Genome-Wide Scan for Estimated Glomerular Filtration Rate in Multi-Ethnic Diabetic Populations

The Family Investigation of Nephropathy and Diabetes (FIND)

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OBJECTIVE—Diabetic nephropathy, the most common cause of end-stage renal disease, aggregates in families and specific ethnic groups. Deconstructing diabetic nephropathy into intermediate, quantitative phenotypes may increase feasibility of detecting susceptibility loci by genetic screens. Glomerular filtration rate (GFR), which characterizes diabetic nephropathy, was employed as a quantitative trait in a preliminary whole-genome scan.

RESEARCH DESIGN AND METHODS—Estimated GFR (eGFR) was calculated for 882 diabetic sibpairs (mean age 57 years) of African-American (25.6% of total), American Indian (8.6%), European-American (14.2%), and Mexican-American (51.6%) descent enrolled in the initial phase of the Family Investigation of Nephropathy and Diabetes (FIND). A whole-genome scan was performed using 404 microsatellite markers (average spacing 9 cM) and model-free linkage analysis.

RESULTS—For all ethnicities combined, strong evidence for linkage was observed on chromosomes 1q43 ($P = 3.6 \times 10^{-3}$), 7q36.1 ($P = 2.1 \times 10^{-4}$), 8q13.3 ($P = 4.6 \times 10^{-4}$), and 18q23.3 ($P = 2.7 \times 10^{-3}$). Mexican-American families, who comprised the major ethnic subpopulation in FIND, contributed to linkage on chromosomes 1q43, 2p13.3, 7q36.1, 8q13.3, and 18q23.3, whereas African-American and American-Indian families displayed linkage peaks on chromosomes 11p15.1 and 15q22.3, respectively.

CONCLUSIONS—We have demonstrated multiple chromosomal regions linked to eGFR in a multi-ethnic collection of families ascertained by a proband with diabetic nephropathy. Identification of genetic variants within these loci that are responsible for the linkage signals could lead to predictive tests or novel therapies for subsets of patients at risk for diabetic nephropathy. ***Diabetes*** **57:235–243, 2008**

End-stage renal disease (ESRD) is the final outcome for multiple chronic kidney diseases (CKD), including one-third of patients with type 1 and type 2 diabetes. Nearly 45% of incident ESRD cases in the U.S. are due to diabetic nephropathy (www.usrds.org/2005/pdf/02_incid_prev_05), and <5% of ESRD cases are caused by Mendelian inheritance of known susceptibility genes. ESRD is therefore considered a complex disease in the remaining 95% of cases, with hereditary and environmental factors contributing to clinical outcomes. Evidence to support a genetic component to diabetic nephropathy is abundant, including concordance of diabetic nephropathy between diabetic siblings (1,2) and clustering of diabetic nephropathy within families and specific ethnic groups (3–5).

Whole-genome screens have been conducted to identify diabetic nephropathy genes in Pima Indian and African-American populations (6,7), and each study employed composite phenotype definitions for diabetic nephropathy. These screens have yielded candidate loci but no major susceptibility genes to date. A potential disadvantage of this study design is that establishing a categorical phenotype definition can be difficult, and inaccurate phenotype classification can reduce the likelihood of identifying candidate loci. An alternative approach is measurement of a quantitative trait, which represents an intermediate phenotype to predict an aspect of the complex disease. Although intermediate, quantitative traits may also be heterogeneous. Some potential advantages to this strategy are that these traits may be more proximal to underlying genes that regulate CKD, controlled by fewer loci, affected by fewer environmental factors, and more likely to reflect

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ARB, angiotensin receptor blocker; CKD, chronic kidney diseases; eGFR, estimated glomerular filtration rate; ESRD, end-stage renal disease; FIND, Family Investigation of Nephropathy and Diabetes; GFR, glomerular filtration rate; IBD, identity by descent; LOD, logarithm of odds; MDRD, Modification of Diet in Renal Disease.

disease pathophysiology (8–10). Importantly, analysis using continuous, quantitative traits can increase power to detect genetic effects. Although most often applied to model organisms, this strategy has been successfully implemented to identify complex human disease loci (8,11–14).

Several groups have assessed heritability (h^2) of estimated glomeruluar filtration rate (eGFR) as a quantitative trait. Hunt et al. (15) reported $h^2 = 0.33$, 0.36, and 0.53 for three successive creatinine clearance examinations in a healthy Utah pedigree. These investigators subsequently analyzed eGFR from a similar (healthy and European-American) population at three time points over a 10-year period, demonstrating h^2 ranging from 0.25 to 0.31 (16). DeWan et al. (17) calculated heritability of 0.17 in African-American and 0.18 in European-American hypertensive cohorts. In the Framingham Heart Study, Fox et al. (18) determined that eGFR heritability was 0.36. In a study of type 2 diabetic European-American subjects, Langefeld et al. (19) calculated h^2 of eGFR, by the Modifican of Diet in Renal Disease equation, to be 0.75 after adjusting for age, sex, blood pressure, medications, and A1C levels. Bochud et al. (20) estimated heritability in families with hypertension, similarly adjusting for age and sex, and revealing $h^2 = 0.41$ for inulin clearance, 0.52 for creatinine clearance, and 0.82 for creatinine clearance estimated by the Cockcroft-Gault formula. More recently, the Joslin group demonstrated significant eGFR heritability ($h^2 = 0.45$) in families enriched for type 2 diabetes (21). Collectively, these data indicate that glomerular filtration rate (GFR) is heritable, and it is reasonable to screen for genes regulating GFR and/or renal disease progression.

