A Locus for Renal Malformations Including Vesico-Ureteric Reflux on Chromosome 13q33–34

Kalyani R. Vats,* Chandra Ishwad,† Ish Singla,* Ashutosh Vats,‡ Robert Ferrell,† Demetrius Ellis,* Michael Moritz,* Urvashi Surti,§ Parul Jayakar,‖ Donald R. Frederick,* and Abhay N. Vats*

**Department of Pediatrics, Children's Hospital of Pittsburgh, †Department of Human Genetics, Graduate School of Public Health, and §Division of Cytogenetics, Department of Pathology, Magee Womens Hospital, University of Pittsburgh School of Medicine, Pittsburgh, Pennsylvania; ‡Division of Bioinformatics, Atharva BioSciences, Herndon, Virginia; and ‖Division of Genetics and Metabolism, Department of Pediatrics, Miami Children's Hospital, Miami, Florida*

Congenital anomalies of kidney and urinary tract (CAKUT), including vesico-ureteric reflux (VUR), are major causes of ESRD in childhood. Herein is reported evidence for a locus on 13q33q34 associated with CAKUT. Deletion mapping of chromosome 13q was performed in four children with CAKUT using 31 microsatellite markers on peripheral blood genomic DNA that was obtained from the patients and their parents. mRNA expression of the positional candidate genes was compared with sequences in electronic databases *in silico* and also studied in adult and fetal mouse kidneys using reverse transcription–PCR. The children (three girls; age range 5 to 17 yr) had varying severity of developmental delay and other organ system involvement. The spectrum of CAKUT included high-grade VUR ($n = 2$), renal dysplasia ($n = 2$), and hydronephrosis ($n = 1$). Both the children with VUR had evidence of renal failure with one of them developing ESRD. Deletion mapping identified a 7-Mb critical region flanked by markers D13S1311 and D13S285. There are 33 genes (12 known; 21 computer predicted) in this region. *In silico* expression studies showed matches for 14 of these genes in the kidneys and 10 in the bladder expressed sequenced tags databases. Mouse kidney studies showed that of the 24 genes examined, several had variable expression through the different stages of renal development, whereas five of the genes were not expressed at all. Herein is reported a new locus on chromosome 13q33q34 that can be associated with VUR with several genes showing mRNA expression patterns that suggest their potential for involvement in renal/urinary tract developmental anomalies.

J Am Soc Nephrol 17: 1158–1167, 2006. doi: 10.1681/ASN.2005040404

Congenital anomalies of the kidney and urinary tract (CAKUT), including vesicoureteral reflux (VUR [MIM 193000]), are major causes of renal failure in childhood (1–4). Although CAKUT accounts for approximately 0.5% of all pregnancies, the true incidence of CAKUT probably is much higher (3–7). Almost 30% of children who present with urinary tract infection (UTI) may have VUR. VUR can cause renal parenchymal scarring, and reflux nephropathy (RN) accounts for up to 15% of end-stage renal failure in children and adults (4,6,8–10). Many of these abnormalities, such as VUR and renal dysplasia, often coexist and may take a familial pattern, showing incomplete and variable penetrance (1,4,5,11). VUR also has been reported in 30 to 50% of siblings of affected children, and it has been suggested that VUR possibly is inherited in an autosomal dominant manner with incomplete penetrance (1,8,11). Although the cause of CAKUT probably is multifactorial, identification of underlying genetic mechanisms can improve our understanding of this complex disease (12–15).

CAKUT including VUR can occur both in isolation and in association with various malformation syndromes. In the past decade, several genes for both syndromic and nonsyndromic forms of CAKUT have been studied (11–19). Various genes have been identified to be associated with CAKUT in several human syndromes, such as branchio-oto-renal and hypoparathyroidism deafness renal anomaly (HDR) syndromes (17–19). A single genome-wide study reported a locus that spanned 20 cM on 1p13 with 78% locus heterogeneity to be associated with primary nonsyndromic VUR/RN in seven European families (20). That study also reported a weaker association of VUR with several other chromosomal loci, including a 13q locus. However, recently, in another study, the linkage of another set of families with familial VUR to various previously reported candidate intervals could not be replicated (21). All of these studies point to the genetic heterogeneity of CAKUT including VUR. We have identified several children with 13q deletion and

Received April 15, 2005. Accepted February 2, 2006.

Published online ahead of print. Publication date available at www.jasn.org.

The software programs described in this study are available free of charge by contacting the authors. We are currently enrolling additional patients with 13q deletion for our studies and would appreciate contact from interested health care providers and patient families. The contact information is provided below.

Address correspondence to: Dr. Abhay N. Vats, Department of Pediatrics, Division of Pediatric Nephrology, Children's Hospital of Pittsburgh, 3705 Fifth Avenue, Pittsburgh, PA 15213. Phone: 412-692-5182; Fax: 412-692-7443; E-mail: abhay.vats@chp.edu

Copyright © 2006 by the American Society of Nephrology

ISSN: 1046-6673/1704-1158

recently reported a CAKUT-associated locus on 13q12–22 (22). We now describe the results of cyto- and molecular genetic studies to identify a second region on chromosome 13 that is located on 13q33–34 and is associated with CAKUT including severe VUR and RN.

Materials and Methods

The study was approved by the Human Rights Committee and the Animal Research and Care Committee of the Children's Hospital of Pittsburgh. Informed consent was obtained from the parents or legal guardians of the children described in the study. The patients were investigated by various biochemical and radiologic tests, including ultrasonography, vesico-cysto-urethrography (VCUG), intravenous pyelography, computerized tomography and magnetic resonance imaging.

Cytogenetic and Deletion Mapping Studies

GTG banding was performed on peripheral blood lymphocytes from the proband and the parents using standard protocols as described previously (22,23). Genomic DNA was extracted from peripheral blood lymphocytes by proteinase K digestion and phenol-chloroform extraction for deletion mapping as described previously (22,24). Thirty-one microsatellite markers that spanned the 13q region were examined. Primer pairs were obtained commercially (Research Genetics, Huntsville, AL) or synthesized using primer sequences that are available in UniSTS database from Integrated DNA Technologies (Coralville, IA). Microsatellite markers were analyzed after amplification by PCR. The PCR reactions were performed as described previously (22,24,25). PCR products were diluted 1:1 with loading buffer (95% deionized formamide, 20 mM EDTA, 0.025% xylene cyanol, and 0.025% bromophenol blue), heat-denatured, and electrophoresed through 7% polyacrylamide gel that contained 5.6 M urea and 32% formamide for 3 h at 55°C. Gels were exposed to x-ray films at −80°C. Genotypes were scored visually, and deletion was mapped by comparing the genotypes of parents with those of the probands. The markers used were D13S787, D13S1493, D13S894, D13S626, D13S784, D13S791, D13S889, D13S891, D13S318, D13S776, D13S800, D13S265, D13S281, D13S167, D13S793, D13S154, D13S1252, D13S159, D13S779, D13S1267, D13S317, D13S174, D13S280, D13S1311, D13S796, D13S286, D13S895, D13S1315, D13S148, D13S261, and D13S285.

