Variants in the Plasmacytoma Variant Translocation Gene (*PVT1*) Are Associated With End-Stage Renal Disease Attributed to Type 1 Diabetes

Meredith P. Millis, Danielle Bowen, Christopher Kingsley, Richard M. Watanabe, and Johanna K. Wolford

OBJECTIVE— End-stage renal disease (ESRD) attributed to diabetes is strongly dependent on genetic factors. We previously reported association between variants in the plasmacytoma variant translocation gene (*PVT1*) and ESRD attributed to type 2 diabetes in Pima Indians. The objective of this study was to evaluate the extent to which these variants mediate susceptibility in other populations.

RESEARCH DESIGN AND METHODS— We genotyped 24 markers showing the strongest evidence for association in Pima Indians in unrelated Caucasians with type 1 diabetes from the Genetics of Kidneys in Diabetes (GoKinD) study. The study sample was comprised of 531 case subjects with ESRD and 564 control subjects with diabetes duration >20 years and a maximum urinary albumin-to-creatinine ratio <150 mg/g.

RESULTS— Markers rs13447075 (odds ratio [OR] 1.47 [95% CI 1.14–1.89] per copy of A allele; $P = 0.003$) and rs2648862 (2.66 [1.19–5.92] per copy of C allele; $P = 0.008$) were strongly associated with ESRD in analyses adjusting for age^2, age^3, duration of diabetes, and smoking status. We further identified a common haplotype containing the C allele at rs10808565 and the A allele at rs13447075 that was associated with ESRD ($P = 0.003$). *PVT1* gene expression yields several isoforms, and rs13447075 is located within the coding region of one of these transcript variants. We identified expression of this isoform in four major human kidney cell types, including mesangial, cortical epithelial, epithelial, and proximal tubule cells.

CONCLUSIONS— These results are the first to provide confirmatory evidence supporting a role for *PVT1* in mediating susceptibility to ESRD attributable to diabetes. ***Diabetes* 56:3027–3032, 2007**

Diabetic nephropathy has become the major cause of end-stage renal disease (ESRD) in developed countries and is associated with substantial morbidity and mortality (1). The prevalence of ESRD attributed to diabetes has increased in recent years, mainly due to improved ascertainment of the disease by the U.S. Renal Data System, the rising prevalence of type 2 diabetes, and the decreasing age of type 2 diabetes onset (1,2).

Genetic factors modulate risk for ESRD in diabetes (3,4), but to date, no genes with significant effects on disease susceptibility have been identified. We recently performed a whole-genome association study to identify single nucleotide polymorphisms (SNPs) associated with ESRD in Pima Indians with type 2 diabetes, and found the strongest evidence for association between seven markers located on 8q24 and disease status (5). Genotyping of an additional 100 SNPs confirmed and strengthened findings of association. The strongest evidence for association with diabetic ESRD was found with marker rs2648875 ($P = 1.8 \times 10^{-6}$; odds ratio [OR] 2.97 [95% CI 1.90–4.65]) (5). All of the variants associated with ESRD were located within the plasmacytoma variant translocation gene (*PVT1*) genomic locus (5).

To begin to understand the molecular mechanisms by which *PVT1* may contribute to ESRD susceptibility in diabetes, we first sought to determine the extent to which variants in this gene were associated with the disease in independent groups of affected individuals. Furthermore, because some of the pathophysiology underlying kidney disease, such as thickening of the glomerular basement membrane and mesangial expansion, may be shared between type 1 and type 2 diabetes (6,7), we considered the possibility that *PVT1* variants may also contribute to ESRD susceptibility in individuals with type 1 diabetes. To address these questions, we genotyped *PVT1* variants previously showing the strongest evidence for association with ESRD in Pima Indians in a group of Caucasian participants from the Genetics of Kidneys in Diabetes (GoKinD) study with known ESRD (8). We also investigated *PVT1* expression in key cell types of the kidney as a first step toward understanding the molecular mechanisms by which variants in this gene may mediate susceptibility to ESRD in diabetes.

RESEARCH DESIGN AND METHODS

All subjects in this study are participants in the GoKinD study, which has been described in detail elsewhere (8). Briefly, the GoKinD study was initiated to identify genes involved in diabetic nephropathy. Study participants are characterized by long-term type 1 diabetes, with or without kidney disease. Recruitment criteria for case participants included type 1 diabetes duration of at least 10 years and severe diabetic nephropathy, defined by either persistent proteinuria or ESRD. An ESRD diagnosis was determined by the presence of either chronic dialysis or kidney transplant. Control participants were selected based on a type 1 diabetes duration for at least 15 years, the presence of normoalbuminuria with no prior history of treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, and no usage of antihypertensive medications at the time of enrollment. Subjects provided

