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A genome-wide linkage scan for genes controlling variation in urinary albumin excretion in type II diabetes

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The main hallmark of diabetic nephropathy is elevation in urinary albumin excretion. We performed a genome-wide linkage scan in 63 extended families with multiple members with type II diabetes. Urinary albumin excretion, measured as the albumin-to-creatinine ratio (ACR), was determined in 426 diabetic and 431 nondiabetic relatives who were genotyped for 383 markers. The data were analyzed using variance components linkage analysis. Heritability (h^2) of ACR was significant in diabetic ($h^2 = 0.23$, $P = 0.0007$), and nondiabetic ($h^2 = 0.39$, $P = 0.0001$) relatives. There was no significant difference in genetic variance of ACR between diabetic and nondiabetic relatives ($P = 0.16$), and the genetic correlation ($r_G = 0.64$) for ACR between these two groups was not different from 1 ($P = 0.12$). These results suggested that similar genes contribute to variation in ACR in diabetic and nondiabetic relatives. This hypothesis was supported further by the linkage results. Support for linkage to ACR was suggestive in diabetic relatives and became significant in all relatives for chromosome 22q (logarithm of odds, LOD = 3.7) and chromosome 7q (LOD = 3.1). When analyses were restricted to 59 Caucasian families, support for linkage in all relatives increased and became significant for 5q (LOD = 3.4). In conclusion, genes on chromosomes 22q, 5q and 7q may contribute to variation in urinary albumin excretion in diabetic and nondiabetic individuals.

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Elevated urinary albumin excretion in the range of microalbuminuria or proteinuria is the hallmark of diabetic nephropathy.[1,2] Research during the last decade established that the quantity of albumin excreted in the urine clusters within families, regardless of whether the families were ascertained through individuals with diabetes or without.[3–6] Familial aggregation of a characteristic so closely related to nephropathy justifies a whole genome scan in search of the chromosomal regions harboring the genes (quantitative trait loci, QTLs) controlling variation in urinary albumin excretion.

Previously, we demonstrated that, if the urinary albumin excretion in family members with diabetes is in the range of microalbuminuria and proteinuria, the urinary albumin excretion in family members without diabetes is also elevated, although an order of magnitude lower (Figure 2 in Fogarty *et al.*[7]). This agrees with earlier findings in nuclear families that the nondiabetic siblings and offspring of probands with diabetes and proteinuria excrete more urinary albumin than a general population without diabetes.[8,9] Further, diabetic siblings of diabetic probands with proteinuria have a greater risk of proteinuria than diabetic siblings of probands with diabetes and normoalbuminuria in type 1[10–13] and type II diabetes.[14,15]

These results suggest a hypothesis that genetic predisposition to nephropathy is reflected in the level of urinary albumin excretion. In the absence of diabetes, the level is in the upper range of normoalbuminuria or lower range of microalbuminuria. With exposure to diabetes, high-normal levels of urinary albumin excretion worsen to microalbuminuria and overt proteinuria.[16] An implication of this hypothesis is that variation in the quantity of albumin excreted in the urine is controlled by the same QTLs in diabetic and nondiabetic individuals.

The current study aimed to identify chromosomal regions containing QTLs that control variation in urinary albumin excretion in 63 extended families. Ascertainment of families was based solely on the availability of many members with type II diabetes and many without.[4,17] The search for these QTLs employed a genetic linkage strategy that combined the use of highly polymorphic markers with pedigree-based multipoint variance component analysis.

RESULTS

The study group consists of 63 extended families with an average of 6.8 diabetic members (range 2–14) and 6.8 nondiabetic members (range 1–18) per family. The clinical characteristics of family members at the time of examination are provided in Table 1 according to diabetes status (426 with and 431 without diabetes). For those with diabetes, age was 57 ± 15 years and diabetes duration 11 ± 11 years (age at diagnosis of diabetes, 46 ± 16 years), whereas the age of those without diabetes was 46 ± 17 years. Treatment for diabetes was insulin for 39%, oral agents for 34% and diet or no treatment for 27%. Those with diabetes were more obese than those without diabetes, and they had higher mean arterial blood pressure (MAP) and were more frequently treated with antihypertensive medications than those without diabetes. End-stage renal disease (ESRD) had developed in 11 diabetic family members.

