

Major Susceptibility Locus for Nephropathy in Type 1 Diabetes on Chromosome 3q

Results of Novel Discordant Sib-Pair Analysis

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Diabetic nephropathy (DN) clusters in families with type 1 diabetes and the degree of clustering suggests that a major gene having a common disease allele may be responsible. To investigate the chromosomal regions containing genes for the renin-angiotensin system, we performed a linkage study using pairs of siblings with type 1 diabetes who were discordant for DN. Theoretical considerations supported by simulation studies indicated that such discordant pairs, rather than the usual concordant pairs, would be more effective in detecting a major susceptibility gene for DN. We applied this novel strategy to test for linkage between DN and chromosomal regions containing genes for the ACE, angiotensinogen (AGT), and angiotensin II type 1 receptor (AT1). Two polymorphic markers were genotyped in the vicinity of each of the three loci in 66 discordant sib pairs and were analyzed with multipoint methods. The regions containing ACE and AGT loci were not linked with DN, while the region containing the AT1 locus showed linkage with DN. As a result of these positive findings, eight additional polymorphic markers spanning a 63-cM region around AT1 locus were genotyped. Linkage was demonstrated between DN and a 20-cM region that includes AT1 ($P = 7.7 \times 10^{-5}$), an obvious candidate gene for DN. To investigate whether AT1 could account for the observed linkage, we sequenced all exons, splicing junctions, and the promoter region and examined the identified polymorphisms/mutations for association with DN using the transmission disequilibrium test. Four new polymorphisms in the gene were found, but neither these nor previously described polymorphisms were associated with DN. Thus, while our study does not implicate AT1 itself in the etiology of DN, it provides very strong evidence that a 20-cM region around AT1 contains a major locus for susceptibility to DN. *Diabetes* 47:1164-1169, 1998

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ACR, albumin-to-creatinine ratio; AGT, angiotensinogen; AT1, angiotensin II type 1 receptor; DN, diabetic nephropathy; DSP, discordant sib pair; ESRD, end-stage renal disease; LOD, logarithm of odds; PCR, polymerase chain reaction; TDT, transmission disequilibrium test; TMAC, tetramethylammonium chloride.

Diabetic nephropathy (DN) is one of the most serious of the late complications of diabetes and accounts for ~35% of new cases of end-stage renal disease (ESRD) in the U.S. (1). It results from hyperglycemia-related glomerular injury, but the underlying mechanisms are unknown (2). Clinically, DN is manifested as persistent proteinuria that progresses to impaired renal function, ESRD, and death due to cardiovascular complications (3). Since only one in three individuals with type 1 diabetes ever develops DN, genetic susceptibility has been postulated as the mechanism that determines who develops hyperglycemia-related glomerular injury (3). After two reports of familial clustering of DN (4,5), a large study was conducted at the Joslin Diabetes Center to assess the magnitude of this clustering (6). This investigation demonstrated that in comparison with a lifetime DN risk of 35% among unrelated individuals with type 1 diabetes, the risk in families to a second diabetic sibling increases to 72% or decreases to 25%, depending on whether the first sibling had DN (6). Since familial clustering of glycemic control could not account for this large disparity (47%) in sibling risk, an autosomal-dominant major gene with a common disease allele was proposed as a plausible explanation (6). To map a major (though not necessarily dominant) gene, we recently showed through a simulation study that among sib pairs with type 1 diabetes, those discordant sib pairs (DSPs) for DN are four times more effective as those concordant for DN (7). This difference is due to the very high risk of DN (72%) in the diabetic siblings of probands with DN.

We applied the DSP strategy to test for linkage between DN and chromosomal regions containing loci that encode for proteins of the renin-angiotensin system, all of which have been considered candidate genes for DN (8-11). The regions containing the angiotensinogen (AGT) gene (chromosome 1q) and the ACE gene (chromosome 17q) were excluded as major loci for DN in this study; however, we found linkage between DN and the region on chromosome 3q containing the angiotensin II type 1 receptor (AT1) gene.