From the Translational Genomics Research Institute, Phoenix, Arizona.
Address correspondence and reprint requests to Johanna K. Wolford, PhD, Translational Genomics Research Institute, 445 North Fifth St., Phoenix, AZ 85004. E-mail: jwolford@tgen.org.
Received for publication 17 May 2007 and accepted in revised form 26 August 2007.
Published ahead of print at http://diabetes.diabetesjournals.org on 19 September 2007. DOI: 10.2337/db07-0675.
Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db07-0675.
ACR, albumin-to-creatinine ratio; ACTB, β-actin; CEC, cortical epithelial cell; ESRD, end-stage renal disease; GoKinD, Genetics of Kidneys in Diabetes; PTEC, proximal tubule epithelial cell; SNP, single nucleotide polymorphism; tv6, transcript variant 6.
© 2007 by the American Diabetes Association.
The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024

TABLE 1
Characteristics of study participants

	Case subjects	Control subjects	*P* value*
n	531	564	
Male	243 (45.8)	264 (46.8)	NS
Age (years)	44.5 ± 6.1	41.6 ± 8.8	<0.0001
Age diabetes onset (years)	11.8 ± 6.7	12.8 ± 8.4	NS
Diabetes duration (years)	32.6 ± 7.2	28.8 ± 7.0	<0.0001
Current smoker	60 (11.3)	61 (10.8)	NS
Ever smoked cigarettes	259 (48.8)	207 (36.7)	0.01

Data are *n* (%) and means ± SD. All study participants were Caucasians with type 1 diabetes. Calculated using *χ^2 or Wilcoxon rank-sums tests.

written informed consent, and the study was approved by the Institutional Review Boards of all participating centers (8). For the current study, we designed case and control groups to have similar distributions of sex, age, age of diabetes onset, diabetes duration, and smoking status. All *PVT1* variants in the present study were genotyped in 531 case subjects with ESRD and 564 control subjects with diabetes duration >20 years, defined as the duration of diabetes at onset of nephropathy for case patients or duration of diabetes at time of enrollment for control participants (8). Mean albumin-to-creatinine ratio (ACR) in the control group was 8.7 mg/g. Of the 564 individuals in the control group, 8 had ACR ranges between 30 and 300 mg/g; however, the likelihood that these individuals will progress to ESRD after 20 years of diabetes duration is considered low. Mean HbA_{1c}, BMI, and systolic/diastolic blood pressure were similar between the case and control groups (6.9 ± 1.9 vs. 7.5 ± 1.2%; 25.2 ± 5.3 vs. 26.2 ± 4.3 kg/m^2; 131 ± 19/73 ± 11 vs. 119 ± 12/71 ± 8 mmHg, respectively). Eighty-six percent of individuals in the case group had retinopathy, compared with 36.7% of those in the control group. All members of the subset used in this study were Caucasian. Characteristics of the study sample are shown in Table 1.

SNP selection and genotyping. Twenty-four markers showing the strongest evidence for association with ESRD in Pima Indians with type 2 diabetes (5) were selected for genotyping in the GoKinD study sample. These included rs7010121, rs11993333, rs10808565, rs3815871, rs13447075, rs2720709, rs2720659, rs2720660, rs2648862 (formerly referred to as chr8:129130967 in 5), rs2720662, rs1499373, chr8:129137686, rs2648875, rs2648876, rs2250888, rs2720666, rs2720667, rs1499368, rs1499367, rs3931283, rs4526320, rs4733595, rs1121947, and rs7465157. These markers spanned a distance of ~285 kb in the *PVT1* genomic locus. All SNPs were genotyped using the iPLEX assay in conjunction with the MassARRAY platform (Sequenom, La Jolla, CA) as previously described (5). Briefly, primers and multiplex conditions were designed using the Assay Design v3.0 software, and DNA amplification and iPLEX primer extension were performed according to the manufacturer's protocol (Sequenom). The observed genotype frequency for each SNP was assessed for deviation from that expected under Hardy-Weinberg equilibrium using χ^2 analysis, and 72 encrypted samples were used to assess data quality. Assays were considered successful and genotype data were subsequently analyzed if *1*) a minimum of 90% of all genotyping calls were obtained, *2*) markers did not deviate significantly ($P \leq 0.05$) from Hardy-Weinberg equilibrium, and *3*) genotyping error results were <3%.

Statistical analyses. Statistical analyses were performed using SAS system v8.02 (SAS Institute, Cary, NC). The association between genotypes and ESRD was assessed using logistic regression under an additive model and adjusting for the effects of age^2 (age × age), age^3 (age × age × age), diabetes duration, and cigarette smoking status, which were the only covariates showing statistically significant differences ($P \leq 0.05$) between case and control subjects using multivariate regression analysis. Because markers were selected due to previous findings of association with ESRD, we considered them to be *a priori*, independent hypotheses, and therefore we did not correct for multiple testing. Linkage disequilibrium between SNP pairs was quantified using both D′ and r^2. The fastPHASE program (9) was used to infer missing genotypes, predict haplotypic phase, and estimate haplotype frequencies for multiple loci within *PVT1*. Haplotypes were then tested for association with ESRD using a logistic regression model as described for the analysis of individual markers.