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

Characteristics	DM	
	Present[a] (*N*=426)	Absent (*N*=431)
Men (%)	43	45
Age at examination (years) (mean ± s.d.)	57 ± 15	46 ± 17
Age at diagnosis of diabetes (years) (mean ± s.d.)	46 ± 16	–
Diabetes duration (years) (mean ± s.d.)	11 ± 11	–
Hypoglycemic Rx at examination (%)		
Insulin	39	–
Oral agents only	34	–
Diet only or no Rx	27	–
% ideal body weight (mean ± s.d.)	140 ± 33	127 ± 26
Mean arterial BP (mmHg) (mean ± s.d.)	98 ± 11	92 ± 13
Antihypertensive Rx (%)	45	14
Treatment with ACE inhibitors (%)	13	3
End-stage renal disease (%)	2.6	0.0
Urinary ACR[b] percentiles (μg/mg)		
25th	6	4
50th	14	6
75th	55	9
90th	791	19
LogACR[c] (mean ± s.d.)	13.9 ± 8.4	8.1 ± 3.8

[a]Includes 44 individuals with impaired glucose tolerance and seven with gestational diabetes.
[b]ACR=albumin (μg) to creatinine (mg) ratio.
[c]LogACR=ACR transformed to the $\log_{10}$ and multiplied by 10. The distribution of logACR in individuals with diabetes and without in the Joslin Family Collection was published previously by Fogarty *et al.*[7]

Albumin-to-creatinine ratio (ACR) levels were higher in family members with diabetes than in those without. While high values occurred in both groups, an ACR greater than 20 μg/mg was significantly more frequent in those with diabetes than those without (41 vs 10%, $P<0.001$). Because of the skewness of the ACR distribution, ACR was transformed for analysis to $\log_{10}$ and multiplied by 10 (logACR in Table 1).

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 5656 relative pairs: 1332 relative pairs concordant for diabetes (diabetes mellitus (DM)–DM pairs); 1849 relative pairs concordant for the absence of diabetes (non-diabetes mellitus (NDM)–NDM pairs) and 2475 relative pairs discordant for diabetes (i.e., one of the pair had diabetes and the other did not, DM–NDM pairs).

Estimates of the heritability of residual (adjusted) logACR were as follows: in relatives with diabetes, $h^2 = 0.23 \pm 0.08$ ($P = 0.0007$ with 16% of the total phenotypic variance explained by covariates); in relatives without diabetes, $h^2 = 0.39 \pm 0.11$ ($P = 0.0001$ with 5% of the total phenotypic variance explained by covariates) and in all family members together, $h^2 = 0.20 \pm 0.05$ ($P = 3 \times 10^{-7}$ with 26% of the total phenotypic variance explained by covariates).

As the main focus of this study was to identify QTLs for logACR in individuals with diabetes, multipoint variance component linkage analyses were conducted first in the 1332 DM–DM relative pairs, and then in all 5656 pairs of relatives (DM–DM, NDM–NDM and DM–NDM pairs). Comparison of the support for linkage coming from these two analyses at a chromosomal region of interest is a measure of the specificity of the effect of that region on variation in adjusted logACR. Three possibilities were considered: (1) the effect may be specific to relatives with diabetes if linkage is supported in DM–DM pairs and decreases or remains constant in all pairs; (2) the effect may be independent of diabetes status if linkage support is present in DM–DM pairs and increases significantly in all pairs and (3) the effect may be specific to nondiabetic relatives if there is no linkage support in DM–DM pairs but it is present in all pairs.

The number of chromosomal regions with potential evidence for linkage (logarithm of odds, LOD $\geqslant$ 1.2) was six

Table 2 | Number 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)	148	145	302	595
Sibling (0.500)	407	243	302	952
Half-sibling (0.250)	14	13	25	52
Grandparental (0.250)	18	19	31	68
Avuncular (0.250)	328	292	749	1369
Cousin, etc. (0.125)	244	607	561	1412
Other (0.063)	173	530	505	1208
All pairs	1332	1849	2475	5656

( ) Relationship coefficient; DM=individual with diabetes; NDM=individual without diabetes.

