Original Article

A Genome-Wide Linkage Scan for Genes Controlling Variation in Renal Function Estimated by Serum Cystatin C Levels in Extended Families With Type 2 Diabetes

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We performed a variance components linkage analysis of renal function, measured as glomerular filtration rate (GFR), in 63 extended families with multiple members with type 2 diabetes. GFR was estimated from serum concentrations of cystatin C and creatinine in 406 diabetic and 428 nondiabetic relatives. Results for cystatin C were summarized because they are superior to creatinine results. GFR aggregates in families with significant heritability (h^2) in diabetic ($h^2 = 0.45$, $P < 1 \times 10^{-5}$) and nondiabetic ($h^2 = 0.36$, $P < 1 \times 10^{-3}$) relatives. Genetic correlation ($r_G = 0.35$) between the GFR of diabetic and nondiabetic relatives was less than one ($P = 0.01$), suggesting that genes controlling GFR variation in these groups are different. Linkage results supported this interpretation. In diabetic relatives, linkage was strong on chromosome 2q (logarithm of odds [LOD] = 4.1) and suggestive on 10q (LOD = 3.1) and 18p (LOD = 2.2). In nondiabetic relatives, linkage was suggestive on 3q (LOD = 2.2) and 11p (LOD = 2.1). When diabetic and nondiabetic relatives were combined, strong evidence for linkage was found only on 7p (LOD = 4.0). In conclusion, partially distinct sets of genes control GFR variation in relatives with and without diabetes on chromosome 2q, possibly on 10q and 18p in the former, and on 7p in both. None of these genes overlaps with genes controlling variation in urinary albumin excretion. *Diabetes* 55: 3358–3365, 2006

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ACR, albumin-to-creatinine ratio; CC-GFR, GFR estimated by cystatin C measured in micrograms per liter multiplied by 100; CG-GFR, Cockcroft-Gault estimate of GFR; ESRD, end-stage renal disease; G × DM, genotype by diabetes; GFR, glomerular filtration rate; LOD, logarithm of odds; logACR, ACR values transformed to a base 10 logarithm and multiplied by 10; MDRD, Modification of Diet in Renal Disease; MDRD-GFR, MDRD estimate of GFR; QTLs, quantitative trait loci.

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Diabetic nephropathy is the major complication of type 2 diabetes and the major cause of new cases of end-stage renal disease (ESRD) in the U.S. population (1). Elevated urinary albumin excretion and declining renal function are its main phenotypes (2,3). Research during the last decade established that both of these phenotypes cluster within families, regardless of whether the families were ascertained through individuals with or without diabetes (4–9). Familial aggregation of diabetic nephropathy phenotypes justifies a search for genes (quantitative trait loci [QTLs]) influencing both phenotypes separately. Previously, we described QTLs linked with variation in urinary albumin excretion in the Joslin Collection of Families With Type 2 Diabetes (10).

In this study, we investigated familial aggregation (heritability) of renal function measured as glomerular filtration rate (GFR) and conducted a genome-wide scan for QTLs that control variation in GFR. GFR was estimated from serum concentrations of creatinine using the Cockcroft-Gault formula (11), the Modification of Diet in Renal Disease (MDRD) equation (12), and from serum cystatin C, a new reliable marker of GFR, particularly in individuals with normal renal function (13).

RESEARCH DESIGN AND METHODS

The families used for this study had been previously recruited for the Joslin Study on the Genetics of Type 2 Diabetes (5,10,14). Briefly, between 1993 and 2003, 104 families were ascertained through Joslin Clinic probands with type 2 diabetes and examined because the pattern of occurrence of type 2 diabetes in family members was consistent with an autosomal dominant mode of inheritance. It is important to emphasize that presence or absence of diabetic nephropathy did not have any impact on the ascertainment and selection of the families into the Joslin Family Collection.

The mean age at diagnosis of type 2 diabetes within a family was "early" in 46 families (aged <35 years). These were considered maturity-onset diabetes of the young families and were not included in this study. The remaining 68 families represent common type 2 diabetes. Within these families, the average mean age of diagnosis was between 35 and 59 years. Included in this study were 63 families, comprising 59 European-American and 4 minority families.

The Human Subjects Committee of the Joslin Diabetes Center approved the study protocol and informed consent procedures. After giving written consent to participate, family members were examined by trained recruiters according to previously described protocols (5,10,14). Diagnosis of diabetes was based on the history of hypoglycemic treatment, results of oral glucose tolerance test, and levels of HbA_{1c}, as previously described (14).

Laboratory methods. Fasting blood was drawn for DNA extraction and biochemical measurements. Serum measurements included cystatin C, which reflects GFR accurately even in individuals with normal or elevated renal

function (13). Cystatin C was measured in thawed serum samples (previously stored in −85°C) by an immunoassay based on rabbit monospecific anti-human cystatin C antiserum (Dade Behring Diagnostics, Newark, DE). The measurements were conducted on a BN Prospec System nephelometer (Dade Behring). The between-assay coefficient of variation in samples from the lowest and highest quartiles of the cystatin C distribution was 3.8 and 3.0%, respectively. GFR measured by ithalamate clearance is closely approximated by the reciprocal of serum cystatin C measured in micrograms per liter multiplied by 100 (CC-GFR) (13). Estimated renal function by CC-GFR is independent from sex and body weight (13).

