Original Article

Genome-Wide Scans for Diabetic Nephropathy and Albuminuria in Multiethnic Populations

The Family Investigation of Nephropathy and Diabetes (FIND)

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The Family Investigation of Nephropathy and Diabetes (FIND) was initiated to map genes underlying susceptibility to diabetic nephropathy. A total of 11 centers participated under a single collection protocol to recruit large numbers of diabetic sibling pairs concordant and discordant for diabetic nephropathy. We report the findings from the first-phase genetic analyses in 1,227 participants from 378 pedigrees of European-American, African-American, Mexican-American, and American Indian descent recruited from eight centers. Model-free linkage analyses, using a dichotomous definition for diabetic nephropathy in 397 sibling pairs, as well as the quantitative trait urinary albumin-to-creatinine ratio (ACR), were performed using the Haseman-Elston linkage test on 404 microsatellite markers. The strongest evidence of linkage to the diabetic nephropathy trait was on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3. In ACR (883 diabetic sibling pairs), the strongest linkage signals were on chromosomes 2q14.1, 7q21.1, and 15q26.3. These results confirm regions of linkage to diabetic nephropathy on chromosomes 7q, 10p, and 18q from prior reports, making it important that genes underlying these peaks be evaluated for their contribution to nephropathy susceptibility. Large family collections consisting of multiple members with diabetes and advanced nephropathy are likely to accelerate the identification of genes causing diabetic nephropathy, a life-threatening complication of diabetes. *Diabetes* 56:1577–1585, 2007

Diabetic nephropathy (Online Mendelian Inheritance in Man [OMIM] no. 603933, available at http://www.ncbi.nlm.nih.gov/omim) is a common microvascular complication of type 1 and type 2 diabetes. Increasing prevalence of diabetic nephropathy and end-stage renal disease (ESRD) attributed to diabetes have been observed, particularly in older adults (1–3). In 1999–2003, the estimated prevalence of type 2 diabetes among all ESRD patients in the U.S. was ~29.1%, but among new ESRD cases during the same period, it was 40.5% (1). Although progression of diabetic nephropathy to ESRD and/or development of a cardiovascular complication is common among patients with advanced chronic kidney disease, aggressive treatment of hyperglycemia, proteinuria, and hypertension may slow its progression (4–6).

Major genes underlying susceptibility to diabetic nephropathy have yet to be identified, despite multiple candidate gene and genome scan investigations (7,8). Six genome screens have examined linkage for ESRD or nephropathy in small- to moderate-sized cohorts containing sibling pairs and nuclear families that were recruited at single centers (9–14). Three of these studies were conducted at a single site (11–13). Although these studies did not provide definitive evidence for linkage that met genome-wide criteria, several putative regions of linkage were reported.

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Received for publication 16 August 2006 and accepted in revised form 20 February 2007.

Published ahead of print at http://diabetes.diabetesjournals.org on 15 March 2007. DOI: 10.2337/db06-1154.

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Additional information for this article can be found in an online appendix at http://dx.doi.org/10.2337/db06-1154.

ACR, albumin-to-creatinine ratio; ESRD, end-stage renal disease; GFR, glomerular filtration rate; IBD, identity-by-descent.

Imperatore et al. (9) performed a genome-wide survey in 98 diabetic Pima Indian sibling pairs concordant for diabetic nephropathy. Suggestive evidence for linkage was observed on chromosomes 3, 7, 9, and 20. A genome scan was performed in 18 large Turkish families who were enriched for the presence of type 2 diabetes and diabetic nephropathy (10). There was a highly significant logarithm of odds score of 6.1 on chromosome 18q22.3–23, between markers D18S469 and D18S58. A genome scan for diabetic nephropathy in African Americans identified loci on chromosomes 7p, 12p, 14q, 16p, 18q, and 21q using ordered subsets analysis (11). A recent genome scan for albuminuria revealed evidence for linkage to 22q, 5q, and 7q in 59 large, predominantly European-American pedigrees enriched for members with type 2 diabetes (14). Additional evidence for linkage to 21p was observed when these analyses were restricted to diabetes-affected relative pairs. Consistent evidence for linkage of diabetic nephropathy (and nondiabetic nephropathy) has also been observed on chromosome 10p (15,16), in the syntenic region of the rodent *Rf1* locus (15–17), and on 3q (11,18).

The Family Investigation of Nephropathy and Diabetes (FIND) consortium was established in 1999 to identify diabetic nephropathy susceptibility genes (19). The FIND consortium consisted initially of eight centers, with three centers added to enhance minority recruitment, a Genetic Analysis and Data Coordinating Center, and the NIDDK (National Institute of Diabetes and Digestive and Kidney Diseases). The consortium is assembling a database of families, as well as case and control subjects, and conducting genome-wide scans using microsatellite loci in this initial set of recruited families from four ethnic groups: European Americans, African Americans, Mexican Americans, and American Indians. FIND has the power to detect linkage to diabetic nephropathy in and among four ethnic groups, as well as the ability to confirm prior positive linkage findings using independent families recruited from different geographic areas under a uniform protocol and disease definition. We report results of the first FIND microsatellite-based genome scan for the traits diabetic nephropathy and urinary albumin-to-creatinine ratio (ACR) in four ethnic groups.

RESEARCH DESIGN AND METHODS

The FIND family study design was described previously (19), and the sample described herein was collected from the eight original FIND centers during the first half of the recruitment period (2001–2003). In brief, families of probands with diabetic nephropathy having a diabetic sibling with or without nephropathy were recruited. Living parents and other relatives (i.e., avuncular, cousin, half-sibling, and grandparental affected pairs) were also recruited, when available. FIND is predominantly a sibling pair study (>90% have no relatives other than sibling pairs). Informed consent was obtained from every subject, and approval for recruitment was secured from the institutional review board at each center, including the coordinating center, and a certificate of confidentiality was filed at the National Institutes of Health before the start of enrollment.

