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SHORT REPORT

Uroplakin III is not a major candidate gene for primary vesicoureteral reflux

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Vesicoureteral reflux (VUR) is the retrograde flow of urine from the bladder into the ureter and towards the kidneys. VUR is the most common cause of end stage renal failure in both children and adults and it is a major cause of severe hypertension in children. VUR is seen in approximately 1–2% of newborn Caucasians. Substantial evidence exists that VUR is a genetic disorder. Uroplakins are integral membrane proteins found in the bladder wall. Knockout studies in mice have suggested uroplakin III (UPK3) as a candidate gene for VUR. We have used parametric and nonparametric linkage analysis and tests for association, to investigate this possibility in a cohort of 126 sibling pairs affected with primary VUR. None of the analyses showed any substantial evidence for linkage or association of markers at the UPK3 locus to VUR. Our results do not support a role for UPK3 in primary VUR.
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Introduction

Vesicoureteral reflux (VUR) is the retrograde flow of urine from the bladder along the ureter and towards the kidneys. The primary form of this condition is relatively common, occurring in an estimated 1–2% of newborn Caucasians.[1] VUR is the most common cause of end-stage renal failure in both children and adults and it is a major cause of severe hypertension in children.[1–3] Primary VUR arises from a submucosal ureter that is of insufficient length, or a ureteric orifice that is too wide to allow for the fulfilment of a proper valvular mechanism.[1,3] VUR occurs frequently in families; approximately half of the offspring will be affected but the mode of inheritance is unknown.[1] The disease may be heterogeneous in nature and it is likely that a number of loci exist.[4]

The uroplakins constitute a group of four integral membrane proteins, UPIa, UPIb, UPII and UPIII. They are expressed at the luminal surface of the urothelium, the transitional epithelium lining the urinary tract.[5–7] Hu *et al* observed VUR in mice in which the gene for UPIII (locus upk3) had been homozygously inactivated.[7] We investigated UPK3 as a candidate locus for VUR in a large cohort of affected sibling pairs.

Materials and methods

In total, 97 families with two or more members affected with primary VUR of any grade, were collected for this

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study. The diagnosis of VUR was confirmed by a postnatal voiding cystourethrography, normally after a child presented with a urinary tract infection. Patients with reflux secondary to another condition (eg neurogenic bladder) were excluded.

Of 467 samples collected, 184 were parents, 204 affected siblings, 77 unaffected siblings, and 2 grandparents. There were 126 sib pairs in total. Additional families were added for the final SNP studies: 63 families consisting of 132 unaffected siblings and 135 affected siblings, giving rise to an additional 82 sib pairs.

Five dinucleotide repeat markers closely flanking UPK3 were selected for analysis (Figure 1).

Linkage data were analysed using Genehunter.[8] Nonparametric analysis was used, as the mode of inheritance for VUR is not yet confirmed. Genehunter 2.0 was used in a sliding-window analysis for the haplotype-TDT test.[9]

An assay was developed for SNP (rs2742631) -G->C 207 bp 5′ to exon 1, from the sequence data. Mismatched primers were used to introduce an *Xho*1 site when the C allele was present. The PCR products were digested with *Xho*1 and analysed on agarose gels. An additional six SNPs were selected to be analysed within and around the UPK3 gene. Genotyping of these SNPs was carried out by KBiosciences, using Amplifluor™ assays. The TDT test (a family-based association test[10]), was used to analyse these SNP data.

Results

All 467 samples were typed for five microsatellites closely flanking the UPK3 locus. The microsatellite data were analysed using Genehunter (Version 2.0). Genehunter tests for an excess of identical-by-descent (IBD) allele sharing between affected subjects within a pedigree. Any families which showed consistent Mendelian errors over three repetitions were removed. Results were also assessed for linkage using the Chapman *et al*[11] genetic model for VUR, assuming a major dominant locus with high penetrance (see Table 1). None of the marker loci tested showed any significant evidence of linkage to VUR by the nonparametric sib-pair model. Negative lod scores of −20 or less exclude linkage at this locus using the dominant model of Chapman.[11] The microsatellite marker haplotype data were also analysed for association with VUR in a sliding-window version of the transmission disequilibrium test as implemented in Genehunter 2.0. After correction for multiple testing using standard Bonferroni methods, no significant excess transmission was observed (data available on request).

A SNP 207 bp 5′ to exon 1, rs2742631 was identified by sequencing. In all, 80 families were typed and the results analysed for association with VUR using the TDT. Overall, 17 families were excluded due to Mendelian error. The results of the TDT also confirmed that UPK3 was not linked to VUR, giving a χ^2 value of 2.314 ($P=0.128214$, 1df).