RESEARCH DESIGN AND METHODS

Families. Two panels of families were collected at the Joslin Diabetes Center: 1) families having multiple siblings with type 1 diabetes and 2) families having a single case of type 1 diabetes, either with or without DN. To limit genetic and phenotypic heterogeneity of diabetes and DN, we included only Caucasian families with cases of type 1 diabetes diagnosed before age 30 and treated continuously

with insulin since diagnosis. Families were selected for the first panel if one sibling had DN and another remained free of DN despite 15 years of diabetes. In total, 47 such families were identified, yielding 60 DSPs (25, 32, and 3 with two, one, and zero parents studied, respectively). The first panel of families was supplemented with DNA samples from five families with type 1 diabetes (six DSPs, all parents studied) from the Human Biological Data Interchange, Philadelphia, PA. The second panel, used for transmission disequilibrium test (TDT) analysis of DNA sequence differences, included family trios, comprising two parents and either a DN⁺ ($n = 147$) or a DN⁻ offspring ($n = 64$), the latter remaining unaffected despite 15 years of diabetes. A detailed medical history was obtained together with urine for urinalysis and blood for DNA extraction. Each person gave written consent to participate in the study. The consent form and study protocol were approved by the Human Subjects Committee at the Joslin Diabetes Center.

Diagnosis of nephropathy. Diabetic nephropathy status in patients with type 1 diabetes was determined on the basis of questionnaires, medical records, and measurements of the albumin-to-creatinine ratio (ACR). Methods for ACR determination have been described previously (6). Individuals with no history of DN and an ACR <20 µg/mg were considered to be free of nephropathy (DN⁻). Patients with renal replacement therapy, persistent proteinuria, or persistently high microalbuminuria were considered (after review of all information for evidence of nondiabetic renal disease) to have nephropathy (DN⁺). Persistent proteinuria was considered present if two out of three sequential urinalyses were positive (reagent strip 1+ or ACR 300 µg/mg). Persistently high microalbuminuria was diagnosed if the ACR was 100 µg/mg or higher in two out of three urinalyses. Patients not falling into one of these categories were considered unable to be classified and excluded.

Genetic markers, genotyping, and sequencing. DSP families were genotyped for two dinucleotide repeat polymorphic markers, D17S807 and D17S795, in the ACE region and for two microsatellites, AGT and D1S459, in the angiotensinogen region. AGT is a (GT)_n dinucleotide repeat polymorphism 0.5 kb apart from the 3' end of the angiotensinogen gene. To investigate the AT1 locus for linkage with DN, we genotyped DSP families for closely linked dinucleotide repeat polymorphic markers, ATCA and D3S1308. ATCA is a dinucleotide repeat polymorphism localized 15.5 kb downstream from the 3' end of the AT1 gene, and D3S1308, a dinucleotide repeat polymorphism, is 1 cM telomeric from the AT1 locus.

As a result of positive findings, we genotyped eight additional markers (D3S3045, D3S2460, D3S1292, D3S1764, D3S1744, D3S1763, D3S3053, D3S2427) spanning a 63-cM chromosomal region around the AT1 locus. Genetic distances among these markers were taken from the sex-averaged genetic map from the Center for Medical Genetics, Marshfield, WI (K.W. Broman, J.C. Murray, V.C. Sheffield, R.L. White, and J.L. Weber, Comprehensive human genetic maps; individual and sex-specific variation in recombination, 1998 submitted article. Data are available at <http://www.marshmed.org/genetics/>).

Marker genotyping was performed using polymerase chain reaction (PCR)-based protocols (12). Genomic DNA (20 ng) was amplified in a 15-µl PCR reaction using a 96-well Thermocycler (MJ Research, Watertown, MA). A single primer in each reaction was end-labeled with either [γ -³²P]dATP or fluorescent dye (Research Genetics, Huntsville, AL). Forty cycles of PCR were performed (94°C for 60 s, 55°C for 60 s, and 72°C for 60 s). For [γ -³²P]dATP products, 2 µl of each reaction was loaded onto 5% polyacrylamide gels, electrophoresed, and exposed to X-ray film. For detection of fluorescence, gels were run using an ABI 373XL Sequencer (PE Applied Biosystems, Perkin Elmer, Norwalk, CT).