Phenotypic evaluation. Participants were interviewed regarding prior diagnoses and treatment of kidney disease and diabetes. Medical information was recorded on standardized questionnaires, family history was collected using a standardized instrument, and information was obtained from medical record review. Participants without ESRD contributed urine for quantification of ACR and protein-to-creatinine ratio, and they contributed blood for cell line creation, DNA extraction, A1C, serum creatinine, blood urea nitrogen, and glucose concentrations; however, those with ESRD provided only blood for cell lines, DNA extraction, and A1C. Blood was obtained in hemodialysis patients before initiation of treatment and before heparin administration. Blood and urine samples were shipped to the Laboratory of Genomic Diversity for centralized processing. Samples for assay were shipped from the Laboratory of Genomic Diversity to the central clinical laboratory (Penn Medical Laboratories, Medstar). De-identified clinical, demographic, and assay information was entered into a centralized database housed at the Genetic Analysis and Data Coordinating Center. BMI was calculated using current weight and height recorded on medical questionnaires.

Definitions. Diabetic participants were considered to have diabetes if they were currently or previously treated with insulin and/or oral hypoglycemic medicines. Subjects reporting diabetes but not treated with these medicines, and those without a history of diabetes, had A1C and fasting plasma glucose concentrations measured at study entry. A1C concentrations >6.0% were considered suggestive of diabetes, and fasting plasma glucose and/or oral glucose tolerance testing was then performed to confirm the diagnosis. American Diabetes Association 1997 criteria (20) were used to define diabetes in participants not previously known to have diabetes. In previously diagnosed individuals, the date of diagnosis of diabetes was obtained from their medical history, with confirmatory medical record review, when possible. Subjects with type 1, type 2, or other types of diabetes were eligible.

Nephropathy. Subjects were considered to have overt proteinuria in the presence of a historical 24-h urine collection with ≥500 mg protein per 24 h or ≥300 mg albumin per 24 h, random protein-to-creatinine ratio ≥0.5 g/g, or random ACR ≥0.3 g/g. ESRD was defined as the need for chronic renal replacement therapy with either dialysis or renal transplantation. Whether an individual was considered affected or unaffected in the analysis depended on the participant category (see eligibility criteria below).

Diabetic retinopathy. The diagnosis required medical record documentation of an ophthalmologic exam demonstrating microaneurysms, proliferative diabetic retinopathy, or macular edema. Alternatively, the subject may have had a history of retinal laser surgery (photocoagulation) for diabetic retinopathy.

Eligibility criteria for probands. Probands met the above criteria for diabetes and had diabetic nephropathy defined by one of the following. The first was kidney biopsy revealing diabetic nephropathy in the presence of overt proteinuria defined by the following: *1*) nodular and/or diffuse increases in mesangial matrix accumulation, *2*) thickened glomerular basement membranes and/or arteriolar hyalinization, and *3*) absence of mesangial immunoglobulin or paraprotein deposits by immunofluorescence microscopy, absence of amyloid deposits by Congo Red staining or electron microscopy, or absence of electron-dense deposits within the glomerular basement membrane or glomerular capillary subendothelial space. The second was ESRD attributed to diabetic nephropathy based on *1*) onset of diabetes ≥5 years before renal replacement therapy with diabetic retinopathy, *2*) onset of diabetes ≥5 years before renal replacement therapy with historic 24-h urine protein ≥3 g (protein-to-creatinine ratio ≥3.0 g/g), or *3*) diabetic retinopathy with historic 24-h urine protein >3 g (protein-to-creatinine ratio >3.0 g/g). The third was chronic kidney disease (non-ESRD) attributed to diabetic nephropathy based on either diabetic retinopathy with historic 24-h urine protein ≥1 g (protein-to-creatinine ratio ≥1.0 g/g) or 24-h urine protein excretion ≥3 g (protein-to-creatinine ratio ≥3.0 g/g) after diabetes duration ≥10 years.

Eligibility criteria for family members. Entry of a proband with diabetic nephropathy into the FIND family protocol required participation of either two living parents (regardless of the presence or absence of diabetes or nephropathy) or at least one full diabetic sibling classified as either diabetic nephropathy affected or lacking diabetic nephropathy. Enrollment of a diabetic nephropathy sibling required one of the following: *1*) kidney biopsy consistent with diabetic nephropathy (regardless of the degree of proteinuria as defined by the biopsy criteria above), *2*) urine albumin excretion ≥30 mg per 24 h (ACR ≥0.03 g/g) regardless of diabetes duration, or *3*) serum creatinine concentration ≥1.6 mg/dl in men or ≥1.4 mg/dl in women or ESRD.

Diabetic siblings were classified as having diabetic nephropathy (forming a diabetic nephropathy concordant sibling pair) if they had elevated urine albumin excretion (≥300 mg per 24 h or ACR ≥0.3 g/g) or ESRD attributed to diabetic nephropathy. Diabetic siblings were classified as unaffected by nephropathy (forming a diabetic nephropathy discordant sibling pair) if they had diabetes duration ≥10 years with normal serum creatinine concentration (male <1.6 mg/dl, female <1.4 mg/dl) and normal urine albumin excretion (<30 mg per 24 h or ACR <0.03 g/g) without historical evidence of kidney disease. Diabetic siblings who lacked ESRD or elevated serum creatinine concentrations and had spot urine ACR values between 0.03 and 0.3 g/g (in the microalbuminuric range) were included in the ACR quantitative trait genome scan but not the dichotomous diabetic nephropathy trait analysis.

