

Genotype by Diabetes Interaction Effects on the Detection of Linkage of Glomerular Filtration Rate to a Region on Chromosome 2q in Mexican Americans

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OBJECTIVE—Glomerular filtration rate (GFR) is used to assess the progression of renal disease. We performed linkage analysis to localize genes that influence GFR using estimated GFR data from the San Antonio Family Diabetes/Gallbladder Study. We also examined the effect of genotype by diabetes interaction ($G \times DM$) on the detection of linkage to address whether genetic effects on GFR differ in diabetic and nondiabetic subjects.

RESEARCH DESIGN AND METHODS—GFR ($N = 453$) was estimated using the recently recalculated Cockcroft-Gault (GFR-CGc) and the simplified Modification of Diet in Renal Disease (GFR-4VMDRD) formulae. Both estimates of GFR exhibited significant heritabilities, but only GFR-CGc showed significant $G \times DM$ interaction. We therefore performed multipoint linkage analyses on both GFR measures using models that did not include $G \times DM$ interaction effects (Model 1) and that included $G \times DM$ interaction effects (Model 2, in the case of GFR-CGc).

RESULTS—The strongest evidence for linkage (Model 1) of both GFR-CGc (logarithm of odds [LOD] 2.9) and GFR-4VMDRD (LOD 2.6) occurred between markers D9S922 and D9S1120 on chromosome 9q. However, using Model 2, the strongest evidence for linkage of GFR-CGc on chromosome 2q was found near marker D2S427 (corrected LOD score [LOD_C] 3.3) compared with the LOD score of 2.7 based on Model 1. Potential linkages (LOD or LOD_C ≥ 1.2) were found only for GFR-CGc on chromosomes 3p, 3q, 4p, 8q, 11q, and 14q.

CONCLUSIONS—We found a major locus on chromosome 2q that differentially influences GFR in diabetic and nondiabetic

environments in the Mexican-American population. *Diabetes* 56:2818–2828, 2007

Chronic kidney disease and declining renal function cause end-stage renal disease (ESRD), which is an important health concern for individuals with hypertension and diabetes. Diabetic nephropathy is the leading cause of chronic kidney disease in patients starting renal replacement therapy and is associated with increased cardiovascular mortality (1). Hispanics are among the fastest-growing ethnic minority populations in the U.S. and are twice as likely to develop kidney failure as non-Hispanic whites, largely because of the increased prevalence of diabetes. Mexican Americans also have a sixfold increased risk of developing ESRD secondary to diabetes compared with Caucasians (2). Glomerular filtration rate (GFR) or creatinine clearance are frequently used as indicators to evaluate renal function.

Diabetic nephropathy and GFR are complex phenotypes that are influenced by genetic and environmental factors and their interactions. Several studies involving humans and animal models have provided evidence for genetic influences on susceptibility to diabetic nephropathy or ESRD and their related phenotypes (3,4). The rate of progression of diabetic nephropathy varies greatly among individuals. A part of this variability could be attributed to genetic influences. In fact, in recent years, various linkage studies have been performed to localize susceptibility genes related to ESRD and diabetic nephropathy in populations such as Caucasians, Mexican Americans, African Americans, and Pima Indians (5–11). In addition, linkage studies have also been conducted to map loci that influence variation in renal function, using data from various ethnic groups and several measures of GFR such as serum creatinine, serum cystatin C, creatinine clearance, and formulae estimating GFR (Cockcroft-Gault [GFR-CG] and Modification of Diet in Renal Disease [GFR-MDRD] study equations) (12–18).

However, there is paucity of such genetic information in Mexican Americans, a population at high risk for type 2 diabetes and its complications. Therefore, the purpose of the present study was to 1) determine the heritability of estimates of GFR, 2) identify susceptibility loci that influence variation in renal function using estimates of GFR, and 3) examine the effect of genotype by diabetes ($G \times DM$) interaction on the detection of linkage of GFR in Mexican Americans.

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Received for publication 14 July 2006 and accepted in revised form 6 August 2007.

Published online ahead of print at <http://diabetes.diabetesjournals.org> on 13 August 2007. DOI: 10.2337/db06-0984.

Additional information for this article can be found in an online appendix at <http://dx.doi.org/10.2337/db06-0984>.

CIDR, Center for Inherited Disease Research; ESRD, end-stage renal disease; $G \times DM$, genotype by diabetes; $G \times E$, genotype by environment; GFR, glomerular filtration rate; GFR-4VMDRD, recalculated simplified four-variable Modification of Diet in Renal Disease (MDRD) study equation; GFR-CC, GFR estimated from serum cystatin C concentrations; GFR-CGc, Cockcroft-Gault equation adjusted for body surface area and corrected for bias in the MDRD data; IBD, identical by descent; LOD, logarithm of odds; QTL, quantitative trait locus; SAFDGS, San Antonio Family Diabetes/Gallbladder Study.

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RESEARCH DESIGN AND METHODS

The phenotypic and demographic information was collected from 741 individuals drawn from 39 large Mexican-American families that were enrolled in the San Antonio Family Gallbladder Study, a follow-up and extension of the San Antonio Family Diabetes Study. These studies are collectively referred to as the San Antonio Family Diabetes/Gallbladder Study (SAFDGS). The recruitment for the San Antonio Family Gallbladder Study was conducted between 1998 and 2001, and the particulars of the SAFDGS recruitment and related material were detailed previously (19,20). Briefly, probands were recruited from a random sample of low-income Mexican-American individuals who had been identified in the earlier San Antonio Heart Study as having type 2 diabetes. All first-, second-, and third-degree relatives of ≥ 18 years of age were invited to participate in the study. Each participant in the study gave informed consent. This study was approved by the Institutional Review Board, University of Texas Health Science Center, San Antonio, Texas.

Phenotype data. For the San Antonio Family Gallbladder Study, several metabolic, hemodynamic, anthropometric, and demographic variables were collected at the General Clinical Research Center Laboratory at the South Texas Veterans Health Care System, Audie L. Murphy Memorial Hospital Division, San Antonio, Texas, using standard procedures. The collection of participant medical histories included information on medication status (e.g., antidiabetic and antihypertensive medication). Blood samples were obtained after a 12-h fast for assessment of various phenotypes including glucose, insulin, total cholesterol, triglycerides, and HDL cholesterol, and they were collected again 2 h after a standardized oral glucose load for plasma glucose. Diabetes status has been defined by both the 1999 criteria of the World Health Organization (i.e., fasting glucose levels ≥ 126 mg/dl and/or 2-h glucose levels ≥ 200 mg/dl) (21). Participants who did not meet these criteria but who reported that they were under treatment with either oral antidiabetic agents or insulin and who gave a history of diabetes were also considered to have diabetes. The duration of diabetes for individuals who were diagnosed before our study was estimated based on their self-reported age-at-onset information. Urine albumin was measured with an immunoturbidimetric method using the COBAS INTEGRA diagnostic reagent system that uses anti-albumin antibody specific to human albumin. Urine creatinine was measured using a kinetic alkaline picrate assay. The ratio of concentration of albumin (mg/dl) to creatinine (mg/dl) in random urine specimen was used as an index of urinary albumin excretion. Albuminuria (micro- or macro-) was defined as an albumin (mg/dl) to creatinine (mg/dl) ratio of ≥ 0.03 . Serum samples were collected for estimation of creatinine, which was measured by alkaline picrate assay. The anthropometric data (height, weight, and waist circumference) were collected using standardized anthropometric protocols (22,23).

