and regulation of the actin cytoskeleton, which could be involved in developmental abnormalities such as VUR (Madaule and Axel 1985; Maekawa et al. 1999). Additional interesting genes for further study in the interval on chromosome 1 include glutathione s-transferase, the absence of which is associated with an increased risk of bladder cancer (Golka et al. 1997), and colony-stimulating factor 1, which is implicated in renal inflammation (Wada et al. 1997).

However, since pedigrees 1 and 2 have negative parametric LOD scores at the locus on chromosome 1, there is evidence for genetic heterogeneity, suggesting that the most likely mode of inheritance for VUR is dominant, with different genes acting in different families. More pedigrees need to be tested to confirm or refute the

regions found in this study and to narrow candidate regions. It may then become apparent that the inheritance of VUR is dominant in only a proportion of families and that the overall inheritance of VUR in the population is polygenic. We have collected 40 additional pedigrees with VUR for investigation, and, of the samples collected, few had such dominant-looking pedigrees as those used in this study. At present, it remains unclear whether the diagnosis of VUR has been missed in older and uninvestigated individuals and inheritance in these pedigrees is dominant or whether these individuals are really unaffected, which suggests polygenic inheritance. Furthermore, a positive diagnosis of VUR and its nephropathy requires invasive radiological investigation, with cystogram, isotope renogram, or intravenous pyelogram, the nature of which may limit the definitive testing of asymptomatic individuals, particularly children, and acts to reduce the power of pedigrees included in a genetic study of VUR.

The testing of additional data sets may also uncover further loci for examination. We did not find positive results in areas that have previously been reported as renal malformation loci, including the short arm of chromosome 6 (Macintosh et al. 1989; Izquierdo et al. 1992; Groenen et al. 1998) and the long arm of chromosome 10, where the gene PAX2 (Sanyanusin et al. 1995) is located. The exclusion of the PAX2 locus is not surprising, because this has been reported by other authors who performed SSCP analysis and sequencing of PAX2 in individuals with nonsyndromic cases of VUR (Choi et al. 1998). Furthermore, there was no evidence of visual defects in the pedigrees analyzed in this study. However, on the X chromosome, there is a region with $P = .04$ that includes the locus of the postulated renal tract malformation gene AT2 (Nishimaura et al. 1999). This locus is worthy of further examination, but the population reported to have an association between an intronic polymorphism of AT2 and renal tract malformations was apparently male infants with sporadic diverse renal tract malformations; conversely, our pedigrees probably represent a different group of older individuals with recurrent UTI and a strong family history of VUR. There are more affected females than affected males in our pedigrees, a fact that has been reported by other authors (MacGregor and Freeman 1975), which might suggest a modifying gene on the X chromosome, although it has been noted that, in individuals who present with UTI, VUR is found with a similar frequency in the two sexes (Kelais 1971).

Acknowledgments

The National Kidney Research Fund assisted with two project grants (R26/2/96; R34/1/98). The Kidney Research Aid Fund and the Northern Counties Kidney Research Fund also provided funding. We thank all the families for donating samples for the study. S.A.F. was a Research Training Fellow funded by Action Research. We thank Cathryn Lewis for helpful advice with linkage analysis and Jennifer Allison for liaison with pedigree 1. We acknowledge the clinical contributions of Prof. T.M. Barratt, Dr. J. Smellie and Prof. S.H. Sacks.

Electronic-Database Information

Accession numbers and URLs for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for VUR [MIM 193000])

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