Creatinine concentration in serum was measured by alkaline picric colorimetry (modified Jaffe reaction) on a Synchron CX5 (Beckman Instrumentation, Brea, CA). The between-assay coefficient of variation in samples from the lowest and highest quartiles of the creatinine distribution was 4.7 and 3.5%, respectively. The Cockcroft-Gault estimate of GFR (CG-GFR) was computed by the modified Cockcroft-Gault formula [(140 − age in years) × (actual weight in kg)/(72 × serum creatinine in mg/dl) × 0.85 (if female)] (11). The MDRD estimate of GFR (MDRD-GFR) was computed by the following equation: 186 × (serum creatinine in mg/dl)$^{-1.154}$ × (age in years)$^{-0.203}$ × 0.72 if female)] (12).

Methods for measuring albumin and creatinine in a random urine sample for determination of the albumin-to-creatinine ratio (ACR) were previously described (5,10). ACR values were transformed to a base 10 logarithm and multiplied by 10 (logACR). Family members with ESRD did not have measurements of GFR or ACR; these individuals were assigned extreme values (GFR = 10 ml/min, ACR = 2,500 μg/mg, and examination date = first dialysis date).

DNA extraction and genotyping. For all 900 examined members of the 63 families, DNA was extracted from buffy-coat specimens using a standard phenol chloroform protocol. A genome-wide scan was performed by the National Heart, Lung, and Blood Mammalian Genotyping Service (available at http://research.marshfieldclinic.org/genetics/) at the Marshfield Medical Research Foundation. The panel of polymorphic markers (screening set 12) consisted of 383 microsatellite markers spaced, on average, 9.1 cM over 22 autosomes, with an average marker heterozygosity of 0.75. Markers on sex chromosomes were not analyzed, as the risk of impaired renal function and ESRD is similar in men and women (1). In total, both phenotype and genotype were available for 834 relatives.

Statistical genetic analyses. SAS (version 8.02) was used for statistical analysis. SOLAR, a pedigree-based variance components software package (version 2.1.4; Southwest Foundation for Medical Research, San Antonio, TX) was used for genetic analyses. Heritability (h^2), as a measure of familial aggregation of GFR, was estimated after adjustment for relevant covariates.

Different covariates were used in the genetic analyses. For CG-GFR, the covariates were logACR, systolic blood pressure, antihypertensive treatment, diabetes, and diabetes duration. Sex, BMI, and age were not included because they are included in the Cockcroft-Gault formula. For MDRD-GFR, the covariates were logACR, systolic blood pressure, antihypertensive treatment, BMI, diabetes, and diabetes duration. Sex and age were not included because they are included in the MDRD equation. For CC-GFR, the covariates were logACR, systolic blood pressure, antihypertensive treatment, sex, BMI, and diabetes duration for diabetic relatives; for nondiabetic relatives, covariates were logACR, systolic blood pressure, antihypertensive treatment, sex, BMI, and age. In diabetic relatives, we did not use age as a covariate because its addition to the other covariates decreased heterability of CC-GFR from h^2 = 0.45 to h^2 = 0.36.

The effect of diabetes on GFR is expected to be significant. To test for genotype-by-diabetes (G × DM) interaction on GFR in all relatives, the variance components approach was extended using information from the covariance between relative pairs under different environments (i.e., diabetic and nondiabetic) (15–18). In this interaction model, two parameters were estimated: *1*) diabetes status–specific genetic variances of estimates of GFR (genetic variances in diabetic and nondiabetic relatives) and *2*) the genetic correlation for estimates of GFR between diabetic and nondiabetic relatives (i.e., in relative pairs discordant for diabetes). In the presence of G × DM interaction, the genetic variances for estimates of GFR in relatives with and without diabetes should be different (i.e., $\sigma_{G\text{-}DM} \neq \sigma_{G\text{-}NDM}$, where σ_G is the additive genetic SD, DM is diabetic, and NDM is nondiabetic), and/or the genetic correlation between diabetic and nondiabetic relatives in relative pairs discordant for diabetes should be significantly lower than 1 [i.e., $r_{G\ (DM,\ NDM)} < 1$]. This approach is extended to estimate environment-specific QTLs effects (19). If there is a significant G × DM interaction, the genetic variance of GFR attributable to the QTLs for individuals living in different environments (i.e., diabetic and nondiabetic) will not be equal ($\sigma_{qDM} \neq \sigma_{qNDM}$).

Pedigree-based variance components linkage analysis implemented in the program SOLAR was used to detect and localize QTLs controlling variation in estimates of GFR (20,21). This approach has been shown to be a powerful linkage analysis technique (21–23). We used the program SOLAR to estimate multipoint IBD matrices using Markov Chain Monte Carlo methods implemented in the program Loki (24), and the number of iterations used was 100,000. Hypotheses were tested by likelihood ratio tests (25,26). Conversion of the $\log_e$ likelihoods to $\log_{10}$ yielded logarithm of odds (LOD) scores. Only multipoint LOD scores are reported. According to Lander and Kruglyak criteria (27), LOD ≥1.9 ($P = 1.7 \times 10^{-3}$) is considered as suggestive and LOD ≥3.3 ($P = 4.9 \times 10^{-5}$) is considered as significant.

Estimation of heritability, and subsequently evaluation of linkage, incorporated a correction for ascertainment bias by conditioning the likelihood for the family data on the phenotype of the proband (28). Multipoint variance component linkage analyses of adjusted estimates of GFR were performed separately in relatives with diabetes and relatives without diabetes, as well as in all relatives combined. Comparison of the support for linkage from these analyses at a chromosomal region of interest can be considered an imprecise measure of the specificity of the QTLs effect in that region on variation in GFR.

Empirical *P* values were approximated using the lodadj procedure within SOLAR. A fully informative marker unlinked to estimates of GFR was simulated, IBD scores estimated, and linkage analyzed. The distribution of LOD scores from 10,000 simulations determined the empirical *P* value. When this was insufficient to elicit five LOD scores greater than that observed, 50,000 simulations were used.