Confirmation of rs13447075 and transcript variant 6 by direct sequencing. The marker showing the strongest evidence for association, rs13447075, is located within a putative *PVT1* transcript isoform, which we refer to as transcript variant 6 (tv6) in this study. To evaluate whether this transcript variant was detectable by direct sequencing, we designed primers using the cDNA clone IMAGE:4366721 (BG110543). The forward primer (5′-ATGGCTAGAGGATCTACATGAACTATTTAG) spanning the putative exon 3, where rs13447075 is located, and the reverse primer (5′-TGTCACCCTAACATTAACTGCTCAG) spanning the putative exon 4/5 boundary were used to amplify cDNA obtained from human kidney cells (renal epithelial cells, renal proximal tubule epithelial cells [PTECs], renal cortical epithelial cells [CECs], and mesangial cells; see below for details), and q-PCR Human Reference Total RNA (Stratagene, Los Angeles, CA). Amplified cDNA product was used as a template for sequencing using the same primers described above but with the addition of M13 tags to facilitate sequencing (forward, 5′-TGTAAAACGACGGCCAGT; and reverse, 5′-CAGGAAACAGCTATGACC). Amplicons were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction kit (Applied Biosystems, Foster City, CA) according to the manufacturer's instructions. Chromatograms were analyzed using Sequencher software v4.7 (Gene Codes, Ann Arbor, MI).

Expression profile of tv6 in human renal cells. To determine expression patterns for tv6, we performed quantitative PCR using total RNA derived from different human primary renal cell types. Renal epithelial cells, PTECs, CECs, and mesangial cells were purchased from Cambrex (Walkersville, MD) and cultured under the conditions recommended by the supplier. Cells were plated in six-well tissue culture plates at a density of 10,000 cells/cm^2. Cells were harvested at ≥85% confluency and treated with TRIzol (Invitrogen, Carlsbad, CA), and total RNA was isolated using the RNeasy mini kit according to the manufacturer's protocol (Qiagen, Valencia, CA). RNA concentration was determined using the NanoDrop ND-1000 (NanoDrop Technologies, Wilmington, DE), and RNA quality was assessed with the Series II RNA 6000 Nano Lapchip kit in conjunction with the 2100 BioAnalyzer (Agilent Technologies, Chicago). First-strand cDNA was synthesized from the total RNA obtained from epithelial cells, PTECs, CECs, and mesangial cells using oligo(dT) priming and SuperScript III reverse transcriptase (Invitrogen). Quantitative PCR was performed using 15 ng total RNA, 1× Power SYBR Green Master Mix (Applied Biosystems), and 4.5 pmol each primer; the ABI Prism 7000 Sequence Detection System (Applied Biosystems); and cycling conditions consisting of an initial denaturation of 95°C for 10 min, followed by 40 cycles of 95°C/15 s and 60°C/1 min. A pool of cDNA from each of the kidney cell types was made and used as a template for generation of a standard curve. β-2-Microglobulin, glyceraldehyde-3-phosphate dehydrogenase, and β-actin (ACTB) were used to normalize expression data using the qBase program (10).

RESULTS

Characteristics of study sample. The 1,095 individuals in this study sample were all recruited as part of the GoKinD study (8). Table 1 contains demographic data for the case and control subjects. All study participants were Caucasian with type 1 diabetes, and age of diabetes onset and sex were closely matched between the two groups. The mean age of individuals with ESRD was 44.5 ± 6.1 years compared with 41.6 ± 8.8 years in the control group ($P < 0.0001$). Duration of diabetes was slightly higher in case subjects (32.6 ± 7.2 years) versus control subjects (28.8 ± 7.0 years; $P < 0.0001$). Although the percentage of current smokers was similar between case and control subjects, a positive history of cigarette smoking was more common among case subjects compared with control subjects (48.8 vs. 36.7%, respectively; $P = 0.01$).