Many of these heritability studies simultaneously demonstrated linkage with quantitative eGFR phenotypes. In the HyperGEN study involving 1,100 hypertensive EA and AA subjects, a locus on 3q27 was identified (17). Follow-up studies by this same group, with added subjects and denser genotyping, revealed linkage to 3p (22) and 7q (23) in the African-American cohort. In two studies by Hunt et al., which examined pedigrees from EA families in Utah, loci on chromosomes 2q21.3 (16) and 10q23.3 (15) were identified. The Framingham Heart Study identified linkage on chromosome 3q26.3 with creatinine clearance and on chromosome 4q23.3 with eGFR (18). However, these populations were largely devoid of diabetic subjects, and mean eGFRs of the study populations were in the normal range. The FIND consortium was established to identify diabetic nephropathy susceptibility genes in four ethnic groups (Hispanic American, African American, European American, and American Indian) (24). In contrast to other studies, families were ascertained for diabetic ESRD or severe diabetic nephropathy, and many participants have low eGFR. A whole-genome scan was performed with microsatellite markers. Here, we report the results of a linkage analysis of eGFR.

RESEARCH DESIGN AND METHODS

The FIND family study population and study design have been described in detail (24). Briefly, samples were collected from the eight participating investigation centers. The predominant recruitment strategy in the family-based linkage portion of FIND was to enroll probands with diabetic nephropathy and diabetic siblings with or without diabetic nephropathy. A total of 941 subjects, comprising 882 sibpairs, were included in this analysis (Table 1). Approval for recruitment was obtained from institutional review boards at each participating institution. Subjects were recruited and samples collected according to the Declaration of Helsinki principles, and a certificate of confidentiality was filed at the National Institutes of Health.

TABLE 1
Description of genotyped families

Ethnicity	Pedigrees	Individuals	Sibpairs
African Americans	96	229	166
American Indians	32	72	69
European Americans	54	133	126
Mexican Americans	196	507	521
Total	378	941	882

Data are *n*.

Diabetes definition. Diabetes was self-reported and corroborated in most subjects by prevalent treatment with insulin and/or oral hypoglycemic agents. Diabetes duration was obtained from the medical history and confirmed by medical record review. In diabetic patients not treated with hypoglycemic agents, diabetes was diagnosed by A1C screening and fasting plasma glucose confirmation at study entry. Subjects with A1C >7.0% were considered diabetic, whereas those with A1C <6.0% were considered nondiabetic. Subjects with A1C concentration in the range from >6.0 to <7.0% underwent fasting plasma glucose and/or oral glucose tolerance testing. Assay results for diabetes diagnosis were interpreted according to American Diabetes Association criteria (25). Subjects with either type 1 or type 2 diabetes were included, though nearly all enrollees were believed to have type 2 diabetes (26).

Proband phenotype criteria. Detailed criteria have been published previously (24). Briefly, parameters for inclusion in the FIND family study required diagnoses of diabetic nephropathy, as defined by kidney biopsy revealing diabetic nephropathy plus overt proteinuria (>0.5 g protein/g creatinine); by CKD due to diabetic nephropathy (but not yet ESRD), based upon either diabetic retinopathy with proteinuria (>1.0 g protein/g creatinine) or diabetes duration ≥10 years with proteinuria (>3.0 g protein/g creatinine); or by ESRD due to diabetic nephropathy (as defined by one of the following: *1*) onset of diabetes ≥5 years before renal replacement therapy plus diabetic retinopathy [documented microaneurysms, proliferative diabetic retinopathy, macular edema, or prior retinal laser photocoagulation], *2*) onset of diabetes ≥5 years before renal replacement therapy with documented proteinuria [>3.0 g protein/g creatinine], or *3*) diabetic retinopathy with proteinuria [>3.0 g protein/g creatinine]). Demographic and clinical characteristics are shown in Table 2.

Diabetic sibling phenotype criteria. Diabetic siblings were considered to be either affected or unaffected by diabetic nephropathy. Criteria for siblings affected for diabetic nephropathy included the following: *1*) kidney biopsy consistent with diabetic nephropathy, *2*) microalbuminuria (>30 mg albumin/g creatinine), or *3*) serum creatinine concentration ≥1.6 mg/dl for men and ≥1.4 mg/dl for women. Diabetic siblings were classified as unaffected by diabetic nephropathy by virtue of diabetes duration ≥10 years, serum creatinine concentration <1.6 mg/dl for men and <1.4 mg/dl for women, and urine albumin–to–creatinine ratio <30 mg/g. Diabetic siblings with normal urine albumin excretion and diabetes duration <10 years were considered indeterminate but included in the linkage analysis.

eGFR. For most subjects, eGFR was calculated by the Modification of Diet in Renal Disease (MDRD) equation (27): eGFR (ml/min per 1.73 m^2) = $186 \times (\text{plasma creatinine})^{-1.154} \times (\text{age})^{-0.203} \times (0.742 \text{ if female}) \times (1.210 \text{ if African American})$. eGFR was assumed to be 5.0 ml/min per 1.73 m^2 for patients receiving hemodialysis or for transplant recipients ($n = 333$).