TABLE 1
Clinical characteristics of examined members of the 63 families according to diabetes status

Characteristics at examination	Diabetes	
	Present*	Absent
n	406	428
Men (%)	43	45
Age (years)	57 ± 16	46 ± 17
Duration of diabetes (years)	11 ± 11	—
Treatment with insulin (%)	39	—
BMI (kg/m^2)	30 ± 7	28 ± 5
Systolic blood pressure (mmHg)	137 ± 20	121 ± 20
Diastolic blood pressure (mmHg)	78 ± 11	75 ± 11
Antihypertensive medication (%)	44	13
Treatment with ACE inhibitors (%)	13	3
Urinary ACR (25th, 50th, and 75th percentiles)†	6, 14, 54	4, 6, 9
ESRD (%)	2.6	—
CC-GFR (ml/min)‡§	104 ± 35	120 ± 24
CG-GFR (ml/min)‡‖	87 ± 37	101 ± 30
MDRD-GFR (ml/min)‡¶	80 ± 29	90 ± 25

Data are means ± SD or percentage, unless otherwise indicated. *This group includes 44 individuals with impaired glucose tolerance and 7 individuals with gestational diabetes. For all these individuals, duration of diabetes was assumed 0 years. †Values of ACR were expressed as urinary ACR (micrograms for albumin per milligrams for creatinine). In the analysis we used logACR ($\log_{10}$ transformed and multiplied by 10). ‡ESRD patients were assigned GFR = 10 ml/min. §CC-GFR: GFR estimated from serum cystatin C levels by formula 100/serum cystatin C (for validation see 13). ‖CG-GFR: GFR estimated from serum creatinine levels by formula provided by Cockcroft and Gault (11). ¶MDRD-GFR: GFR estimated from serum creatinine levels by the MDRD formula (12).

RESULTS

Characteristics of examined families. The 63 extended families included in this study were recruited and examined for the Joslin Study on Genetics of Type 2 Diabetes and previously were used to identify QTLs for urinary albumin excretion (10). Clinical data and genotypes from 834 members of these families (406 with diabetes and 428 without diabetes) were analyzed. On average, 6.4 relatives (range 3–14) with diabetes and 6.8 relatives (1–12) without diabetes were studied in each family. Clinical characteris-

TABLE 2
Distribution of pairs of relatives in the 63 families according to genetic relationship and diabetes status

Relationship	Pairs of relatives by diabetes status			
	DM-DM	NDM-NDM	DM-NDM	All pairs
Parental (0.500)	129	127	267	523
Sibling (0.500)	372	220	293	885
Half-sibling (0.250)	11	12	19	42
Grandparental (0.250)	13	15	22	50
Avuncular (0.250)	304	263	714	1,281
Cousin, etc. (0.125)	224	564	527	1,315
Other (<0.125)	149	477	465	1,091
All pairs	1,202	1,678	2,307	5,187

Data in parentheses are relationship coefficients. DM, individuals with diabetes; NDM, individuals without diabetes.

tics of the relatives included in the study are provided by diabetes status in Table 1. At the time of examination, the mean age of relatives with diabetes was 57 ± 16 years compared with 46 ± 17 years for those without diabetes. The mean age at diagnosis of diabetes in these families was 46 ± 16 years (mean diabetes duration 11 ± 11 years). At the time of examination, 39% of the diabetic relatives were treated with insulin and the rest were treated with oral agents or diet. On average, family members with diabetes were more obese, had higher systolic and diastolic blood pressure, and more were treated with antihypertensive medications than those without diabetes. Because the majority of patients were recruited into the study in the mid-1990s, only a small proportion of them was treated with ACE inhibitors (13% diabetic and 3% nondiabetic subjects).

Family members with diabetes had higher ACR values and a much higher proportion in the microalbuminuria or proteinuria range (≥20 μg albumin/mg urinary creatinine) than those without diabetes (41 vs. 10%, $P < 0.001$). At the time of examination, ESRD was present in 11 (2.7%) family members with diabetes. Excluding these 11 individuals, mean serum cystatin C was 1.08 ± 0.60 μg/l in diabetic and 0.87 ± 0.22 μg/l in nondiabetic subjects, and mean serum creatinine was 1.0 ± 0.5 mg/dl in diabetic and 0.9 ± 0.3 mg/dl in nondiabetic subjects. The two measurements were only moderately correlated (Spearman's correlation = 0.61 and 0.44 in diabetic and nondiabetic relatives, respectively).

Using serum concentrations of cystatin C and creatinine, three estimates of GFR were computed for each individual. The mean values of these estimates are shown in the bottom part of Table 1. The SDs for the GFR estimates were similar, but the mean values differed. In diabetic and nondiabetic subjects, means were the highest for CC-GFR, lower for CG-GFR, and the lowest for MDRD-GFR. This pattern is in agreement with our recent report (13), where the discrepancy between these GFR estimates and iothalamate clearance was least for mean CC-GFR (only 5 ml lower), more discrepant for mean CG-GFR (15 ml lower), and most discrepant for mean MDRD-GFR (20 ml lower).

The distribution of relative pairs in this study according to genetic relationship and diabetes status is shown in Table 2. The 63 extended families generated 5,187 relative pairs: 1,202 pairs concordant for diabetes (DM-DM pairs), 1,678 pairs concordant for "absence of diabetes" (NDM-NDM pairs), and 2,307 pairs discordant for diabetes (DM-NDM pairs).

