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A Recessive Gene for Primary Vesicoureteral Reflux Maps to Chromosome 12p11-q13

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ABSTRACT

Primary vesicoureteral reflux (pVUR) is one of the most common causes of pediatric kidney failure. Linkage scans suggest that pVUR is genetically heterogeneous with two loci on chromosomes 1p13 and 2q37 under autosomal dominant inheritance. Absence of pVUR in parents of affected individuals raises the possibility of a recessive contribution to pVUR. We performed a genome-wide linkage scan in 12 large families segregating pVUR, comprising 72 affected individuals. To avoid potential misspecification of the trait locus, we performed a parametric linkage analysis using both dominant and recessive models. Analysis under the dominant model yielded no signals across the entire genome. In contrast, we identified a unique linkage peak under the recessive model on chromosome 12p11-q13 (D12S1048), which we confirmed by fine mapping. This interval achieved a peak heterogeneity LOD score of 3.6 with 60% of families linked. This heterogeneity LOD score improved to 4.5 with exclusion of two high-density pedigrees that failed to link across the entire genome. The linkage signal on chromosome 12p11-q13 originated from pedigrees of varying ethnicity, suggesting that recessive inheritance of a high frequency risk allele occurs in pVUR kindreds from many different populations. In conclusion, this study identifies a major new locus for pVUR and suggests that in addition to genetic heterogeneity, recessive contributions should be considered in all pVUR genome scans.

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Vesicoureteral reflux (VUR; OMIM no. 193000) is the retrograde flow of urine from the bladder to the ureters and the kidneys during micturition. Uncorrected, VUR can lead to repeated urinary tract infections, renal scarring and reflux nephropathy, accounting for up to 25% of pediatric end stage renal disease.^{1,2} VUR is commonly seen as an isolated disorder (primary VUR; pVUR), but it can also present in association with complex congenital abnormalities of the kidney and urinary tract or with specific syndromic disorders, such as renal-coloboma and branchio-otorenal syndromes.^{3–8}

pVUR has a strong hereditary component, with monozygotic twin concordance rates of

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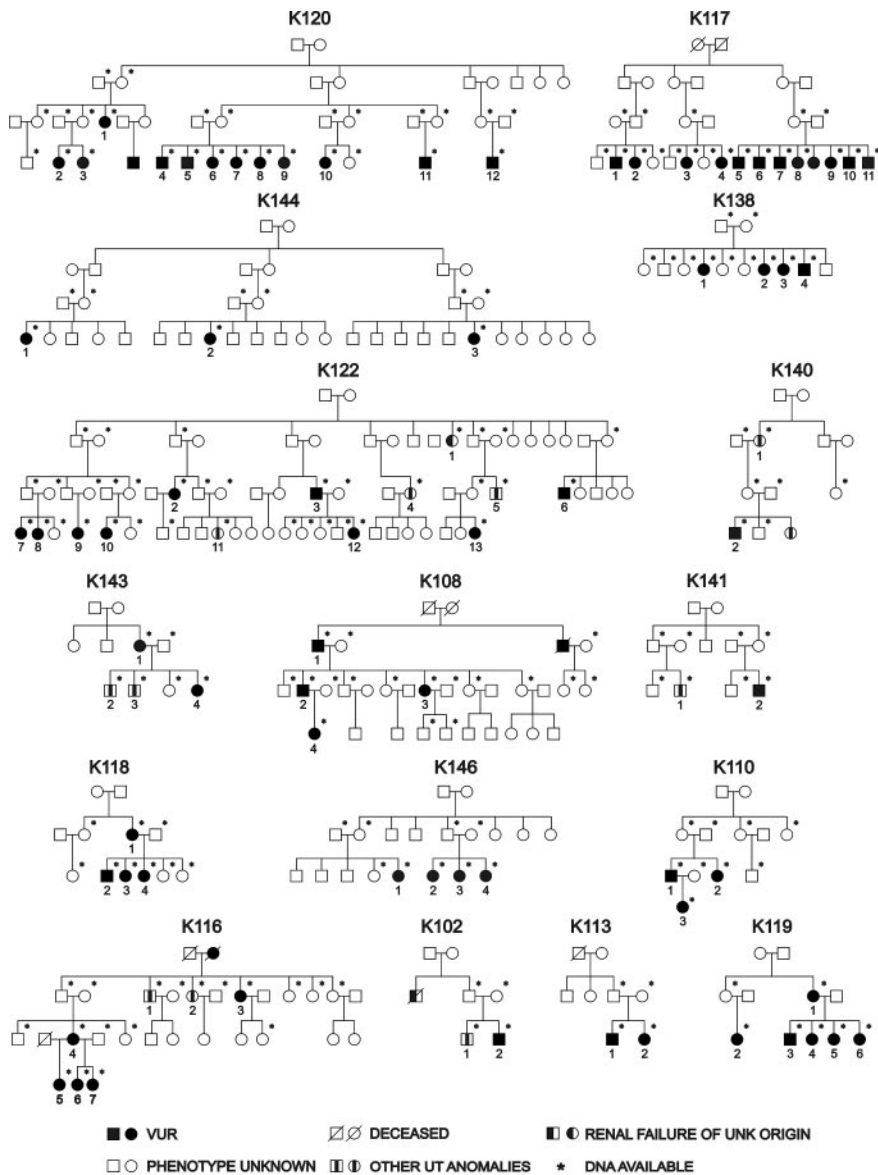