Genotyping and analytic methods. Genotyping and linkage analyses were conducted on 882 diabetic sibpairs. DNA was extracted for genotyping from either lymphoblast cell lines or leukocyte buffy coats. Genotyping was performed at the Center for Inherited Disease Research, including 404 microsatellite markers on 22 autosomes and two sex chromosomes, using the Marshfield Genetics, version 8, marker set from Research Genetics, with an average marker spacing of 9 cM.

All computer programs noted below are part of the S.A.G.E (Statistical Analysis for Genetic Epidemiology), version 5.0, program package. Differences between groups (probands vs. affected siblings and unaffected vs. affected siblings) were tested allowing for a sibling correlation, as implemented in ASSOC, using two-sided tests. Mendelian inconsistencies were identified with the MarkerInfo program. No departure from Hardy-Weinberg proportions was observed at any marker at a 1% significance level. Maximum likelihood estimation was performed with the FREQ program to estimate separate marker allele frequencies in all four ethnic groups (African American, European American, American Indian, and Mexican American). Before performing linkage analyses, errors in relationship specification were identified with the program RELTEST, using the entire genome scan. Multipoint identity by descent (IBD) allele sharing estimates were computed using the

TABLE 2
Description of genotyped participants by phenotype

	Probands	Affected siblings	Unaffected siblings	Siblings with diabetes <10 years	*P**	*P*†
n	347	360	146	88		
Male	169 (48.7)	165 (45.8)	43 (29.5)	29 (33.0)	<0.0001	0.001
Age, years (SD)	57 (10.7)	57 (11.0)	59 (10.0)	54 (10.9)	0.141	0.143
Ethnicity					0.322	0.047
African American	89 (25.6)	90 (25.0)	32 (21.9)	18 (20.5)		
American Indian	30 (8.6)	34 (9.4)	7 (4.8)	1 (1.1)		
European American	49 (14.2)	41 (11.4)	27 (18.5)	16 (18.2)		
Mexican American	179 (51.6)	195 (54.2)	80 (54.8)	53 (60.2)		
ESRD	282 (80.8)	51 (13.6)	0 (0.0)	0 (0.0)	<0.0001	<0.0001
Diabetes diagnosis age (years)	34 ± 12.3	41 ± 13.0	42 ± 11.5	52 ± 11.4	<0.0001	0.657
Diabetes duration (years)	23 ± 8.4	17 ± 9.4	17 ± 7.0	3.2 ± 2.6	<0.0001	0.443
BMI (kg/m^2)	30 ± 7.2	32 ± 9.1	32 ± 7.3	33 ± 7.8	0.014	0.578
A1C (%)‡	7.8 ± 1.8	8.6 ± 2.5	7.8 ± 2.0	7.6 ± 1.8	0.651	0.0008
Serum creatinine (mg/dl)‡	2.4 ± 1.4	1.7 ± 1.3	1.0 ± 0.2	1.0 ± 0.2	<0.0001	<0.0001
Blood urea nitrogen (mg/dl)‡	43.1 ± 22.7	25.5 ± 18.9	15.7 ± 4.9	14.1 ± 4.4	<0.0001	<0.0001
Urine albumin-to-creatinine ratio (g/g)‡	2.1 ± 1.9	1.2 ± 1.1	0.01 ± 0.01	0.01 ± 0.01	<0.0001	<0.0001
Urine protein-to-creatinine ratio (g/g)‡	4.5 ± 4.3	2.7 ± 5.1	0.2 ± 0.2	0.1 ± 0.1	<0.0001	<0.0001
eGFR (ml/min per 1.73 m^2)‡	40.6 ± 27.9	72.3 ± 37.3	85.6 ± 24.5	94.6 ± 24.2	<0.0001	0.008

Data are *n* (% phenotype group) or means ± SD unless otherwise indicated. *Comparison between probands and unaffected siblings. †Comparison between affected and unaffected sibs. ‡Data exclude subjects with ESRD.

program GENIBD for each of the four ethnic groups. Linkage analyses were conducted with eGFR as a quantitative trait and the Haseman-Elston regression linkage test (within the program SIBPAL), using multipoint IBD sharing estimates. SIBPAL performs linear regression modeling of sibpair traits as a function of marker allele IBD sharing. With this method, a weighted average of the squared mean-corrected trait sum, minus the squared trait difference, was used as the dependent variable, the regression allowing for the nonindependence of sibpairs and the nonindependence of squared trait sums and differences (28). For regions suggestive of linkage, asymptotic *P* values from the Haseman-Elston test were validated by obtaining empirical *P* values in SIBPAL. To do this, we permuted the allele sharing among the sibling pairs within each sibship and across all sibships of the same size. For each permutation, we recalculated the test statistic to generate a distribution of test statistics. We determined the proportion of the replicates that are equal to or more extreme than the original test statistic and report this as the empirical *P* value. Based on the settings used in SIBPAL for the number of permutations, the empirical *P* value is estimated to be within 20% of the true value, with a 95% CI.