Familial aggregation of estimated GFR. Estimates of the heritability (h^2) of the GFR estimates after adjustment for relevant covariates are shown in Table 3, according to the three groupings of relative pairs. Heritability estimates of renal function were highest and most statistically significant in diabetic relatives (h^2 ranging from 0.29 to 0.47). Heritability estimates were much lower in nondiabetic relatives, with CC-GFR ($h^2 = 0.36$) and MDRD-GFR ($h^2 = 0.22$) being statistically greater than zero, while CG-GFR ($h^2 = 0.15$) was not. In all relative pairs combined, estimates of heritability of renal function were significant and intermediate in magnitude among the separate groups ($h^2 = 0.28$ for CC-GFR, $h^2 = 0.31$ for CG-GFR, and $h^2 = 0.28$ for MDRF-GFR).

To examine whether the same genetic factors contribute to variation in renal function in diabetic and nondiabetic relatives, G × DM interaction models were developed for the three GFR estimates. The genetic variance of CC-GFR was larger in diabetic ($\sigma_{G\text{-}DM} = 18.8 \pm 3.3$ ml/min) than nondiabetic ($\sigma_{G\text{-}NDM} = 12.1 \pm 2.4$ ml/min) relatives ($P = 0.1$). Furthermore, genetic correlation [$r_{G\,(DM,\,NDM)} = 0.35 \pm 0.21$] between the adjusted CC-GFR of diabetic and nondiabetic relatives in pairs discordant for diabetes ($n = 2,307$) was significantly less than one ($P = 0.01$). The above findings are consistent with a G × DM interaction on variation in CC-GFR and suggest that the set of genes controlling variation in GFR in diabetic and nondiabetic relatives are not identical. For the creatinine-based estimates of renal function (CG-GFR and MDRD-GFR), the genetic variance was not statistically different between diabetic and nondiabetic relatives (Table 3). Also, genetic correlations between the adjusted GFRs of diabetic and nondiabetic relatives in pairs discordant for diabetes were not significantly different from one (data not shown).

TABLE 3
Heritability (h^2) of different estimates of GFR in various groups of relatives

Groups of relatives	Relative pairs (n)	Estimates of GFR		
		CC-GFR*†	CG-GFR*†	MDRD-GFR*†
Diabetic subjects only	1,202	0.45 ± 0.11 ($P < 1 \times 10^{5}$)	0.47 ± 0.12 ($P < 1x10^{-5}$)	0.29 ± 0.11 ($P < 0.001$)
Nondiabetic subjects	1,678	0.36 ± 0.13 ($P < 1 \times 10^{-3}$)	0.15 ± 0.11 (NS)	0.22 ± 0.12 ($P < 0.05$)
All relatives	5,187	0.28 ± 0.06 ($P < 1 \times 10^{-7}$)	0.31 ± 0.07 ($P < 1 \times 10^{-7}$)	0.28 ± 0.07 ($P < 1 \times 10^{-6}$)

Data are h^2 ± SE (P value). *Covariates used for adjustment (see RESEARCH DESIGN AND METHODS). †Genetic (σ_G ± SE) and environmental (σ_E ± SE) SDs relating to the h^2 ± SE estimates by group of relatives and estimate of GFR. **Diabetic subjects only:** CC-GFR = $\sigma_G = 18.77 \pm 2.68$, $\sigma_E = 20.58 \pm 1.96$; CG-GFR = $\sigma_G = 22.53 \pm 3.33$, $\sigma_E = 23.70 \pm 2.48$; and MDRD-GFR = $\sigma_G = 13.77 \pm 2.74$, $\sigma_E = 21.56 \pm 1.64$. **Nondiabetic subjects only:** CC-GFR = $\sigma_G = 12.09 \pm 2.35$, $\sigma_E = 16.25 \pm 1.56$; CG-GFR = $\sigma_G = 11.12 \pm 4.22$, $\sigma_E = 26.77 \pm 1.86$; and MDRD-GFR = $\sigma_G = 10.69 \pm 3.01$, $\sigma_E = 20.29 \pm 1.57$. **All relatives:** CC-GFR = $\sigma_G = 13.23 \pm 1.63$, $\sigma_E = 21.12 \pm 0.95$; CG-GFR = $\sigma_G = 17.23 \pm 2.10$, $\sigma_E = 25.52 \pm 1.25$; and MDRD-GFR = $\sigma_G = 12.95 \pm 1.73$, $\sigma_E = 20.81 \pm 0.98$.