Genotyping and genetic analytic methods. Genotyping and linkage analyses were conducted on 883 full sibling pairs with diabetes from 378 families. DNA was extracted from either lymphoblastoid cell lines or buffy coats at the Genetic Analysis and Data Coordinating Center and shipped to the Center for Inherited Disease Research (CIDR) for genotyping. The Center for Inherited Disease Research genotyped 404 markers on 22 autosomes and two sex chromosomes using a marker set based on the Marshfield Genetics version 8 screening set from Research Genetics, with an average marker spacing of 9 cM. Mendelian inconsistencies were identified using the MARKERINFO

TABLE 1
Clinical characteristics of the genotyped individuals stratified by proband and diabetic nephropathy status

	Diabetic nephropathy probands	Diabetic nephropathy relatives	Diabetes without nephropathy relatives
n	349	390	147
Male	169 (46.0)	180 (46.2)	43 (29.3)
Age (years)	57 ± 10.7	56 ± 11.4	59 ± 10.0
BMI (kg/m^2)	30 ± 7.2	32 ± 8.6	32 ± 7.3
Ethnicity			
European American	50 (14.3)	42 (10.8)	27 (18.4)
African American	90 (25.8)	95 (24.4)	33 (22.4)
Mexican American	179 (51.3)	210 (53.8)	80 (54.4)
American Indian	30 (8.6)	43 (11.0)	7 (4.8)
ESRD	282 (80.8)*	53 (13.6)	0 (0.0)
Diabetes			
Age diagnosed (years)	34 ± 12.3	41 ± 13.1	42 ± 11.5
Duration (years)	23 ± 8.4	16 ± 10.1	17 ± 7.0
Biochemistry†			
A1C (%)	7.8 ± 1.8	8.6 ± 2.4	7.8 ± 2.0
Serum creatinine (mg/dl)	8.58 ± 3.02	2.6 ± 3.2	0.99 ± 0.16
Blood urea nitrogen (mg/dl)	72.9 ± 7.2	29.9 ± 25.5	15.7 ± 4.9
Urine protein-to-creatinine ratio (g/g)	3.29 ± 0.69	1.38 ± 1.4	0.17 ± 0.26
Urine ACR (g/g)			
Mean	2.75 ± 0.7	0.93 ± 1.2	0.01 ± 0.01
Median	3 (3–3)	0.1 (0.05–0.8)	0.009 (0.006–0.01)
GFR (ml/min per 1.73 m^2)			
Mean	10.6 ± 15.0	68.5 ± 42.0	85.6 ± 24.5
Median	5 (5–5)	76.2 (47.4–98.0)	89.8 (73.9–105.1)

Data are *n* (%), means ± SD, or median (interquartile range). *Three probands are not on dialysis and have no urine results but were classified as ESRD for the analyses; †laboratory values in participants with ESRD (either on dialysis or after renal transplant) were set to: serum creatinine 10 mg/dl, blood urea nitrogen 80 mg/dl, protein-to-creatinine ratio 3.5 g/g, ACR 3 g/g, and GFR 5.0 ml/min per 1.73 m^2.

program from the S.A.G.E. (Statistical Analysis for Genetic Epidemiology) (21) software package. All programs are part of the S.A.G.E. suite of programs, unless otherwise specified. Errors in relationship specification were identified through the use of all markers with the program RELTEST. A total of 98 individuals in 54 full sibships were reclassified as unrelated, and 65 individuals in 54 full sibships were reclassified as half-siblings. We tested for deviation from Hardy-Weinberg proportions separately for each ethnicity, and no significant departure was observed at a 1% significance level. The number of Mendelian inconsistencies blanked because of genotyping error in consistent pedigrees was 260 (0.05%).

We evaluated information in four ethnic groups containing diverse genomes. Maximum likelihood estimation was used, as implemented in the program FREQ, to estimate the marker allele frequencies separately in each ethnic group. Multipoint identity-by-descent (IBD) allele sharing estimates were computed separately within each of the four ethnic groups using the program GENIBD. For the IBD calculations, 378 pedigrees with 1,227 individuals, 1,337 full sibling pairs (of whom 883 were diabetes concordant), 147 half-sibling pairs, 226 parent-offspring pairs, 97 avuncular pairs, and 28 cousin pairs were used.

Linkage analyses were performed in two ways. First, diabetic nephropathy was examined as a binary variable (affected versus unaffected, excluding individuals with microalbuminuria) and as quantitative traits (urine ACR and protein-to-creatinine ratio). Diabetic nephropathy was dichotomized based on affection status (affected [with both diabetes and diabetic nephropathy] versus unaffected [without diabetic nephropathy after diabetes duration ≥10 years]), thus modeling affection status as a function of diabetes with or without kidney disease. This stringent definition of diabetic nephropathy was based on a random urine ACR ≥0.3 g/g, and the analyses were based on the binary outcome of presence or absence of diabetic nephropathy.

Urine ACR and protein-to-creatinine ratio were evaluated in linkage analysis as quantitative traits. Because urine assays are difficult to interpret in individuals with ESRD, we evaluated linkage to ACR and protein-to-creatinine ratio by either excluding or including ESRD/transplant participants in two separate models. The model that included ESRD participants fitted each ESRD individual with an ACR of 3.0 g/g (or total protein-to-creatinine ratio of 3.5 g/g). Individuals with chronic renal failure not yet on dialysis whose measured ACR was >3.0 g/g or protein-to-creatinine ratio was >3.5 (n = 83) were set to (Winsorized) values of 3.0 and 3.5, respectively, to ensure that all values of ACR and protein-to-creatinine ratio were set on a similar scale of measurement. However, the linkage analysis results obtained without Winsorizing these values did not materially alter the results (data not shown).

For binary and quantitative traits, the Haseman-Elston regression linkage test (22), as extended for sibships (23), available in SIBPAL, was performed separately for each ethnicity using the multipoint IBD sharing estimates. SIBPAL performs linear regression–based modeling of sibling pair traits as a function of marker allele IBD sharing. Under the latest version of this method, the weighted combination of squared trait difference and squared mean-corrected trait sum was used, further adjusted for the nonindependence of sibling pairs and the nonindependence of squared trait sums and differences (23). For the binary trait, sex was added as a covariate in the Haseman-Elston regression as a 0,1 variable and included as the sum, which assumes that the effect of being a male-female pair is halfway between the effects of the two same-sex pairs. Quantitative traits were adjusted for sex and age at diabetes diagnosis before linkage analysis. The residuals were used as the trait values in the Haseman-Elston regression. For regions suggestive of linkage, asymptotic P values were validated by obtaining empirical P values in SIBPAL.

For the binary trait, "mean" tests and "proportion" tests were performed using SIBPAL, separately for concordant affected pairs (pairs who had diabetic nephropathy and diabetes), discordant pairs (one member of this pair had diabetic nephropathy and diabetes, whereas the other had long-standing diabetes but no diabetic nephropathy), and concordant unaffected pairs (individuals with long-standing diabetes but no nephropathy). These tests determine whether affected pairs share more alleles IBD and discordant pairs share fewer alleles IBD, a pattern expected at a true disease susceptibility locus.