Quantitative eGFR was evaluated according to two different models. In the first model, the covariates diabetes duration, ACE inhibitor or angiotensin receptor blocker (ARB) use, BMI, and A1C concentration were incorporated. In the second model, a linear regression was performed before linkage, and only covariates that were significant at the 5% level (diabetes duration and ACE inhibitor or ARB use) were used. A separate linkage analysis was performed for each ethnicity, and *P* values were combined across ethnicities according to Fisher's method (29). The Fisher meta-analysis technique was utilized because it was not necessarily valid to treat all of the ethnic groups as a single sample owing to allele frequency and demographic differences. To corroborate pooled results obtained with Fisher's method, we also calculated a weighted average of the Haseman-Elston regression coefficients, with weights inversely proportional to the estimated variances of the coefficients, and analyzed the combined ethnicities together, with membership in ethnic group included in the Haseman-Elston regression as covariates (30).

RESULTS

Subjects were enrolled and samples collected from 378 families (Table 1); 52% were Mexican American (196 pedigrees), 25% African American (96 pedigrees), 14% European American (54 pedigrees), and 9% American Indian (32 pedigrees). As previously reported (30), African-American probands had significantly lower male-to-female ratio and higher BMI compared with the other groups (Table 2). Otherwise, probands had similar demographic characteristics among the four ethnic groups.

Within the 378 FIND families, diabetic siblings were enrolled and classified as probands, siblings with diabetic nephropathy, siblings unaffected by diabetic nephropathy, or siblings with insufficient diabetes duration to meet unaffected sibling criteria. Demographic and clinical data are shown for each of these groups in Table 2. Of note, unaffected siblings were significantly older than probands or affected siblings, and probands had significantly younger age of onset and shorter diabetes duration than unaffected siblings. As expected, mean eGFR was markedly lower in probands than in other groups (Table 2), especially if winsorized (at 5 ml/min per 1.73 m^2) eGFR values were used for probands and affected siblings with ESRD (data not shown).

Because eGFR decline characterizes and predicts diabetic nephropathy, eGFR derived from the MDRD equation was employed in the quantitative trait linkage analysis. The mean eGFR value for the entire population was 77.5 ± 32.4 ml/min per 1.73 m^2 when ESRD subjects were excluded from the calculation and 56.8 ± 43.9 ml/min per 1.73 m^2 when winsorized eGFR values were included for ESRD subjects. Data obtained from the entire population (882 diabetic sibpairs) and analyzed using eGFR as a quantitative trait according to the first model (adjusted for covariates diabetes duration, ACE inhibitor/ARB use, BMI, and A1C) yielded suggestive evidence for linkage using multipoint IBD estimates on chromosomes 1q43, 7q36.1, 8q13.3, and 11p15.1 and a broad peak on 18q13–21 and 20p12.2 (not shown). Mexican-American families, who comprised the majority of the families, predominantly contributed to the 1q, 7q, 8q, 18q, and 20p linkage peaks. In the ethnicity-specific analysis of Mexican Americans, peaks on 8q (nominal $P = 8.70 \times 10^{-6}$) and 18q (nominal $P = 6.40 \times 10^{-6}$) reached Lander-Kruglyak threshold for significant linkage (31).

Although BMI has been implicated as an independent risk factor for ESRD (32), it was not a statistically significant covariate in the regression analysis of either the total or ethnicity-specific samples in this study. In addition, A1C is not considered a reliable measure of blood glucose control in ESRD patients (33). Therefore, the linkage