Gene Expression Studies

Gene Expression Analyses *In Silico*. We used computational techniques that were developed in our laboratory to perform *in silico* analyses to study the expression of 13q candidate genes in kidneys and urinary tract. We first created various local BLAST searchable databases for all of the expressed sequenced tags (EST) and reference sequences (RefSeq) that are expressed in either kidneys or urinary tract (renal and urinary tract transcriptome). The sequences were retrieved from the National Center for Biotechnology (NCBI) BLAST ftp site (ftp://ftp.ncbi.nlm.nih.gov/blast/db/) using a set of queries and programs. A program called nucSearch.pl was written in PERL script to submit queries to NCBI that retrieved the GenInfo Identifier (GI) numbers for various EST. Unique queries were used to create different BLAST searchable databases. For example, to create the database for genes that are expressed in lower urinary tract (ureter, bladder, and trigone transcriptome), we used queries "ureter AND mRNA," "trigone AND mRNA," and "urinary bladder AND mRNA." To create the "kidney transcriptome" database, the queries were "sapiens AND kidney AND mRNA," "norvegicus AND kidney AND mRNA," and "musculus AND kidney AND mRNA." Each set of files then was concatenated and used to create their respective databases using the NCBI "formatdb" program with the -F and -L options to alias the entire EST and RefSeq database and restrict the search to only those GI numbers that were a result of the queries. A second program, called giJobe.pl, also written in PERL script, was used to submit the GI numbers sequentially to NCBI (http://www.ncbi.nlm.nih.gov/entrez/batchentrez.cgi?db = Nucleotide) and to retrieve the FASTA sequence corresponding to the numbers. The retrieved FASTA sequences were formatted into four local BLAST databases that were created for the renally expressed genes of humans, mice, and rats and also a common database for all of the genes that are expressed in ureter, bladder, and trigone. All of the GI numbers of EST that lie on chromosome 13 between the two given microsatellite markers were retrieved from the NCBI. These FASTA sequences then were compared against various local BLAST databases separately using the -I option. The output files then were run through a parser program (called "BLAST Result Parser" available at http://cbi.swmed.edu/Computation/).

Gene Expression in Mouse Kidneys. Primers for amplifying cDNA were synthesized (Integrated DNA Technologies, Coralville, IA) for the genes that lie in the 13q33q34 candidate region, and their expression was studied in FVB mice fetal (embryonic day of life 16 [E16]), neonatal, and adult (21 d old) kidneys by reverse transcription–PCR (26). FVB mice (The Jackson Laboratory, Bar Harbor, ME) are so named because they carry the $Fv1^b$ allele for sensitivity to the B strain of Friend leukemia virus. This strain is useful for many fetal/neonatal experiments as they have a vigorous reproductive performance with large litters. All reagents, including enzymes, were obtained from Invitrogen Corp. (Carlsbad, CA). Briefly, 2 μg of total RNA was reverse transcribed into cDNA using Superscript II reverse transcriptase with oligo dT priming. Amplification of cDNA was performed (35 to 40 cycles) by PCR using the following condition: The cycles were initiated by denaturing the DNA at 94°C for 30 s, followed by annealing reaction for 30 s at 58°C and extending at 72°C for 45 s. The PCR products were resolved on 2% agarose gel and photographed using KODAK EDAS-2 gel documentation system (Eastman Kodak, New Haven, CT).

Results

We identified five children with 13q deletion in the 13q33–34 region. Four of these five children had a renal anomaly identified by renal ultrasound or clinical history, and incomplete clinical data were available on one child. Further studies were done on the remaining three patients and are presented here. One of the patients (patient 1) attended the clinics at Children's Hospital of Pittsburgh, and the others were identified through the Chromosomal Deletion Outreach organization (www.chromodisorder.org). Table 1 shows a summary of findings in the children studied, including the karyotype findings identified with GTG banding. Patient 1 had the most severe renal involvement with CAKUT as well as ESRD that led to dialysis. All of the children had involvement of other organ systems besides the kidneys/urinary tract and had varying severity of developmental delay.

Patient 1

This 17-yr-old girl in foster care presented with seizure disorder and mild developmental delay at the age of 12 yr. Investigations showed total calcium 4.3 mg/dl with ionized calcium 0.68 mmol/L, phosphate 7.7 mg/dl, HCO_3 18 mEq/L, and serum creatinine 10.5 mg/dl, suggesting end-stage renal failure. Correction of hypocalcemia ameliorated the seizure disorder. A VCUG showed outpouching of bladder dome, presumed

Table 1. Summary of clinical findings in children with 13q deletion and renal involvement[a]

Patient	Current Age	Gender	Age at Diagnosis	Karyotype	Kidney Abnormalities	Other Abnormalities
1	17	F	12 yr	46, XX, del(13)q32.3	VUR (grade 5), renal dysplasia, ESRD	Mild developmental delay, seizures
2	5	F	8 mo	46, XX, del(13)q33q34	VUR (grade 5), mild renal failure, recurrent UTI	Neurodevelopmental delay, hypotonia; seizures
3	13	F	5 wk	46, XX, del(13)q32q34	Hydronephrosis, hydroureter, small kidneys, recurrent UTI, hematuria and proteinuria	Abnormal anal opening (anteriorly placed), constipation, spinal curvature, hemivertebrae, cognitive delay, neurodevelopmental delay

[a]VUR, vesico-ureteric reflux;UTI, urinary tract infection.

to be urachal remnant, a single left-sided bladder diverticulum, and a right sided grade 4 VUR (Figure 1, A and B). A renal ultrasound showed left echogenic kidney that measured 5 cm in length (Figure 1C). The right kidney was caudally displaced and hyperechoic and measured 5.5 cm in length. Urinalysis showed 1+ proteinuria with two to three white cells per high-power field and no red blood cells. A computerized tomography scan that was obtained at 2 yr of age had shown no structural malformations of brain. She was found to have a 13q32.3 deletion (Figure 2A). She began dialysis soon after initial presentation and has been seizure-free since initiation of dialysis except for one brief episode, lasting 30 s, approximately 18 mo after initiating hemodialysis.

Patient 2

This 5-yr-old girl was born full term. Her postnatal course was complicated by hypotonia, feeding difficulties, seizure disorder, and poor weight gain. She had mild facial dysmorphism and developmental delay (Figure 1D). She was found to have chromosome 13q33q34 deletion at 8 mo of age (Figure 2B). Subsequently, she developed recurrent UTI, including several episodes of severe pyelonephritis. A renal ultrasound at 2 yr of age showed slightly small kidneys with normal shape and echogenicity. A VCUG showed bilateral VUR (grade 4 to 5). A magnetic resonance imaging scan showed no structural malformations of brain. She underwent a right cutaneous ureterostomy and left uretero-ureterostomy at 30 mo of age. Her serum creatinine at 3 yr of age was elevated at 1.1 mg/dl.