A separate linkage analysis was performed for each ethnicity, and we combined P values across ethnicity using a method proposed by Fisher (24). Fisher's method written as

$$-2 \sum_{i=1}^{4} \log_e(p_i),$$

where p_i is the P value for the ith ethnicity, compared with a χ^2_8. Fisher's method was used because it was not desirable to treat all the ethnic groups as a single sample, with the attendant allele frequencies and demographic differences. This method enables us to combine information on all ethnic groups after accounting for group-specific differences.

TABLE 2
Sample size for diabetic nephropathy as dichotomous trait genome scan and urine ACR as quantitative trait genome scan

		Dichotomous trait								Quantitative trait
		Full sibling pairs			Half-sibling pairs			Avuncular	Cousin	Full sibling pairs
Ethnicity	Pedigrees	Concordant affected	Discordant	Concordant unaffected	Concordant affected	Discordant	Concordant unaffected	Concordant affected	Concordant affected	NA
African American	96	51	27	2	4	6	0	4	0	168
American Indian	32	27	15	1	2	2	0	0	0	69
European American	54	17	29	3	1	2	0	0	0	126
Mexican American	196	122	87	16	4	3	1	10	4	520
Total	378	217	158	22	11	13	1	14	4	883

Concordant affected means that both individuals in the sibling pair have diabetes and diabetic nephropathy. Discordant means that both individuals in the sibling pair have diabetes, one has diabetic nephropathy, and the other is unaffected with diabetic nephropathy after diabetes duration of at least 10 years. Concordant unaffected means that both individuals in the sibling pair have diabetes with a duration of at least 10 years, and neither has diabetic nephropathy. NA, not applicable to quantitative traits.

Molecular genetic quality control analyses were performed by comparing a forensic marker panel for DNA samples collected from buffy coat and cell line sources in a 5% random sample of FIND participants. A total of 2.5% of the 400 FIND participants tested were found to have mismatched DNA from the buffy coat and cell line sources, equating to a ~2.5% overall error rate in the genome scans. Clinical quality control was performed with an independent auditing agency site visiting each center.

RESULTS

Eight centers contributed samples for this microsatellite genome scan. Of the families, 14% were European American (54 pedigrees), 25% were African American (96 pedigrees), 52% were Mexican American (196 pedigrees), and 9% were American Indian (32 pedigrees). A one-way ANOVA was used to compare ethnic-specific variation in proband characteristics. Differences between the groups included sex and BMI ($P < 0.017$ and 0.014, respectively, on the ANOVA with 3 degrees of freedom). African Americans had the lowest male-to-female ratio and highest BMI. The difference in BMI paralleled the nationally reported trends of higher BMI among African Americans (25). Remaining clinical characteristics were similar between probands in the four ethnic groups.

We also compared the clinical characteristics of the participants based on presence/absence of kidney disease and their ascertainment status (Table 1). The study included 349 probands, of which 81% were on dialysis or had a renal transplant. The remaining probands had severe proteinuria with reduced glomerular filtration rate (GFR), indicating a high likelihood of rapid progression to renal replacement therapy. Of the 390 relatives in this report, 53 (13.6%) also had ESRD. The age ranges of the probands and their affected relatives were similar; however, the diabetic nephropathy–affected relatives were younger than the diabetic relatives without nephropathy. Glycemic control assessed by A1C was poor in all three groups, with probands and diabetic siblings without nephropathy having slightly better control than diabetic nephropathy–affected relatives. Diabetes duration was longest in the probands (mean 23 ± 8.4 years), differing from other affected relatives who had shorter diabetes duration (mean 16 ± 10 years). As anticipated, the probands and relatives had different biochemical parameters (GFR, serum creatinine, blood urea nitrogen, ACR, and protein-to-creatinine ratio).

Diabetic nephropathy was evaluated as a binary trait using the Haseman-Elston regression. The linkage analysis used full sibling pairs who were either concordantly affected, discordant, or concordantly unaffected for diabetic nephropathy. There were 49 European-American, 80 African-American, 225 Mexican-American, and 43 American Indian sibling pairs (Table 2). After pooling P values using Fisher's method, the most significant linkage signals ($P \leq 0.003$) were detected on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3. The entire genome scan in all ethnicities is shown in Fig. 1. The analogous plots for the two other methods of pooling results for ethnicities are given in the supplementary material, which can be found in an online appendix (available at http://dx.doi.org/10.2337/db06-1154).

We next evaluated which ethnic group(s) contributed to the linkage signals on chromosomes 7, 10, 14, and 18. African-American families had a suggestive linkage peak on chromosome 7 at 106 cM ($P \leq 0.000022$) (Fig. 2). The small sample of American Indian families showed a significant linkage peak on chromosome 10 at 0 cM ($P \leq$