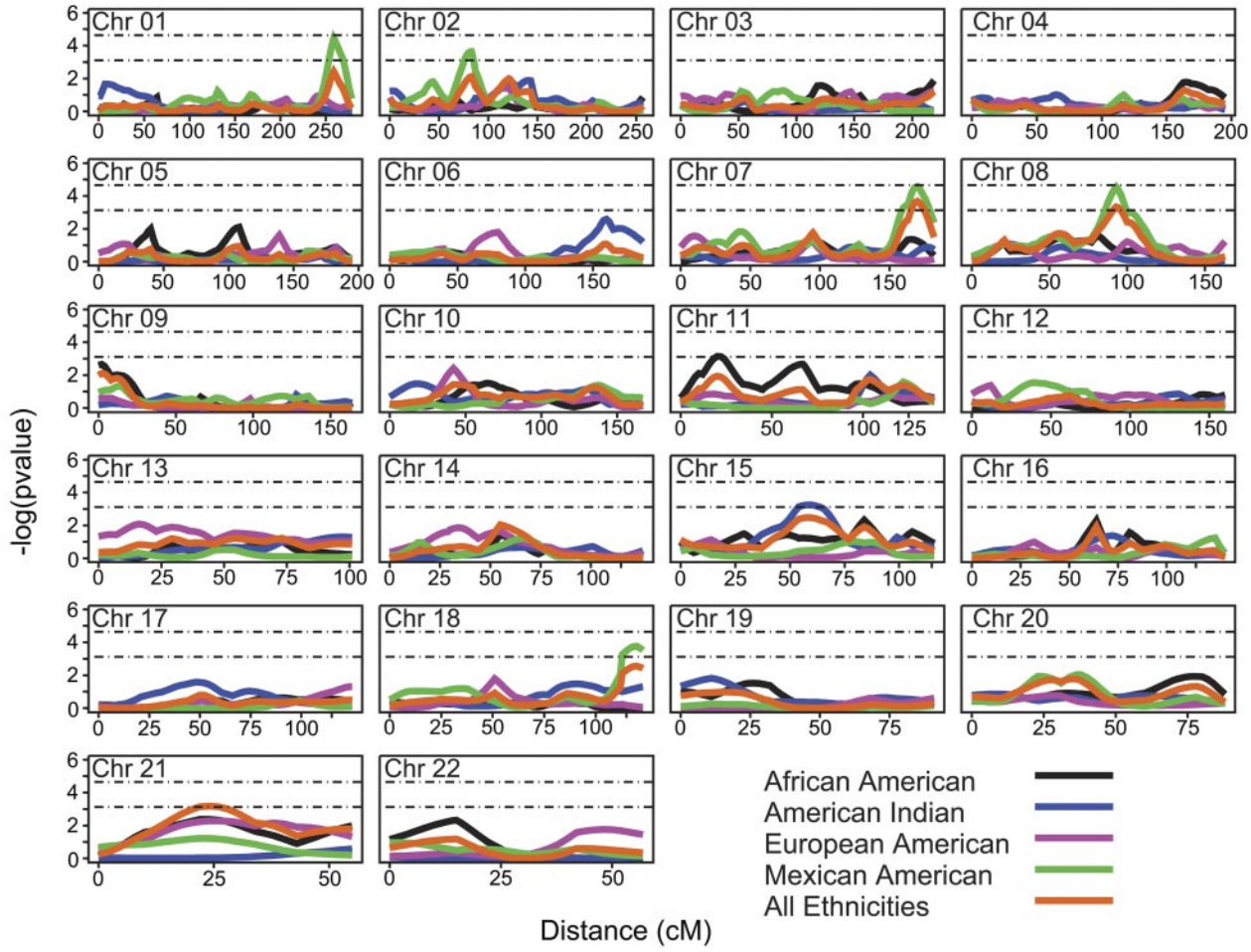

FIG. 1. Multipoint results of the genome-wide linkage scan for eGFR on 22 autosomes after adjusting for covariates diabetes duration and ACE inhibitor or ARB use. For each chromosome, genetic distance (cM) is plotted on the *x*-axis against −log10 (*P* value) on the *y*-axis, with corresponding LOD score scale. Data are presented for each ethnicity separately and for ethnicity-combined analyses. Lower horizontal dotted lines represent suggestive evidence ($P = 7.4 \times 10^{-4}$), and upper horizontal lines show threshold for evidence ($P = 2.2 \times 10^{-5}$) of linkage by Lander-Kruglyak criteria (ref. 31).

analysis was repeated after adjusting only for diabetes duration and ACE inhibitor/ARB use. In these analyses, suggestive evidence for linkage in the entire population persisted at 1q43, 7q36.1, 8q13.3, 11p15.1, 18q22.3 (Fig. 1 and Table 3), and 2p13.3. As seen in Fig. 1 and Table 3, Mexican Americans primarily contributed to the linkage signals on chromosomes 1q, 2p, 7q, 8q, and 18q, while African-American and American Indian families contributed to linkage peaks on 11p and 15q22.3, respectively.

Linkage results are shown for eGFR in ethnicity-specific diabetic populations (Fig. 2). Data are presented without covariate adjustment (baseline model) and with pair-mean diabetes duration and ACE inhibitor/ARB use as covariates (adjusted model). For all chromosome regions displayed, covariate adjustments resulted in increased evidence for linkage.

DISCUSSION

In the current report, several major chromosomal intervals, the most prominent at 1q43, 7q36.1, 8q13.3, and 18q23.3, significantly contributed to eGFR phenotype. The findings were influenced primarily by Mexican Americans, who comprised the largest subpopulation in our cohort. The 1q locus appears to be unique, with no obvious overlap with previous diabetic nephropathy genome scans (Table 4). As discussed in more detail below, the 18q23.3 peak replicated our prior findings in FIND (30) based on a composite diabetic nephropathy definition that included criteria other than eGFR (24). Interestingly, the 7q peak was not detected in the FIND population diabetic nephropathy scan (30), and the locus on 8q showed modest support but was not sufficient to meet Lander-Kruglyak evidence for significance (31). Taken together, because there are few common linkage peaks between the two FIND analyses, we speculate that GFR and other aspects of the diabetic nephropathy phenotype may be regulated by different genes. However, our failure to replicate these diabetic nephropathy linkage signals from FIND or from other collections of diabetic nephropathy patients may reflect the modest sample size used in this study (see power analysis in the online appendix [available at http://dx.doi.org/10.2337/db07-0313]) and the differences in phenotypes and ascertainment strategies (34).

Several studies have identified 7q susceptibility loci in scans employing diabetic nephropathy phenotypes defined by albuminuria (6–35), and a very recent genome scan of eGFR demonstrated linkage to 7q37 in African Americans (23) (Table 4). Krolewski et al. (35) analyzed albuminuria as a quantitative trait and discovered a linkage peak at 7q36.2, which yielded a logarithm of odds (LOD) score in excess of 3.0. Despite the difference in phenotype definitions, the 7q peak described by