Patient 3

This 14-yr-old girl was born full term and received a diagnosis of chromosome 13q32q34 deletion at 5 wk of age. She had recurrent UTI until 3 yr of age and required frequent antibiotic courses. A renal ultrasound that was performed at 15 mo of age showed grade 2 hydronephrosis of the left kidney with dilation of the left proximal ureter. The left kidney measured 5.1 cm, and the right measured 5.3 cm in length at that time. She was not found to have VUR on a VCUG that was performed at approximately the same time, but it did show a large-capacity bladder with a volume of 250 ml and significant residual volume. She also had dysfunctional urinary voiding until approximately 3 yr of age. Serum blood urea nitrogen and creatinine that were obtained at 10 yr of age were 11 and 0.7 mg/dl, respectively. Subsequent renal ultrasound at 12 yr of age (weight 40 kg) showed resolution of urinary tract dilation but showed small-sized kidneys (left 7.5 cm and right 7.8 cm) with normal echogenicity. She also had hematuria and proteinuria on urinalyses (generally performed with UTI episodes), which resolved. She developed enuresis at 13 yr of age, which spontaneously resolved during the next year. Her other problems include a T7 hemivertebra and malformed vertebrae from T8 to T12 and a clinically visible spinal curvature and scoliosis at T12/L1 level. She also has an abnormal anteriorly placed anal opening and severe constipation. She underwent an Achilles tendon release at the age of 3 yr and can walk and ambulate well now. She has had significant cognitive delay and currently functions at a 2.5- to 3-yr-old level (Figure 1E). She has no spoken language except for a few words but has good motor skills.

Cytogenetic and Deletion Mapping Studies

The details of the karyotype for patients 1 and 2 as well as an ideogram of the deleted region is shown in Figure 2, C through E. Table 2 shows the details of deletion mapping studies. The PCR product sizes for each marker pair and the visually ascertained alleles for each individual studied (arbitrarily labeled alphabetically) are shown. The deleted chromosomal region for each patient is shown in boldface type in Table 2. The order of the markers is based on the May 2004 assembly of the human genome available through University of California Santa Cruz genome browser (http://genome.ucsc.edu). The genetic distances for the markers were derived from the genetic location database (ftp://cedar.genetics.soton.ac.uk/pub/chrom13/map.html). Patient 1 had only maternal blood sample available, and deletion studies were performed using a smaller

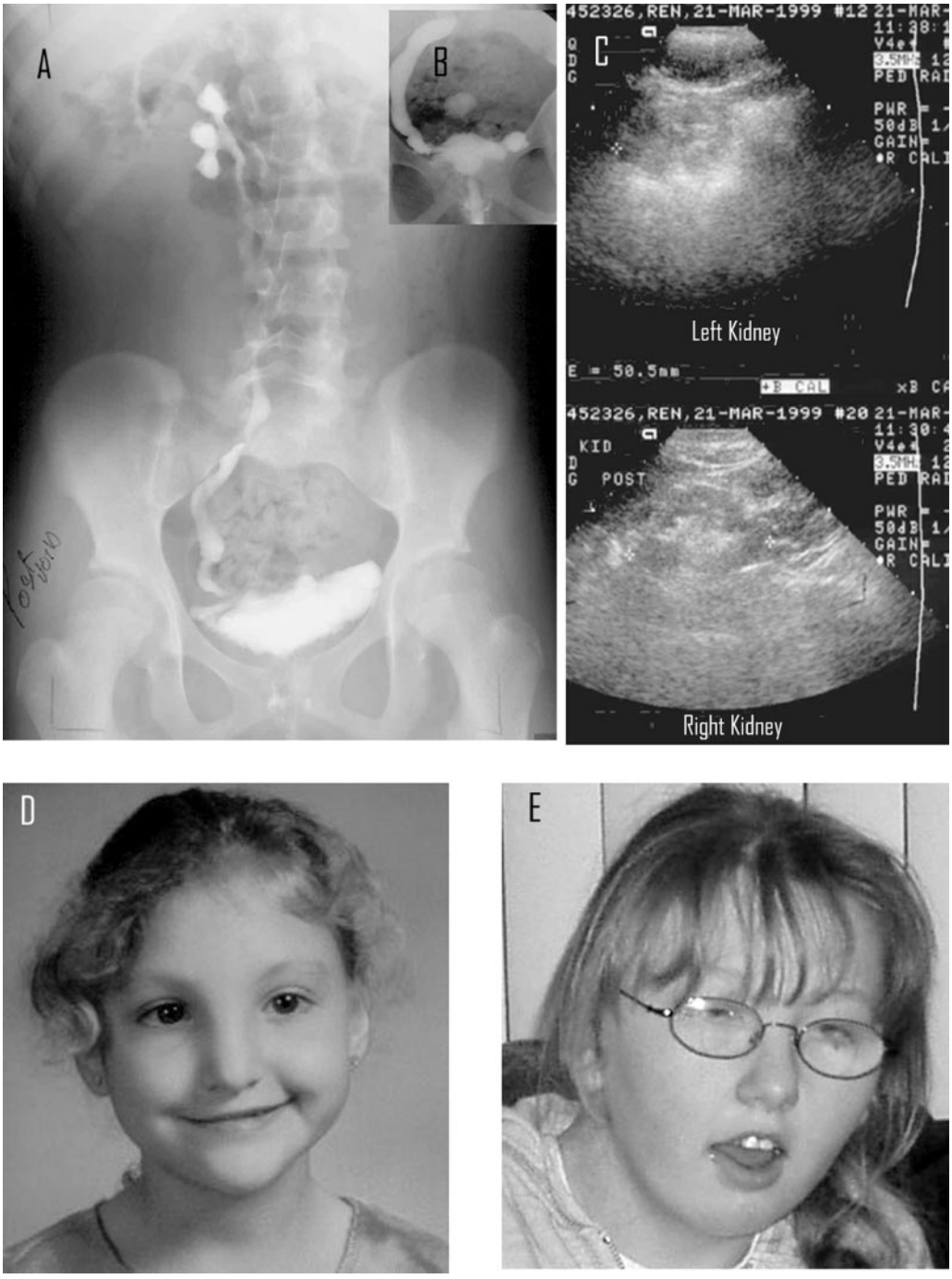

Figure 1. Clinical findings of patients with 13q deletion. (A) Vesico-cysto-urethrography (VCUG) of patient 1 showing a right-sided grade 4 vesico-ureteric reflux (VUR). (B) An outpouching of bladder dome, presumed to be urachal remnant is shown; a single left-sided bladder diverticulum is shown in inset. (C) Renal ultrasound showing echogenic kidneys. The left kidney measured 5 cm in length, and the right kidney measured 5.5 cm in length. (D and E) Mild facial dysmorphic features of patient 2 (D) and patient 3 (E). The facial pictures are published with signed consent of the parents of both children.

number of microsatellite markers because of limited DNA availability as she was in foster care. Paternal blood sample was not available for patient 1, and her deletion mapping studies were interpreted to show a deletion that extended from D13S779 to D13S285 because this region coincided with the deleted region that was visible on the karyotype. She had apparent loss of paternal alleles. Patient 2 showed a deletion from D13S1311 to at least D13S285 with a loss of paternal alleles. The distal limit of the deletion was not mapped for patient 2. Patient 3 also showed a deletion that extended from D13S779 to D13S285 with loss of maternal alleles. Our studies suggest existence of a critical region in the 13q33q34