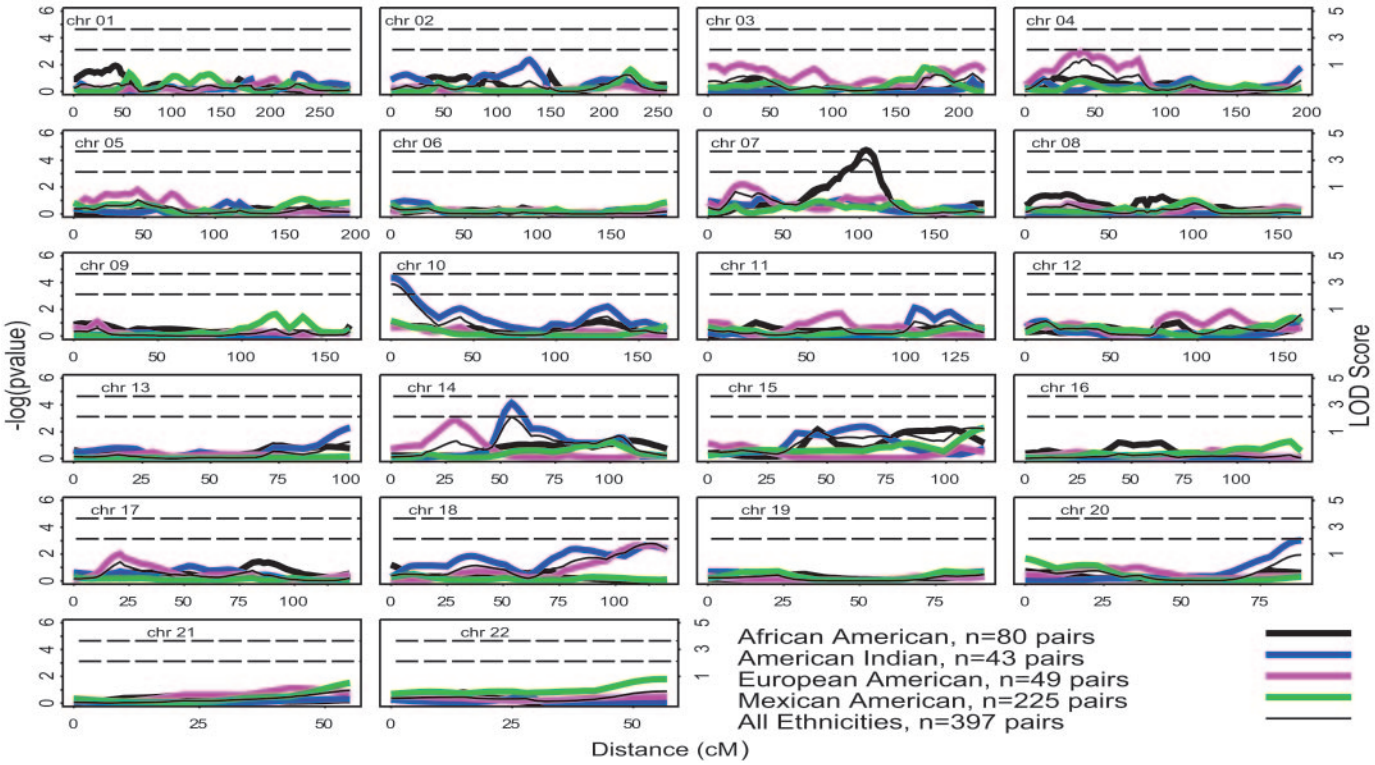

FIG. 1. Plot of the genome scan for diabetic nephropathy as a dichotomous trait. For each autosome (1–22), the genetic distance along the chromosome is plotted on the *x*-axis, and the $-\log_{10}(P$ value) is plotted on the *y*-axis. On the right side of chromosomes 4, 8, 12, 16, and 20, the corresponding logarithm of odds (LOD) score is plotted on the *y*-axis. The dashed lines represent *P* values of 0.000022 and 0.000744, which meet the Lander-Kruglyak criterion (33) of significant and suggestive linkage, respectively. Logarithm of odds scores of 3.7 and 2.1 equate to the Lander-Kruglyak *P* values of significant and suggestive linkage, respectively.

0.000022) (Fig. 2) and chromosome 14 at 55 cM ($P = 7.23 \times 10^{-5}$) (Fig. 2). Finally, the small sample of European-American families showed evidence for linkage on chromosome 18 at 116 cM ($P = 2.17 \times 10^{-3}$) (Fig. 2). Details of the peaks are provided in Table 3.

The mean allele sharing at the linkage peaks are provided in Table 4. For the peaks on chromosomes 7, 10, 14, and 18 there was significantly decreased allele sharing ($P \leq 0.03$) among the discordant sibling pairs for the African-American, American Indian, and European-American ethnicities, respectively, indicating that the discordant pairs proved most of the evidence for linkage. However, significant excess sharing was also observed among the affected sibling pairs on chromosome 7 in African Americans ($P = 0.0248$) and chromosome 10 in American Indians ($P = 0.0498$), and suggestive excess sharing was also observed in American Indians on chromosome 14 ($P = 0.1733$) and European Americans on chromosome 18 ($P = 0.1959$). The loci that best follow the anticipated patterns of allele sharing are located on chromosomes 7 and 10.

We conducted a quantitative trait linkage analysis of ACR (Table 2). There were 942 individuals making up a total of 883 sibling pairs used in the analysis. Values of urine ACR were imputed for ESRD participants as 3.0 g/g (as described in the section on genotyping and genetic analytic methods, above). Median GFR and ACR (interquartile range) for the 942 subjects used in the analysis was 79.7 (56.0–103.7) and 0.05 (0.01–0.38), respectively. For urine ACR, suggestive evidence for linkage was observed on chromosomes 2q14.1 in American Indian families, 7q21.1 in European-American families, and 15q26.3 in African-American families (Fig. 3 and Table 5). The linkage results for protein-to-creatinine ratio confirmed the results for ACR on 2p and 7q, with smaller *P* values observed for protein-to-creatinine ratio (not shown).

DISCUSSION

This report contains the first-phase genome scan results for diabetic nephropathy and for the quantitative traits ACR and protein-to-creatinine ratio in the multiethnic FIND study. FIND families contain large numbers of sibling pairs concordant for diabetes and either concordant or discordant for the presence of diabetic nephropathy. The current linkage analysis for the discrete trait diabetic nephropathy was performed using 397 informative full sibling pairs, and 883 sibling pairs were used for the quantitative trait urine ACR. Because of the stringent criteria for the dichotomous trait (and thus the smaller sample size), this group has less power to detect linkage than the analysis of the quantitative trait (see the supplementary material). This interim report used a microsatellite marker–based genome scan. In contrast, the final FIND genome scan will be performed using a dense map of single nucleotide polymorphisms with a spacing of 0.64 cM and will include all the FIND families recruited through 2005.