Figure 1. Pedigree structure of the 16 families studied. Asterisks (*) mark the individuals from whom DNA was available for the study. Patients with other urinary tract (UT) anomalies are indicated by a blackened rectangle within the symbol.

80%.^{9–12} Sibling recurrence rates of 30% to 65% have suggested segregation of a single gene or oligogenes with large effects.^{9,12–14} Interestingly however, the three published genome-wide linkage scans of pVUR have strongly suggested multifactorial determination.^{15–17} Two pVUR loci have been identified with genome-wide significance on chromosomes 1p13 and 2q37 under an autosomal dominant transmission with locus heterogeneity.^{15,16} Multiple suggestive signals have also been reported, but remarkably, these studies show little overlap.^{15–17} These data suggest that pVUR may be extremely heterogeneous, with mutations in different genes each accounting for a fraction of cases. The genes underlying pVUR loci have not yet

been identified, but two recent studies have reported segregating mutations in the *ROBO2* gene in up to 5% of pVUR families.^{18,19}

Despite evidence for genetic heterogeneity and different subtypes of disease, genetic studies have all modeled pVUR as an autosomal dominant trait.^{15–17,20} Recessive inheritance has generally not been considered because the absence of affected parents can be explained by spontaneous resolution of pVUR with older age. However, many pVUR cohorts are composed of affected sibships or pedigrees compatible with autosomal recessive transmission, suggesting the potential for alternative modes of inheritance.^{9–12,16,17,20–22} Systematic family screening to clarify the mode of inheritance is not feasible for pVUR because the standard diagnostic tool, the voiding cystourethrogram (VCUG), is invasive and would expose participants to radiation. Formal assessment of a recessive contribution in sporadic pVUR has also been difficult because studies have been conducted in populations with low consanguinity rates.^{9–12,16,17,20–22} However, recent studies have identified an unexpected recessive contribution to several complex traits such as ductus arteriosus or autism.^{23,24} Thus, in addition to genetic heterogeneity, genes with alternative modes of transmission may segregate among pVUR families, and misspecification of the inheritance model may complicate mapping studies of this trait.

Several approaches can be considered to address the difficulties imposed by complex inheritance, variable penetrance, and genetic heterogeneity. Studying large, well characterized cohorts with newer single-nucleotide polymorphism (SNP)-based technologies can maximize inheritance information across the genome and increase the power of linkage studies.²⁵ In addition, in the setting of locus heterogeneity and uncertainty about the mode of transmission, analysis under a dominant and a recessive model has greater power compared with nonparametric methods and more often results in detection of the correct mode of transmission without incurring a significant penalty for multiple testing.^{26–29} We combined these approaches in this study and successfully localized a major gene for VUR, which unexpectedly demonstrates autosomal recessive transmission.