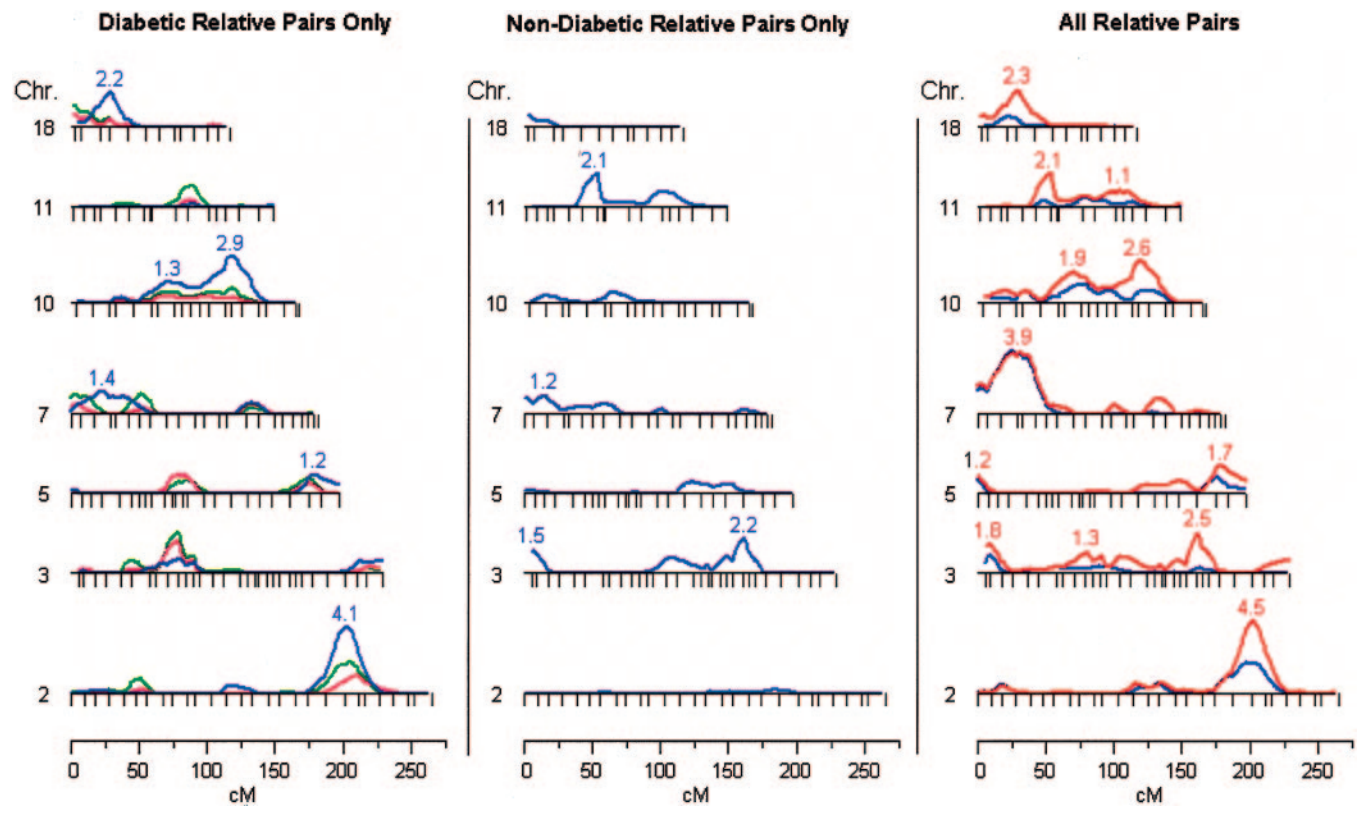

FIG. 1. ***Blue lines*****: Genome scan results for linkage to variation in CC-GFR in DM-DM relative pairs, in NDM-NDM relative pairs, and in all relative pairs. The results for all relative pairs consist of the sum of the two former groups of relatives, as well as DM-NDM relative pairs for which the results are not displayed by SOLAR. Only chromosomes with LOD ≥1.0 are shown. The numbers indicate maximum LOD scores. Chromosomal positions correspond to the Marshfield map. After adjustment for relevant covariates, the residuals for CC-GFR have the following kurtosis and skewness: 0.09 and 0.05 in diabetic subjects, 0.67 and −0.19 in nondiabetic subjects, and 0.56 and −0.06 in all relatives.** ***Green lines*****: Genome scan results for linkage to variation in CG-GFR in DM-DM relative pairs on chromosomes that had LOD ≥1.0 for CC-GFR. Chromosomal positions correspond to the Marshfield map. After adjustment for relevant covariates, the residuals for CG-GFR in diabetic subjects have kurtosis 0.70 and skewness 0.63.** ***Pink lines*****: Genome scan results for linkage to variation in MDRD-GFR in DM-DM relative pairs are shown on chromosomes that had LOD ≥1.0 for CC-GFR. Chromosomal positions correspond to the Marshfield map. After adjustment for relevant covariates, the residuals for MDRD-GFR in diabetic subjects with kurtosis 0.33 and skewness 0.22.** ***Red lines*****: Genome scan results for linkage to variation in CC-GFR in all relative pairs after QTLs × DM interaction was incorporated. The numbers indicate maximum LOD scores. Chromosomal positions correspond to the Marshfield map.**

Genome scan for QTLs controlling variation in estimated GFR based on serum concentration of cystatin C. Multipoint variance component linkage analyses of adjusted CC-GFR was performed separately in diabetic relatives and nondiabetic relatives, as well as in all relatives combined. Chromosomes with at least potential evidence for linkage (LOD >1.0) in any of these three analyses are shown in Fig. 1 (blue lines). For three chromosomal regions (2q, 10q, and 18p) evidence for linkage was present in DM-DM but not NDM-NDM relative pairs. For three other chromosomal regions (3p, 3q, and 11p), it was present in NDM-NDM but not DM-DM relative pairs. For 2q it was present in DM-DM and all relative pairs, and for 7p it was present in all three groupings of relative pairs.