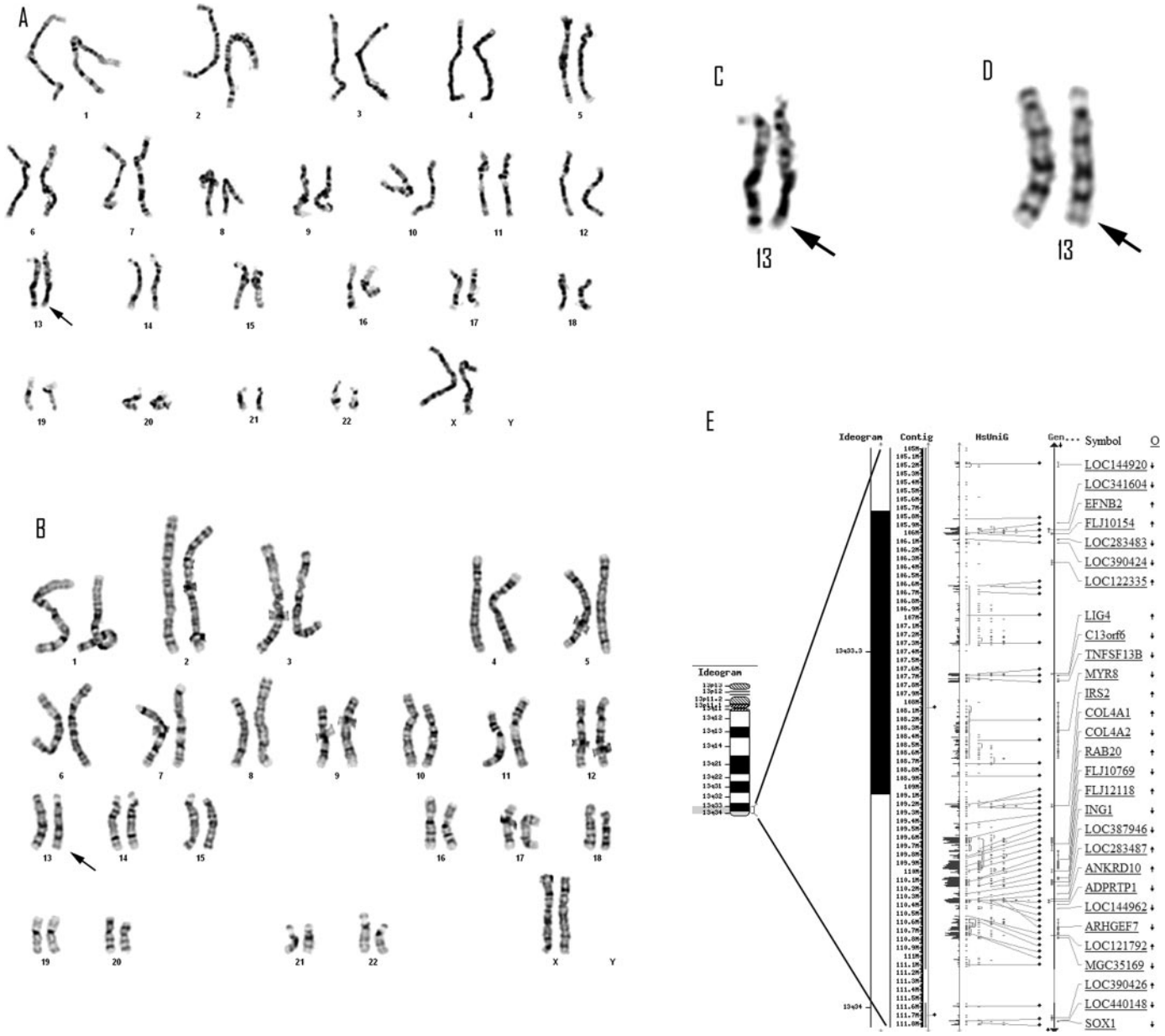

Figure 2. (A and B) Karyotype of patient 1 (A) and patient 2 (B) showing deleted 13q region (arrows). (C and D) Magnified banding pattern of chromosome 13 of patient 1 (C) and patient 2 (D) showing loss of bands in the 13q33q34 region. (E) The critical 13q33q34 region shown in an ideogram and some of the genes in the region displayed as symbols.

area that is involved in the development of CAKUT, especially VUR. The region is defined by markers D13S1311 proximally and D13S285 distally extending approximately 7 Mb.

Gene Expression Studies

We identified 33 genes (12 known; 21 computer predicted) in the critical region based on the May 2004 assembly of human genome. *In silico* gene expression comparison with rodent and human renal and urinary bladder EST databases was performed to identify whether any of the 13q candidate genes have been identified to have renal expression. We downloaded a total of 472,765 unique sequences to create the three renal "transcriptomes" for human, mouse, and rat using query terms described earlier. The database for genes that are expressed in urinary bladder, ureter, and trigone had 123,802 sequences. We performed local BLAST match for the 33 genes in the 13q33 region against these databases. All of the genes with a significant match (an e value score of <-10) and the match scores are presented in Figure 3; in this figure, a higher score represents a better match. There were 11 matches in the human kidney transcriptome database, nine of which were near perfect. There were 12 matches in the mouse kidney, five in the rat kidney, and 10 in the bladder databases. The matches in the nonhuman and bladder databases had lower similarity scores than the human database matches, and also