Table 1. Pairwise HLOD scores for Chr12 p11-q13

Marker	Mb	pVUR Phenotype	Broad pVUR Phenotype
rs2054436	21.5	0.9	0.7
rs725124	23.7	0.7	0.4
D12S1591	24.0	2.2	1.2
TSC54265	25.1	0.5	0.8
D12S1596	25.8	1.5	1.2
rs2343866	26.2	0.2	0.0
rs1388659	27.1	0.4	0.3
TA27A06P	27.5	0.6	0.2
D12S1643	29.2	1.4	0.7
rs958478	29.3	0.1	0.0
D12S1681	30.4	0.9	0.3
D12S1584	31.6	0.7	0.1
D12S345	33.0	2.6	1.9
D12S2080	33.3	0.9	0.4
rs1352123	37.4	0.6	0.9
rs721709	39.0	1.6	1.4
D12S1048	39.3	1.8	1.5
rs2215456	40.6	1.6	0.9
D12S1589	40.7	1.6	1.5
D12S291	41.7	2.8	2.8
rs721483	42.0	0.2	0.3
TA91H06M	42.4	0.8	0.5
D12S1687	43.0	1.3	0.7
rs1377002	43.2	0.3	0.2
D12S85	45.6	0.3	0.2
rs215389	46.1	1.3	1.4
rs2254210	46.6	0.3	0.3
D12S2196	47.0	1.3	1.2
rs953673	47.4	1.4	1.1
D12S1627	48.1	1.9	1.7
rs4133070	48.4	1.6	1.8
rs1316607	49.3	0.9	1.2
D12S361	49.8	1.6	2.2
D12S1712	50.7	2.0	1.9
UT5029	50.9	1.3	1.4
rs686339	51.3	0.1	0.1
D12S398	51.5	3.8	3.1
D12S1586	52.4	1.6	1.6
D12S1707	53.3	1.0	1.2
rs2371455	55.1	0.5	0.6
D12S1644	55.8	1.0	0.4

Bolded numbers indicate HLOD scores >2. Broad phenotype includes individuals with pVUR or other urinary tract abnormalities.

RESULTS

We identified 16 large Caucasian pedigrees from the United States ($n = 8$) and Italy ($n = 8$) ascertained through an index case with pVUR documented by positive VCUG and obtained DNA from 200 individuals (Figure 1 and Supplemental Table S1). Of the United States kindreds, six were of Hasidic Jewish origin (K117, K120, K122, K138, K144, K146), and two were of Irish American origin (K118, K119). Among the 184 relatives of the probands, 56 individuals (30%) had pVUR based on a positive VCUG without other renal or urologic defects, and 11

individuals (6%) had urinary tract abnormalities other than pVUR (Supplemental Table S1). The prevalence of pVUR and other urinary tract abnormalities among relatives was approximately 30-fold and 60-fold higher, respectively, than the reported prevalence in the general population.^{14,30} These data are consistent with the known familial aggregation of pVUR and other urinary tract defects, indicating a strong genetic effect on these traits. The remaining 117 individuals were considered as phenotype unknown. Based on our primary phenotype of pVUR without other urologic abnormalities, 12 families were informative for linkage (72 affected individuals, 22 males and 50 females). If individuals with non pVUR urinary tract defects were also considered as affected, all 16 families were informative (broad pVUR phenotype, 83 affected individuals, 28 males and 55 females).