The following two recently recalculated GFR prediction equations (24) have been used for this study: the recalculated simplified four-variable Modification of Diet in Renal Disease (MDRD) study equation (25) and the Cockcroft-Gault equation (26) adjusted for body surface area and corrected for the bias in the MDRD data. Briefly, the recalculation was performed with serum creatinine levels calibrated to an enzymatic assay traceable to an isotope-dilution mass spectrometry assay. For the purpose of discussion, GFR recalculated by the four-variable MDRD formula was referred to as GFR-4VMDRD and GFR estimated by the recalculated Cockcroft-Gault formula as GFR-CGc. The GFR-4VMDRD was estimated using the following equation: $\text{GFR} = 175 \times [\text{serum creatinine (mg/dl)}]^{-1.154} \times [\text{age (years)}]^{-0.203} \times [0.742, \text{ if female}] \times [1.212, \text{ if African American}]$. The GFR-CGc was estimated as follows: $\text{creatinine clearance} = 0.8 \times [140 - \text{age (years)}] \times [\text{weight (kg)}] \times [0.85, \text{ if female}] \times 1.73/72$ serum creatinine \times body surface area (defined m^2). In these prediction equations, GFR-4VMDRD and GFR-CGc are expressed as ml/min per 1.73 m^2 . For the genetic analyses, for both phenotypes, only values within ± 4 SDs from the mean were considered. The coefficients of skewness and kurtosis for GFR-CGc and GFR-4VMDRD were 0.48 and 0.54 and 0.48 and 0.86, respectively. When log-transformed, the coefficients of skewness and kurtosis for GFR-CGc and GFR-4VMDRD were -0.60 and 0.90 and -0.42 and 1.43 , respectively. Because the transformation increased kurtosis, we have used the original data for all genetic analyses.

Genotype data and genetic map. The Center for Inherited Disease Research (CIDR) has performed a 10-cM genome scan using SAFDGS data. DNA was prepared from lymphocytes for genotyping. The CIDR performed a genome scan using automated fluorescent microsatellite analysis, and its marker set was composed primarily of trinucleotide and tetranucleotide repeats across the genome. The CIDR genetic map is similar to the genetic map provided by the Center for Genetics at the Marshfield Medical Research Foundation. For the present study, we used CIDR genotypic data on 382 highly polymorphic autosomal markers. The CIDR routinely checked for genotype errors and possible pedigree relationship errors. However, as an added precaution, the microsatellite marker data were used to further correct potential errors. The program PREST (27) was used to resolve pedigree discrepancies. The data

were checked for Mendelian inconsistencies using the PEDSYS (28) programs INFER and GENTEST to eliminate typing errors. If the discrepancies continued to exist, the program SimWalk2 (29), which used Markov Chain Monte Carlo and simulated annealing algorithms to assign probabilities of mistyping to each genotype, was used to make decisions about the appropriate genotypes to blank (exclude).

Maximum likelihood techniques that account for pedigree structure were used to estimate allele frequencies. Frequency estimates obtained using samples containing related individuals can be significantly biased unless pedigree structure is taken into account (30). For each genetic marker locus, the estimates of the allele frequencies and their standard errors were obtained using SOLAR (31). To perform linkage analysis, we constructed sex-averaged genetic maps using our SAFDGS genotypic data and pedigree structures and the programs Multimap and CRI-map (32,33). The map distances between the marker loci were obtained by Kosambi's mapping function, and these maps were similar to the Marshfield genetic maps. Locus-specific identical-by-descent (IBDs) were calculated using the program SOLAR (31), and multi-point IBD matrices given a number of genetic markers (map distance in Haldane cM) were estimated using Markov Chain Monte Carlo methods implemented in the program Loki (34). For the purpose of comparison with other studies, we discussed the genetic locations of our linkage findings according to the Marshfield map.

Statistical genetic analyses. To estimate heritabilities of GFR traits and to test potential linkages between marker loci and GFR traits, variance components linkage techniques were used (31,35,36). This approach uses information from all possible biological relationships simultaneously in an attempt to disentangle the genetic architecture of a quantitative trait. Linkage of a genetic location with a given trait (e.g., GFR-CGc) was tested using a general variance components method, which allows for marker locus-specific effects, residual genetic effects, and random environmental effects. In our approach, the sampling unit is a pedigree, and the covariance matrix for a given pedigree (Ω) is given by: $(\Omega) = \Pi\sigma_q^2 + 2\Phi\sigma_g^2 + I\sigma_e^2$, where Π is a matrix with elements (π_{ij}) providing the estimated proportion of alleles that individuals i and j share IBD at a marker (m) hypothesized to be linked to a quantitative trait locus (QTL), σ_q^2 is the additive genetic variance due to the marker locus, Φ is the kinship matrix; σ_g^2 is the genetic variance due to residual additive genetic effects, I is an identity matrix, and σ_e^2 is the variance due to random environmental effects. Both variance components and covariate effects were estimated simultaneously by maximum likelihood techniques. For example, the hypothesis of no linkage was tested by comparing the likelihood of a model in which the parameter h^2 is estimated, but h^2_q is constrained to a value of 0 with a model in which both h^2 and h^2_q are estimated, where $h^2 = (\sigma_q^2 + \sigma_g^2)/\sigma_p^2$, $h^2_q = \sigma_q^2/\sigma_p^2$, and $\sigma_p^2 = \sigma_q^2 + \sigma_g^2 + \sigma_e^2$. Twice the difference in the log-likelihoods of these models yields a test statistic that is asymptotically distributed as $1/2:1/2$ mixture of χ^2_1 and a point mass at 0, denoted by $\chi^2_{0,1}$, where the df is equal to the difference in the number of parameters estimated between the two competing models (37). After including all covariates for a given phenotype in the initial analysis, the null hypotheses of no influence of a given covariate (e.g., diabetes covariate β parameter = 0) were tested using likelihood ratio tests. Each of these tests involved comparison for a given parameter and had 1 df. A statistically significant test ($P < 0.05$) was considered as evidence of a significant nonzero estimate for a given parameter. After evaluating the significance of various covariate β parameter estimates, variance component and significant covariate effects were estimated simultaneously in the final analysis. The following covariates were considered for the initial covariate screening analyses. For GFR-CGc, type 2 diabetes, duration of diabetes (i.e., log-transformed), albuminuria (micro- and macro-), systolic blood pressure, and antihypertensive treatment with ACE inhibitors or AT1R antagonists were considered as covariates. For GFR-4VMDRD, all of the above covariates were used and BMI (weight [kg]/height [m^2]) added. Since age and sex information were used for the definition of both traits, these covariates were not considered for the genetic analyses. Similarly, BMI was excluded as a covariate for GFR-CGc, since its related information is used in the definition of this trait. Logarithm of odds (LOD) scores were obtained by converting the \ln likelihood values into values of \log_{10} . All analytical procedures are incorporated in the program SOLAR (31).