in DM–DM pairs and ten in all pairs (Figure 1). In the latter analysis, with the greatly increased number of relative pairs, the linkage support at the six regions identified in DM–DM pairs changed as follows: the support for linkage (nominal LOD) increased in three regions, becoming strong on 22q (1.9 became 3.7) and 7q (2.2 became 3.1) and suggestive on 5q (1.2 became 2.6). The LOD increased slightly on 17p (1.5 became 1.6), decreased slightly on 21p (2.5 became 2.1) and decreased below the potential linkage range for the second region on 5q (1.5 became 0.5). The nominal LOD in five new regions with potential evidence for linkage included 16p (0.8 became 2.0) and four others: chromosomes 2p (0.8 became 1.4), 6p (0.5 became 1.6), 6q (0.6 became 1.6) and 17q (0.6 became 1.6). No chromosomal regions reached potential evidence for linkage with logACR in analyses of NDM–NDM pairs (data not shown).

The four regions that provide strongest support for linkage with logACR are summarized in Table 3. The evidence supporting linkage on chromosome 5q was derived in large proportion from DM–DM pairs and DM–NDM pairs with a small contribution derived from NDM–NDM pairs. The evidence supporting linkage on chromosome 7q was derived from DM–DM pairs and DM–NDM pairs with no evidence for linkage coming from NDM–NDM pairs. The evidence supporting linkage on chromosome 21q is derived almost exclusively from DM–DM pairs. The evidence supporting linkage on chromosome 22q appears to be derived primarily from DM–DM pairs and DM–NDM pairs; a small contribution is also derived from NDM–NDM pairs.

There were 59 Caucasian families and four minority families (one African-American and three Hispanic). To examine the impact of these four minority families on the

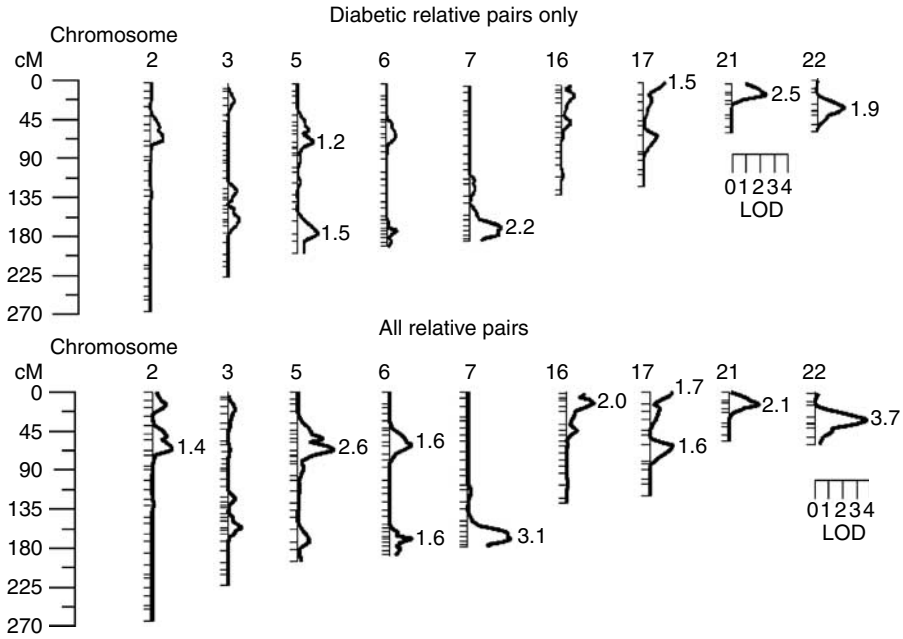

Figure 1 | Genome scan for linkage with logACR (adjusted for relevant covariates) obtained with SOLAR multipoint linkage analysis in diabetic relative pairs only (upper panel) and in all relative pairs (lower panel). Only chromosomes with nominal LOD $\geqslant$ 1.2 are shown. Chromosome 3 is included because the LOD at position 161 cM was 3.2 in our previous study in type I diabetes.[18] Chromosomal positions correspond to the Marshfield map. After adjustment of logACR for covariates (age, gender, diabetes, diabetes duration, %IBW and MAP), the mean $\pm$ s.d. of the residuals according to study group were 0.0 $\pm$ 7.7 and 0.0 $\pm$ 6.1 in diabetic relatives and all relatives, respectively. Estimates of skewness and kurtosis were 0.8 and -0.08 in diabetic relatives and 0.9 and 1.3, in all relatives, respectively.