Table 2. Deletion mapping results of chromosome 13q

Marker	Position (cM)	Position (Kb)	PCR Product Size	Patient 1 (VUR+ESRD)		Patient 2 (VUR)			Patient 3 (Hydronephrosis)		
				Patient	Mother	Patient	Mother	Father	Patient	Mother	Father
D13S787	7	23,278,738	251	AB		AB	AB	BB	BC	BD	BC
D13S1493	25.8	32,906,926	250	DE		AB	AB	BF	DF	DF	FF
D13S894	33	37,636,509	190	CE		DD	CD	CD	BB	BC	BB
D13S626	56.77	52,241,153	339			GH	GH	FG	AC	BC	AD
D13S784	57.06	NA	195 to 196	AC		AB	AB	AB	AA	AA	AC
D13S889	60.43	NA	294 to 295	BC	AB	BE	EE	BE	BC	CD	AB
D13S776	67.58	66,325,302	142	CC	CC	DD	BD	DD	BB	BC	BB
D13S318	65.23	69,473,651	284	BD	BB	DD	DD	AD	AA	AD	AA
D13S791	72.56	NA	293 to 294			BC	BC	CD	AC	CD	AD
D13S800	69.82	72,772,693	295 to 296	AB	AC	BC	BC	CD	AC	CD	AD
D13S891	63.76	NA	200	AB	AA	BC	BC	BC	BC	AC	BB
D13S317	85.07	81,620,060	200	AC		AE	DE	AC	BF	CF	AB
D13S265	70.6	89,170,946	293 to 295			BD	AD	BD	BC	BB	BC
D13S281	74.3	93,361,572	238	AD	AC	BB	AB	AB	AB	AC	BC
D13S167	75.8	93,474,236	184 to 192			DD	AD	DD	CC	CC	AC
D13S154	77.1	95,060,285	243 to 277	AD	DD	DG	DG	GH	AE	AC	BE
D13S793	76	NA	262	CD	CC	AD	AD	AA	CC	BC	AC
D13S1252	79..3	97,335,405	194 to 238			CE	CE	CE	BB	AB	BB
D13S159	81.5	97,851,595	168 to 203			AG	AE	BG	DE	EF	DF
D13S1267	83.6	99,695,156	184 to 216			CD	CD	CD	BC	BC	AB
D13S779	83	100,301,956	191	CE	NA	BB	BB	AB	BC	AC	BC
D13S174	86.9	101,752,077	173 to 199	**DD or D**	BD	AC	AD	CE	**A**	CD	AB
D13S280	87.5	102,346,021	138	**DD or D**	CD	EG	EG	CG	**CC**	CF	AC
D13S1311	92	104,990,121	141 to 149	**CC or C**	BC	BD	BD	BC	**BB**	BC	BC
D13S763	93.52	105,496,202	222	**CC or C**		**CC**	CC	BC	**B**	AD	BD
D13S286	95.9	105,735,872	175 to 195	**BB or B**	BB	**A**	AF	BD	**D**	BB	BD
D13S173	96	106,404,546	166 to 178			**DD**	DE	CD	**BB**	BC	BE
D13S796	94	106,686,966	167			**B**	AB	AD	**C**	AD	BC
D13S778	96.7	107,072,622	157 to 175			**CC**	CC	AC	**CC**	BC	CC
D13S783	NA	107,262,681	269 to 270	**DD or D**	BD	**CC**	CC	CC	**CC**	BC	CC
D13S895	99	NA	160	**GG or G**	CG	**BB**	BD	NA	**BB**	AB	BB
D13S285	111	111,843,433	92 to 106	AD	AE	**C**	AC	BB	BC	AC	BB

Boldface type denotes deleted alleles of chromosome 13q microsatellite markers.

four matches were nonoverlapping with the human renal transcriptome. These *in silico* expression studies, therefore, showed matches for a total of 14 genes in the kidneys and 10 in the bladder EST databases.

Finally, we studied the expression patterns of all of the known genes and a majority of the computer-predicted genes in mouse renal tissue. A total of 24 13q33 candidate genes and two housekeeping genes (β-actin and glyceraldehyde-3-phosphate dehydrogenase) were analyzed (26 total). We designed primers to amplify the cDNA for all of these genes (shown in Figure 3). The primers were designed to amplify regions of genes/EST that were homologous for both human and mouse sequences (if available). The reverse transcription–PCR expression profiles in mouse kidneys of these genes are summarized in the last columns of Figure 3 and shown in Figure 4. Several of the genes had bands at slightly different sizes than expected (marked by an asterisk in Figure 3). This could be explained by alternate splicing or incomplete characterization of the computer-predicted genes in the GenBank. Overall, these mouse kidney expression studies generally confirmed the expression pattern identified in the *in silico* gene expression analyses. Nine genes had renal expression that was not identified by the *in silico* analyses. In addition, it showed that of the 24 genes examined, several had variable expression through the different stages of renal development. Five of the genes were not expressed at all in kidneys at any stage. Although one possible explanation for this observation is that the PCR primers did not work at all for those genes, but given that the housekeeping genes (β-actin and glyceraldehyde-3-phosphate dehydrogenase) were well expressed at the same time in the same tissues, it is likely that these genes truly are not expressed in developing or mature mouse kidney. Also, two of the genes had expression in embryonic or neonatal kidney but not in the adult kidney.

Local BLAST Results (in-silico gene expression)

13q Genes			Human Kidney			Mouse Kidney			Rat Kidney			Ureter Bladder Trigone All Species			Mouse Kidney Gene Expression						
Serial No.	Start	Symbol	Best Match Sequence GI	Source of cDNA	e Score Value	Best Match Sequence GI	Source of cDNA	e Score Value	Best Match Sequence GI	Source of cDNA	e Score Value	Best Match Sequence GI	Source of cDNA	e Score Value	Forward Primer	Reverse Primer	PCR SIZE (bp)	E18	NB (day1)	Adult	NO of the Band (Fig 3)
1	105940099	EFNB2	52189030	Unspecified	100							8774419	Adult Male Bladder Mouse	37	ATTTGCCCCAAAGTGGACTC	TTGATCCAGCAGAACTTGCAT	340	+	+	+	1
2	105993677	FLJ10154				9472076	Newborn	27				8759093	Adult Male Bladder Mouse	31							
3	106068159	LOC283483																			
4	106113786	LOC390424	1992958	Fetal	20	1865579	Newborn	85	3019783	Unspecified	100										
5	106314165	LOC122335	52118346	Embryonic	100	7316047	Unspecified	98	3709494	Unspecified	54										
6	107657795	LIG4	52136840	Epithelial	62	16149964	Adult Male	22							TTCACGAGGTGGCATGATGT	TCGGTCTTGGAGATGGGCTTC	482	+	+	+	2
7	107679846	C13orf6							4253062	Unspecified	29	8775075	Adult Male Bladder Mouse	26	TTTTCTTTTTGGCCGTTCCT	AGTCCGAGATGGGGAGAGTT	301	+	+	-	3
8	107719978	TNFSF13B										27135107	Adult Male Bladder Mouse	14	CGACACGCCGACTATACGAA	GGCACCAAAGAAGGTGTCGT	385	-	-	+	4
9	108000380	MYR8													CATCCCTGAAAACCCCATGA	TGGCATGTCCAAAGGCTTCT	491	-	-	*+	5
10	109204187	IRS2										27135272	Adult Male Bladder Mouse	10	TGGCTGCGATGACAACTACG	CCGGACGACTGACTCTTGCT	397	+	+	+	6
11	109599312	COL4A1	1993319	Fetal	100	33278781	Newborn	100				26069308	Unspecified Bladder Mouse	57	TGCCAGGACCAAGTGGAAGA	GTCTCCTTTGTCACCTTTGAG	428	+	+	+	7
12	109757632	COL4A2	52171140	Epithelial	100	33279590	Newborn	94							TCCTGGCCTTGATGGAGAAA	GCCAAACAGGAAGCCATCTG	436	+	+	+	8
13	109973415	RAB20				1656952	Unspecified	41							AGCCGGATGGGAAGATCGTA	CCGAGGTCAGGTCCACTTTG	351	*+	*+ faint	*+	9
14	110066009	FLJ10769													AGCACAAAGGGCAAGATGGA	TGTCCCTGGCCTTTGACACT	324	+	+	+	10
15	110091760	FLJ12118	52130532	Embryonic	100	32241482	Unspecified	49	4255551	Unspecified	19				AGCCAGCGGACTCTGACAAG	GCATGGCGCTGTCACTGTAG	431	+	-	*+	11
16	110163084	ING1	52189816	Unspecified	23	5749362	Unspecified	52				15408098	Adult Male Bladder Mouse	34							
17	110261945	LOC387946													GGATGGGCTGTTTTGCAGAT	TGGTCTCGATTCCTCTGTGC	303	-	-	+	12
18	110319561	LOC283487	52172023	Epithelial	50	16149589	Adult Male	16							CTCAGCCAAGAGGAGGTTCG	ATGTCCAGGGCAGCTACCAG	303	-	-	-	13
19	110328889	ANKRD10	52136806	Epithelial	100	31575387	Newborn	51	4253070	Unspecified	11	16961252	Unspecified Bladder Mouse	28	GACTCCTTCTACGGCTGGAC	CCTCACAGTCCGGTTTGTTAAT	216	+	+	+	14
20	110387051	ADPRTP1	52135300	Epithelial	100	40379232	Newborn	100				17109635	Unspecified Bladder Mouse	67	CCGTGCATCAGCACTAAAGA	TGCAGAGTGTTCCAGACCAG	282	-	-	-	15
21	110433655	LOC144962													GTGATGTTCGTTGCACTGCT	AGATTCAGAAGCCTCAGCAAA	263	-	-	-	16
22	110565783	ARHGEF7													AAACCTTTCAGCTCAGTGTCAAG	AGCTTTGTGATTGTCATTCCTGT	283	faint+	faint+	+	17
23	110757566	LOC121792													TGGGAAAGCATCACAATGAA	GGAAGCCATTGATTCCAAAA	239	-	-	+	18
24	110771016	MGC35169													CTTCGTCATCACCATCATCTACA	CTCAGTTTCTTCGGCTTCTGTTA	235	-	-	-	19
25	111700644	LOC390426																			
26	111759963	LOC440148																			
27	111769914	SOX1													AGGAGAACCCCAAGATGCAC	GCCAGCGAGTACTTGTCCTT	202	+	+	+	20
28	111857470	LOC400161													CCCAAACAAATGAACATGGA	GCTGGAACGGTGAAGGAGTA	305	-	-	-	21
29	111866406	LOC401740																			
30	111959043	LOC400163													GACTCTCCACGGCTCTTCAG	CCTGTGTCAGGGACCTTGTT	200	-	-	faint *	22
31	112015782	LOC400164																			
32	112078670	LOC122258													CCTGGCATTGAGGACAAAAT	TCTAGCAGGCTCCTCTCCTG	314	*+	*+	-	23
33	112187326	TUBGCP3										8775448	Adult Male Bladder Mouse	17	TGCTTATTTTGAGACCAGCAAA	TAATCCAGGCTGAAGACATCC	350	+	+	+	24
House keeping		b-Actin													AGGAGCGAGACCCCACTAAC	GTGGTTCACACCCATCACAA	179	+	+	+	25
House keeping		GAPDH													AAACTGGAACGGTGAAGGTG	AGTGGGGTGGCTTTTAGGAT	166	+	+	+	26