Association of *PVT1* variants with ESRD. We determined the haplotype block structure for *PVT1* using the method of Gabriel et al. (11) given the SNPs genotyped, and identified five haplotype blocks (Fig. 1*A*). Blocks 1–4 are situated within *PVT1*, and block 5 is located in the adjacent 3′-flanking region of the gene. None of the haplotype blocks encompassed the coding region of the main *PVT1* mRNA isoform, which extends from 128972056–129021982. Block 1 starts within intervening sequence between alternative *PVT1* exons and ends in an alternative exon. Blocks 2, 3, and 4 are situated within

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024

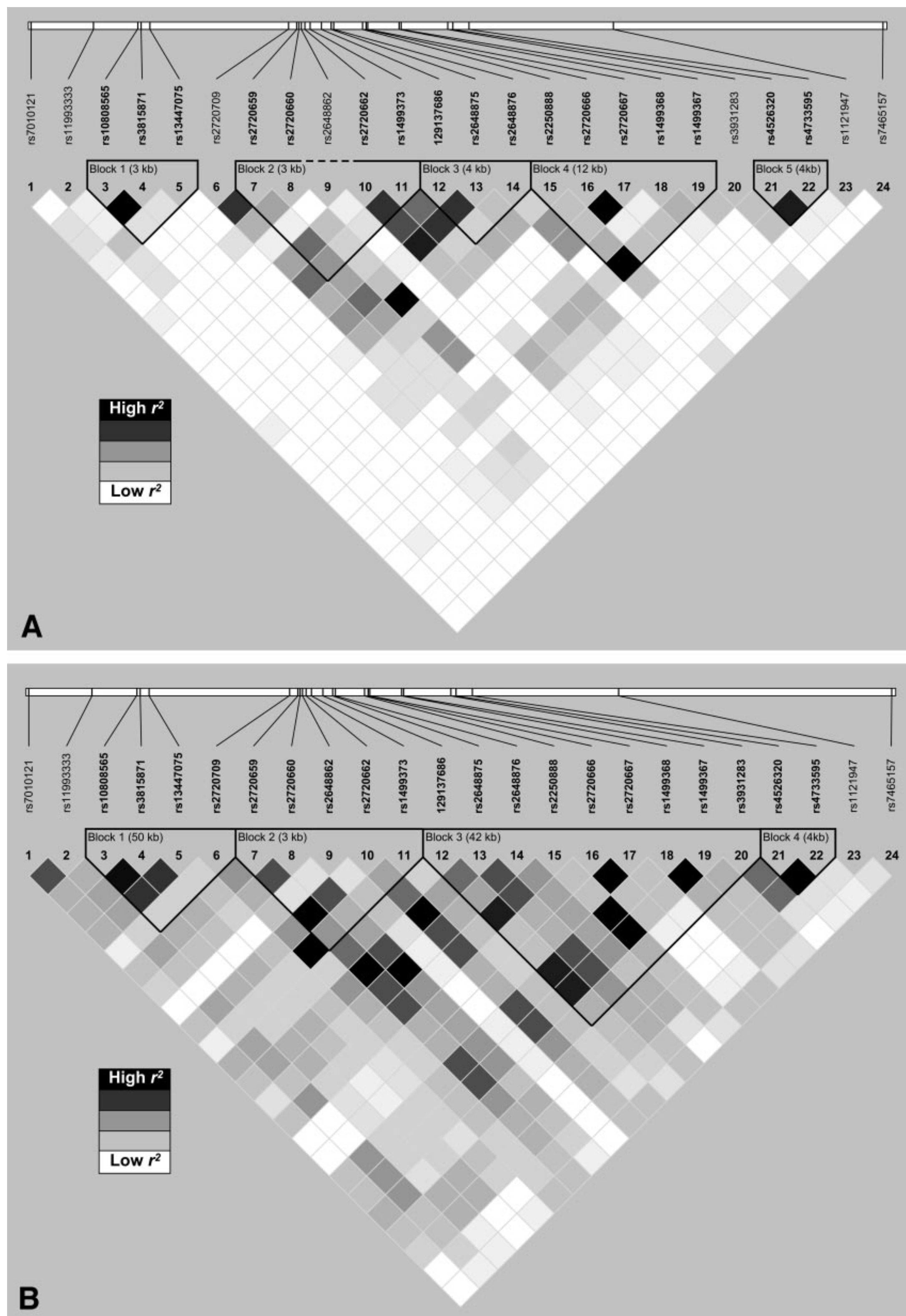

FIG. 1. Estimation of pairwise linkage disequilibrium between *PVT1* SNPs in GoKinD study participants (*A*) and Pima Indians (*B*). Linkage disequilibrium is shown in terms of r^2, which is a measure of concordance between markers. Haplotype blocks were estimated using the method of Gabriel et al. (11).

intervening sequence, and all contain an alternate exon. Block 4 was the largest block and spanned 12 kb. In contrast, haplotype blocks in Pima Indians ranged in size from 3 to 50 kb (Fig. 1*B*). In general, linkage disequilibrium between *PVT1* variants was substantially greater and extended over a larger range in Pima Indians compared with GoKinD study participants (see Supplementary Fig. 1, which is available in the online appendix at http://dx.doi.org/10.2337/db07-0675).