Suggestive evidence for linkage to the dichotomous trait of diabetic nephropathy was observed on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3. The linkage peak at 102.0 cM on chromosome 7q was driven predominantly by the African-American families, although there was strong evidence for linkage in ethnicity-combined FIND families. Diabetic nephropathy was also linked to 10p, 14q, and 18q in all FIND families; however, the American Indian families made major contributions to the chromosome 10p and

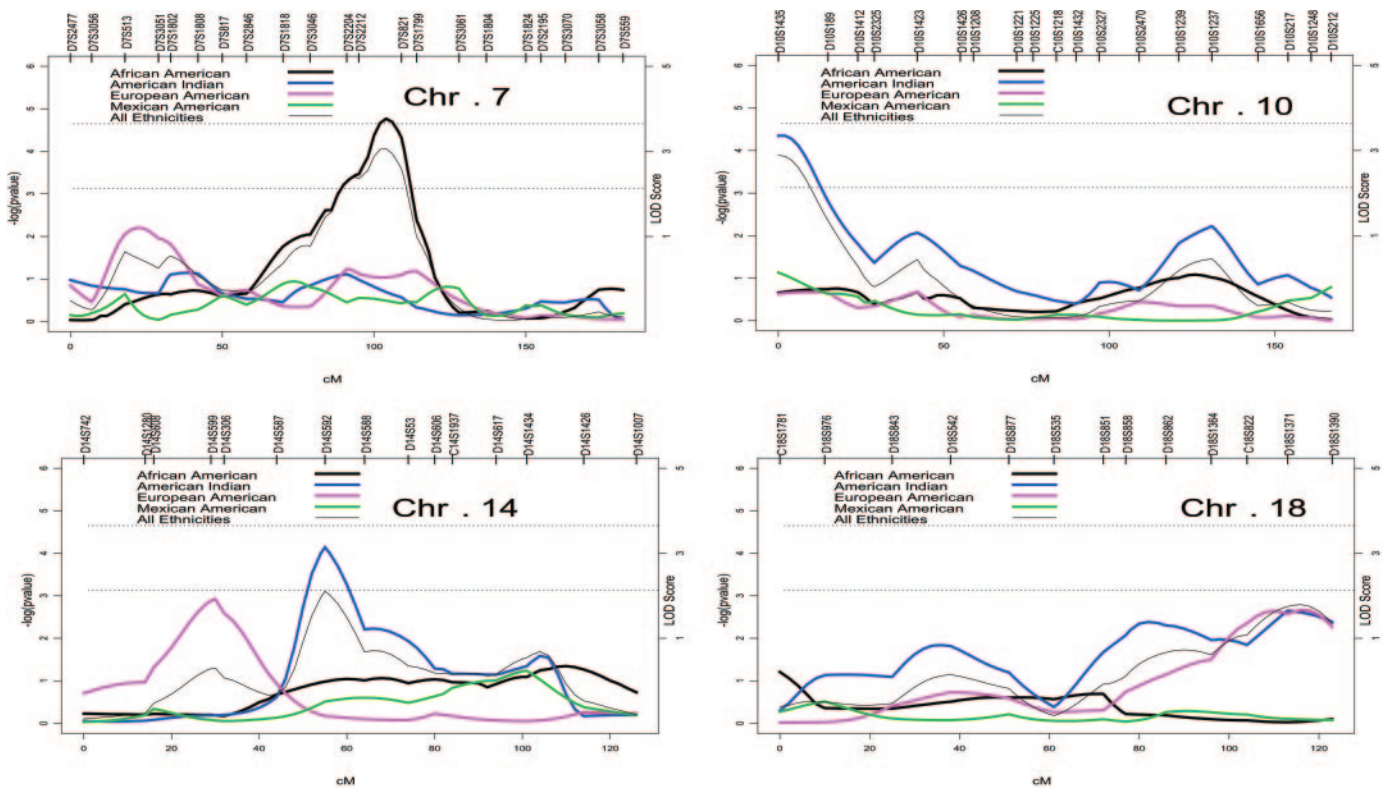

FIG. 2. Chromosomes 7, 10, 14, and 18 linkage results for diabetic nephropathy as a dichotomous trait displayed by ethnic group. The genetic distance along the chromosome is plotted on the *x*-axis, and the $-\log_{10}$(*P* value) is plotted on the *y*-axis. The markers that were genotyped are labeled along the top of the graph. On the right side, the corresponding logarithm of odds (LOD) score is plotted on the *y*-axis. The dashed lines represent *P* values of 0.000022 and 0.000744, which meet the Lander-Kruglyak criterion (33) of significant and suggestive linkage, respectively. Logarithm of odds scores of 3.7 and 2.1 equate to the Lander-Kruglyak *P* values of significant and suggestive linkage, respectively.

14q peaks, and both European Americans and American Indians contributed to the evidence for linkage to 18q.

When ACR was evaluated as a quantitative trait, suggestive evidence for linkage was observed on chromosomes 2q, 7q, and 15q. Again, the relative ethnic contributions to each peak differed, with the relatively small number of American Indian families predominantly contributing to the chromosome 2 peak, European Americans to the 7q peak, and African Americans to the 15q peak.

These results should be considered in the context of other genome scans that have evaluated albuminuria, GFR, overt nephropathy, and ESRD in diabetic and nondiabetic families. Although the majority of African-American families in this report were recruited by the Wake Forest University School of Medicine, the FIND families were different from those contained in the previously published diabetic ESRD genome scan from that institution (11–13). Several Pima families reported previously (9) were included in this FIND analysis, although they are a small component of the total participants in this report. The FIND 7q, 10p, and 18q diabetic nephropathy linkage regions replicate peaks previously observed in type 2 diabetic nephropathy and type 2 diabetic ESRD in Turkish, African-American, and Pima families. The novel FIND peaks on 2, 14, and 15 likely reflect the inclusion of additional ethnic groups or possibly different FIND inclusion criteria for diabetic nephropathy. The FIND inclusion criteria for probands required the presence of severe

TABLE 3
Summary of linkage peaks for the dichotomous diabetic nephropathy trait where the nominal *P* value reached ≤0.003