Genotype by environment interaction. We have extended the variance components approach to test for genotype by environment [$G \times E$] (i.e., the two environmental exposure groups are diabetic and nondiabetic individuals) interaction using information from variance-covariance of GFR between relative pairs under different environments (i.e., diabetic and nondiabetic) (38–41). Specifically, $G \times E$ interaction refers to the environmental (diabetic and nondiabetic) influences on the genes contributing to variation in GFR. In this interaction model, two things were modeled: 1) diabetes status-specific genetic variances (genetic variances in diabetic and nondiabetic individuals, respectively) and 2) a genetic correlation between groups of individuals living in different environments (i.e., diabetic-nondiabetic pairs). In the absence of G

TABLE 1
 Characteristics of 453 individuals with GFR values by diabetes status

	Diabetes	No diabetes
Sample size (<i>n</i>)	130	323
Sex (% females)	62	59
Age (years)	56.7 ± 13.1	42.6 ± 15.3
Duration of diabetes (years)	7.9 ± 9.4	—
Albuminuria	36	4
Hypertension	60	22
Antihypertensive medication*	22	4
Systolic blood pressure (mmHg)	137.7 ± 21.7	124.9 ± 15.2
Diastolic blood pressure (mmHg)	72.6 ± 10.1	70.7 ± 9.4
GFR-CGc (ml/min per 1.73 m ²)	87.1 ± 30.7	91.6 ± 25.6
GFR-4VMDRD (ml/min per 1.73 m ²)	87.9 ± 20.6	89.1 ± 17.9
Serum creatinine (mg/dl)	0.80 ± 0.2	0.84 ± 0.2
BMI (kg/m ²)	32.9 ± 6.7	30.6 ± 7.1
Weight (kg)	85.9 ± 20.5	81.9 ± 21.7

Data are means ± SD or % unless otherwise indicated. *Treatment with ACE inhibitors or AT1R antagonists.

× DM interaction, the genetic variances for GFR living in different exposure groups (diabetic [DM] and nondiabetic [NDM]) should be equal (i.e., $\sigma_{DM} = \sigma_{NDM}$), and/or the genetic correlation (i.e., GFR) between diabetic and nondiabetic pairs should be 1 [i.e., $\rho_{G(DM, NDM)} = 1$]. To estimate residual environment-specific QTL effects, the expected covariance (COV) between individuals *i* and *j* can be defined as:

$$COV_{(DM, NDM)} = \pi_{mij} \sigma_{qDM} \sigma_{qNDM} + 2\Phi_{ij} \sigma_{gDM} \sigma_{gNDM} \rho_g$$

If there is a significant QTL × diabetes interaction, the genetic variance of GFR attributable to the QTL for individuals living in different exposure groups (e.g., diabetic and nondiabetic) will not be equal ($\sigma_{qDM} \neq \sigma_{qNDM}$). In all G × E interaction models, the genetic and environmental variances were estimated as the genetic and environmental SDs, respectively. Because this G × E interaction technique had an additional QTL variance compared with the standard linkage model, we corrected for the increase in degrees of freedom and referred to it as the corrected LOD score (LOD_C). The LOD_C assumes that environment-specific QTL effects are independent under the null and that the corresponding test statistic is asymptotically distributed as a 1/4:1/2:1/4 mixture of χ^2_{2c} , χ^2_{2c-1} , and χ^2_{2c} . Although this assumption may be overly conservative, we used the corrected LOD scores instead of the (uncorrected) observed LOD scores under the G × E interaction model for the presentation of our data.

Because the SAFDGS families were ascertained on diabetic probands, as a conservative approach, all genetic analyses were performed using SOLAR by correcting for the ascertainment by conditioning the likelihood for the family data on the phenotype (i.e., GFR) of the proband (30). To verify the findings from the multipoint linkage analyses (with and without G × DM interaction), we conducted simulation analyses to determine the trait-specific empirical *P* values using the following procedure (SOLAR). A fully informative marker was simulated, which was not linked to a given phenotype. For this marker, IBD information was calculated, and then linkage analysis was performed using the simulated genotypes and the observed phenotype data. For a given phenotype, we generated the empirical *P* values on the basis of LOD score distribution obtained from the 100,000 replicates. In addition, to control for the overall false-positive rate in the genome-wide linkage analysis of a given phenotype, we used an approach based on the work of Feingold et al. (42), as implemented in the program Gauss 5.0.29 (Aptech Systems, Maple Valley, WA), and estimated the genome-wide *P* values for the strongest linkage findings (i.e., LOD ≥ 2.0). This approach takes into account the finite marker density in the linkage map used in the multipoint QTL screens and the mean recombination rate for the pedigreed population studied.

RESULTS

Characteristics of 453 individuals with GFR data in SAFDGS families. The clinical characteristics of 453 individuals for whom GFR and covariate data were available are reported in Table 1 by diabetes status. These individuals were distributed across 29 families. On average, there were 15.6 individuals in each family, and the average number of diabetic individuals per family was 4.5. As can be seen in Table 1, ~29% of the individuals were

affected with diabetes. The 453 individuals generated 23 types of relative pairs, and there were 3,833 relative pairs (diabetic-diabetic = 261, diabetic-nondiabetic = 1,442, and nondiabetic-nondiabetic = 2,130) in our data (Table 2). About 60% of the subjects were females. The mean (± SD) age of the diabetic individuals at the clinic examination was 56.7 ± 13.1 years, compared with 42.6 ± 15.3 years for those without diabetes. Of the 130 diabetic subjects, 27 subjects (21%) were clinically diagnosed at the time of their SAFDGS examination. The remaining 103 diabetic subjects (79%) were diagnosed before the study, and their age-at-onset information was based on self-reporting. The mean age at onset for all 130 affected subjects was 48.8 ± 12.9 years. The mean age at onset for the subjects diagnosed at the time of their SAFDGS clinic examination was 49.9 ± 14.2 years, compared with 48.5 ± 12.6 years for subjects diagnosed before the study. As can be seen in Table 1, on average, individuals with diabetes had higher systolic and diastolic blood pressure and were obese compared with those without diabetes. The individuals with diabetes had higher incidence of albuminuria (36% of subjects with diabetes vs. 4% without diabetes), and more individuals with diabetes were treated with antihypertensive medication (22%) compared with the subjects without diabetes (4%).

Heritability estimates for the two GFR phenotypes. Before conducting the linkage analyses, we estimated heritabilities (*h*²) for the two phenotypes GFR-4VMDRD and GFR-CGc. These analyses involved trait-specific screening for significant covariate effects as described previously. All covariates were found to be significant (*P* ≤ 0.05), excepting albuminuria and hypertension medication status. However, antihypertensive medication was included in the genetic analyses, since systolic blood pressure was used as a covariate irrespective of the hypertension medication status of an individual. After adjustment for the trait-specific covariates (i.e., GFR-CGc = diabetes, diabetes duration, systolic blood pressure, and antihypertensive medication; GFR-4VMDRD = diabetes, diabetes duration, systolic blood pressure, antihypertensive medication, and BMI), the coefficients of kurtosis for GFR-CGc and GFR-4VMDRD residuals were 0.79 and 1.35, respectively. The overall heritability estimates including both diabetic and nondiabetic individuals were as follows: GFR-CGc *h*² (± SE) = 0.40 ± 0.09, *P* <

TABLE 2
Numbers and types of relative pairs in 29 SAFDGS families consisting of 453 individuals with GFR data by diabetes status