The new polymorphisms/mutations in the AT1 gene were found by direct sequencing. The genomic PCR products from the promoter region, all exons, and splicing sites from six DN⁺ siblings and six DN⁻ siblings were electrophoresed on agarose gel and purified using the QIAEX II gel Extraction Kit (Qiagen, Valencia, CA). Sequencing was performed with an ABI 377 sequencer and dye terminator chemistry according to the manufacturer's protocol (Perkin Elmer). Heterozygosity was identified using the program AutoAssembler, version 1.4.0 (PE Applied Biosystems), and the sequence was also compared with the one published in GenBank, accession number S80189 (available at <http://www.ncbi.nlm.nih.gov/>).

We genotyped 147 DN⁺ trios and 64 DN⁻ trios for the following previously known polymorphisms: T573C (13), A1166C (14,15), and ATCA (16). The additional AG-180CC and T-679G polymorphisms, found in the promoter region, were genotyped by PCR-based protocols using primers AG-180CCF (5'-CTC-CGCAGGAAATGATACTC-3'), AG-180CCR (5'-GTCCAGACGTCCTGCACTCG-3'), T-679GF (5'-GCAATTGGCATATCCATCAC-3'), and T-679GR (5'-TCCCAACT-CATGCTATGAGG-3'). The amplified fragments were digested with restriction enzymes and electrophoresed on agarose gels. The CC-180 allele has an *Eco*0109I restriction site, and the 337-bp PCR product yields 70 bp and 267 bp fragments after digestion. The G-679 allele has a *Fok*I restriction site, and the 353-bp PCR product yields 228 bp and 125 bp fragments after digestion.

The third polymorphism found in the promoter region (T-776A) and the fourth found in exon 4 (ex4_T56C) were genotyped by allele-specific oligonucleotide (ASO) hybridization using the following primers for amplification: Ex4F (5'-GCTGAC-TACTGGAGTTGTATCC-3'), Ex4R (5'-GGGCTGCACAAGCCATGC-3'), T-776AF

(5'-GCAATTGGCATATCCATCAC-3'), and T-776AR (5'-TCCCAACTCATGCTAT-GAGG-3'). The following allele specific probes were used for hybridization: ex4_T56 (5'-CTGGTAGAGCAATAGGA-3'), ex4_C56 (5'-CTGGCAGAGCAATAGGA-3'), T-776 (5'-TATCTAACATTAATTGA-3'), and A-776 (5'-TATCAAACATTAATTGA-3'). The genotyping was performed using the standard protocol (17). Briefly, the dot-blot membranes were incubated overnight in 3 mol/l tetramethylammonium chloride (TMAC) hybridization solution at 52°C containing the allele-specific probe (0.15 pmol/ml hybridization solution, end-labeled with [γ -³²P]dATP) and washed for 20 min in 3 mol/l TMAC wash solution at room temperature followed by 20 min of washing in 3 mol/l TMAC wash solution at 52°C. The membranes were exposed to X-ray film for 12 h.

Analytic methods. Genetic model-free linkage analysis was performed on all 66 DSPs using a modified version of MAPMAKER/SIBS, version 4 (18), and verified using the ASPEX program (D. Hinds and N. Risch, <ftp://lahmed.stanford.edu/pub/aspx>). Allele frequencies were calculated from the genotypes of available parents and used to aid determination of allele-sharing in DSPs who are missing one parent ($n = 32$) or both parents ($n = 3$). Sensitivity analysis was performed to ensure that results were robust with respect to these values. For the initial analysis of ATCA and D3S1308, allele-sharing probabilities were left unconstrained to assess descriptively the degree of deviation from the null hypothesis (i.e., 0.25, 0.50, and 0.25 sharing 0, 1, and 2 haplotypes, respectively). Subsequently, an assumption of no dominance variance was added to ensure a highly powerful statistic over a wide range of underlying genetic models (19). Consequently, *P* values for the full analysis are based on 1 df.

Sequence differences were analyzed for excess/diminished transmission from heterozygous parents to DN⁺/DN⁻ offspring using the TDT (20). Unlike case-control analysis, this test for allelic association with disease cannot be biased by population stratification (20); however, it does require that both parents be genotyped (21). The TDT *P* values were found by comparing McNemar's statistic (with continuity correction) with a 1-df χ^2 distribution. DN⁻ offspring, which must necessarily be considered separately from DN⁺ offspring, were included in the TDT analysis to rule out both segregation distortion (20) and the effects of genes responsible for susceptibility to type 1 diabetes.