A further four SNPs within the coding sequence of UPIII were genotyped over 134 VUR families, 630 individuals, giving 293 trios. The data were again analysed for association to VUR using the TDT. These data were analysed in three groups (see Table 2).

There was one significant result for rs2075951 with a *P*-value of 0.006 observed in the second set of families, but the original set and the combined family sets do not confirm this result. Power calculations using the method of Purcell *et al*[12] and based on the model of VUR inheritance proposed in Chapman *et al*[11] indicated that this sample would have up to 86% power to detect an effect at this locus with 95% confidence when the variant is causative of VUR, or in near-complete LD with the causative variant.

Discussion

Hu *et al*[7] observed VUR among other features in mice in which the gene for UPIII had been homozygously inactivated. They suggested that UPK3 be treated as a candidate locus for VUR in humans. We investigated this possibility

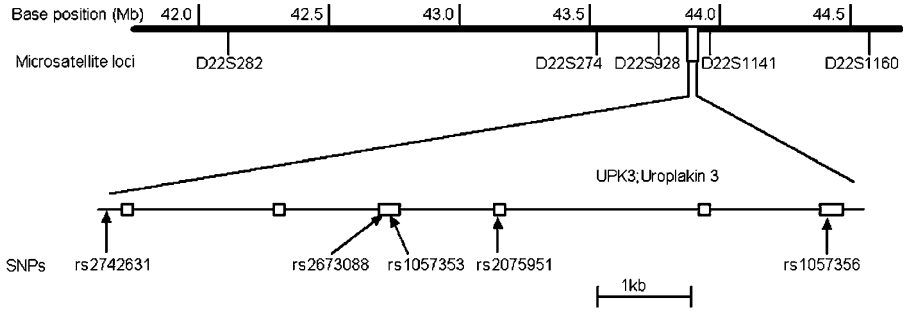

Figure 1 UPK3-linked microsatellite and SNP markers used in this study. Physical map of the microsatellite and SNP marker selected for this study. Five microsatellite markers span 5 cM of chromosome 22. Five SNP markers are also shown, four of which are located in the coding sequence of the UPIII gene. All positions are derived from the UCSC Genome Browser[14] using NCBI Build 34, July 2003 freeze.

Table 1 Results of linkage analysis

Marker	*cM*[a]	*NPL*[b]	P-*value*	*LOD*[c]
D22S282	48.19	−0.0006	0.4990	−20.404
D22S274	51.54	0.5819	0.2750	−22.786
D22S928	52.08	0.1958	0.4193	−25.286
UPK3				
D22S1141	52.61	−0.3395	0.6341	−26.767
D22S1160	53.16	−0.8758	0.8137	−29.855

[a]Position in Kosambi cM on chromosome 22; [b]Nonparametric LOD score; [c]LOD score based on the model of Chapman *et al*.

Table 2 *P*-values for TDT results for four SNPs within UPK3

Rs number	*Original families*	*Newer families*	*Combined families*
Rs2673088	0.144	0.351	0.087
Rs1057353	0.397	0.463	0.263
Rs2075951	1.000	0.006	0.074
Rs1057356	0.863	0.046	0.161

in a cohort of 208 sibling pairs from 160 families affected with VUR, the largest such cohort assembled to date.

Nonparametric linkage analysis using highly informative microsatellite markers flanking the UPK3 locus showed no significant evidence of linkage of primary VUR. Parametric analysis, using a dominant model with one major locus, strongly rejected linkage of all markers to VUR.

TDT analysis of five SNPs in the promoter and coding regions of the UPK3 gene and haplotype-TDT for the flanking microsatellite markers showed no significant evidence for linkage of VUR to UPK3.

Other studies have come to similar conclusions: Feather *et al*, who carried out a whole-genome scan in VUR, did not detect linkage in this area.[4] Recently, Giltay *et al* sequenced the UPK3 coding sequence in 25 patients with primary VUR and found no pathogenic mutations. However, the authors concede that their results do not rule out mutations in regulatory elements affecting gene expression or function.[12] Our results indicate that genetic alterations at UPK3 make little or no contribution to primary VUR in humans.

The close interactions of the uroplakins, maintaining bladder wall integrity and the evidence that there is a relationship between bladder function and VUR prognosis[13] suggest that genes for other members of the uroplakin family should now be investigated as candidates in primary VUR.

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