	Relationship coefficient	Relative pairs by diabetes status			All pairs
		Diabetic-diabetic	Nondiabetic-nondiabetic	Diabetic-nondiabetic	
Parent-offspring	0.5000	27	148	163	338
Sibs	0.5000	45	273	146	464
Grandparent-grandchild	0.2500	3	33	32	68
Avuncular	0.2500	60	255	270	585
Half-sibs	0.2500	5	28	27	60
Double first cousins	0.2500	1	—	2	3
First/second cousins	0.1562	3	1	4	8
Grand avuncular	0.1250	2	66	85	153
Half avuncular	0.1250	6	27	36	69
First cousins	0.1250	81	364	223	668
Double first cousins, once removed	0.1250	2	4	9	15
First cousins, once removed, and second cousins, once removed	0.0781	—	6	12	18
Great grand avuncular	0.0625	—	6	—	6
Half grand avuncular	0.0625	—	6	—	6
First cousins, once removed	0.0625	21	431	294	746
Double second cousins	0.0625	—	6	6	12
Half first cousins	0.0625	—	39	12	51
Second cousins and third cousins	0.0391	—	9	—	9
First cousins, twice removed	0.0312	—	21	28	49
Half first cousins, once removed	0.0312	—	24	—	24
Second cousins	0.0312	5	249	71	325
Second cousins, once removed	0.0156	—	113	22	135
Third cousins	0.0078	—	21	—	21
Total		261	2,130	1,442	3,833

0.0001, and GFR-4VMDRD $h^2 (\pm SE) = 0.36 \pm 0.09$, $P < 0.0001$. The heritability profiles were more or less similar. The heritability estimates of GFR-CGc (adjusted for diabetes duration, systolic blood pressure, and hypertension medication) and GFR-4VMDRD (adjusted for diabetes duration, systolic blood pressure, hypertension medication, and BMI) were significant in diabetic subjects: GFR-CGc $h^2 (\pm SE) = 0.54 \pm 0.17$, $P = 0.0002$, and GFR-4VMDRD $h^2 (\pm SE) = 0.43 \pm 0.21$, $P = 0.0116$. The estimates in nondiabetic individuals only were also significant: GFR-CGc $h^2 (\pm SE) = 0.56 \pm 0.14$, $P < 0.0001$, and GFR-4VMDRD $h^2 (\pm SE) = 0.36 \pm 0.14$, $P = 0.0024$.

The correlation between GFR-CGc and GFR-4VMDRD measures. To our knowledge, the two estimates of GFR have not been validated in the Mexican-American population. In the absence of such information, we have assessed the extent of concordance between these two measures for proper interpretation of the data. We conducted bivariate genetic analysis of these two estimates after adjusting for the trait-specific covariate effects to examine their phenotypic (ρ_P), genotypic (ρ_G), and environmental (ρ_E) correlations before conducting linkage analyses. All of the estimated correlations ($\pm SE$) were high and significant as follows: $\rho_P = 0.79 \pm 0.02$, $P < 0.0001$; $\rho_G = 0.80 \pm 0.07$, $P < 0.0001$; and $\rho_E = 0.77 \pm 0.04$, $P < 0.0001$. However, the phenotypic and genetic correlations between the two phenotypes indicated that only 62–64% of the variation is commonly shared by GFR-4VMDRD and GFR-CGc. Thus, the two estimates were highly correlated, but there was a distinct sizable proportion of variance between the two estimates, in turn suggesting the possibility of some loci differentially influencing these surrogate measures of GFR.

Evidence for G \times DM interaction effects on GFR-CGc. After the estimation of heritabilities, we analyzed the data to detect G \times DM interaction effects on variation in GFR measures after adjusting for the trait-specific covariate effects. Of the two estimates of GFR, only the GFR-CGc exhibited significant G \times DM interaction effects. The difference in genetic SDs of diabetic ($\sigma_{DM} \pm SE = 20.33 \pm 3.62$) and nondiabetic ($\sigma_{NDM} \pm SE = 18.17 \pm 3.05$) individuals was statistically not significant ($P = 0.642$). However, the genetic correlation [$\rho_{g(DM, NDM)} = 0.53 \pm 0.25$, $P = 0.034$] between relative pairs discordant for diabetes was statistically different from 1 ($P = 0.039$), indicating that different genes might influence GFR-CGc in diabetic and nondiabetic individuals.

Findings from multipoint linkage analyses. As reported above, both estimates of GFR exhibited significant heritabilities, but only GFR-CGc showed significant G \times DM interaction. We therefore performed linkage analyses on both GFR measures, after adjusting for the trait-specific covariate effects, using models that did not include G \times DM interaction effects (Model 1, both GFR-CGc and GFR-4VMDRD) and that included G \times DM interaction effects (Model 2, only GFR-CGc). Using the standard linkage analysis (Model 1, without G \times DM interaction), the strongest evidence for linkage of GFR-CGc (LOD 2.9, empirical $P = 7.6 \times 10^{-4}$, genome-wide $P = 0.045$) occurred at 86 cM in-between markers *D9S922* (80 cM) and *D9S1120* (89 cM) on chromosome 9q and the 1 – LOD support interval around the linkage peak spanned an ~18 cM-long region (76–93 cM) on the Marshfield map (Table 3, Figs. 1 and 2). The strongest evidence for linkage of GFR-4VMDRD also occurred at the same location (LOD 2.6, empirical $P = 2.3 \times 10^{-4}$, genome-wide $P = 0.083$), and it slightly improved when adjustment for BMI was not

TABLE 3

Chromosomal regions potentially linked (LOD or LOD_C ≥ 1.2) to GFR-CGc and GFR-4VMDRD based on multipoint linkage analyses

Phenotype*	Nearest marker region	Distance from p-ter† (cM)	Chromosome location	1 - LOD interval†	Maximum LOD or LOD _C score			
					Model 1‡		Model 2‡	
					LOD	Empirical P	LOD _C	Empirical P
GFR-CGc	D2S1363	227	2q36.3	209–249	2.7	1.1 × 10 ⁻³	3.3§	3.2 × 10 ⁻⁴
	D3S2387	6	3p26.3	6–24	1.4	1.4 × 10 ⁻²	1.7	9.6 × 10 ⁻³
	D3S1764–D3S1744	153–161	3q23–q24	137–165	1.4	1.4 × 10 ⁻²	1.6	1.2 × 10 ⁻²
	D4S2632–D4S1627	51–60	4p15.1–p13	30–92	1.3	1.7 × 10 ⁻²	(1.0)	4.1 × 10 ⁻²
	D8S373	164	8q24.3	160-qter	(0.0)	—	1.2	2.7 × 10 ⁻²
	D9S922–D9S1120	80–89	9q21.31–q21.33	76–93	2.9	7.6 × 10 ⁻⁴	2.4	1.9 × 10 ⁻³
	D11S912–D11S968	131–148	11q24.3–q25	122-qter	2.4	1.8 × 10 ⁻³	1.9	5.7 × 10 ⁻³
	D14S1426–D14S1007	126–138	14q32.2–q32.33	106-qter	1.7	7.9 × 10 ⁻³	1.2	2.7 × 10 ⁻²
GFR-4VMDRD¶	D9S922–D9S1120	80–89	9q21.31–q21.33	76–92	2.6	2.3 × 10 ⁻⁴	—	—
GFR-4VMDRD#	D9S922–D9S1120	80–89	9q21.31–q21.33	77–91	3.0	1.0 × 10 ⁻⁴	—	—