* = Size discrepancy between predicted and observed size of PCR fragment (possible alternate splicing)

Figure 3. Candidate genes in the 13q33q34 region and their expression in kidneys and urinary tract. *Size discrepancy between predicted and observed size of PCR fragment (possible alternate splicing).

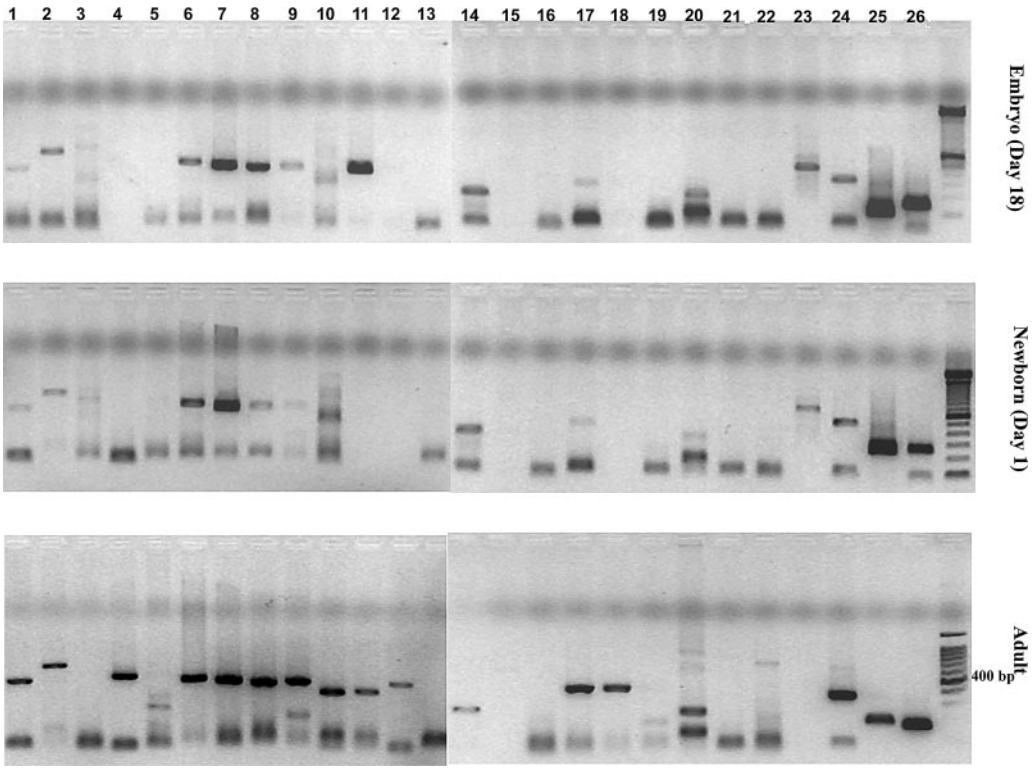

Figure 4. Candidate gene expression from 13q33 location in fetal, neonatal, and adult mouse kidneys. The numbers on top of each lane refer to the genes in the last column of Figure 3, and the last lane shows a DNA ladder.

Discussion

Congenital renal anomalies, including VUR, are a major cause for morbidity in the pediatric age group and are becoming a significant cause of ESRD in adulthood (1,4–6). Previous studies have shown that VUR can occur in families, and genetic factors likely play an important role in the pathogenesis (9–11,16). Different modes of inheritance of VUR have been suggested, including a dominant single gene and polygenic inheritance (27–29). Although cases of 13q deletion syndrome have been identified occasionally with renal malformations, VUR and ESRD previously have not been associated with 13q deletion (30–33). Also, before our studies, no information was available on the critical regions of 13q associated with renal development. We had previously reported association of interstitial deletion of 13q12q22 with CAKUT (22) and now present evidence for a second locus on 13q33q34 associated especially with severe VUR, RN, and ESRD.

Our studies suggest presence of at least one gene in the 7-Mb region on 13q33–34 that is likely to be responsible for *in utero* renal and urinary tract development. We hypothesized that for a gene to be involved in CAKUT, it has to be expressed in the kidney. Therefore, we conducted *in silico* and mouse kidney mRNA expression studies of all of the genes in this region. This chromosomal region has 33 genes (including some computer predicted) and include several interesting genes, such as collagen IV subunits 1 and 2 (COL4A1 [MIM 120130] and COL4A2 [MIM 120090]). COL4A1 and A2 are not currently associated with any human renal disease but showed strong renal expression. As other COL4 chains are associated with Alport syndrome and its variants (34,35), it is intriguing to speculate a role for these genes in renal development. The mRNA expression studies also identified several genes to have no renal or urinary tract expression, therefore making them less likely to be involved in renal ontogeny. In contrast, the genes with a high renal expression, especially in the embryonic phase of development, would be good candidates for further exploring their role in CAKUT.