Table 3 | Maximum LOD scores for linkage with urinary logACR[a] according to chromosomal location, type of relative pair, and ethnicity of families

			All families			Caucasian families only[b]		
Chromosome	Location (cM)	Marker	DM pairs	NDM pairs	All pairs	DM pairs	NDM pairs	All pairs
5	69	GATA67D03	1.2	0.5	2.6	1.7	0.6	3.4
7	172	GATA30D09B	2.2	0.0	3.1	1.6	0.0	2.3
21	15	GGAA3C07	2.5	0.0	2.1	2.6	0.0	2.3
22	33	GATA11B12	1.9	0.6	3.7	1.8	0.5	3.4

[a]logACR was adjusted: for age, gender, diabetes, diabetes duration, MAP and %IBW in the analysis of all relative pairs; for age, gender, diabetes duration, MAP and %IBW in the analysis of DM–DM pairs; and for age, gender, MAP and %IBW in NDM–NDM pairs.
[b]Exclusion of the four minority families reduced the families to 59, the DM–DM pairs to n=1257, the NDM–NDM pairs to n=1825, and all pairs to n=5458.

linkage results, we repeated the analysis after excluding the minority families. The nominal LOD changed significantly for two of the four regions with the strongest linkage signals in the analysis of all families (Table 3). The nominal LOD for linkage with logACR on chromosome 5q increased significantly in DM–DM pairs (1.2 became 1.7) and in all pairs (2.6 became 3.4). These increased LOD scores occurred despite the reduced number of DM–DM pairs (from 1332 to 1257) and all pairs (from 5656 to 5558) after the exclusion of minority families. The evidence for linkage with logACR on chromosome 7q declined significantly in DM–DM pairs (2.2 became 1.6) and in all pairs (3.1 became 2.3). The evidence for linkage with logACR on 21p increased slightly, and evidence on 22q decreased slightly. The contribution of NDM–NDM pairs to the evidence for linkage on chromosome 5q and 22q remained minimal.

In order to examine the effect of ESRD on the linkage results, the analyses were repeated with the ACR values 'Winsorized', so that the phenotypic value of ACR in those with ESRD was reduced from 2500 to 800. An ACR value of 800 represents the 90th percentile of the distribution in diabetic relatives. The effect of this change in ACR among those with ESRD was minimal. The support for linkage declined by 0.1, remained the same, or increased by 0.1 in diabetic relatives. In all relatives, the support for linkage declined by 0.4 on 5q and 0.3 on 22q in all families; in Caucasian families, the decline was 0.3 on 5q and 0.2 on 22q. Support for linkage on 7q and 21p was unchanged.

We have extended the variance components method to model genotype-by-diabetes ($G \times DM$) interaction for logACR values. First, the analyses of the total sample provided no evidence for $G \times DM$ on logACR. The difference in genetic variances of logACR in diabetic ($\sigma_{DM} \pm s.e. = 3.49 \pm 0.65$) and nondiabetic ($\sigma_{NDM} \pm s.e. = 2.40 \pm 0.36$) relatives was not statistically significant ($P = 0.16$), and the genetic correlation (r_G) between relative pairs discordant for diabetes ($r_{G(DM, NDM)} \pm s.e. = 0.64 \pm 0.29$; $P = 0.03$) was not statistically different from 1.00 ($P = 0.12$). Second, there was no improvement in the strength of linkage support with the chromosomal regions reported in Table 3 when the data were subjected to a $QTL \times DM$ interaction model (data not shown). Thus, the $G \times DM$ interaction analyses suggest that the genetic determinants of variation in logACR are similar in diabetic and nondiabetic relatives.

The linkage plots of linkage analysis of logACR for chromosomes 22q, 5q, 7q and 21p together with nominal and empirical *P*-values are shown in Figure 2. The locations of the linkage peaks are very similar in DM–DM pairs as well as in all pairs. The support for linkage in genetic regions on 22q, 5q and 7q was much stronger in all pairs than in the subset that included only DM–DM pairs. Nominal *P*-values for evidence for linkage in all relatives on 22q, 5q and 7q were lower than empirical, indicating that the nominal *P*-values may have slightly overstated the evidence for linkage to these regions. In contrast, the support for linkage of logACR to chromosome 21p in DM–DM pairs was stronger than in the group of all pairs, despite the larger number of relative pairs in the latter analysis.