Interpretation of modes of inheritance in pVUR is complicated as a result of incomplete penetrance of the trait as well as spontaneous resolution of disease with increasing age. Examination of the pedigree structure revealed that seven families (44%) demonstrated parent-child transmission, suggestive of autosomal dominant inheritance. Absence of parent-child transmission in the remaining families was compatible either with dominant transmission with incomplete penetrance or recessive transmission of a high frequency gene. In the setting of genetic heterogeneity and uncertain mode of inheritance, parametric, LOD-based analysis under two simple models (dominant and recessive) is more powerful than allele sharing linkage methods.^{26–28} Therefore, our primary analysis was performed by computing heterogeneity LOD scores under both dominant and recessive models, using pVUR as our primary phenotype (12 informative families: K108, K110, K113, K116, K117, K118, K119, K120, K122, K138, K144, K146).

As with previous pVUR genome scans, analysis under genetic homogeneity did not identify any significant linkages under the dominant or recessive models.^{15–17} Under genetic heterogeneity and dominant transmission, the highest genome-wide heterogeneity LOD (HLOD) was on chromosome 8 (multipoint HLOD = 1.7, $\alpha = 0.6$, nonparametric linkage [NPL] = 1.3, $P = 0.05$). However, after saturating this locus with 16 microsatellite markers, the HLOD decreased to zero. Combining microsatellite with SNP data has been shown to increase information content and improve the resolution of genome scans.²⁵ Therefore, to exclude low marker informativeness as a cause of false negative results, we performed genome-wide SNP genotyping (Affymetrix 10K arrays) in 95 individuals in the most informative families. This analysis did not reveal novel loci across the genome, indicating that the absence of linkage under the dominant model is not due to lack of marker informativeness. *Post hoc* analysis of the seven pedigrees with parent-child transmission also did not reveal any promising signals. Hence, we found no significant or suggestive loci across the genome under the dominant model (supplemental Figure S1).

In contrast, heterogeneity analysis under the recessive model identified a single promising signal on chromosome 12

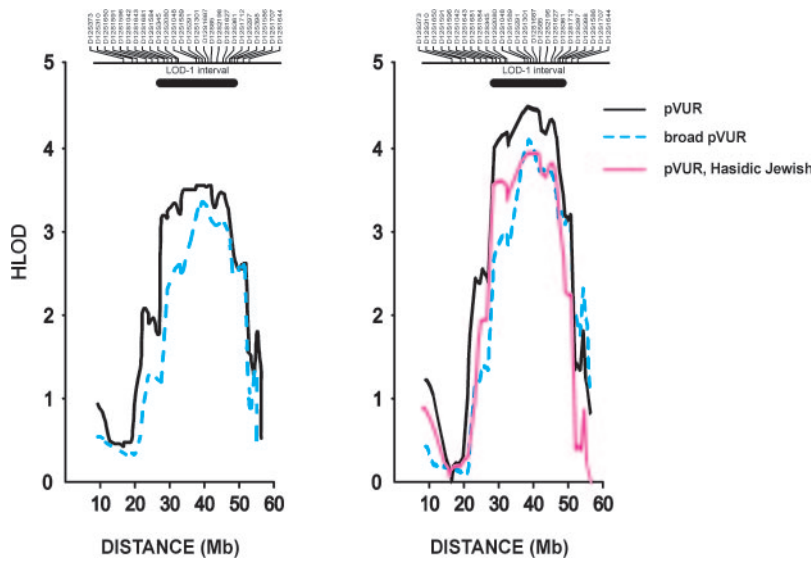


Figure 2. (A) HLOD plot of chromosome 12p11-q13 locus in the full cohort. The multipoint HLOD scores for the pVUR and broad pVUR phenotypes are shown on the y-axis. The x-axis denotes Mb distance based on the NCBI human physical map build 36.3. The location of the microsatellite markers genotyped is shown above the graph. The LOD-1 interval is indicated by the horizontal bar above the HLOD curve. (B) HLOD plot after *post hoc* exclusion of K117 and K122.