We first assessed individual SNP associations with

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024

ESRD. As shown in Table 2, three SNPs (rs11993333, rs13447075, and rs2648862) were associated with ESRD at statistically significant levels ($P < 0.05$) in analytical models assuming an additive allele effect on the logarithm of odds and adjusting for the effects of diabetes duration, smoking status, age^2, and age^3. Concordance between these markers was $r^2 = 0.16$ (rs11993333-rs13447075), 0.01 (rs11993333-rs2648862), and 0.00 (rs13447075-rs2648862).

We next examined the association between *PVT1* haplotype frequencies and ESRD. Because rs10808565 and rs3815871, both located in the first block, were strongly concordant (i.e., $D' = 1.00$; $r^2 = 0.98$), genetic information in block 1 was represented by two markers: rs10808565 and rs13447075. In block 1, we identified three major haplotypes, including haplotype C-A, which had a frequency of 17.5% in case subjects and 13.4% in control subjects and was associated with ESRD (OR 1.47 [95% CI 1.14–1.90]; $P = 0.003$). We observed four major haplotypes with a frequency >10% in both blocks 2 and 3 but found no statistically significant evidence for association with ESRD with any of the haplotypes in these blocks. Within block 4, represented by markers rs2250888, rs2720666, and rs1499368, there were four common haplotypes with frequencies >5%. The most common haplotype in this block, C-A-G, showed a trend toward association with ESRD but at levels that did not reach statistical significance ($P = 0.08$).

***PVT1* tv6 is expressed in human renal cells.** The *PVT1* genomic locus spans 128972017–129182681 on human chromosome 8q based on the latest genome assembly (http://genome.ucsc.edu [March 2006]) and through use of alternative splicing and alternate exons, gives rise to at least six different mRNA isoforms. Markers rs11993333, rs13447075, and rs2648862 are located at 129061669, 129079772, and 129130967, respectively, and all lie downstream from the last coding exon of the major *PVT1* transcript, XM_001129539. SNP rs13447075, which showed the strongest association with ESRD, is uniquely located within *PVT1* tv6.

To initiate characterization of the rs13447075 marker and the tv6 sequence, we amplified cDNA from epithelial cells, PTECs, CECs, mesangial cells, and Q-PCR Human Reference Total RNA (Stratagene) using primers flanking the putative exon 3 of the transcript where marker rs13447075 is located. Using direct sequencing, we verified the presence of rs13447075 and found that each of the cell lines had the CC genotype at this locus. We also confirmed the presence of the putative exon, including junction boundaries for flanking exons.

We performed quantitative PCR using RNA isolated from epithelial cells, PTECs, CECs, and mesangial cells to evaluate tissue-specific expression of tv6. As shown in Fig. 2, tv6 expression was detected in each cell type examined, with CECs and PTECs showing the highest levels of expression. In general, tv6 expression was higher in these renal cell types relative to other *PVT1* transcript variants (data not shown), suggesting that the product encoded by tv6 plays a role in the kidney.

DISCUSSION

This study represents the first attempt to replicate statistically significant findings of association between markers in the *PVT1* gene and ESRD resulting from diabetes in Pima Indians. In the current study, we observed association between ESRD and *PVT1* variants in an independent and ethnically distinct population, suggesting that this gene may be a key determinant of ESRD across populations.

One of the most interesting findings of this study pertains to the association between *PVT1* markers and ESRD due to type 1 diabetes. In our original study (5), we observed association between *PVT1* markers and ESRD attributed to type 2 diabetes. Although type 1 and type 2 diabetes are not known to share a common etiology and are not dependent on similar risk factors, complications resulting from either disease often follow a similar course of progression. For example, in renal complications of diabetes, pathophysiological features such as thickening of the glomerular basement membrane and expansion of the mesangium are hallmark events in patients affected with either type 1 or type 2 diabetes (6,7). The current findings suggest that genetic determinants of kidney disease may also be shared between type 1 and type 2 diabetic individuals.

Through the use of alternative exon usage, *PVT1* expression gives rise to numerous transcript variants. We found the strongest evidence for association with *PVT1* marker rs13447075, which is uniquely located within one of these mRNA isoforms: tv6. Preliminary characterization confirms both the identity of this marker and exon junction boundaries in the transcript. Interestingly, the first two ATG sequences in the currently available EST sequence for tv6 (BG110543) occur at 130 and 147 bp. If either sequence represents a methionine start codon, then the C-to-A substitution represented by rs13447075 is predicted to cause a Gln17Stop or a Ser23Stop mutation. Such a substitution is expected to produce a truncated protein, which may impact on PVT1 function or activity. We are presently characterizing the full-length tv6 sequence to address possible functional consequences of rs13447075. However, findings of relatively abundant tv6 expression in four different human renal cell lines are consistent with a role for this gene in the development of kidney disease.