*GFR-CGc and GFR-4VMDRD are the recalculated Cockcroft-Gault equation and simplified four-variable MDRD study equation (please see text). †Marshfield data (Kosambi cM) for the purpose of comparison. ‡Model 1 = model without G × DM interaction and Model 2 = model with G × DM interaction. §The maximum LOD score of 2.7 based on Model 1 occurred at 225 cM (1 - LOD interval = 209–249 cM) near marker D2S1363 (227 cM); however, according to Model 2, the maximum LOD_C score of 3.3 occurred at 236 cM (1 - LOD interval = 216–249 cM) near marker D2S427 (237 cM). The locations where the maximum LOD scores occurred were separated by ~11 cM, but the 1 - LOD intervals were found to be strongly overlapped. ||Corresponding LOD scores in comparison with the other model. ¶GFR-4VMDRD analysis included BMI as a covariate. #GFR-4VMDRD analysis did not include BMI as a covariate.

made (LOD 3.0, empirical P = 1.0 × 10⁻⁴, genome-wide P = 0.031). For GFR-4VMDRD (with or without BMI as a covariate), there were no other loci that exhibited potential evidence for linkage (LOD ≥ 1.2) (Table 3 and supplemental Figs. S1 and S2 of the online-only appendix available at <http://dx.doi.org/10.2337/db06-0984>), while several loci were found for GFR-CGc with potential evidence for linkage on chromosomes 2q, 3p, 3q, 4p, 11q, and 14q (Table 3, Fig. 1). For example, the second strongest evidence for linkage of GFR-CGc occurred near the

marker *D2S1363* region on chromosome 2q (LOD 2.7, empirical P = 1.1 × 10⁻³, genome-wide P = 0.075). A genetic location between the markers *D11S912* and *D11S968* was also found to exhibit moderate evidence for linkage (LOD 2.4, empirical P = 1.8 × 10⁻³, genome-wide P = 0.139).

After the detection of significant G × DM interaction effects, we performed multipoint linkage analysis of GFR-CGc phenotype using Model 2 (with G × DM interaction effects). After incorporating G × DM interaction, we found

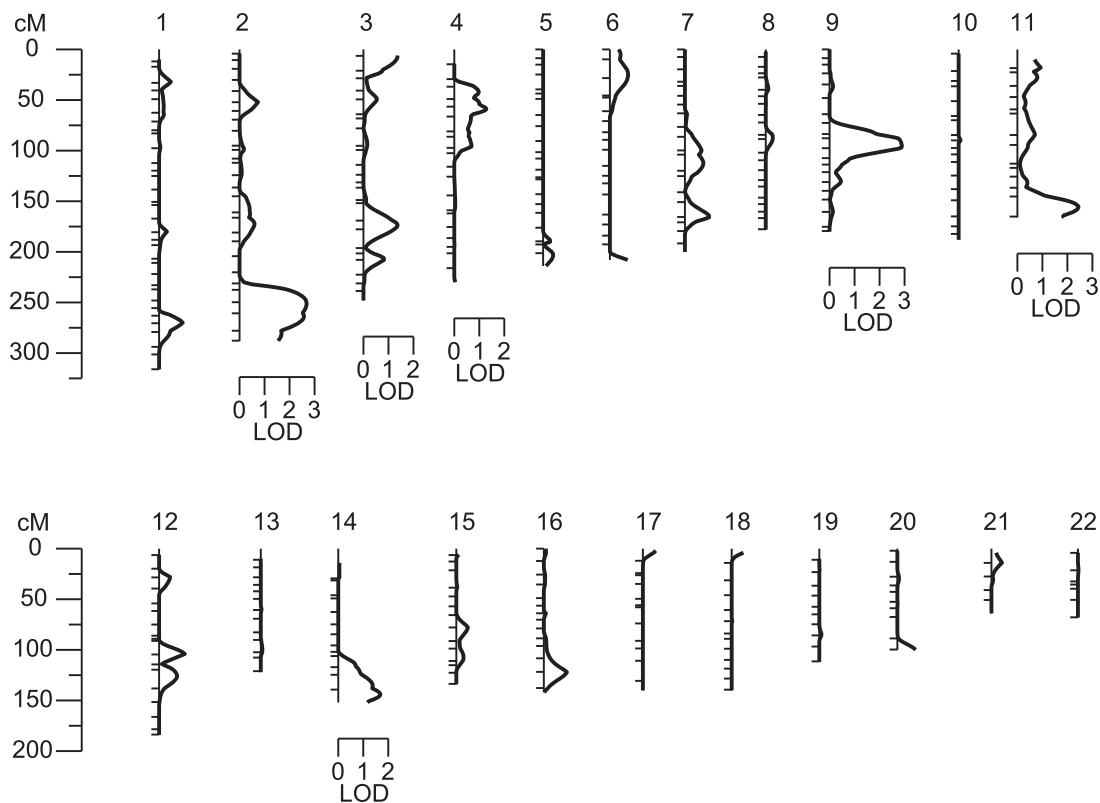


FIG. 1. Multipoint linkage analysis of GFR-CGc without G × DM interaction (Model 1). Map distance is in Haldane cM.

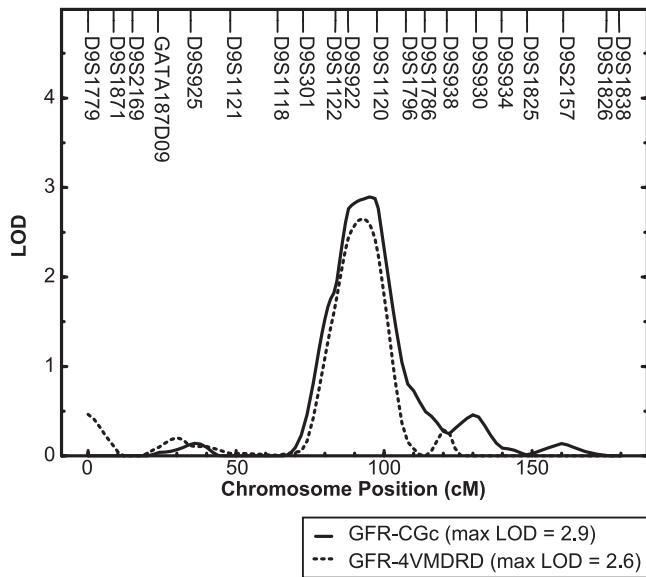


FIG. 2. Multipoint linkage profiles for GFR-4VMDRD and GFR-CG_c (Model 1) on chromosome 9q. Map distance is in Haldane cM.

the strongest evidence for linkage of GFR-CG_c on chromosome 2q near the marker *D2S427* region, where the LOD score of 2.7 (near marker *D2S1363*) based on the standard linkage analysis (without $G \times DM$ interaction) improved greatly to an uncorrected LOD score of 3.8 (LOD corrected for additional degrees of freedom [LOD_c] 3.3, empirical $P = 3.2 \times 10^{-4}$, genome-wide $P = 0.019$) (Figs. 3 and 4). Henceforth, only corrected LOD scores are discussed. As reported in Table 3, it should be noted that the locations where the maximum LOD scores occurred

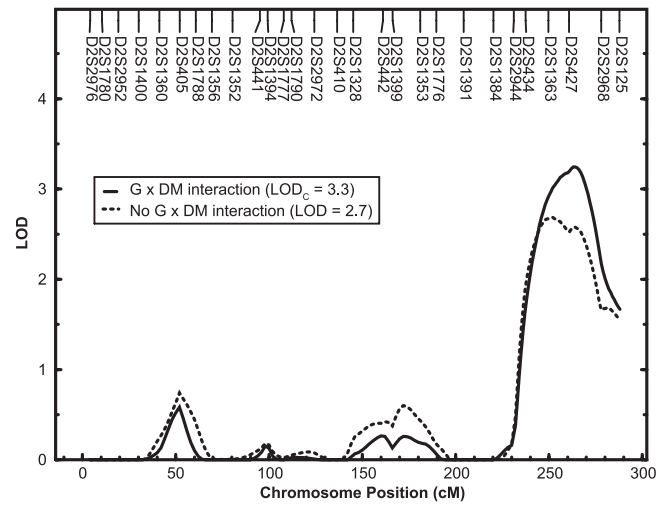


FIG. 4. Multipoint linkage profiles for GFR-CG_c on chromosome 2q, obtained from Models 1 and 2. Map distance is in Haldane cM.