Chromosome	Ethnic group	Asymptotic *P* value	Empirical *P* value	Peak location (cM)	Flanking markers†
7	African American	1.71×10^{-5}	6.00×10^{-5}	7q21.3 (104)	D7S2212-D7S821
7	All*	8.60×10^{-5}		7q21.3 (104)	D7S2212-D7S821
10	American Indian	4.45×10^{-5}	1.40×10^{-4}	10p15.3 (0)	D10S1435-D10S189
10	All*	1.29×10^{-4}		10p15.3 (0)	D10S1435-D10S189
14	American Indian	7.23×10^{-5}	2.00×10^{-5}	14q23.1 (55)	D14S587-D14S588
14	All*	7.80×10^{-4}		14q21.1 (55)	D14S587-D14S588
18	European American	2.17×10^{-3}	3.15×10^{-2}	18q22.3 (116)	D18S1371-D18S1390
18	All*	1.61×10^{-3}		18q22.3 (116)	D18S1371-D18S1390

Empirical *P* value is the permutation *P* value computed for 50,000 replicates. *Linkage evidence for the full sample was determined by post hoc pooling of the *P* values for all the ethnic groups using Fisher's method, as described in RESEARCH DESIGN AND METHODS. Ethnic-specific *P* values are reported only for those groups that showed significant evidence of linkage. †The flanking markers represent the nearest marker on either side of the linkage peak where there was a 1-unit drop in the *P* value to define the peak.

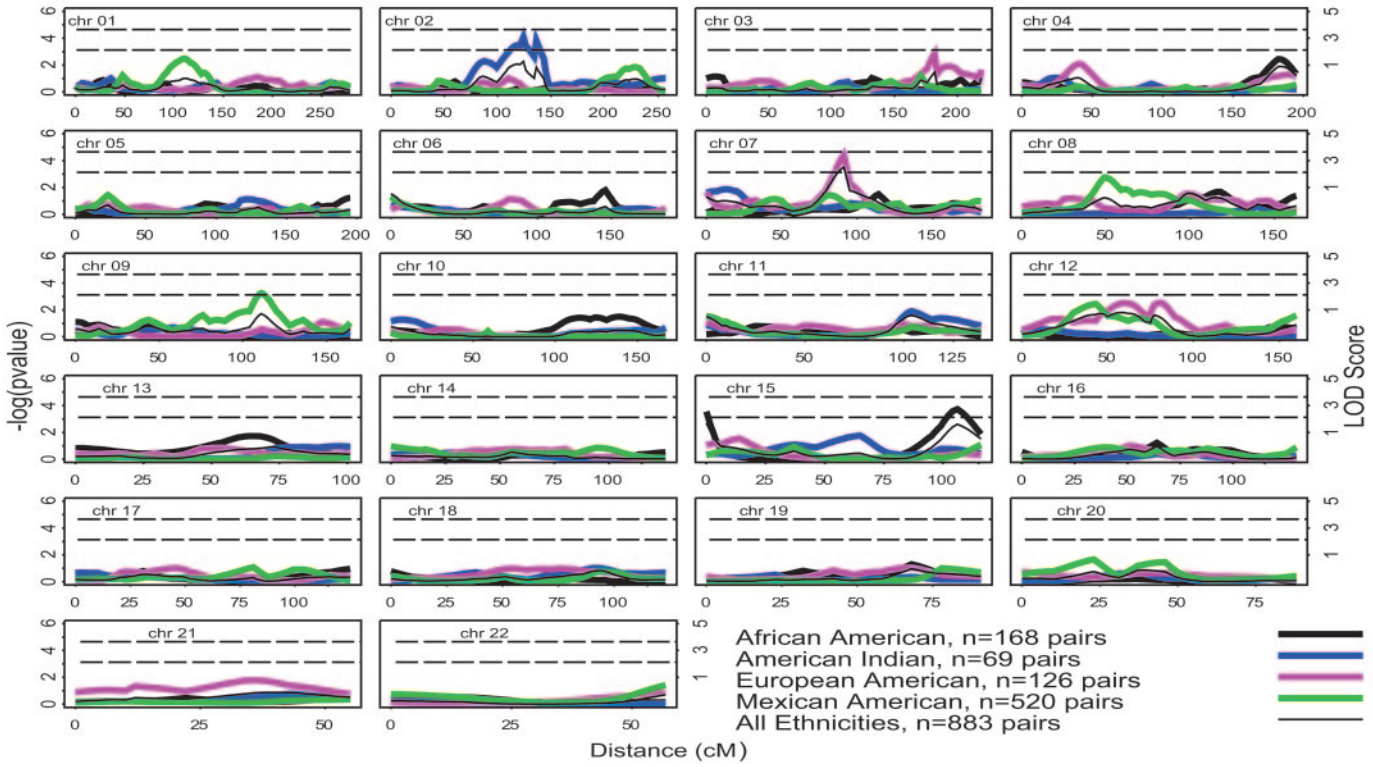

FIG. 3. Plot of the genome scan for urine ACR in all diabetic participants. For each autosome (1–22), the genetic distance along the chromosome is plotted on the *x*-axis, and the $-\log_{10}(P$ value) is plotted on the *y*-axis. On the right side, the corresponding logarithm of odds (LOD) score is plotted on the *y*-axis. The dashed lines represent *P* values of 0.000022 and 0.000744, which meet the Lander-Kruglyak criterion (33) of significant and suggestive linkage, respectively. Logarithm of odds scores of 3.7 and 2.1 equate to the Lander-Kruglyak *P* values of significant and suggestive linkage, respectively.

diabetic nephropathy, either ESRD or proteinuria >1 g/day, which is likely to progress to ESRD.

It is possible that the susceptibility to albuminuria and to chronic kidney disease with reduced GFR may not share many genetic determinants and that the genes regulating renal function may differ from those controlling proteinuria (26). However, longitudinal studies will ultimately be required to reach this conclusion. The FIND diabetic nephropathy linkage result on 10p replicates linkage to diabetic and nondiabetic ESRD previously observed in reports evaluating African-American families (15,16). Ewens et al. (27) and McKnight et al. (17) used association-based methods in type 1 diabetic populations of modest size, and they observed associations with the neuropilin 1 and the D10S1435 marker, respectively. This genomic stretch is also orthologous to the *RF-5* locus of the rat reported by Brown et al. (28). Therefore, it is possible that a general "renal failure" susceptibility gene exists on 10p, a gene promoting renal failure in the presence of hyperglycemia, as well as other systemic insults, including high blood pressure. The chromosome 18q peak has been observed previously in diabetic nephropathy in several different ethnic groups (10,11), and it is reportedly due to polymorphisms in the carnosinase gene (CNDP1) (29,30). Individuals homozygous for the 5 leucine repeat (CNDP1 Mannheim allele) were at reduced risk for development of diabetic nephropathy. CNDP1 and other positional candidate genes under the 18q peak should be evaluated for their role in susceptibility to diabetic nephropathy because multiple independent studies show convergence for linkage results in this region.