The LOD-1 support intervals for the four chromosomal regions are shown in Figure 2. The LOD-1 support interval on chromosome 22q spans a genetic region of 11 cM, which corresponds to 9.1 Mb on the physical map. The LOD-1 support interval in Caucasian families on chromosome 5q spans a genetic region of 10 cM, which corresponds to 11.6 Mb on the physical map. The LOD-1 support interval on chromosome 7q spans a genetic region of 16 cM, which corresponds to 4.7 Mb on the physical map. The LOD-1 support interval on chromosome 21p spans a genetic region of 14 cM, which corresponds to 6.1 Mb on the physical map.

DISCUSSION

Substantial literature has accumulated on the familial aggregation of elevated urinary albumin excretion as the main phenotype of diabetic nephropathy. However, the genetic architecture underlying familial aggregation of this phenotype remains unknown. The current study is the first to search for chromosomal regions harboring genes controlling variation in urinary albumin excretion in large, predominantly Caucasian families with type II diabetes. Using a genome scan approach, we found evidence for linkage with variation of urinary albumin excretion on chromosome 22q, 5q and 7q. The patterns of linkage results obtained in DM–DM and in all relative pairs indicate that these chromosomal regions harbor QTLs that control variation in urinary albumin excretion in diabetic as well as in nondiabetic relatives. An additional QTL on 21p may exert its effect on urinary albumin excretion, but only in the presence of diabetes.

To interpret the results of this study, some new aspects of our investigation should be considered. First, previous attempts to map susceptibility genes for diabetic nephropathy in families defined nephropathy as overt proteinuria, a discrete phenotype. In contrast, our effort was to identify QTLs linked with variation in a cardinal quantitative phenotype of diabetic nephropathy, urinary albumin excretion. Second, by selecting large families that included many members with and without type II diabetes, we were able to test whether the genetic determinants of variation in urinary albumin excretion are the same in diabetic as in nondiabetic relatives. Third, the use of extended families and an analytic approach that could use all family members gave us a robustly powered sample of 5656 relative pairs.

The present study confirms the significant role of genetic factors in controlling variation in urinary albumin excretion. Our estimates of the heritability of urinary albumin excretion, ranging from 0.20 to 0.39 in diabetic and nondiabetic groups of relatives, are similar in magnitude to heritability estimates reported previously.[3–6]

In this study, we found a high genetic correlation between the urinary albumin excretion of diabetic and nondiabetic relatives in pairs discordant for diabetes. Also, there was no statistically significant difference in the genetic variances of