Two interesting differences were noted between the current study and the initial one conducted in Pima Indians with type 2 diabetes. First, different markers in the *PVT1* locus showed the strongest evidence for association with ESRD between the two study samples. In Pima Indians, rs2648875 was most strongly associated with ESRD, whereas in the GoKinD study participants, the most significant evidence for association was observed with marker rs13447075. This finding is likely attributed to the varying patterns of linkage disequilibrium between the two populations. For example, linkage disequilibrium between rs13447075 and rs2648875 was vastly different between study samples ($D' = 0.32$ and 0.91 in GoKinD study participants and Pima Indians, respectively). In general, linkage disequilibrium was stronger and extended over much greater distances in Pima Indians compared with the GoKinD study participants. This observation implies that identification of the causal variant in Pima Indians may be hampered by the substantial linkage disequilibrium between SNPs in the *PVT1* locus in this population. In contrast, concordance between genotyped markers was much lower in GoKinD study participants compared with Pima Indians, indicating that susceptibility alleles for diabetic ESRD may be more feasibly identified in this population. It is thus possible that rs13447075 or a marker in strong linkage disequilibrium with rs13447075 may represent a causal variant. However, it is also possible that

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024

TABLE 2
Association of *PVT1* markers with ESRD in GoKinD study participants

Marker	Position	Genotype	Case subjects	Control subjects	OR (95% CI)	*P*
rs7010121	129040083	GG	174 (34.7)	184 (34.8)		
		GA	277 (55.3)	292 (55.2)		
		AA	50 (10.0)	53 (10.0)	1.00 (0.82–1.22)	0.997
rs11993333	129061669	TT	145 (27.5)	120 (21.5)		
		TC	266 (50.6)	295 (52.9)		
		CC	115 (21.9)	143 (25.6)	1.23 (1.03–1.46)	0.020
rs10808565	129076594	CC	232 (45.0)	230 (41.9)		
		CT	222 (43.0)	251 (45.7)		
		TT	62 (12.0)	68 (12.4)	1.08 (0.90–1.29)	0.408
rs3815871	129077760	CC	232 (44.4)	236 (42.4)		
		CG	224 (42.8)	250 (45.0)		
		GG	67 (12.8)	70 (12.6)	1.04 (0.87–1.24)	0.684
rs13447075 (rs10283090)	129079772	CC	361 (68.8)	413 (74.3)		
		CA	142 (27.0)	135 (24.3)		
		AA	22 (4.2)	8 (1.4)	0.74 (0.58–0.93)	0.003
rs2720709	129127538	GG	269 (51.3)	283 (50.9)		
		GA	210 (40.1)	230 (41.4)		
		AA	45 (8.6)	43 (7.7)	0.99 (0.82–1.19)	0.914
rs2720659	129129986	GG	233 (44.5)	241 (43.3)		
		GA	219 (41.9)	258 (46.3)		
		AA	71 (13.6)	58 (10.4)	0.96 (0.80–1.14)	0.648
rs2720660	129130424	GG	205 (48.5)	218 (47.8)		
		GA	215 (50.8)	234 (51.3)		
		AA	3 (0.7)	4 (0.9)	1.03 (0.80–1.33)	0.813
rs2648862	129130967	CC	515 (98.3)	531 (95.3)		
		CA	9 (1.7)	26 (4.7)		
		AA	0 (0.0)	0 (0.0)	2.80 (1.30–6.04)	0.008
rs2720662	129132203	CC	315 (60.0)	335 (59.9)		
		CT	184 (35.0)	200 (35.8)		
		TT	26 (5.0)	24 (4.3)	0.98 (0.80–1.20)	0.868
rs1499373	129133847	CC	351 (70.5)	365 (68.5)		
		CG	132 (26.5)	147 (27.6)		
		GG	15 (3.0)	21 (3.9)	1.10 (0.88–1.38)	0.388
chr8:129137686	129137686	GG	362 (69.0)	392 (70.4)		
		GA	143 (27.2)	155 (27.8)		
		AA	20 (3.8)	10 (1.8)	0.88 (0.70–1.11)	0.283
rs2648875	129141343	GG	320 (60.9)	341 (61.1)		
		GA	180 (34.3)	192 (34.4)		
		AA	25 (4.8)	25 (4.5)	0.99 (0.80–1.21)	0.901
rs2648876	129142148	GG	206 (39.3)	219 (39.4)		
		GA	224 (42.8)	243 (43.7)		
		AA	94 (17.9)	94 (16.9)	0.98 (0.83–1.16)	0.801
rs2250888	129151559	CC	328 (62.4)	362 (65.0)		
		CT	177 (33.6)	178 (32.0)		
		TT	21 (4.0)	17 (3.0)	0.89 (0.72–1.10)	0.291
rs2720666	129152641	AA	181 (35.6)	213 (39.1)		
		AG	237 (46.7)	237 (43.5)		
		GG	90 (17.7)	95 (17.4)	0.93 (0.78–1.10)	0.396
rs2720667	129152766	AA	184 (35.2)	209 (37.7)		
		AG	251 (48.0)	252 (45.4)		
		GG	88 (16.8)	94 (16.9)	0.95 (0.80–1.13)	0.581
rs1499368	129163771	GG	463 (88.0)	493 (88.5)		
		GA	60 (11.4)	63 (11.3)		
		AA	3 (0.6)	1 (0.2)	0.93 (0.65–1.31)	0.668
rs1499367	129164160	GG	326 (62.1)	358 (64.3)		
		GA	178 (33.9)	182 (32.7)		
		AA	21 (4.0)	17 (3.0)	0.90 (0.73–1.12)	0.357
rs3931283	129179915	TT	128 (24.4)	145 (26.1)		
		TC	264 (50.3)	296 (53.2)		
		CC	133 (25.3)	115 (20.7)	0.88 (0.74–1.04)	0.133
rs4526320	129182269	GG	138 (26.3)	146 (26.4)		
		GC	262 (50.0)	290 (52.3)		
		CC	124 (23.7)	118 (21.3)	0.95 (0.80–1.13)	0.575
rs4733595	129186837	AA	131 (24.9)	139 (24.9)		
		AG	260 (49.4)	291 (52.0)		
		GG	135 (25.7)	129 (23.1)	0.95 (0.80–1.13)	0.550
rs1121947	129234261	CC	405 (77.7)	413 (75.3)		
		CT	114 (21.9)	133 (24.3)		
		TT	2 (0.4)	2 (0.4)	1.13 (0.86–1.49)	0.378
rs7465157	129324588	GG	479 (91.1)	514 (92.1)		
		GA	46 (8.7)	44 (7.9)		
		AA	1 (0.2)	0 (0.0)	0.86 (0.56–1.31)	0.470