Table 2. Peak multipoint LOD scores for each individual pedigree at the chromosome 12p11-q13

Pedigree	Ethnicity	LOD pVUR Phenotype	LOD Broad Phenotype
120	Ashkenazi Jewish	1.9	1.9
138	Ashkenazi Jewish	1.5	1.5
119	Irish-American	1.0	1.0
108	Italian	0.8	0.8
116	Italian	0.5	-1.0
146	Ashkenazi Jewish	0.5	0.5
144	Ashkenazi Jewish	0.1	0.1
110	Italian	-0.5	-0.5
113	Italian	-0.6	-0.6
118	Irish-American	-0.7	-0.7
117	Ashkenazi Jewish	-1.2	-1.2
122	Ashkenazi Jewish	-1.4	-1.6
143	Italian	N.I.	0.6
140	Italian	N.I.	0.1
141	Italian	N.I.	-0.1
102	Italian	N.I.	-0.4

Broad phenotype includes individuals with pVUR or other urinary tract abnormalities. N.I., pedigree is not informative for pVUR phenotype.

with an HLOD of 1.4 on pairwise analysis (D12S297, $\alpha = 0.64$). Multipoint analysis augmented the HLOD to 2.7 across this interval ($\alpha = 0.65$, peak at D12S1301). Importantly, this was the only peak with multipoint HLOD ≥ 2 across the entire genome under either the dominant or recessive model (Supplemental Figures S1 and S2). To confirm this finding, we performed high resolution mapping with 22 additional microsat-

elite markers and combined these with the SNP data, resulting in a mean intermarker distance of 0.77 Mb across the chromosome 12p11-q13 region. We observed positive pairwise HLODs across all markers within this interval, including one marker with genome-wide significance (D12S398, HLOD = 3.8, $\alpha = 0.82$, Table 1).³¹ Next, multipoint analysis of linkage confirmed these results (multipoint HLOD 3.6 at marker D12S1048, $\alpha = 0.6$, Figure 2A). This result exceeds traditional genome-wide significance thresholds.^{31,32} Furthermore, based on 1000 simulated genome-scans of the pedigrees under the assumption of no linkage, the empiric genome-wide significance thresholds for testing two models under genetic heterogeneity were 2.7 and 3.3 at $P = 0.05$ and 0.01, respectively. The HLOD of 3.6 therefore corresponds to an empiric genome-wide P value of 0.005, confirming genome-wide significance of findings by empiric criteria. The LOD-1 interval spans 22.7 Mb between markers rs1388659 and D12S361 and includes 161 genes. We next performed comparison of haplotypes across the chromosome 12 linkage regions to ex-

lore the possibility of a founder mutation, but found no evidence of shared segments among Hasidic or among Italian pedigrees (Supplemental Table S2). However, since these pedigrees were not closely related, the shared segments may be below the resolution of our typed markers.

Alternative analyses were also performed to determine whether varying model parameters, analytic algorithm, or phenotype assignment impacted the chromosome 12 linkage results. Examination of pedigree LOD scores revealed that both Hasidic and nonHasidic pedigrees contributed to the linkage signal on chromosome 12. In addition, multipoint analysis in the six Hasidic Jewish pedigrees using marker allele frequencies from ethnicity matched controls revealed a peak HLOD of 2.6 (marker D12S291, $\alpha = 0.6$). This further confirmed that most, but not all of the linkage signal originated from this subgroup (Table 2). Varying gene frequency (0.01 to 0.1) had negligible effects on overall linkage findings, with the best LOD scores achieved by modeling a high frequency risk allele (Table 3). Nonparametric analyses were also conducted and yielded a NPL score of 4.0 and $P = 1 \times 10^{-4}$ (at D12S1301), which falls just below the genome-wide significance threshold. Finally, to determine the impact of a broader phenotype assignment, we repeated the linkage analysis after including as affected all individuals with other renal and uro-

Table 3. Peak multipoint HLOD scores at chromosome 12p11-q13 with varying disease gene frequency

Disease Gene Frequency	0.01	0.05	0.10
HLOD (α), pVUR phenotype	3.4 (0.7)	3.6 (0.6)	3.4 (0.65)
HLOD (α), broad pVUR phenotype	3.2 (0.6)	3.4 (0.5)	3.0 (0.5)