There is considerable variability in the renal and extrarenal manifestations of 13q deletion syndrome (32,33). It generally is thought that 13q deletions have three major phenotypic associations. Proximal 13q deletions (especially those involving 13q14) show mild to moderate developmental delay and an almost 80% risk for development of retinoblastoma (31). More distal deletions, involving 13q32, have associated holoprosencephaly, severe developmental delay, growth deficiency, and limb anomalies. Finally, the most distal deletion, involving 13q33q34, is associated with severe developmental delay but no gross malformation or growth deficiency (30,31). We identified children with severe VUR and renal failure from this last group that involves 13q33q34. Our previous studied had identified a region on 13q12q22 that is centromeric to the current critical region on 13q33q34, which was associated with CAKUT manifesting as obstructive uropathy but not VUR (22). There is no apparent overlap between these two distinct 13q loci associated with CAKUT. Taken together with our earlier report of a CAKUT locus on 13q12–22 region, we believe that at least two loci for genes on chromosome 13q are associated with renal/genitourinary development *in utero*.

CAKUT most likely is a polygenetic condition. In the only genome-wide study of seven families with nonsyndromic VUR, a linkage to chromosome 1 was reported by Feather *et al.* (20). It is interesting that two of the seven pedigrees in that report did not link to this locus, suggesting genetic heterogeneity for VUR. Recently, Sanna-Cherchi *et al.* (21) analyzed another seven large European families with VUR. They studied markers on chromosomes 1p13, 3p12, 6p21, 10q26, and 19q13, but no linkage to any of these loci was found. These data demonstrate substantial genetic heterogeneity of VUR, and our findings of a 13q33 locus lend further credence to their observation. There may be additional but only moderate support for a 13q33 locus for renal diseases if we analyze some of the published reports more carefully (20,36). It is interesting that Feather *et al.* (20) in their report also identified 12 additional possible loci for VUR with a $P < 0.05$ including a locus on 13q. The cumulative parametric logarithm of odds (LOD) score for the 13q locus (in the region of 96 to 113 cM) in that study was 2.08 (nonparametric linkage score = 2.37; $P = 0.02$) when all of the families that were not linked to chromosome 1 were analyzed. Although these LOD scores were small, their data are at least suggestive of a possible locus on chromosome 13 that seems to overlap with the locus described in this report. Finally, in an attempt to map the genes that predispose to the common causes of ESRD, a genome-wide scan in 1023 individuals with chronic kidney disease from 483 black families was performed by Freedman *et al.* (36). Their results showed a modest evidence of linkage to 13q33.3 near D13S796 on multipoint nonparametric linkage analysis (LOD = 1.72). Incidentally, marker D13S796 is in the middle of the critical 13q33 region found in our study (Table 2). Although there are no data available on the presence of VUR in the Freedman *et al.* study, it is conceivable that gene(s) in this region may contribute to both renal function and anatomy. VUR was documented recently to be not an infrequent finding in adults with incidentally diagnosed hypertension from an Indian cohort of patients (6). This locus therefore may be worthy of further examination in both syndromic and nonsyndromic VUR.

Conclusion

We report association of interstitial deletion of 13q33q34 with VUR and ESRD. This region contains several genes that show renal expression, and these would be good candidate genes for further study. More patients or pedigrees with 13q deletion and with inherited CAKUT/VUR will need to be tested to identify the role of this region in both syndromic and nonsyndromic VUR.

Acknowledgments

A.V. is supported by National Institutes of Health grants DK02854 and DK064933 and is a consultant for ViraCor Laboratories.

We thank the children described in this study and their parents and caregivers for participation and help. We thank Linda Sorg from Chromosome Deletion Outreach for help in identification of patients with

13q deletion. We also thank Suzanne Betera, PhD, for helping with the mouse kidney tissue.