Pairwise linkage disequilibrium coefficients and corresponding *P* values were calculated by assembling parental allele and haplotypes estimates from the computer program EH (X. Xie and J. Ott, <ftp://linkage.rockefeller.edu/software/eh>) (22) to form the statistic suggested by Thompson et al. (23). In addition, the percentage of the maximum possible disequilibrium coefficient (conditional on allele frequencies) (23) was also calculated for each marker pair.

RESULTS

To test the chromosomal regions containing the gene of renin-angiotensin system for linkage with DN, we assembled 52 multiplex families with type 1 diabetes, containing 66 diabetic sib pairs discordant for DN. The clinical characteristics of the siblings are shown in Table 1. In general, DN⁻ and DN⁺ siblings had similar body weight, diabetes duration, age at onset, and insulin dose. The DN⁺ siblings had poorer glycemic control (higher HbA_{1c}) than DN⁻ siblings (Table 1). The mean HbA_{1c} among DN⁻ siblings, however, was not different from that in the total population of patients with type 1 diabetes attending the Joslin Diabetes Center. At the time of the examination, 37% of the DN⁺ siblings had ESDR, another 53% had persistent proteinuria, and only 10% had high microalbuminuria. DN⁺ siblings also had elevated blood pressure, and the majority of them were treated with antihypertensive drugs.

Three chromosomal regions, although a part of the whole genome scanning, were screened first because they contain genes encoding proteins of the renin-angiotensin system: the genes for AGT, ACE, and AT1. We genotyped the 66 DSPs for two markers in the vicinity of each of these three genes: D17S807 and D17S795 in the ACE region; AGT and D1S459 in the AGT region; and ATCA and D3S1308 in the AT1 region. A multipoint linkage analysis was performed under the assumption of no linkage, and the results are presented in Table 2. There were no significant differences between estimated and expected probabilities of shared alleles for markers in the ACE and AGT regions. The results of multipoint linkage analysis for marker ATCA in the AT1 region (Table 2)

TABLE 1
Clinical characteristics of families used for sib-pair analysis according to nephropathy status

	DN ⁻ siblings	DN ⁺ siblings
<i>n</i> *	60	58
Age at examination (years)	36.9 ± 9.0	37.2 ± 8.9
Age at diabetes onset (years)	14.9 ± 8.2	11.3 ± 8.3
Duration of diabetes (years)	22.1 ± 8.8	25.9 ± 8.8
Percentage of ideal body weight	117.1 ± 23.0	113.3 ± 18.7
Insulin dose (U)	44.8 ± 17.2	45.8 ± 23.4
Average HbA _{1c} for 1988–1994 (mmHg)†	8.8 ± 1.8	9.8 ± 2.3
ESRD (%)	—	37
Persistent proteinuria (%)	—	53
High microalbuminuria (%)	—	10
Blood pressure (mmHg)		
Mean systolic	120.7	131.8
Mean diastolic	73.6	77.9
Antihypertensive medication (%)	13.3	60.3

Data are means ± SD, *n*, or %. *52 families (38 families DN⁺/DN⁻, 6 families 2DN⁺/DN⁻, 8 families DN⁺/DN⁻) totaling 66 DSPs (31, 32, and 3 with two, one, and zero parents, respectively); †available for 36 DN⁻ and 46 DN⁺ siblings who were attending the Joslin Diabetes Center.

revealed an excess of sharing 0 alleles (0.53 estimated probability as compared with 0.25 expected) and deficiencies of sharing 1 allele (0.36 estimated probability as compared with 0.50 expected) or 2 alleles (0.11 estimated probability as compared with 0.25 expected). Similar results were obtained for marker D3S1308. The difference between estimated and expected probabilities of shared alleles was statistically significant for both markers ATCA (*P* < 0.0001) and D3S1308 (*P* < 0.0002). Note that, in contrast to a concordant sib-pair study, where increased allele-sharing is evidence of linkage, positive results in the discordant study are characterized by diminished sharing.