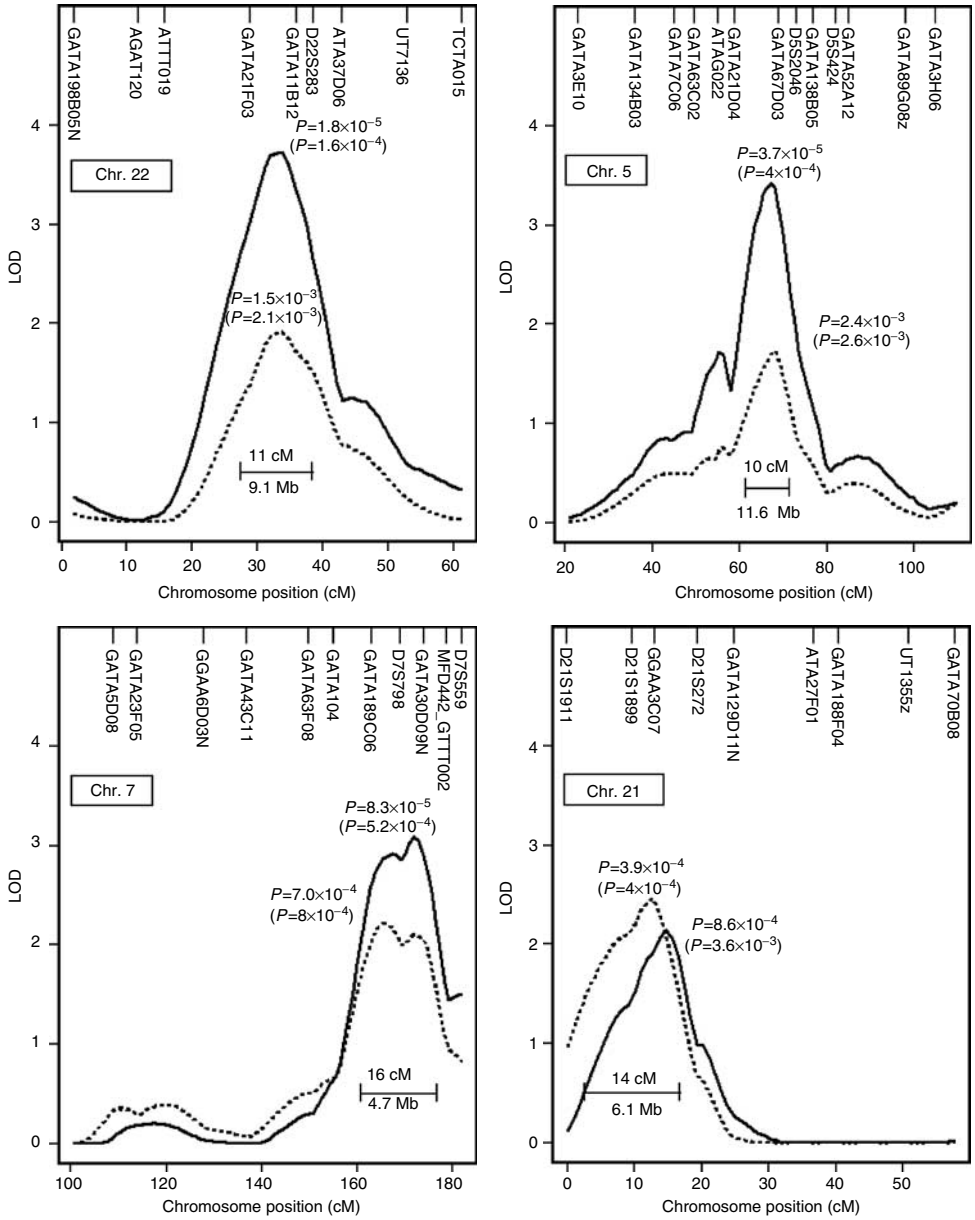

Figure 2 | Detailed genome scan plots of LOD for linkage with logACR on chromosome 22, 5, 7 and 21 for DM–DM pairs only (broken line) and all relative pairs (DM–DM, NDM–NDM and DM–NDM pairs) (solid line). Nominal and empirical (in parentheses) *P*-values and LOD-1 unit support intervals are shown. Chromosomal positions correspond to the Marshfield map. Data for chromosome 5q are for the 59 Caucasian families only.

urinary albumin excretion in diabetic and nondiabetic relatives. These findings suggest that QTLs contributing to variation in urinary albumin excretion in diabetic and nondiabetics relatives are similar, as proposed in our previous publications.[4,7] This hypothesis is supported further by our finding of strong evidence for linkage to variation of urinary albumin excretion on three chromosomes in the genome scan. The first genetic region is on chromosome 22q with a peak at 36 cM. The second genetic region is on chromosome 7q with a peak at 169 cM. The third genetic region is on chromosomal 5q with a peak at 69 cM. The evidence for this genetic region became stronger when the four minority families were excluded, suggesting that this region may control variation in urinary albumin excretion primarily in Caucasian families.

Although the support for linkage to these chromosomal regions from the NDM–NDM relative pairs was minor and that from the DM–DM and DM–NDM relative pairs was major, we concluded that these three chromosomal regions contain QTLs controlling variation in urinary albumin excretion in diabetic as well as in nondiabetic individuals. There are three reasons for our conclusion. First, the weak

evidence for linkage in NDM–NDM relative pairs results from the large proportion of pairs consisting of distant relatives, a feature that effectively diminishes the power to identify linkage in this subgroup alone. Second, the evidence for linkage coming from the DM–NDM relative pairs appeared to be true and it was only minimally influenced by the high values of urinary albumin excretion assigned to individuals with ESRD. Third, when G × DM interaction was incorporated in the analyses, there was no evidence of different gene(s) that influence variation in urinary albumin excretion values in diabetic and nondiabetic relatives.