Additional details regarding linkage to CC-GFR variation

TABLE 4
Support for linkage to variation in CC-GFR* in different groups of relatives

Chromosome	Closest marker†	Location (cM)‡	Max. LOD score§	Nominal *P*	Empirical *P*	LOD-1 interval (Mb)
Diabetic relative pairs						
2q	D2S1384	202	4.1	6.2×10^{-6}	2×10^{-5}	195–213
10q‖	D10S2470–D10S677	114	3.6	2.5×10^{-5}	1.2×10^{-4}	85–101
18p	D18S843	28	2.2	7.0×10^{-4}	2.4×10^{-3}	6–10
Nondiabetic relative pairs						
3q	D3S1744	161	2.2	6.6×10^{-4}	1.2×10^{-3}	138–152
11p	D11S1993	52	2.1	8.0×10^{-4}	1.6×10^{-3}	36–53
All relative pairs						
7p	D7S3047–D7S3051	23	4.0	9.8×10^{-6}	4×10^{-5}	6–26

*Covariates used for adjustment of CC-GFR (see RESEARCH DESIGN AND METHODS). †Name of one marker is provided if it coincides with the peak of the maximum (Max.) LOD score, names of two markers are provided if the peak of the maximum LOD score is located between markers. ‡Position according to Marshfield map. §Multipoint LOD score. ‖Results from the set of Caucasians only.

in different groups of relatives are summarized in Table 4. In diabetic relatives, evidence for linkage with GFR was significant on 2q (LOD = 4.1, empirical $P = 2.0 \times 10^{-5}$) and suggestive on chromosomes 10q (LOD = 3.6, empirical $P = 1.2 \times 10^{-4}$) and 18p (LOD = 2.2, empirical $P = 2.4 \times 10^{-3}$). The LOD-1 support intervals for these regions varied in size, ranging from 4 to 18 Mb. In nondiabetic relatives, evidence for linkage with variation in GFR was suggestive on chromosomes 3q (LOD = 2.2, empirical $P = 1.2 \times 10^{-3}$) and 11p (LOD = 2.1, empirical $P = 1.6 \times 10^{-3}$). The LOD-1 support intervals for these regions were 14 and 16 Mb. When all relatives were analyzed together, evidence for linkage was significant on chromosome 7p (LOD = 4.0, empirical $P = 4 \times 10^{-5}$). This LOD score approximates the sum of the LOD scores in DM-DM, NDM-NDM, and DM-NDM relative pairs. The LOD-1 support interval on chromosome 7p spans a region of 20 Mb on the physical map.

The second set of results shown in Fig. 1 for all relative pairs (red lines) was obtained with a model incorporating a G × DM interaction. The evidence for linkage to CC-GFR variation in all relatives became significant on chromosome 2q (LOD = 4.5), remained significant on chromosome 7p (LOD = 3.9), and increased to the level of suggestive on chromosomes 3q, 10q, 11q, and 18p. Importantly, of the findings with respect to G × DM interaction, the evidence for linkage (LOD = 4.5) for CC-GFR on chromosome 2q improved greatly. The magnitude of difference in diabetes status–specific QTLs effects ($\sigma q_{DM} = 20.9 \pm 2.3$ and $\sigma q_{NDM} = 1.5 \pm 3.1$) was found to be highly statistically significant ($P = 0.00003$). However, the strength of the linkage evidence on chromosome 7p remained similar with (LOD = 3.9) or without (LOD = 4.0) G × DM interaction. Not surprisingly, the magnitude of difference in diabetes status–specific QTLs effects ($\sigma q_{DM} = 15.7 \pm 2.3$ and $\sigma q_{NDM} = 9.8 \pm 2.0$) failed to be significant ($P = 0.09$). Thus, it appears that the QTLs on chromosomes 2q and 7p differentially influence the variation in GFR in diabetic versus nondiabetic relatives.

Our family collection includes 59 European-American families and 4 minority families (1 African-American and 3 Hispanic families). To examine the impact of removing these four minority families, we repeated the linkage analyses of CC-GFR. Two of the linkage signals in DM-DM relative pairs changed significantly. The evidence for linkage on chromosome 2q declined from LOD = 4.1 to LOD = 2.4 but increased on chromosome 10q from LOD = 2.9 to LOD = 3.6. Support for linkage was unaffected at other locations in DM-DM and NDM-NDM relative pairs. In all relative pairs, the support for linkage on 7p has diminished only slightly from LOD = 4.0 to LOD = 3.7 when the minority families were removed.

Genome scan for QTLs controlling variation in estimated GFR based on serum concentration of creatinine. We repeated the multipoint variance component linkage analysis of GFR in the same three groupings of relative pairs using CG-GFR and MDRD-GFR instead of CC-GFR. The results for CG-GFR in DM-DM relatives are shown in Fig. 1 (green lines). Suggestive evidence for linkage on chromosome 2q (LOD = 2.0) colocalized with the region that strongly supported linkage for CC-GFR (LOD = 4.1). Suggestive evidence for linkage was also present on chromosome 3q (LOD = 2.6) at 85 cM, where evidence for linkage to CC-GFR was minimal. All other signals for linkage to CG-GFR were weak and most colocalized with signals for linkage to CC-GFR. No suggestive or strong evidence for linkage to CG-GFR was present in NDM-NDM relative pairs or all relative pairs. In the former, the highest evidence was on chromosome 7q (LOD = 1.3) at 141 cM, and in the latter the highest evidence was on chromosome 6p (LOD = 1.7) at 52 cM (data not shown). The results for MDRD-GFR for DM-DM relatives are shown in Fig. 1 (pink lines). Suggestive evidence for linkage was present on chromosome 3q (LOD = 2.0) at 85 cM, the same location as suggestive evidence for linkage to CG-GFR. All other signals for linkage to MDRD-GFR were weak and some colocalized with signals for linkage to CC-GFR. No suggestive or strong evidence for linkage to MDRD-GFR was present in NDM-NDM relative pairs or all relatives. In the former, the highest evidence was on chromosome 19q (LOD = 1.2) at 116 cM, and in the latter the highest evidence was on chromosome 6p (LOD = 1.8) at 56 cM (data not shown).