Data are *n* (%). Genotypes for each marker were evaluated in 531 diabetic case subjects with ESRD and 564 diabetic control subjects from the GoKinD study. The number of individuals (*n*) per genotype is shown with the frequency (%) for each group. ORs were calculated under an analytical model assuming an additive allele effect on the logarithm of odds and is expressed per copy of the major allele, which is listed first in the Genotype column. Results shown were adjusted for the effects of diabetes duration, smoking status, age^2, and age^3.

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024

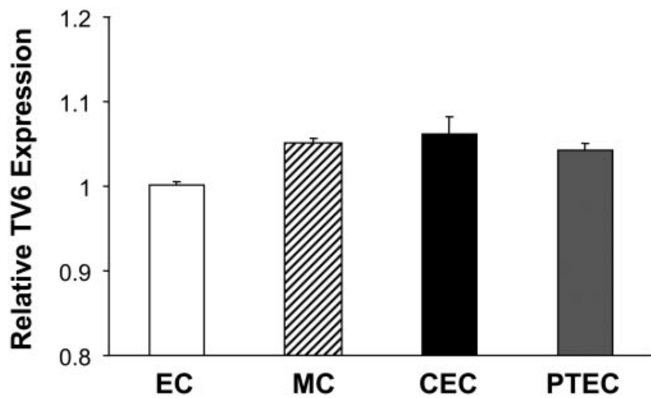

FIG. 2. Levels of tv6 expression in human renal cells. Quantitative PCR was performed in triplicate using tv6 primers and RNA extracted from epithelial cells (EC), mesangial cells (MC), renal CECs, and PTECs, as described in RESEARCH DESIGN AND METHODS. Results shown represent tv6 expression levels normalized to ACTB. Expression levels in each cell type have been normalized to expression in epithelial cells.

distinct variants within *PVT1* may differentially affect susceptibility to disease in the two samples.

We observed that the A allele of rs13447075 was more frequently present in diabetic individuals with ESRD from the GoKinD study, whereas the C allele was associated with diabetic ESRD in Pima Indians. Association of opposite alleles at the same marker (i.e., flip-flops [12]) are somewhat common in studies involving case-control samples and may be either the result of spurious findings of association or true confirmations of locus effects. Observations of flip-flop occur across study samples of different ethnic compositions and may be explained by variable effects of the same biallelic locus due to differences in genetic background and/or environmental exposures (12). Flip-flop associations may represent spurious findings when the minor allele frequency is $<5\%$ (12). At the rs13447075 locus, the A allele in Pima Indians and GoKinD study participants was present at frequencies of 11.5 and 15.6%, respectively, suggesting that rare allele frequencies are not a major determinant of flip-flop association at this marker. Flip-flop associations may also be due to a lack of consideration for effects of multiple loci on disease susceptibility (12). Diabetic ESRD likely results from complex interactions among genetic and environmental factors. In the present study, we investigated a single locus, and it is possible that findings of flip-flop association are attributable to our not accounting for multilocus effects jointly with the *PVT1* markers. However, few loci for diabetic ESRD have been identified, much less confirmed, and it is not yet feasible to account for such multilocus effects in investigations of this disease. Systematic investigation of this gene and testing of *PVT1* variants in multiple independent populations are necessary to verify whether rs13447075 flip-flop association is spurious or confirmatory of a true locus effect.