The pathogenesis of type 1 versus type 2 diabetic nephropathy and their respective genetic bases remain unknown. Although common genes for these disorders have been postulated, the loci that were linked with diabetic albuminuria and nephropathy in the Joslin Diabetes Clinic population (type 1 diabetes) are clearly different from those identified in this, and other, type 2 diabetic

TABLE 4
Mean allele sharing for affected and discordant sibling pairs at the peak locations for diabetic nephropathy

Ethnic group	Chromosome	Discordant sibling pairs mean sharing*	*P* value	Affected sibling pairs mean sharing*	*P* value
African American	7	0.38	0.0013	0.58	0.0248
American Indian	10	0.27	0.0007	0.58	0.0498
American Indian	14	0.30	0.0287	0.56	0.1733
European American	18	0.40	0.0308	0.57	0.1959

*Under the null hypothesis, sibling pairs should share 50% of their genome identical by descent. At a disease locus, discordant siblings should share <50%, and affected siblings should share >50%.

TABLE 5
Summary of linkage peaks for urine ACR where the nominal *P* value reached ≤0.006

Chromosome	Ethnic group	Asymptotic *P* value	Empirical *P* value*	Peak location (cM)	Flanking markers†
2	American Indian	8.24×10^{-5}	0.0015	2q14.1 (124)	D2S410-D2S1328
2	All	5.58×10^{-3}		2q14.1 (124)	D2S410-D2S1328
7	European American	3.76×10^{-5}	0.0006	7q21.1 (91)	D7S3046-D7S2212
7	All	3.04×10^{-4}		7q21.1 (91)	D7S3046-D7S2212
15	African American	1.85×10^{-4}	0.0055	15q26.3 (106)	D15S657-D15S642
15	All	2.48×10^{-3}		15q26.3 (106)	D15S657-D15S642

*Empirical *P* value = permutation *P* value computed for 50,000 replicates; †the flanking markers represent the nearest marker on either side of the linkage peak where there was a 1-unit drop in the *P* value to define the peak.

nephropathy cohorts. Therefore, it remains possible that different genetic loci are implicated in these two forms of diabetic nephropathy.

Issues concerning heterogeneity of the diabetic nephropathy disease phenotype, typically expressed as either loss of GFR (e.g., presence of chronic kidney disease or ESRD) versus albuminuria per se, abound. It is clear that the "classic" diabetic nephropathy phenotype typically encompasses loss of GFR in concert with excess albuminuria. Therefore, the FIND study attempted to define a severe diabetic nephropathy phenotype for affected probands, which would be present in those with diabetic nephropathy as their cause for ESRD (or advanced chronic kidney disease) and also those at high risk for rapid progression to diabetic ESRD (as defined by proteinuria >1 g/day). The association between high-level proteinuria and rapid loss of GFR in diabetic nephropathy is well established (31,32). However, there is heterogeneity of disease states when referring to diabetic subjects with milder levels of albuminuria (e.g., microalbuminuria) and when comparing them to subjects with diabetic ESRD. This relates to the fact that microalbuminuria may regress to normal levels and is independently associated with risk for cardiovascular events far more strongly than to development of diabetic nephropathy. This heterogeneity led the FIND investigators to perform independent genome scans for: *1*) the amalgamated dichotomous phenotype of diabetic nephropathy, *2*) the quantitative trait of urine ACR, and *3*) the quantitative trait of GFR.

In summary, the initial preliminary phase of the FIND linkage analysis detected evidence for linkage to diabetic nephropathy on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3 and to albuminuria on 2q14.1, 7q21.1, and 15q26.3. The first-phase FIND results replicate the evidence for linkage to diabetic nephropathy on chromosomes 7q, 10p, and 18q in diverse ethnic groups, as well as on 10p in diabetic and nondiabetic ESRD. The final-phase FIND genome scan with a single nucleotide polymorphism–based marker set will be performed in ~5,000 individuals in >1,200 families. Genomic regions revealing significant and consistent evidence for linkage to diabetic nephropathy in large family-based studies, such as the FIND study, should be fine mapped to identify diabetic nephropathy susceptibility genes. Our strategy for prioritizing ultrafine mapping of linkage regions entails ranking the regions by the best *P* value and their allele-sharing characteristics. These genes have the potential to improve our understanding of the pathogenesis of diabetic nephropathy and hold great promise for the identification of novel therapeutic strategies.

ACKNOWLEDGMENTS

This study was supported by Research Grant U01DK57292-05 from the National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK) and, in part, by the Intramural Research Program of the NIDDK. This work was supported by the National Center for Research Resources for the General Clinical Research Center (GCRC) grants: Case Western Reserve University M01-RR-000080, Wake Forest University M01-RR-07122, Harbor-UCLA Medical Center M01-RR-00425, College of Medicine—University of California Irvine M01-RR-00827-29, University of New Mexico HSC M01-RR-00997, and Frederic C. Bartter M01-RR-01346. Genotyping was performed by the Center for Inherited Disease Research (CIDR). CIDR is fully funded through a federal contract from the National Institutes of Health to the Johns Hopkins University, contract no. N01-HG-65403. The results of this analysis were obtained by using the S.A.G.E. package of genetic epidemiology software, which is supported by a U.S. Public Health Service resource grant (RR03655) from the National Center for Research Resources.

We kindly thank all FIND participants.

APPENDIX

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Electronic database information: Online Mendelian Inheritance In Man (OMIM), available at http://www.ncbi.nlm.nih.gov/omim (for diabetic nephropathy).

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