Broad pVUR phenotype includes individuals with pVUR or other urinary tract abnormalities. The α value indicates the percent of families linked.

logic clinical disorders. This expanded the cohort to 16 pedigrees and 83 affected individuals. Despite the broader phenotype, linkage to chromosome 12p11-q13 was confirmed, with a peak HLOD score of 3.4 ($\alpha = 0.5$, Figure 2A). This locus remained the only suggestive or significant signal across the genome under either phenotype assignment scheme, with the next best multipoint HLOD score being 1.0 on chromosome 8. Altogether, these data demonstrate that the chromosome 12 linkage results were robust to varying analytic parameters and phenotype assignment criteria.

Finally, we scrutinized the genome in two large families (K117 and K122) that did not demonstrate linkage to chromosome 12 and were each large enough to achieve genome-wide significance (LOD >3). Remarkably, there was no evidence for linkage across the entire genome in either kindred under both dominant and recessive inheritance, even after incorporation of genome-wide SNPs into microsatellite data. The best signals were multipoint LOD scores of 1.3 with the dominant model on chromosome 8 (D8S592) for K117 and 1.2 with the recessive model for K122 on chromosomes 2 (SraP) and 10 (D10S1412), which are all below the suggestive significance threshold. Given the large size of these pedigrees and the comprehensive analyses performed across the genome, the absence of any linkage signals cannot be attributed to genetic heterogeneity or low power. These data suggest that problems such as complex structure or bilinear inheritance likely confound the analysis of linkage in these pedigrees. Because these two pedigrees did not map anywhere across the genome and consequently could only obscure linkage signals in our study, we performed *post hoc* analysis of the chromosome 12 locus after exclusion of these two complex kindreds (Figure 2B). This resulted in HLOD scores of 4.5 ($\alpha = 0.8$) and 4.1 ($\alpha = 0.7$) on chromosome 12p11-q13 for the pVUR and broad pVUR phenotypes, respectively (Figure 2B). Significantly, the remaining four Hasidic Jewish pedigrees demonstrate a LOD score of 3.9 with genetic homogeneity across the same region, indicating that most of the linkage signal originates from this subgroup (Figure 2B).

DISCUSSION

In the present study, we combined multiple approaches to overcome problems such as complex inheritance, incomplete penetrance, and genetic heterogeneity to localize pVUR susceptibility loci. Instead of studying sib pairs with nonparametric methods, we ascertained uniquely large families and analyzed the genome under both dominant and recessive transmission, because this approach avoids potential misspecification of the genetic model and maximizes power in the analysis of complex traits.^{26,29} The genome scan under the dominant model provided no signals across the entire genome. On the other hand, analysis under a recessive model localized a major susceptibility gene to an approximately 22-Mb interval on chromosome 12p11-q13, with a peak HLOD score of 3.6 ($\alpha = 0.6$, NPL = 4.0, $P = 1 \times 10^{-4}$). This linkage peak was the

only positive signal across the entire genome, exceeding genome-wide and empiric significance thresholds, and was not significantly changed by alternative analytic models or incorporation of a broader phenotype. These data emphasize the utility of parametric models for detection of major genes underlying complex traits.

The localization of a major gene with the recessive model may seem surprising because the literature has primarily focused on VUR segregating as a dominant trait.^{15,16,19,33–35} The absence of disease transmission across multiple generations has usually been attributed to incomplete penetrance or undetectable VUR due to spontaneous resolution in older individuals. However, given that many pVUR families consist of affected sib pairs, and considering the high degree of genetic heterogeneity of this trait, it is likely that genes with different modes of inheritance segregate among different pVUR pedigrees. The lack of parent-offspring transmission may therefore represent true recessive inheritance in some kindreds. Low penetrance recessive alleles imparting large effects have been implicated in several other complex traits such as patent ductus arteriosus, Hirschsprung disease, or autism.^{23,24,36} For these traits, gene localization was achieved by homozygosity mapping in consanguineous populations that were enriched for recessive disorders.^{23,24,36,37} Although we did not set out to study consanguineous populations, our cohort included six Hasidic Jewish kindreds, which contributed a significant proportion (but not all) of the linkage signal on chromosome 12 (Figure 2B). This population has many characteristics of a classic isolate, such as a limited set of founders, high endogamy, and recent expansion.^{38,39} In such a population, a common predisposing allele can achieve a high frequency due to founder effects, selection, or drift, enhancing the probability of recessive disorders.^{39,40} However, because several other pedigrees also contributed to the chromosome 12 linkage signal, these data suggest that recessive transmission applies to pVUR families of varying ethnicity.