In summary, we observed association between *PVT1* markers and ESRD, previously identified in Pima Indians, in an independent and ethnically distinct population of diabetic individuals. Furthermore, because linkage disequilibrium between markers is much weaker in this population relative to that in Pima Indians, we have narrowed the region of interest to a specific *PVT1* transcript isoform. Preliminary characterization indicates that this transcript is abundantly expressed in kidney cells, which is strongly consistent with a potential role in metabolic dysregulation in this tissue preceding the development of renal failure in diabetes. Together, these findings suggest that *PVT1* may be a key factor in mediating susceptibility to this disease.

ACKNOWLEDGMENTS

D.B. has received support from the Dimensions program of Cornell College. The GoKinD study sample collection is supported by the Juvenile Diabetes Research Foundation in collaboration with the Joslin Diabetes Center and George Washington University and by the U.S. Centers for Disease Control and Prevention.

We thank the members of the GoKinD study for participating in this research.

REFERENCES

1. U.S. Renal Data System: *United States Renal Data System Annual Data Report: Atlas of End-Stage Renal Disease in the United States.* Bethesda, MD, National Institutes of Health, National Institute of Diabetes and Digestive and Kidney Diseases, 2002, p. 296–322
2. Jones CA, Krolewski AS, Rogus J, Xue JL, Collins A, Warram JH: Epidemic of end-stage renal disease in people with diabetes in the United States population: do we know the cause? *Kidney Int* 67:1684–1691, 2005
3. Freedman BI, Tuttle AB, Spray BJ: Familial predisposition to nephropathy in African-Americans with non-insulin-dependent diabetes mellitus. *Am J Kidney Dis* 25:710–713, 1995
4. Pettitt DJ, Saad MF, Bennett PH, Nelson RG, Knowler WC: Familial predisposition to renal disease in two generations of Pima Indians with type 2 (non-insulin-dependent) diabetes mellitus. *Diabetologia* 33:438–443, 1990
5. Hanson RL, Craig DW, Millis MP, Yeatts KA, Kobes S, Pearson JV, Lee AM, Knowler WC, Nelson RG, Wolford JK: Identification of PVT1 as a candidate gene for end-stage renal disease in type 2 diabetes using a pooling-based genome-wide single nucleotide polymorphism association study. *Diabetes* 56:975–983, 2007
6. Tsilibary EC: Microvascular basement membranes in diabetes mellitus. *J Pathol* 200:537–546, 2003
7. Caramori ML, Mauer M: Diabetes and nephropathy. *Curr Opin Nephrol Hypertens* 12:273–282, 2003
8. Mueller PW, Rogus JJ, Cleary PA, Zhao Y, Smiles AM, Steffes MW, Bucksa J, Gibson TB, Cordovado SK, Krolewski AS, Nierras CR, Warram JH: Genetics of Kidneys in Diabetes (GoKinD) study: a genetics collection available for identifying genetic susceptibility factors for diabetic nephropathy in type 1 diabetes. *J Am Soc Nephrol* 17:1782–1790, 2006
9. Scheet P, Stephens M: A fast and flexible statistical model for large-scale population genotype data: applications to inferring missing genotypes and haplotypic phase. *Am J Hum Genet* 78:629–644, 2006
10. Hellemans J, Mortier G, De Paepe A, Speleman F, Vandesompele J: qBase relative quantification framework and software for management and automated analysis of real-time quantitative PCR data. *Genome Biol* 8:R19, 2007
11. Gabriel SB, Schaffner SF, Nguyen H, Moore JM, Roy J, Blumenstiel B, Higgins J, DeFelice M, Lochner A, Faggart M, Liu-Cordero SN, Rotimi C, Adeyemo A, Cooper R, Ward R, Lander ES, Daly MJ, Altshuler D: The structure of haplotype blocks in the human genome. *Science* 296:2225–2229, 2002
12. Lin PI, Vance JM, Pericak-Vance MA, Martin ER: No gene is an island: the flip-flop phenomenon. *Am J Hum Genet* 80:531–538, 2007

Downloaded from http://diabetesjournals.org/diabetes/article-pdf/56/12/3027/386722/zdb01207003027.pdf by guest on 19 June 2024