TABLE 3
Linkage analysis results for eGFR with nominal significance

Chromosome	Flanking markers*	Ethnicity	cM†	LOD-1 coordinates	Nominal P‡	Empirical P‡	LOD score§
1	D1S235, D1S1609	MA	259	233,960,389–242,132,680	3.45×10^{-5}	5.99×10^{-5}	3.78
		All			3.63×10^{-3}	6.65×10^{-3}	1.87
2	D2S1352, D2S441	MA	82	50,687,218–68,092,661	2.17×10^{-4}	3.80×10^{-4}	3.02
		All			7.59×10^{-2}	9.47×10^{-3}	1.73
7	7S3070, D7S3058	MA	170	151,198,047–154,198,999	2.68×10^{-5}	2.00×10^{-5}	4.23
		All			2.06×10^{-4}	2.02×10^{-4}	3.28
8	D8S1136, D8S1119	MA	93	66,230,599–87,241,316	2.81×10^{-5}	3.99×10^{-5}	3.95
		All			4.60×10^{-4}	7.52×10^{-4}	2.75
11	D11S1981, D11S348	AA	20	17,042,780–24,077,587	6.95×10^{-4}	2.29×10^{-3}	2.30
		All			1.10×10^{-2}	2.93×10^{-2}	1.29
15	D15S1507, D15S131	AI	58	63,127,063–68,971,161	5.65×10^{-4}	4.20×10^{-4}	2.98
		All			3.31×10^{-3}	3.98×10^{-3}	2.08
18	D18S1371, D18S1390	MA	120	71,239,736–75,953–069	1.70×10^{-4}	3.77×10^{-3}	2.10
		All			2.67×10^{-3}	2.24×10^{-2}	1.40

*For peaks at an exact marker location (chromosomes 1 and 8), flanking markers are on either side of the peak marker. If the most significant P value was not at a marker (chromosomes 2, 7, 11, 15, and 18), then the markers closest to the peak are defined as the flanking markers. †Chromosomal positions correspond to the Marshfield map. Coordinate base pairs obtained from www.ensembl.org. ‡Nominal P values from SIBPAL assume that the regression coefficients are normally distributed; empirical P values were obtained using a permutation test with sufficient permutations to ensure with 95% confidence of being within 1.2-fold of the true P value, with up to 50,000 replicate permutations. §Calculated from the empirical P value according to the following: $\Phi^{-1}(1 - P)/2 \log_e(10)$, where Φ is the distribution function of a χ^2 variable with 1 d.f. AA, African American; AI, American Indian; MA, Mexican American.

Krolewski et al. is replicated in our study (Table 4) and coincides with the locus for the *NOS3* gene (36–38). In the genome scan involving the Pima Indian population, a single peak of linkage was identified at 7q33 (6), which is considerably more centromeric.

The Mexican-American cohort also exhibited linkage at 18q23, consistent with linkage peaks in genome scans for type 2 diabetes (39) and diabetic nephropathy (7,40) in European-American and African-American populations, as well as the FIND linkage scan for a composite diabetic nephropathy phenotype (30). In addition, Ewens et al. (41) found three polymorphisms in the *BCL2* gene, which resides at 18q21.33, that were significantly associated with diabetic nephropathy in European-American families. Although European-American and African-American families did not contribute significantly to the 18q peak in this study (Fig. 1), the data from all of the studies collectively suggest the presence of race-independent diabetes and diabetic nephropathy genes in this region. Of note, our peak is centered ~900 kb from the carnosinase gene (*CNDP1*) (Table 4). Polymorphisms in *CNDP1* have been associated with resistance to diabetic nephropathy in European Americans (42,43).

Of the few whole-genome scans for eGFR that have been conducted, overlap between chromosome regions identified, including the results described in this study, is limited. The first scan by Hunt et al. identified linkage of creatinine clearance to chromosome 10 in families from Utah ascertained for cardiovascular disease risk, with relatively normal GFR (15). A second scan by the same group, which analyzed three creatinine-based GFR estimates (by the MDRD equation) over a 10-year period, showed linkage to chromosome 2q but only for the third examination (16). DeWan et al. (17) analyzed creatinine clearance but in a mixed African-American and European-American population ascertained for hypertension and at increased risk for cardiovascular diseases. These investigators identified linkage with chromosome 3q27 but not chromosomes 2 or 10. Fox et al. (18) used multipoint variance component linkage analysis in 330 families from the community-based Framingham Heart Study offspring cohort, using a 10-cM genome-wide scan, for serum creatinine, eGFR, and creatinine clearance measured from 1998 to 2001. eGFR was estimated using the simplified MDRD equation, and creatinine clearance was estimated using the Cockcroft-Gault equation. The peak LOD scores for serum creatinine, eGFR, and creatinine clearance were 2.28 at 176 cM on chromosome 4, 2.19 at 78 cM on chromosome 4, and 1.91 at 103 cM on chromosome 3, respectively. This group recently reported the results of linkage analysis with 11,200 markers in this same cohort. A marker (rs10489578) near 1q43 peak in the FIND genome scan was linked to both eGFR (LOD 3.08) and serum creatinine (LOD 3.35) (44). Finally, Placha et al. (21) reported a genome scan of eGFR, estimated by serum cystatin C, MDRD, and Cockroft-Gault equations, in 63 extended Caucasian pedigrees (406 individuals with type 2 diabetes and 428 without diabetes) with normal eGFR values. This group detected strong evidence for linkage among diabetic relatives on chromosome 2q and suggestive evidence on 10q in proximity to the region previously identified by Hunt et al. (15) and 18p. When diabetic and nondiabetic relatives were combined in the analysis, evidence for linkage was also found on chromosome 7p. Linkage peaks in the current study may not replicate previous findings owing to differences in ethnic composition of the populations studied and relatively modest sizes of most prior studies. In contrast to earlier reports, the FIND study ascertained for subjects with diabetes and renal insufficiency.