The support for linkage of urinary albumin excretion to chromosome 21p with a peak at 13 cM is in contrast with the findings on chromosomes 5q, 7q and 22q. The evidence for linkage came from the diabetic relative pairs and not at all from the other subgroups. These results are consistent with the possibility that this QTL controls variation in urinary albumin excretion but only in the presence of exposure to diabetes. However, owing to the lack of support for this hypothesis from the test for QTL × DM interaction and only suggestive evidence for linkage, this hypothesis needs to be tested further in different family collections.

The identities of the putative QTLs located in the chromosomal regions linked to variation in urinary albumin excretion in diabetic and nondiabetic relatives are unknown. We compared the four regions to NCBI's genome annotation (GenBank build 35, version 1), found at http://www.ncbi.nlm.nih.gov/. In the 9.1 Mb critical region on 22q, there are 115 known or highly probable genes, none representing an obvious candidate. In the 4.7 Mb critical region on 7q, there are 50 known or highly probable genes and one, *eNOS*, is a strong candidate. Association of polymorphisms in this gene with the risk of ESRD in diabetic and nondiabetic individuals has been reported by our group[19] and by others.[20–22] However, there are studies that did not find any association.[23–25] Finally, in the 11.6 Mb critical region on 5q and the 6.1 Mb critical region on 21p, there are 71 and 16 known or highly probable genes, respectively, but no obvious candidates.

Only a few genome screens have been conducted in search of chromosomal regions harboring susceptibility genes for diabetic kidney disease. A genome scan was performed in Pima Indian families using 93 diabetic sib-pairs concordant for overt proteinuria.[26] Three chromosomal regions were identified with at least potential evidence for linkage with diabetic nephropathy. Only one region provided evidence that was sufficiently strong to be considered suggestive, a region in proximity to our region on chromosome 7q (LOD = 2.0 at 144 cM). The other regions in the Pima study occurred on chromosome 3q (LOD = 1.5 at 181 cM) and on chromosome 20p (LOD = 1.8 at 18 cM). A partial genome scan was performed in 18 extended Turkish families with type II diabetes.[27] The investigators obtained strong evidence for linkage with 'proteinuria only' or 'proteinuria with microalbuminuria' on chromosome 18q. Assuming a dominant mode of inheritance in the Turkish families provided a LOD = 6.6 at 110 cM, while assuming a recessive mode of inheritance yielded a LOD = 2.2 at the same location.

A genome scan in search of regions linked with variation in urinary albumin excretion was also conducted in 805 sib-pair families collected by the Hypertension Genetic Epidemiology Network.[5] In the 1727 nondiabetic relative pairs in that study, suggestive evidence for linkage was found on 19p (LOD = 2.7 at 9 cM) and on 12q (LOD = 2.0 at 112 cM). Weak evidence for linkage was found on 7q (LOD = 0.9) and 22q (LOD = 1.2) at locations corresponding to the two chromosomal regions identified in our study.[5] In the Framingham Heart Study, which had 1497 nondiabetic relative pairs, evidence for linkage with urinary albumin excretion was found on chromosome 8q (LOD = 2.2 at 135 cM).[6] In our previous report, based on 66 sib-pairs with type I diabetes and discordance for diabetic nephropathy, three candidate chromosomal regions were examined for linkage. In that study, strong evidence for linkage was observed on chromosome 3q (LOD = 3.2 at 161 cM).[18] In the present study, the evidence for linkage at the same location was weak (LOD = 0.8 in diabetic pairs and LOD = 1.0 in all pairs).