DISCUSSION

Although substantial literature has accumulated on susceptibility to nephropathy in type 2 diabetes (4–9), the genetic architecture underlying this susceptibility has not been resolved. To investigate this issue, we used a genome-wide scan of the Joslin collection of extended families ascertained for multiple members with type 2 diabetes. Previously, we reported that genes located on chromosomes 5q (LOD = 3.4), 7q (LOD = 3.1), and 22q (LOD = 3.7) account for the majority of the genetic variance of urinary albumin excretion in both diabetic and nondiabetic relatives (10). We now report that variation in estimated GFR also has significant heritability, but the responsible genes localize to chromosomal regions (2q, 7p, and possibly 10q and 18p) that are different from those responsible for the heritability of urinary albumin excretion. Furthermore, the contribution of the chromosomal regions controlling variation in GFR is somehow different in diabetic and nondiabetic relatives.

Certain novel aspects of our investigation need to be emphasized. First, in contrast to previous linkage studies (29–34) that defined nephropathy as a discrete trait (i.e., the presence of proteinuria or ESRD), we searched for genes controlling variation in GFR, a quantitative trait that may be genetically distinct from variation in urinary albumin excretion. This approach, in comparison with the earlier studies, increases the power to detect QTLs controlling renal function and permitted a search for QTLs for GFR independently of QTLs for urinary albumin excretion. Second, the selection of large families enabled us to test whether the QTLs controlling variation in GFR are the same in diabetic and nondiabetic relatives. Third, lacking direct measurements of GFR for genetic analysis, we used three different indirect measures that are based on serum concentrations of endogenous metabolites. Two of these, based on serum creatinine and limited accuracy if renal function is normal or high, have been widely used in the literature (11,12). The third, based on serum cystatin C, is novel (13). Of the three measures, GFR estimates based on cystatin C provided the strongest support for genetic determinants of renal function and the most revealing indications of the chromosomal locations of putative QTLs controlling GFR.

The present study confirmed the role of genetic factors contributing to variation of renal function findings previously reported by others (rev. in 35). Our estimates of heritability of GFR, range from 0.35 to 0.45 in nondiabetic and diabetic groups of relatives. The genetic variance of

GFR was larger in diabetic than in nondiabetic relatives, an indication that a diabetic environment magnifies the impact of genetic factors on variation in renal function. Furthermore, the low genetic correlation between the GFR of diabetic and nondiabetic relatives in pairs discordant for diabetes (significantly less than one) indicates that with the development of diabetes, different genes assume control of variation in GFR. Localization of QTLs on different chromosomes in these two groups of relatives is consistent with this finding.

In diabetic relative pairs, support for linkage with variation in GFR on chromosome 2q is strong. While it is also strong in European Americans on chromosome 10q, it did not reach the threshold of significance; on chromosome 18p the evidence is only suggestive. In nondiabetic relative pairs, the lack of support for linkage of GFR with these chromosomal regions favors the interpretation that the QTLs effects in these three chromosomal regions are specific to a diabetic environment. Only for the region on chromosome 7p is the support for linkage to variation in GFR significant in all relative pairs. Support for linkage with GFR in nondiabetic relatives is suggestive on chromosomes 3q and 11p, while it is absent in these regions in diabetic relative pairs and all relative pairs. The interpretation of these findings is not clear.

The results of our study cannot be compared directly with other reports in the literature, as this is the first linkage study of variation in GFR in predominantly European-American families with diabetes. However, they can be compared with the findings obtained in three studies of minority families. A study of 39 extended Mexican-American families, in which one-third of the relatives had diabetes, identified a region on chromosome 2q that was linked to variation in GFR estimated by the Cockcroft-Gault formula (36). Support for linkage significantly increased (from LOD = 2.6 to LOD = 4.0) when G × DM interaction was incorporated into the analysis, suggesting that the influence of this region on GFR is different in diabetic and nondiabetic environments. These findings are similar to the ones observed in our data. The locations for peak LOD scores in these two studies are separated by ~23 cM, but low linkage resolution does not exclude the possibility that the results of both studies indicate the same QTLs.

The second linkage study was carried out in 166 African-American families with type 2 diabetes with ESRD (34). In this study, support for linkage with ESRD was found at three chromosomal regions. In an ordered subset analysis, the strongest support for linkage (LOD = 4.6) was on chromosome 3q in the 29% of families with the youngest age at onset of ESRD. This region is located in between the region for suggestive linkage to CG-GFR in diabetic relatives and the region of suggestive linkage to CC-GFR for nondiabetic relatives in our study. In the same analysis, there was also significant evidence for linkage (LOD = 3.6) with ESRD on chromosome 7p in the 37% of families with the longest duration of diabetes and on chromosome 18q (LOD = 3.7) in the 64% of families with the youngest age at onset of diabetes. The support for linkage with ESRD on chromosome 7p in these African-American families colocalizes almost exactly with support for linkage to GFR in our families (Fig. 2). The evidence for linkage on 18q, however, does not overlap with our findings on chromosome 18p.