As a result of these preliminary positive findings, we extended the genotyping to include eight additional polymorphic markers spanning a 63-cM region around the AT1 locus. Multipoint analysis on all ten markers again showed evi-

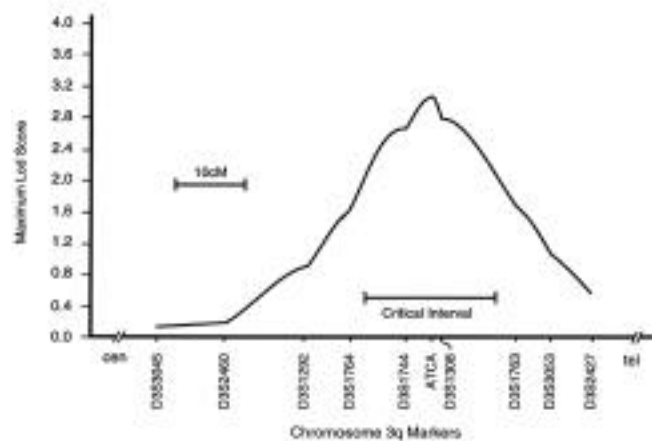


FIG. 1. Results of multipoint linkage analysis between 10 markers on chromosome 3q and diabetic nephropathy in 66 discordant sib pairs.

TABLE 2
Initial multipoint discordant sib-pair linkage analysis for markers

	Number of DSPs	Estimated allele sharing*			<i>P</i> value
		z0	z1	z2	
ACE region					
D17S807	66	0.28	0.49	0.23	NS
D17S795	66	0.28	0.50	0.22	NS
AGT region					
AGT	66	0.30	0.44	0.26	NS
D1S459	66	0.28	0.46	0.26	NS
AT1 region					
ATCA	66	0.53	0.36	0.11	<0.0001
D3S1308	66	0.51	0.37	0.12	<0.0002

Analysis for the following markers: ATCA and D3S1308 in AT1 region; D17S807 and D17S795 in ACE region; AGT and D1S459 in AGT region; *z0, z1, z2 are the estimated probabilities of sharing 0, 1, or 2 alleles identical by descent among sib pairs.

dence of significant linkage. The peak maximum logarithm of odds (LOD) score of 3.1 (*P* = 7.7 × 10⁻⁵) occurred at the ATCA locus, and the region of the chromosome over which the maximum LOD score was within 1 unit of this peak value encompassed 20 cM (Fig. 1). This is the critical interval within which the putative DN susceptibility gene presumably resides.

The most obvious candidate gene for DN in this critical interval was the AT1 gene. To investigate whether polymorphisms/mutations in this gene could explain the linkage results, we sequenced all exons, splicing junctions, and 2,600 bp of the promoter region in six DSPs (six siblings DN⁺ and six siblings DN⁻).

In addition to three previously reported polymorphisms, we found four new polymorphisms, three in the promoter region and one in exon 4 (Fig. 2). The linkage disequilibrium between polymorphisms of AT1 genes is presented in Table 3. Alleles of these polymorphisms were assessed for non-random transmission from heterozygous parents to DN⁺ offspring using the TDT analysis (20). A similar analysis was done with DN⁻ offspring to rule out both segregation distortion and effects of genes that predispose to type 1 diabetes.

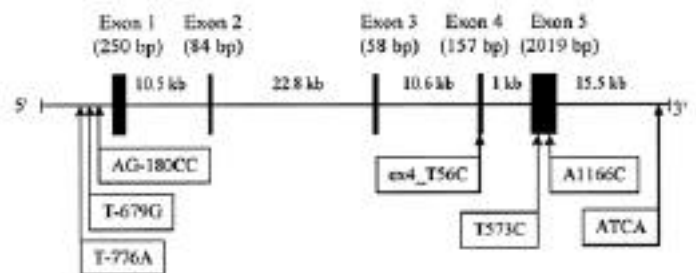


FIG. 2. Exon-intron structure of the human AT1 gene (35). DNA sequence differences examined by the TDT are indicated by arrows. Frequency of the T-776A, AG-180CC, ex4_T56C, T573C, and A1166C polymorphisms are 20, 21, 12, 49, and 30%, respectively (estimated from 316 unrelated nondiabetic individuals who are parents of patients with type 1 diabetes). The exons are presented as black boxes.