based on Models 1 and 2 were separated by an ~ 11 cM-long region, but the 1 – LOD support intervals were found to be strongly overlapped (Model 1: LOD_c 2.7 at 225 cM [1 – LOD interval = 209–249 cM] near marker *D2S1363* [227 cM] versus Model 2: LOD_c 3.3 at 236 cM [1 – LOD interval = 216–249 cM] near marker *D2S427* [237 cM] on Marshfield map). Aside from the 2q chromosomal region, the 3p, 3q, 8q, 9q, 11q, and 14q chromosomal regions exhibited potential evidence for linkage ($LOD_c \geq 1.2$) using Model 2 (Fig. 3 and Table 3). However, only a few chromosomal regions (i.e., 3p, 3q, and 8q) exhibited some improvement in LOD scores. Importantly, the genetic location on chromosome 9q, where we had strong

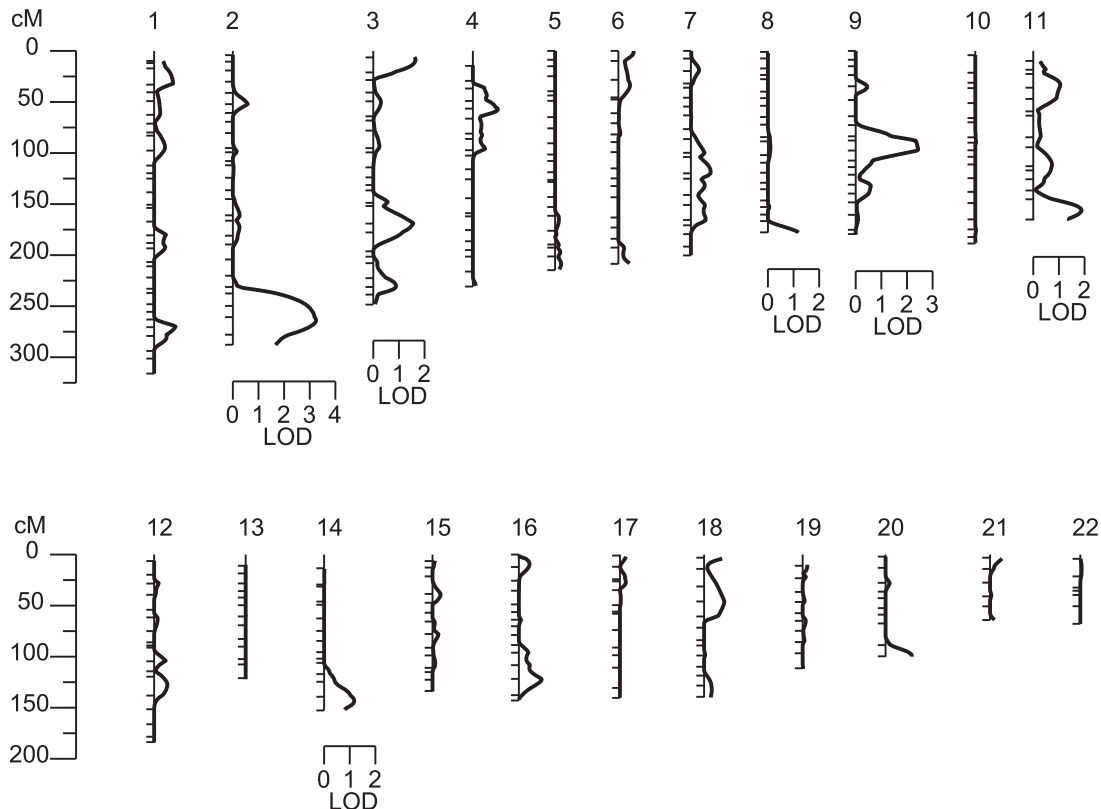


FIG. 3. Multipoint linkage analysis of GFR-CG_c with $G \times DM$ interaction (Model 2, only the LOD_c values are reported). Map distance is in Haldane cM.

evidence for linkage of both measures of GFR using Model 1, failed to exhibit evidence for potential $G \times DM$ interaction influences on GFR-CGc (i.e., Model 1: LOD 2.9, empirical $P = 7.6 \times 10^{-4}$, genome-wide $P = 0.045$, versus Model 2: LOD 2.4, empirical $P = 1.9 \times 10^{-3}$, genome-wide $P = 0.143$). Overall, the implicated marker region on chromosome 2q appears to harbor a major susceptibility locus for variation in GFR-CGc in Mexican Americans, and it appears that the QTL at the marker *D2S1363/D2S427* region on chromosome 2q differentially influences the variation in GFR in diabetic and nondiabetic individuals.

The magnitude of difference in diabetes status-specific QTL effects at the marker *D2S427* region ($\sigma_{qDM} \pm SE = 12.16 \pm 4.82$ and $\sigma_{qNDM} \pm SE = 19.87 \pm 2.39$) appears to be appreciable. However, it failed to reach statistical significance ($P = 0.194$). The genetic SDs due to the QTL in each exposure group were estimated to be statistically different from 0 ($\sigma_{qDM} = 12.16$, $P = 1.0 \times 10^{-2}$, and $\sigma_{qNDM} = 19.87$, $P = 1.5 \times 10^{-5}$), in turn suggesting that the estimation of diabetes-specific (σ_{qDM}) effect size is imprecise based on the strength of the significance level, perhaps because of the smaller sample size of diabetic individuals compared with that of the nondiabetic individuals.

DISCUSSION

The prevalence rates of type 2 diabetes and its complications including diabetic nephropathy have been rapidly increasing globally, especially in the western world. In the U.S., the occurrence of type 2 diabetes and its complications is high in minority populations such as Mexican Americans, the largest subgroup of the U.S. Hispanic population. Because these disease conditions are influenced by genetic and environmental factors and their interaction, numerous studies have attempted to localize susceptibility genes for nephropathy and its related conditions such as GFR in type 2 diabetes, although the specific genes that underlie such susceptibility have yet to be identified.

In this study, we used data from a Mexican-American population that is at high risk for type 2 diabetes and its complications to examine the genetic determinants of GFR and to determine the influence of $G \times DM$ interaction on variation in GFR. We found that the phenotypes GFR-CGc and GFR-4VMDRD are significantly heritable ($h^2 = 40$ and 36%, respectively) in this population and that their heritability profiles are more or less similar. Given that the heritability estimates are population sample specific, several studies have found that GFR is under strong genetic influence. For example, GFR measured as GFR-CG and GFR-MDRD was reported to be heritable (38 and 44%) in Mennonites (43). The phenotypes GFR-CG and GFR-MDRD exhibited heritabilities of 29 and 46% in the Framingham Heart Study (44). Recently, Langefeld et al. (45) reported heritability of GFR to be 75% estimated according to the modified MDRD formula in Caucasian families. Another study on GFR estimated by the MDRD formula from multigenerational families in Utah reported a heritability (for three different GFR examinations) ranging from 37 to 42% (12). Thus, our GFR heritability findings are consistent with other heritability reports of GFR.