The results of our study support a hypothesis that genetic predisposition to diabetic kidney disease is reflected in the level of urinary albumin excretion, which is controlled by (at least) four QTLs. In the absence of diabetes, three of them determine where urinary albumin excretion falls within the low and the high-normal range. With exposure to diabetes, individuals with low levels of urinary albumin excretion remain free of diabetic kidney disease whereas those with the high-normal levels of urinary albumin excretion are at risk of worsening to microalbuminuria and overt proteinuria.[4,7] The risk of the latter is influenced also by another locus specific to diabetes exposure. The nature of abnormalities in the kidney that determine the level of urinary albumin excretion and are impacted by the QTLs is unknown. Two processes may be influenced, the passage of serum proteins through the glomerular filtration barrier, and reabsorption and degradation of these proteins by renal tubular cells.[28]

The main limitation of our study is the lack of precision inherent in an assessment of urinary albumin excretion based on a single ACR measurement. This would bias the results toward the null hypothesis, reducing heritability estimates for urinary ACR and reducing the evidence for linkage with specific chromosomal regions. Moreover, a proportion of family members were treated with angiotensin converting enzyme (ACE) inhibitors. The reduction in urinary albumin excretion by this treatment is expected to dampen variation in urinary albumin excretion and bias the evidence for linkage toward the null hypothesis. We examined the impact of ACE inhibitor treatment, first by considering it as a covariate in analyses of heritability, and second by sensitivity analyses evaluating the effect of different reductions in albumin excretion in those treated with ACE inhibitions (data not shown). In both circumstances, the impact of treatment with ACE inhibitors on LOD scores was insignificant.

MATERIALS AND METHODS

Collection of families with type II diabetes

The families used for this study had been recruited previously for the Joslin Study on the Genetics of Type 2 Diabetes.[4,17] Briefly, between 1993 and 2003, families were ascertained through probands with type II diabetes and examined because the pattern of occurrence of type II diabetes in family members was consistent with an autosomal dominant mode of inheritance. An additional selection criterion was the willingness of a large number of family members (with and without diabetes) to participate in the study. Using these criteria, 104 families were ascertained. The mean age at diagnosis of type II diabetes for a family was 'early' in 46 families (<35 years) and 'middle-age' in 68 (35–59 years). Of the families selected for this study, 63 out of the 68 families with diagnosis in 'middle-age' represent common, type II diabetes. This set of families included 59 European Caucasian families and four minority families.

Examination of study participants

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 living in New England were examined by trained recruiters according to previously described protocols.[4,17] Subjects with impaired glucose tolerance (IGT, $n=44$) or gestational diabetes (GDM, $n=7$) were considered to be 'diabetic' with 0 years duration of diabetes.

Laboratory methods

Methods for measuring albumin and creatinine in a random urine sample for determination of the ACR as an index of urinary albumin excretion have been described previously.[29,30] The value of ACR, expressed in micrograms of albumin and milligrams of creatinine, approximates the value of the corresponding albumin excretion rate measured in a timed urine collection and expressed in μg/min.[30] Individuals with ESRD were assigned ACR values of 2500 μg/mg. In order to approximate a normal distribution for analyses, ACR values were transformed to the logarithm_{10} and multiplied by 10 (logACR).

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 NHLBI Mammalian Genotyping Service (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. Genotypes from 857 individuals were used in the reported analyses.

Statistical genetic analyses

Heritability of logACR was estimated by pedigree-based variance components methods as implemented in the program SOLAR (version 2.1. 4). Initially, logACR was adjusted for gender, age, diabetes, diabetes duration, percent ideal body weight (%IBW), MAP, and ACE inhibitor treatment. ACE inhibition did not contribute significantly to the model and was omitted from the adjustment of logACR for linkage analyses.

Pedigree-based variance component linkage analysis implemented in the program SOLAR was used to detect and localize QTLs controlling variation in logACR.[31,32] This approach has been shown to be a powerful linkage analysis technique.[32–34] Multipoint IBD probabilities were calculated using the Markov chain Monte Carlo methods implemented in the program Loki.[35]

Hypotheses were tested by likelihood ratio tests.[36,37] Conversion of the $\log_e$ likelihoods to $\log_{10}$ yielded LOD scores. Empirical *P*-values were based on simulations performed within SOLAR. A fully informative marker unlinked to logACR was simulated, IBD scores were estimated and linkage was analyzed. The distribution of LOD scores from 10 000 or 50 000 simulations determined the empirical *P*-value.

The estimation of heritability, and subsequently the evaluation of linkage, incorporated a correction for ascertainment bias by conditioning the likelihood for the family data on the phenotype of the proband.[38] To test for a G $\times$ DM interaction on logACR in the total sample, we extended the variance components approach by examining the covariance between relative pairs under different environments (i.e., DM and NDM relatives).[39–41]

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