References

1. Mak RH, Kuo HJ: Primary ureteral reflux: Emerging insights from molecular and genetic studies. *Curr Opin Pediatr* 15: 181–185, 2003
2. Thompson M, Simon SD, Sharma V, Alon US: Timing of follow-up voiding cystourethrogram in children with primary vesicoureteral reflux: Development and application of a clinical algorithm. *Pediatrics* 115: 426–434, 2005
3. Cohen-Overbeek TE, Wijngaard-Boom P, Ursem NT, Hop WC, Wladimiroff JW, Wolffenbuttel KP: Mild renal pyelectasis in the second trimester: Determination of cut-off levels for postnatal referral. *Ultrasound Obstet Gynecol* 25: 378–383, 2005
4. Ataei N, Madani A, Esfahani ST, Kejbafzadeh A, Ghaderi O, Jalili S, Sharafi B: Screening for vesicoureteral reflux and renal scars in siblings of children with known reflux. *Pediatr Nephrol* 19: 1127–1131, 2004
5. Houle AM, Cheikhelard A, Barrieras D, Rivest MC, Gaudreault V: Impact of early screening for reflux in siblings on the detection of renal damage. *BJU Int* 94: 123–125, 2004
6. Barai S, Bandopadhayaya GP, Bhowmik D, Patel CD, Malhotra A, Agarwal P, Singhal T: Prevalence of vesicoureteral reflux in patients with incidentally diagnosed adult hypertension. *Urology* 63: 1045–1048, 2004
7. Anderson NG, Allan RB, Abbott GD: Fluctuating fetal or neonatal renal pelvis: Marker of high-grade vesicoureteral reflux. *Pediatr Nephrol* 19: 749–753, 2004
8. Ichikawa I, Kuwayama F, Pope JC 4th, Stephens FD, Miyazaki Y: Paradigm shift from classic anatomic theories to contemporary cell biological views of CAKUT. *Kidney Int* 61: 889, 2002
9. Pope JC 4th, Brock JW 3rd, Adams MC, Stephens FD, Ichikawa I: How they begin and how they end: Classic and new theories for the development and deterioration of congenital anomalies of the kidney and urinary tract, CAKUT. *J Am Soc Nephrol* 61: 2018–2028, 1999
10. Kincaid-Smith PS, Bastos MG, Becker GJ: Reflux nephropathy in the adult. *Contrib Nephrol* 39: 94–99, 1984
11. Eccles MR, Bailey RR, Abbott GD, Sullivan MJ: Unravelling the genetics of vesicoureteric reflux: A common familial disorder. *Hum Mol Genet* 5: 1425–1429, 1996
12. Kelly H, Ennis S, Yoneda A, Bermingham C, Shields DC, Molony C, Green AJ, Puri P, Barton DE: Uroplakin III is not a major candidate gene for primary vesicoureteral reflux. *Eur J Hum Genet* 13: 500–502, 2005
13. Zagradisnik B, Bracic K, Varda NM, Kokalj Vokac N, Gregoric A: G-protein beta3 subunit gene C825T polymorphism in patients with vesico-ureteric reflux. *Ann Genet* 47: 209–216, 2004
14. Yu OH, Murawski IJ, Myburgh DB, Gupta IR: Overexpression of RET leads to vesicoureteric reflux in mice. *Am J Physiol Renal Physiol* 287: F1123–F1130, 2004
15. North RA, Taylor RS, Gunn TR: Pregnancy outcome in women with reflux nephropathy and the inheritance of vesico-ureteric reflux. *Aust N Z J Obstet Gynaecol* 40: 280–285, 2000
16. Nishimura H, Yerkes E, Hohenfellner K, Miyazaki Y, Ma J, Hunley TE, Yoshida H, Ichiki T, Threadgill D, Phillips JA 3rd, Hogan BM, Fogo A, Brock JW 3rd, Inagami T, Ichikawa I: Role of the angiotensin type 2 receptor gene in congenital anomalies of the kidney and urinary tract, CAKUT, of mice and men. *Mol Cell* 3: 1–10, 1999
17. Van Esch H, Groenen P, Nesbit MA, Schuffenhauer S, Lichtner P, Vanderlinden G, Harding B, Beetz R, Bilous RW, Holdaway I, Shaw NJ, Fryns JP, Van de Ven W, Thakker RV, Devriendt K: GATA3 haplo-insufficiency causes human HDR syndrome. *Nature* 406: 419–422, 2000
18. Sanyanusin P, Schimmenti LA, McNoe LA, Ward TA, Pierpont ME, Sullivan MJ, Dobyns WB, Eccles MR: Mutation of the PAX2 gene in a family with optic nerve colobomas, renal anomalies and vesicoureteral reflux. *Nat Genet* 9: 358–363, 1995 [published erratum appears in *Nat Genet* 13: 129, 1996]
19. Abdelhak S, Kalatzis V, Heilig R, Compain S, Samson D, Vincent C, Weil D, Cruaud C, Sahly I, Leibovici M, Bitner-Glindzicz M, Francis M, Lacombe D, Vigneron J, Charachon R, Boven K, Bedbeder P, Van Regemorter N, Weissenbach J, Petit C: A human homologue of the *Drosophila* eyes absent gene underlies branchio-oto-renal (BOR) syndrome and identifies a novel gene family. *Nat Genet* 15: 157–164, 1997
20. Feather SA, Malcolm S, Woolf AS, Wright V, Blaydon D, Reid CJ, Flinter FA, Proesmans W, Devriendt K, Carter J, Warwicker P, Goodship TH, Goodship JA: Primary, nonsyndromic vesicoureteric reflux and its nephropathy is genetically heterogeneous, with a locus on chromosome 1. *Am J Hum Genet* 66: 1420–1425, 2000
21. Sanna-Cherchi S, Reese A, Hensle T, Caridi G, Izzi C, Kim YY, Konka A, Murer L, Scolari F, Ravazzolo R, Ghiggeri GM, Gharavi AG: Familial vesicoureteral reflux: Testing replication of linkage in seven new multigenerational kindreds. *J Am Soc Nephrol* 16: 1781–1787, 2005
22. Vats AN, Ishwad C, Vats KR, Moritz M, Ellis D, Mueller C, Surti U, Parizhskaya MZ, Meza MP, Burke L, Schneck FX, Saxena M, Ferrell R: Steroid resistant nephrotic syndrome and congenital anomalies of kidneys: Evidence of locus on chromosome 13q. *Kidney Int* 64: 17–24, 2003
23. McPherson EW, Laneri G, Clemens MM, Kochmar SJ, Surti U: Apparently balanced t(1;7)(q21.3;q34) in an infant with Coffin-Siris syndrome. *Am J Med Genet* 71: 430–433, 1997
24. Vats A, Nayak A, Ellis D, Randhawa PS, Finegold DN, Levinson KL, Ferrell RE: Familial nephrotic syndrome: Clinical spectrum and linkage to chromosome 19q13. *Kidney Int* 57: 875–881, 2000
25. Ishwad CS, Ferrell RE, Rossie KM, Appel BN, Johnson JT, Myers EN, Law JC, Srivastava S, Gollin SM: Loss of heterozygosity of the short arm of chromosomes 3 and 9 in oral cancer. *Int J Cancer* 69: 1–4, 1996
26. Vats A, Katayama H, Fish A, Mauer M, Kim Y, McGlennen RC: Modified method for competitive reverse transcription-polymerase chain reaction (RT-PCR) for rapid and automated quantitation of multiple mRNA messages in multiple samples. *Mol Diagn* 2: 235–240, 1997
27. Noe HN, Wyatt RJ, Peeden JN Jr, Rivas ML: The transmission of vesicoureteric reflux from parent to child. *J Urol* 148: 1869–1871, 1992
28. Chapman CJ, Bailey RR, Janus ED, Abbott GD, Lynn KL: Vesicoureteral reflux: Segregation analysis. *Am J Med Genet* 20: 577–584, 1985

29. de Vargas A, Evans K, Ransley P, Rosenberg AR, Rothwell D, Sherwood T, Williams DI, Barratt TM, Carter CO: A family study of vesicoureteric reflux. *J Med Genet* 15: 85–92, 1978
30. Brown SA, Warburton D, Brown LY, Yu CY, Roeder ER, Stengel-Rutkowski S, Hennekam RC, Muenke M: Holoprosencephaly due to mutations in ZIC2, a homologue of *Drosophila* odd-paired. *Nat Genet* 20: 180–183, 1998
31. Baud O, Cormier-Daire V, Lyonnet S, Desjardins L, Turleau C, Doz F: Dysmorphic phenotype and neurological impairment in 22 retinoblastoma patients with constitutional cytogenic 13q deletion. *Clin Genet* 55: 478–482, 1999
32. Brown S, Russo J, Chitayat D, Warburton D: The 13q- syndrome: The molecular definition of a critical deletion region in band 13q32. *Am J Hum Genet* 57: 859–866, 1995
33. Kuhnle U, Bartsch O, Werner W, Schuster T: Penoscrotal inversion, hypospadias, imperforate anus, facial anomalies, and developmental delay: Definition of a new clinical syndrome. *Pediatr Surg Int* 16: 396–399, 2000
34. Longo I, Porcedda P, Mari F, Giachino D, Meloni I, Deplano C, Brusco A, Bosio M, Massella L, Lavoratti G, Roccatello D, Frasca G, Mazzucco G, Muda AO, Conti M, Fasciolo F, Arrondel C, Heidet L, Renieri A, De Marchi M: COL4A3/COL4A4 mutations: From familial hematuria to autosomal-dominant or recessive Alport syndrome. *Kidney Int* 61: 1947–1956, 2002
35. Kashtan CE: Familial hematuric syndromes: Alport syndrome, thin glomerular basement membrane disease and Fechtner/Epstein syndromes. *Contrib Nephrol* 136: 79–99, 2001
36. Freedman BI, Bowden DW, Rich SS, Valis CJ, Sale MM, Hicks PJ, Langefeld CD: A genome scan for all-cause end-stage renal disease in African Americans. *Nephrol Dial Transplant* 20: 712–718, 2005