After determining that the GFR phenotypes are heritable, we performed genome-wide multipoint linkage screening to identify susceptibility loci that influence variation in GFR and to examine the potential effects of $G \times DM$ interaction on the detection of linkage. The stan-

dard linkage analyses (without $G \times DM$ interaction effects) showed strong evidence for linkage of both GFR-CGc and GFR-4VMDRD to a genetic location on chromosome 9q in-between markers *D9S922* (80 cM, 9q21.31) and *D9S1120* (89 cM, 9q21.33). In fact, the evidence for linkage to GFR-CGc (LOD 2.9) or GFR-4VMDRD (without adjustment for BMI, LOD 3.0) approximates genome-wide significance (46). For GFR-4VMDRD, we failed to find any other potential linkages, whereas GFR-CGc exhibited potential evidence for linkage on chromosomes 2q, 3p, 3q, 4p, 11q, and 14q. Since we observed that GFR-CGc exhibited significant $G \times DM$ interaction effects, we examined the effects of $G \times DM$ interaction on the detection of linkage for GFR-CGc. Using the $G \times DM$ interaction model, we found a corrected LOD of 3.3 near marker *D2S427* on chromosome 2q37.1. The evidence for linkage was found with a suggestive LOD score of 2.7 (near marker *D2S1363*, 2q36.3) using the standard linkage analysis (without $G \times DM$ interaction); however, it improved greatly to a corrected LOD score of 3.3 using the $G \times DM$ interaction model. Although the model-specific LOD curves peaked at different locations separated by an ~ 11 cM-long region, the 1 – LOD support intervals were found to be completely overlapping. The evidence for linkage of GFR to 2q chromosomal region based on the $G \times DM$ interaction model is significant at the level of a genome-wide scan (46). The 3p, 3q, 8q, 9q, 11q, and 14q chromosomal regions exhibited potential evidence for linkage of GFR-CGc, using the $G \times DM$ interaction model.

The ~ 18 cM-long 1 – LOD support interval region (76–93 cM) around the GFR linkage peak (86 cM) on chromosome 9q is flanked by markers *D9S1122* (9q21.13) and *D9S777* (9q22.1) on the Marshfield map, and it contains ~ 52 genes (NCBI Build 36). Although there are no known genes for renal function in this region, several studies have implicated this region or its nearby chromosomal regions to contain susceptibility loci for diabetes-related phenotypes. For example, some evidence for linkage of diabetic nephropathy and retinopathy in the Pima Indian population was found near markers *D9S1120* (89 cM) and *D9S910* (104 cM) (47), and this chromosomal region strongly overlaps with our 9q chromosomal region of interest. Using data from an African-American population, a major susceptibility locus influencing hypertensive nephropathy was mapped to the 9q31.2–q31.3 chromosomal region flanked by markers *D9S172* (112 cM) and *D9S105* (120 cM) (48). As reviewed by Arya et al. (49) and Cai et al. (50), several studies found genetic locations near the marker *D9S301* region (66 cM) that are linked with diabetes (Finnish and Chinese populations) or its correlates such as HDL cholesterol, a compound measure of adiposity and insulin, and fasting glucose (Mexican Americans) and fasting insulin (Pima Indians). Also, infantile nephronophthisis was reported to be linked to a genetic location near markers *D9S280* (102 cM) and *GGAT3G09* (117 cM) in a Bedouin kindred (51).

Given that the 1 – LOD support intervals around the GFR linkage peaks obtained from models with (216–249 cM) or without (209–249 cM) $G \times DM$ interaction effects on chromosome 2q overlap strongly, the markers *D2S2944* (210 cM, 2q34) and *D2S2202* (249 cM, 2q37.2) flank the ~ 40 cM-long critical region. Several studies reported linkages related to type 2 diabetes, ESRD, and GFR-related phenotypes on chromosome 2q. However, the implicated 2q chromosomal region harboring the potential

susceptibility genes is broad, covering the chromosomal locations from 2q21.3 to 2q37.3 (6,7,12,52–57). Such a scenario could relate to the possibility of more than one susceptibility locus for type 2 diabetes, ESRD, and GFR on chromosome 2q. Of the linkage reports, the findings of GFR (GFR estimated from serum cystatin C concentrations [GFR-CC]) from the Joslin collection of families with type 2 diabetes appear to be interesting within the context of the present findings (7). These investigators found strong evidence for linkage (LOD 4.1) of GFR-CC to a genetic location near the marker *D2S1384* region (at 202 cM with 195–213 Mb-long 1 – LOD support interval) in diabetic relative pairs. Importantly, the evidence for linkage (LOD 4.5 and LOD_C 3.9) of GFR-CC near the same marker region on chromosome 2q improved greatly in the total sample of both diabetic and nondiabetic individuals, when the model considered the G × DM interaction effects. This marker region is ~23 cM centromeric to the genetic location linked with GFR-CGc (i.e., standard linkage analysis), and its 1 – LOD support interval slightly overlaps with the 1 – LOD support interval found in our study. Given the uncertainties in the location estimates of QTLs for complex phenotypes across the studies, these two regions on chromosome 2q may correspond to the same putative QTL that influences variation in GFR in diabetic and nondiabetic individuals differentially.

Several studies identified some adjacent 2q chromosomal regions (2q35–q37.3) to contain type 2 diabetes susceptibility loci, and these regions are close to our 2q chromosomal region of interest. Importantly, Hanis et al. (57) found strong evidence for linkage of type 2 diabetes on chromosome 2q37.3 near marker *D2S125* (260 cM), called NIDDM1, in a Mexican-American population. Subsequently, Horikawa et al. (58) identified the calpain 10 (*CAPN10*) gene in the NIDDM1 region by positional cloning. Some potential replications of this finding have been reported in populations such as Caucasians (56). Also, Li et al. (55) found some evidence for linkage of fasting plasma glucose concentrations near the marker *D2S434* (216 cM) region, which is not that far from our 2q chromosomal region of interest. Although our GFR linkage finding on chromosome 2q overlaps with the region(s) reported to be linked to type 2 diabetes in other studies, it should be noted that there is no evidence for linkage of type 2 diabetes in our data. As discussed earlier, all genetic analyses in this study included type 2 diabetes as a covariate. Importantly, we reported type 2 diabetes genome scan/linkage findings in our SAFDGS data twice (the second time with increased sample size and different genome scan data generated by CIDR) (59,60) and found no evidence for linkage of type 2 diabetes to the location on chromosome 2q (i.e., NIDDM1 region). We repeated the linkage analysis of type 2 diabetes using the present data, and there was no evidence for linkage of type 2 diabetes (LOD 0) at the genetic location where we found the strongest evidence for linkage of GFR-CGc. These findings together suggest that the 2q locus we identified is not likely to be a type 2 diabetes susceptibility locus and that it has differential influence on GFR in diabetic and nondiabetic environments.

The 1 – LOD support interval flanked by the markers *D2S2944* and *D2S2202* contains ~222 genes (NCBI Build 36). There are several positional candidate genes in this region with potential functional relevance to kidney diseases such as insulin receptor substrate 1 (*IRS1*) (MIM 147545); collagen type IV α -3 (*COL4A3*) (MIM 120070);

collagen type IV α -4 (*COL4A4*) (MIM 120131); protein-tyrosine phosphatase, receptor-type, N (*PTPRN*), a member of the protein tyrosine phosphatase (PTP) family (MIM 601773); and *SMARCA1* (SWI/SNF-related matrix-associated actin-dependent regulator of chromatin, subfamily A–like protein 1) (MIM 606622).