TABLE 3

Degree of linkage disequilibrium between polymorphisms in the AT1 gene as measured by percentage of the maximum possible disequilibrium coefficient

Polymorphism	T-776A	AG-180CC	ex4_T56C	T573C
AG-180CC*	97.8 (<10 ⁻⁴)†	—	—	—
ex4_T56C	53.5 (<10 ⁻⁴)	55.3 (<10 ⁻⁴)	—	—
T573C	20.1 (0.078)	17.8 (0.107)	62.7 (<10 ⁻⁴)	—
A1166C	3.8 (>0.5)	3.1 (>0.5)	10.5 (>0.5)	90.7 (<10 ⁻⁴)

Data are % (*P* values). Disequilibrium coefficient is estimated from 316 unrelated nondiabetic individuals who are parents of patients with type 1 diabetes. *The genotypes for T-679G determined in 96 individuals were completely concordant with AG-180CC, indicating that these two polymorphisms are in complete linkage disequilibrium. †*P* values are based on $(h-pq) / \sqrt{Npq(1-p)q(1-q)}$, where *p* and *q* are allele frequencies at the two loci, *h* is the corresponding haplotype frequency, and *N* is the sample size.

None of the alleles at the six examined biallelic polymorphisms were preferentially transmitted to DN⁺ or DN⁻ offspring (Table 4). Similar results were obtained for ATCA alleles (data not shown). Stratification of offspring according to sex, duration of diabetes, or severity of DN did not change the results (data not shown).

DISCUSSION

This study provides direct evidence for a major susceptibility locus that predisposes Caucasians with type 1 diabetes to diabetic nephropathy and places that locus within a 20-cM region on the long arm of chromosome 3. These important findings substantiate the growing body of circumstantial evidence for a genetic basis of DN and provide a well-defined chromosomal region for further study.

In the past, epidemiological data have consistently shown that only a subset of those with type 1 diabetes ever develops nephropathy (3). Although this particular phenomenon may, in theory, be due to either environmental or genetic factors, recent studies demonstrating familial clustering of DN strengthen the likelihood of the latter hypothesis (4–6). Moreover, the consistency of our most recent results (6) with a genetic model having a major gene with a common disease allele add further compelling, though not definitive, evidence for a genetic susceptibility to DN.

Through simulation, we have confirmed that a common disease allele at a single locus could account for the observed familial clustering of diabetic nephropathy (7). Under this assumption, we have also shown that the putative DN susceptibility locus would be more readily detected in a study of discordant sib pairs than in one of commonly used affected sib pairs (7). Specifically, a study based on 66 sib pairs concordant for type 1 diabetes but discordant for DN would have over 70% power (at the 0.001 pointwise significance level) to detect linkage with a major locus (7).

Using this powerful linkage design, we continued our previously initiated study of essential hypertension-related genes as candidates for DN susceptibility. In particular, we examined three chromosomal regions that contain genes encoding for proteins of the renin-angiotensin system. While the findings were negative for the angiotensinogen locus on chromosome 1q and the ACE gene locus on chromosome 17q, we demonstrated significant linkage of DN with a 20-cM region on the long arm of chromosome 3 that contains the AT1, although we found no association with the receptor itself. This serendipitous finding simultaneously confirms the epidemiological evidence for genetic determinants of DN and adds support, specifically, to the hypothesis that DN susceptibility in type 1 diabetes is due to a major gene effect.

TABLE 4

Transmission of alleles from parents heterozygous for biallelic markers to DN⁺ and DN⁻ offspring and two-sided *P* values based on McNemar's statistic with correction for continuity

Polymorphism	Heterozygosity	Allele	Transmitted to DN ⁺ offspring	Transmitted to DN ⁻ offspring
T-776A	0.32	T	41	20
		A	41 (0.912)	24 (0.651)
AG-180CC*	0.33	AG	41	19
		CC	46 (0.668)	26 (0.371)
ex4_T56C	0.21	T	25	15
		C	30 (0.590)	13 (0.850)
T573C	0.49	T	66	36
		C	64 (0.930)	39 (0.817)
A1166C†	0.42	A	67	26
		C	54 (0.275)	28 (0.892)

Data are *n* (*P* values). *The genotypes for T-679G determined in 96 individuals were completely concordant with AG-180CC, indicating that these two polymorphisms are in complete linkage disequilibrium. †One-sided *P* values exceed 0.5 if 1166C is considered to be the risk allele.