The third study (31), a targeted investigation of a region on human chromosome 10 that is syntenic with the region

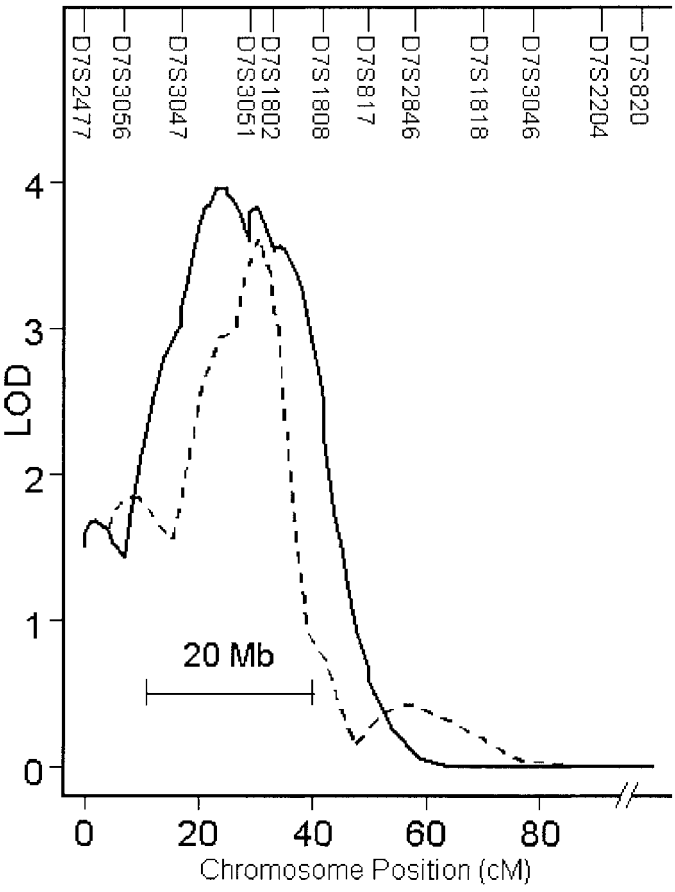

FIG. 2. Detailed LOD score plot for linkage to variation in CC-GFR on chromosome 7p in all relatives in this study (solid line) and LOD-1 unit support intervals. Broken lines represent the LOD score plot for linkage to ESRD reported by Bowden et al. (34) obtained in African-American sibpairs with type 2 diabetes. Chromosomal positions correspond to the Marshfield map.

of the rat renal failure-1 gene (*Rf1*) was conducted in 356 African-American sibpairs affected with ESRD. Evidence supporting linkage to a region centromeric of the *Rf1* locus (LOD = 3.4) was strong and overlaps very closely with our findings.

The identities of the putative QTLs that control variation in GFR in diabetic relatives are unknown. We compared the regions on 2q, 7p, and 10q with the National Center for Biotechnology Information genome annotation (GenBank build 35, version 1; available at http://www.ncbi.nlm.nih.gov/). In the 18-Mb critical region on 2q, there are 129 known and highly probable genes, none representing an obvious candidate. The 20-Mb critical region on 7p contains 116 known and hypothetical genes, and two of them, interleukin-6 (*hIL-6*) and neuropeptide Y (*hNPY*), may be selected as candidate genes for the development of proteinuria on the basis of available literature (37,38). However, the effect of genetic variation within these genes on progression of diabetic nephropathy is unknown. The 16-Mb critical region on 10q contains 154 known and hypothetical genes, but none of them seems to be a good candidate gene predisposing to impaired renal function.

The specific kidney abnormalities that determine variation in renal function and are controlled by QTLs on 2q and 7p and possibly 10q and 18p are unknown. From our genetic analysis, variation in GFR has a strongly negative genetic correlation with variation in systemic blood pres-

sure, suggesting common genetic (but unknown) determinants for the two phenotypes (data not shown). However, these correlations are not determined by QTLs that control variation in the GFR, as none of these QTLs are linked to variation in systemic blood pressure in our families (data not shown).

Limitations of our study should be acknowledged. The first limitation is the lack of a direct measurement of renal function, such as iothalamate clearance. However, such measurements were not feasible in family study due to the high cost and associated inconvenience to study subjects. Therefore, the only option was to use GFR estimates based on serum concentrations of creatinine or cystatin C. Although all three methods used in our study have been recognized as moderately accurate for an individual assessment of renal function (11–13), the CC-GFR seems to be the best for linkage study. While CG-GFR and MDRD-GFR are reliable estimates of GFR when renal function is impaired, their reliability is diminished if renal function is normal. The latter impediment most likely biased the results toward the null hypothesis, reducing the evidence for linkage when CG-GFR and MDR-GFR were used.

The second limitation is that significant proportions of family members in the study were on antihypertensive drugs. Since this treatment most likely slowed declining renal function, one would expect a dampening of the variation in estimated GFR and, therefore, a biasing of the linkage results toward the null hypothesis. As a partial remedy, we included antihypertensive treatment as covariate in the linkage analyses. This resulted in an increase of maximum LOD scores at several locations. However, in these analyses we did not include treatment with ACE inhibitors as an independent covariable for two reasons. One was that use of these drugs was very infrequent, the majority of participants having been recruited into the study by mid-1990s. The other was that inclusion of ACE inhibition as an independent covariate did not change the results of the study.

The third limitation of our study is a lack of statistical power to address the issue of genotype-by-age interaction effects on GFR in diabetic relatives. In our study, we found that adjustment for age, in addition to other covariates in diabetic relatives, resulted in a significant reduction in heritability of CC-GFR and evidence for linkage, specifically on chromosome 2q (from LOD = 4.1 to LOD = 3.1). Such results would be expected when genetic factors modify the effect of age on a quantitative trait (e.g., CC-GFR).

Finally, although we excluded the maturity-onset diabetes of the young families, there may be some concern about whether the results in our extended families with typical adult-onset type 2 diabetes and an autosomal dominant pattern of inheritance of diabetes can be generalized to other diabetic families. In a recent study, we determined that 47% of the patients at the Joslin Clinic with type 2 diabetes diagnosed between the ages of 40 and 60 years reported having a parent with diabetes (39). This result suggests that an autosomal dominant pattern of inheritance is a frequent feature of type 2 diabetes and that the families used in the present study are representative of the large segment of general diabetic population.

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