Of the other regions found to be linked to GFR-CGc, the evidence for linkage was suggestive at the genetic location between markers *D11S912* (131 cM, 11q24.3) and *D11S968* (148 cM, 11q25) on chromosome 11q. Several studies have implicated this region to contain susceptibility genes for diabetes and obesity-related phenotypes (61–64). For example, the diabetes susceptibility locus found in the Pima population occurred between markers *D11S4464* (123 cM, 11q24.1) and *D11S912* (61), and the marker region *D11S912* exhibited some evidence for linkage of diabetes in the Mexican-American population (63). A significant evidence for linkage of autosomal-dominant progressive nephropathy, with features of both focal segmental glomerulosclerosis and Alport syndrome, was found near the marker *D11S4464* on the chromosome 11q24 region in an Asian Indian family (65).

A number of linkage studies have been performed to localize susceptibility genes for diabetic nephropathy or its related phenotypes such as GFR, and Iyengar et al. (11) provided a brief summary of the diabetic nephropathy linkage findings across the studies. We found modest evidence for linkage of GFR-CGc between markers *D3S1764* (153 cM, 3q23) and *D3S1744* (161 cM, 3q24) on chromosome 3q. Interestingly, the same *D3S1744* marker was suggestively linked with GFR-CC in nondiabetic individuals in the Joslin data (7). In the Finnish population, a susceptibility locus for type 1 diabetic nephropathy was identified on chromosome 3q between markers *D3S3606* (144 cM) and *D3S3694* (154 cM) (66), and this interval strongly overlaps our 3q chromosomal region. Several nearby 3q chromosomal regions were reported to be linked to diabetic nephropathy-related phenotypes in populations such as Caucasian Americans (~166 cM) (67), African Americans (~135 cM) (5), and Pima Indians (~177 cM) (47). A positional candidate gene in this region is angiotensin receptor 1 (*AGTR1*) (3q21–q25) (MIM 106165). Of the regions found with the strongest evidence for linkage of diabetic nephropathy (i.e., 7q21.3, 10p15.3, 14q23.1, and 18q22.3) in the FIND data, we found weak evidence for linkage of GFR-CGc (LOD 0.7) at a location between markers *D7S2212* (7q21.11) and *D7S821* (7q21.3) in our data. Interestingly, in our SAFDGS data, this same marker region was significantly linked with the metabolic syndrome (as defined by National Cholesterol Education Program/Adult Treatment Panel [NCEP/ATP] III criteria) (data not shown). It is possible that some loci, such as the one on chromosome 7q21.11–q21.3 across the genome, have potential common genetic influences on type 2 diabetes and GFR. Such traits that are genetically correlated could exhibit overlapping linkage profiles.

Although our linkage findings of the estimates of GFR in this study are interesting, there are some limitations. To identify the specific genetic determinants of renal function across the genome, we used phenotypic information from two common surrogate measures of GFR, instead of using data from direct measures of renal function such as iothalamate clearance. To our knowledge, the two estimates of GFR (i.e., GFR-CG and GFR-MDRD) have not been validated in the Mexican-American population. Although there has been a continued dialogue about their

relative accuracy (24,68,69), these GFR estimates are reasonably accurate in samples from patients with chronic kidney disease, especially the estimates from the GFR-4VMDRD equation. These equations may underestimate GFR in individuals with normal kidney function such as those in the SAFDGS sample. In addition, as remarked by Stevens et al. (68), GFR-CG appears to be less accurate than the GFR-MDRD study formula in older and obese people. Given attention to such observations, we found that the two GFR estimates correlated in our dataset but that there was a distinct sizable proportion of variance between the two estimates, in turn suggesting the possibility of some loci differentially influencing these surrogate measures of GFR.

Obesity is a risk factor for kidney disease (70). We have included BMI as a covariate in the analysis of GFR-4VMDRD. However, incorporation of BMI as an additional covariate slightly attenuated the evidence for linkage (LOD 2.6 with BMI as a covariate vs. LOD 3.0 without BMI as a covariate) on chromosome 9q. Such a scenario cautions about the problems of adjustment for genetically correlated traits as they relate to power to detect linkage, given that BMI is genetically correlated with GFR-4VMDRD in our data (data not shown). In regard to GFR-CGc, BMI was not considered as a covariate, since related information was included in the GFR-CGc formula. We examined whether BMI and weight were linked to the genetic location on chromosome 2q, where we found strong evidence for linkage of GFR-CGc and found that the evidence for linkage of the two obesity measures was none or negligible (BMI LOD 0.08 and weight LOD 0.05). These findings reassure that the major GFR-CGc linkage finding on chromosome 2q is not primarily an obesity locus.

The variance components approach has been shown to be sensitive to violations of the assumption of multivariate normal distribution. Although this approach is shown to be robust to such violations, leptokurtosis in particular could result in inflated type I error rates (36,71). Based on data simulations, it has been shown that trait distributions with a coefficient of kurtosis <2.0 do not appear to result in grossly inflated type I error. In this study, after adjustment for the trait-specific covariates, the coefficients of kurtosis for GFR-CGc and GFR-4VMDRD residuals were 0.79 and 1.35, respectively. Thus, it is possible that the linkage findings from this study may have been slightly inflated. However, for proper interpretation of the data, our study provided the empirical P values together with the genome-wide P values for the strongest GFR linkage findings. Aside from the above issues, the issue of correction for multiple testing in regard to multiple correlated traits is not straightforward, since the corresponding models are not independent, which is the case in this study. Therefore, no attempt was made to correct for multiple testing in regard to the correlated GFR measures. In reference to the $G \times DM$ interaction model used in this study, it is possible that such a model could reflect gene \times gene interactions.

In summary, by conducting a genome-wide linkage scan for GFR susceptibility loci in Mexican Americans, we found strong evidence for susceptibility genes influencing GFR on chromosomes 2q and 9q. The linkage finding on chromosome 2q exhibited genome-wide significance only after incorporating $G \times DM$ interaction effects. This putative gene appears to have differential influence on the variation in GFR in diabetic and nondiabetic individuals in the Mexican-American population. The finding of a GFR

susceptibility locus on chromosome 2q in the Joslin family data with similar features of diabetes status-specific QTL effects on variation in GFR is promising.

Confirmation of our findings in other populations is warranted. This study also provides potential evidence for linkage of GFR on chromosomes 3p, 3q, 4p, 8q, 11q, and 14q. Future GFR gene discovery efforts on chromosomes 2q and 9q may help to pave the way for prevention and treatment of diabetic patients at risk for developing renal diseases.

ACKNOWLEDGMENTS

This study was supported by grants from the National Institute of Diabetes, Digestive and Kidney Diseases (DK53889, DK42273, DK47482, and P50DK061597) and George O'Brien Kidney Research Center.

We are grateful for the nursing and dietetic care provided by the staff of the Frederic C. Bartter General Clinical Research Center at the South Texas Veterans HealthCare Systems—Audie Murphy Division, San Antonio, Texas, and CIDR for performing the genome scan. We warmly thank the families from the SAFDGS for their kind generosity and cooperation in taking part in the study.

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