Although this localization reduces the search for a gene to <1% of the entire genome, the 20-cM critical region on chromosome 3q is still a very large interval to screen systematically for the genetic culprit. However, obvious candidate genes in the region can be examined. The AT1 gene is such a candidate. By sequencing exons, splicing junctions, and the promoter region, we identified several new and several previously known polymorphisms. None, however, were associated with diabetic nephropathy. Because levels of linkage disequilibrium can vary even within a gene (see Table 3), we cannot fully exclude AT1 on the basis of these negative results, although the likelihood of involvement is clearly lessened by this data. Other known genes located in the 20-cM critical region are the sodium/potassium ATPase β -3 subunit, TRPC1, glycogenin, and glucose transporter type 2 (24). Their relevance to DN, however, is less obvious.

It is quite unlikely that our findings are spurious. Since our genotyping was limited to three chromosomal regions, a P value < 0.00008 for markers on chromosome 3q should be considered as significant evidence for linkage (and, in fact, resampling analysis currently under development [J.J.R., E. Wei, X. Xu, unpublished observations] suggests that LOD scores similar to ours will occur by chance in just over 5 per 100 scans of the entire genome). This evidence is strengthened further by the fact that linkage was detected with markers covering quite a broad chromosomal region (25) and by the fact that the results were insensitive to changes in allele frequency estimates (data not shown). At the same time, analysis using the TDT (20), a sensitive and specific family-based design for detecting allelic associations, provided no evidence for a significant role of the AT1 gene itself in the development of DN.

Before a large-scale effort is made to narrow the critical region and clone the DN susceptibility gene, our linkage results must be replicated in another population of sib pairs with type 1 diabetes. An important consideration in planning a replication study is that sib pairs with type 1 diabetes discordant for DN may be several times as informative as those concordant for DN. For example, to achieve power equal to the present study, more than 250 sib pairs with type 1 diabetes concordant for nephropathy would be required. This number of concordant sib pairs would be difficult to assemble because of the high mortality in those with nephropathy (7).

Whether our findings can be generalized to individuals with type 2 diabetes is unclear. Although there is evidence for familial clustering of DN in type 2 diabetes (26–29), its etiology may be more heterogeneous than in type 1 diabetes (30). Also, the lifetime risk of DN is higher in type 2 than in type 1 diabetes, particularly in African-Americans and Pima Indians (31,32). So far, two studies have searched for DN susceptibility loci in type 2 diabetes using relatively small numbers of sib pairs concordant for nephropathy. Studying 38 African-American sib pairs concordant for both type 2 diabetes and DN, Yu et al. (33) did not find any evidence for linkage in the region where the AT1 receptor is located. Imperatore et al. (34) reported results of a total genome screen for DN susceptibility loci in 98 Pima Indian sib pairs concordant for both type 2 diabetes and DN. They found four regions that showed some evidence for linkage: 3q ($P < 0.01$), 7q ($P < 0.001$), 9q ($P < 0.01$), and 20q ($P < 0.01$) (34). Coming from a total genome screen, where the number of statistical comparisons is large, none of these P val-

ues can be considered as significant evidence for linkage. Furthermore, their sib pairs were concordant for both type 2 diabetes and nephropathy, so the weak signals of linkage may be due to type 2 diabetes rather than DN. Their chromosome 3q finding was localized about 23 cM telomeric of the peak maximum LOD score in our study. Such a large distance makes it unlikely that our study and the finding in Pima Indians point to the same major locus of DN susceptibility. This possibility, however, needs to be investigated further.

A possible shortcoming of the present study is the lack of information about the level of exposure to hyperglycemia in the past. At the time of examination, the mean HbA_{1c} in the DN⁺ siblings was significantly higher than the mean in DN⁻ siblings. If that difference accurately reflects a difference in glycemic exposure in the past, some of the DN⁻ individuals may have been misclassified; that is, they were susceptible but did not manifest it due to good glycemic control. In our study, we observed an excess of those sharing 0 alleles and deficiencies of those sharing 1 or 2 alleles among sib pairs discordant for DN. Misclassification of patients with regard to susceptibility to DN due to good glycemic control would have decreased the number of sib pairs sharing 0 alleles and increased the number sharing 1 or 2 alleles.

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