The complexity of pVUR is further demonstrated by the lack of compelling signals across the genome in two high-density Hasidic Jewish kindreds (K117 and K122). These kindreds contained a total of 21 pVUR cases and were each predicted to be large enough to exceed LOD >3 under dominant (K117 and K122) or recessive (K117) models. Thus, the absence of linkage cannot be attributed to low power and genetic heterogeneity. Moreover, phenotyping error is unlikely, since all affected had VCUG-documented VUR. Such high-density pedigrees are commonly enriched for intrafamilial heterogeneity (the situation where affected individuals in a family have different risk alleles in the same gene or different genetic forms of disease).⁴¹ Therefore, the most likely alternative explanation is that these pedigrees have a complex hidden genealogical structure, such that risk alleles segregate across multiple lines of descent. This is a common phenomenon in population isolates, and the presence of all affected sibships in K117 would further support this possibility. Although intrafamilial heterogeneity reduces power in linkage analysis of complex traits, it does not produce

false positive signals nor necessarily lead to false exclusion of linkage.⁴¹ The effects of such confounders can be mitigated by studying large cohorts and applying systematic analytic approaches, as demonstrated by the successful localization of a pVUR gene in our study.

There are 161 genes in the conservative LOD-1 interval on the chromosome 12 p11-q13 locus (National Center for Biotechnology Information [NCBI] Map Viewer). Among these, 19 genes have been implicated in human traits (OMIM). Based on a recently published study, 83 positional candidates have murine homologs and detectable expression in the murine metanephric mesenchyme and ureteric bud tip and stalk (Supplemental Table S3).⁴² These positional candidates may be pursued by systematic sequence analysis; however, it would be preferable to achieve further reduction of this locus through an interval-specific association study.^{43–45} Since the *post hoc* analysis indicated a strong linkage signal with homogeneity among the Hasidic Jewish families, association mapping may be especially suitable in this population because of its limited number of founders.^{38,39,46,47} Furthermore, one would expect enrichment for homozygous segments surrounding susceptibility genes among pVUR cases from this population. We did not detect regions of homozygosity within the chromosome 12 locus in the Hasidic Jewish patients, but this may be due to the lack of close consanguinity among families studied. Therefore, a higher resolution analysis may be required to detect autozygous segments in these kindreds.

These findings have many important implications for future genetic studies of pVUR. Because the chromosome 12 signal originated from pedigrees of varying ethnicity, recessive transmission may be applicable to pVUR kindreds from many different populations. Thus, in addition to genetic heterogeneity, variable modes of transmission should be considered in all pVUR linkage scans, and analysis under recessive transmission is henceforth warranted. It is also worth noting that sporadic disease cannot be differentiated from recessive transmission in the absence of an affected family member or consanguinity. Consequently, the recessive contribution to pVUR may have been underestimated in nonfamilial cases as well. If recessive transmission accounts for a significant fraction of sporadic pVUR, this offers a potentially powerful setting for a genome-wide association study. As demonstrated by recent studies of age-related macular degeneration, modest sized case/control cohorts may be quite successful in such situations.⁴⁸ Thus, association scans in sporadic pVUR may offer an additional promising approach for resolving the genetic basis of this trait.