Another difference between prior eGFR genetics studies and this study is the method of GFR estimation. We chose the MDRD equation to estimate GFR because serum creatinine was easily obtained for all study subjects and because it is currently the most accurate creatinine-based index of eGFR (45). This formula was originally derived from a study population of 1,628 predominantly Caucasian patients with multiple causes of CKD (27) and has subsequently been validated in African-American (46) and American Indian (47) cohorts. In several recent reports, accuracy of the equation was analyzed in patients with relatively normal GFR (48) and in patients with diabetes

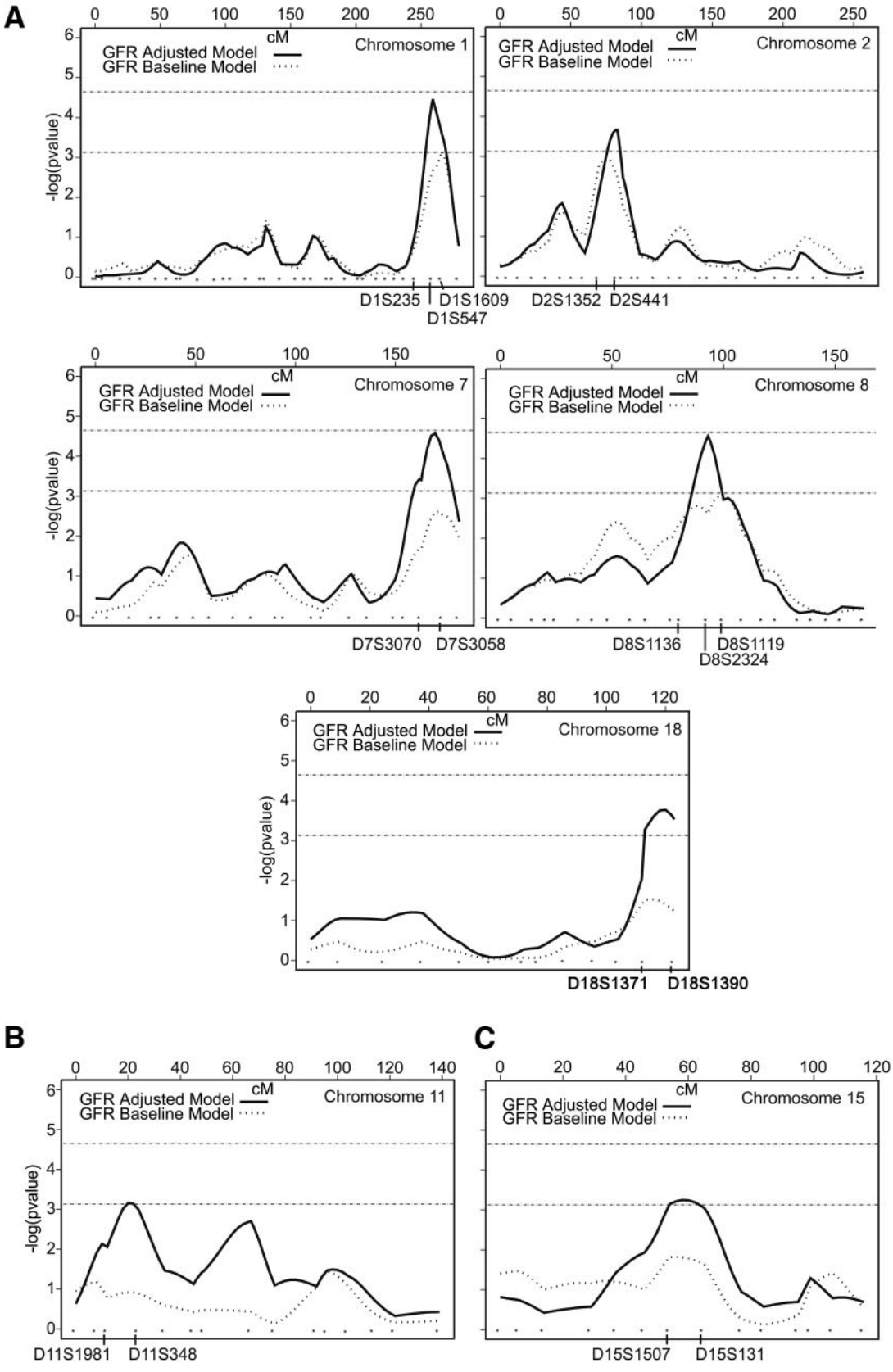

FIG. 2. Detailed genome scans for linkage with eGFR. *A*: Chromosomes 1, 2, 7, 8, and 18 data for Mexican Americans. *B*: Chromosome 11 for African Americans. *C*: chromosome 15 for American Indians. Curves representing data adjusted for the covariates diabetes duration and ACE inhibitor/ARB use are shown with solid lines, whereas curves for unadjusted data are shown with broken lines. Chromosomal position in cM is shown along the upper *x*-axis, microsatellite marker locations are shown along the lower *x*-axis, and −log *P* values are shown along the *y*-axis, with corresponding LOD score scale. Lower horizontal dotted lines represent suggestive evidence ($P = 7.4 \times 10^{-4}$), and upper horizontal lines show threshold for evidence ($P = 2.2 \times 10^{-5}$) of linkage by Lander-Kruglyak criteria (ref. 31).