CONCISE METHODS

Patients and Phenotypes

All families were ascertained through an index case diagnosed with primary VUR by a VCUg. Index cases and family members had no evidence of secondary causes of VUR or syndromic abnormalities. In addition, we conducted extensive family history interviews and

searched medical records to identify family members diagnosed with pVUR documented by VCUg or any clinical or urologic abnormalities that may be pathogenically related to pVUR (e.g., other urologic anomalies or a diagnosis of ESRD in absence of any obvious cause such as glomerulonephritis and/or diabetes mellitus). This led to the identification of 56 relatives with pVUR and 11 relatives with other renal or urologic abnormalities (Supplemental Table S1). For linkage analysis, we considered all individuals with pVUR diagnosed with VCUg as affected, leaving all others as phenotype unknown. Subsequently, we performed linkage analysis by also including the 11 additional family members with clinically related abnormalities in the affected cohort. Because VUR is known to resolve with age, we classified individuals that did not undergo a VCUg and those with a negative VCUg as phenotype unknown in all analyses (affected only analysis). All individuals gave informed consent, and the study protocol adhered to the Declaration of Helsinki and was approved by the Western Institutional Review Board for Columbia University and the ethics committees at the University of Brescia and at the Gaslini Institute.

Genotyping

Total genomic DNA was isolated from peripheral white blood cells of the patients and relatives using standard procedures. We performed genome-wide scans using both microsatellites and SNPs. The microsatellite scan was performed with 393 microsatellites (intermarker distance approximately 10 cM) genotyped across the genome in all 200 individuals (Marshfield Mammalian Genotyping Service). To maximize inheritance information across the genome, we also typed 95 individuals (65 affecteds) in the most complex families with 10,204 SNPs using the GeneChips Mapping 10K 2.0 Arrays (Affymetrix, Santa Clara, California). DNA processing and gene-chip hybridization were performed as suggested by the manufacturer. We fine-mapped two loci suggestive of linkage on chromosome 8 and chromosome 12 (with 16 and 22 polymorphic microsatellite markers, respectively). Integration of the most informative SNPs from 10K GeneChip data (minor allele frequency [MAF] ≥ 0.2) with the fine mapping microsatellites on the chromosome 12 locus yielded an average marker spacing of 0.77 Mb and average information content of 0.9 (standard deviation = 0.1).

Analysis of Linkage

We performed pairwise and multipoint analyses of linkage, using FASTLINK4.1,⁴⁹ and SimWalk2 2.90,⁵⁰ respectively. Since pVUR is known to be genetically heterogeneous, we computed parametric LOD scores under a dominant and a recessive model, with disease gene frequencies of 0.01 and 0.05, respectively. We used penetrance of 75% and phenocopy of 0.01 for both models (since affected only analysis was performed, penetrance parameters did not affect LOD statistics). For comparison, we concurrently computed nonparametric statistics with the SimWalk2 program (NPL_{pairs} score and associated exact *P* value). We calculated allele frequencies on the basis of the frequencies observed in the dataset for the genome-wide microsatellites, whereas for the SNP data, we based frequencies on Caucasian allele frequencies provided by Affymetrix. In the fine mapping experiments, we obtained control allele frequencies from 40 Ashkenazi Jew-

ish individuals. We used published thresholds for significant linkage under heterogeneity ($LOD = 3.3$).^{31,32} Moreover, we estimated empiric thresholds of significance by performing 1000 genome scans with microsatellites spaced every 10 cM across the genome under the hypothesis of no linkage, using the same structure as our pedigrees, and performing pairwise genome scans under the dominant and recessive models described above.

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The URLs for data presented herein are as follows:

NCBI Map Viewer, <http://www.ncbi.nlm.nih.gov/mapview> (build 36.3).

Online Mendelian Inheritance in Man (OMIM), <http://www.ncbi.nlm.nih.gov/Omim> (vesicoureteral reflux).

NCBI HomoloGene, <http://www.ncbi.nlm.nih.gov/homologene> (release 63)

DISCLOSURES

None.

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