TABLE 4
Comparison with other diabetic nephropathy and diabetic nephropathy–related genome scans and candidate gene studies

Cytogenetic locus	Gene	Phenotype	Marker/SNP ID	Physical distance (Mb)	Source
1q43		**DN/eGFR**	**D1S547**	**240**	
2p13.3		**DN/eGFR**	**D2S441**	**68**	
7q36.1		**DN/eGFR**	**D7S3070**	**151**	
7q33		DN	D7S500	135	Ref. 6
7q34		eGFR		138	Ref. 23
7q36	*NOS3*	DN,ESRD		150	Refs. 36–38
7q36.2		DN/Ualb	D7S798	152	Ref. 35
			GATA30D09N	153	
8q13.3		**DN/eGFR**	**D8S2324**	**74**	
11p15.1		**DN/eGFR**	**D11S348**	**18**	
11p13	*CAT*	DN	rs1049982	34	Ref. 41
			rs560807		
			rs12594610		
15q22.3		**DN/eGFR**	**D15S131**	**69**	
15q22.33	*SMAD3*	DN	rs4776890	65	Ref. 41
			rs12594610		
15q25.3		DN	D15S996	89	Ref. 30
18q22.3		**DN/eGFR**	**D18S1371**	**71**	
18q21.33	*BCL2*	DN	rs1481031	59	Ref. 41
			rs12457700		
			rs2062011		
18q22.3-q23		DN	D18S58	70	Ref. 40
18q22.3	*CNDP1*	DN	D18S880	70	Refs. 42, 43
18q22.3		Diabetes	D18S1371	71	Ref. 39
18q22.3		DN	D18S1371	71	Ref. 30

Loci and associated data identified in this study are shown in bold. Diabetic nephropathy (DN) defined by composite phenotype parameters; *BCL2*, B-cell leukemia/lymphoma 2; *CAT*, catalase; *CNDP1*, carnosinase; *NOS3*, nitric oxide synthase-3; *SMAD3*, mothers against decapentaplegic 3; SNP, single nucleotide polymorphism.

(49). Each concluded that the MDRD study underestimated GFR in the normal range. However, our cohort has a relatively depressed mean eGFR, approaching the range in the population from which the formula was derived. Furthermore, statistical analyses for genetic studies rely on phenotype ranking. Therefore, to the extent that the MDRD equation uniformly underestimates the absolute GFR value, the rank order is unlikely to change; thus, one would expect the results to be unaffected. The definitive study, which includes the entire FIND family sample with approximately four times as many participants, is ongoing. In this analysis, a single nucleotide polymorphism genotyping platform will replace the microsatellite technology.

A potential limitation of this study is that eGFR was determined on the basis of a single random sample collection. Although validation with serial measures would be ideal, quality control studies demonstrated negligible variability on repeat creatinine measurement of the same serum sample (data not shown). An additional point is that we ascertained for diabetic nephropathy according to criteria that included diabetes duration. It is therefore possible that we omitted subjects with the most severe eGFR phenotypes, who may have died before achieving duration criteria. It has been suggested that such a study design may bias results toward discovery of survival rather than disease genes (50). Selection bias in relation to the participating centers and subsequently enrolled diabetic sibpairs cannot be ruled out. Finally, data were adjusted for a number of confounding variables, such as diabetes duration, antihypertensive therapy, BMI, and A1C concentration, but other potentially relevant parameters, such as blood pressure and cardiovascular diseases, were not available. Like many common diseases, diabetic nephropathy is caused by a combination of genetic and environmental risk factors. Both linkage and association designs have been used to identify common disease genes. Linkage studies are not as powerful as association studies for identification of genes contributing to risk for common, complex diseases, which are caused by variants with effect sizes in the order of odds ratios of 1.15–1.4. FIND was initiated when whole-genome association studies were not feasible. At that time, family-based study designs had the advantage of searching the whole genome in an unbiased manner without predetermining the identity of specific candidates, and association designs were only used to evaluate candidate disease genes. Given recent successes in gene mapping for common disease using whole-genome association, the FIND consortium is now also performing a whole-genome association. The results of linkage analysis reported in this paper will be valuable in the analyses of whole-genome association data and should accelerate identification of genetic variants that regulate diabetic nephropathy pathogenesis.

In conclusion, several linkage peaks for eGFR were identified from the multi-ethnic FIND cohort, which represents the largest diabetic nephropathy genetics study to date. After adjustment for diabetes duration and ACE inhibitor/ARB use, the peaks on chromosomes 1q43, 7q36.1, and 8q21.3 are very close to attaining Lander-Kruglyak significance for linkage (31) within the Mexican-American cohort and achieve genome-wide significance at the 5% level, strongly suggesting that unique diabetic nephropathy alleles cluster within these loci. We speculate that fine-mapping and candidate gene and sequencing analyses should permit identification of mutations within these loci, which could ultimately lead to specific predictive tests and/or therapies for a subset of patients with diabetic nephropathy. Further studies investigating the

association of GFR and genetic loci in large U.S. populations of patients with diabetic kidney disease will be necessary to confirm the findings.

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APPENDIX

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