

Genome-wide scans for microalbuminuria in Mexican Americans: The San Antonio Family Heart Study

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Purpose: Microalbuminuria, defined as urine albumin-to-creatinine ratio of 0.03 to 0.299 mg/mg, is a major risk factor for cardiovascular disease. Several genetic epidemiological studies have established that microalbuminuria clusters in families, suggesting a genetic predisposition. **Method:** We estimated heritability of microalbuminuria and performed a genome-wide linkage analysis to identify chromosomal regions influencing urine albumin-to-creatinine ratio in 486 Mexican Americans from 26 multiplex families. **Results:** Significant heritability was demonstrated for urine albumin-to-creatinine ratio ($h^2 = 24\%$, $P < 0.003$) after accounting for age, sex, body mass index, triglycerides, and hypertension. Genome scan revealed significant evidence of linkage of urine albumin-to-creatinine ratio to a region on chromosome 20q12 (LOD score of 3.5, $P < 0.001$) near marker *D20S481*. This region also exhibited a LOD score of 2.8 with diabetes status as a covariate and 3.0 with hypertension status as a covariate suggesting that the effect of this locus on urine albumin-to-creatinine ratio is largely independent of diabetes and hypertension. **Conclusion:** Findings indicate that there is a gene or genes located on human chromosome 20q12 that may have functional relevance to albumin excretion in Mexican Americans. Identifying and understanding the role of the genes that determine albumin excretion would lead to the development of novel therapeutic strategies targeted at high-risk individuals in whom intensive preventive measures may be most beneficial. **Genet Med 2007;9(2):80–87.**

Key Words: Microalbuminuria, albumin to creatinine ratio, cardiovascular diseases, Mexican Americans, family genetic study

Microalbuminuria is the excretion of low but abnormal levels (≥ 30 mg/day) of albumin in the urine. Microalbuminuria is defined as albumin-to-creatinine ratio of 0.03 to 0.299 mg/mg on a random sample of urine.¹ Microalbuminuria is an important independent risk factor for the development of cardiovascular disease (CVD).² Several studies have shown that microalbuminuria, in addition to being an independent CVD risk factor, is a significant predictor of CVD, and all-cause mortality in the diabetic and nondiabetic general population.^{3–5} This predictive effect of microalbuminuria is independent of other known CVD risk factors such as being overweight, hypertension, hypercholesterolemia, and smoking.⁶ Results from the Third Copenhagen City Heart Study indicate that even very low levels of microalbuminuria are associated with increased independent risk of CVD.⁵ The European Pro-

spective Investigation into Cancer in Norfolk study examined the relationship between microalbuminuria and the incidence of CVD in individuals aged 40 to 79 years without prevalent baseline CVD. Among the participants with baseline CVD, the independent risk of all-cause mortality associated with microalbuminuria increased by 60%, when compared with normoalbuminuric participants,⁷ confirming that microalbuminuria is of prognostic value in patients with established CVD.⁸ Similarly, results from the San Antonio Heart Study showed that normotensive subjects with microalbuminuria had significantly higher triglyceride concentrations and insulin levels than normotensive Mexican American and non-Hispanic white subjects without microalbuminuria, suggesting that an increased atherogenic risk factor pattern exists even in normotensive subjects with microalbuminuria.⁹ In addition, population-based observational studies demonstrated that over the years microalbuminuria may progress to macroalbuminuria (>0.3 mg albumin/mg creatinine) and clinical proteinuria with increased risk of cardiovascular mortality and progression to end stage renal disease.¹⁰

In the United States, it is estimated that the prevalence of microalbuminuria is roughly 11%.¹¹ Jones et al.¹¹ showed that Mexican American race/ethnicity was independently associated with the risk of microalbuminuria after adjusting for diabetes, hypertension, cardiovascular disease, and chronic kid-

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ney disease. A significantly higher percentage (26%) of Mexican Americans with type 2 diabetes have microalbuminuria compared with only 9% in non-Hispanic whites with diabetes ($P = 0.015$).¹² Mexican American type 2 diabetic subjects with microalbuminuria also have elevated CVD risk factors such as increased blood pressure and severe hyperglycemia compared to subjects without microalbuminuria.¹³

Several genetic epidemiological studies have established that microalbuminuria clusters in families, suggesting that there is a genetic predisposition. Urine albumin-to-creatinine ratio (ACR) is a widely accepted quantitative measure for microalbuminuria. A genetic analysis of ACR in Pima Indians with diabetes suggested the presence of a major gene effect with mendelian inheritance.¹⁴ Similarly, Fogarty et al.¹⁵ showed that the levels of urine ACR in white families with type 2 diabetes were determined by a mixture of genes after controlling for environmental covariates. Familial clustering of microalbuminuria has been seen in siblings of subjects with diabetes,¹⁶ and albuminuria has been shown to be heritable among the offspring of diabetic subjects.¹⁷ For example, results from the Insulin Resistance Atherosclerosis Family Study showed a familial aggregation of UACR in nondiabetic Mexican American and African American populations after adjusting for age, sex, and body mass index (BMI).¹⁸ Heritabilities of urine ACR, systolic blood pressure (SBP), and diastolic blood pressure (DBP) were 11%, 26%, and 28%, respectively. The phenotypic correlations between urine ACR and SBP or DBP in Hispanics ($r = 0.17$ and $r = 0.16$, respectively) and African Americans ($r = 0.26$ and $r = 0.16$, respectively) were significant. A genome-wide scan to identify susceptibility loci for urine ACR in Pima Indians with type 2 diabetes revealed suggestive evidence of linkage on chromosomes 3, 7, and 20.¹⁹ The Hypertension Genetic Epidemiology Network (HyperGen) Study also established evidence of suggestive linkage to urine ACR on chromosomes 12 and 19 among African American and non-Hispanic white family members diagnosed with essential hypertension.²⁰ Recently, Fox et al.²¹ performed a genome-wide linkage analysis to investigate susceptibility loci for urine ACR in the Framingham Heart Study (FHS) consisting predominantly of white family members. They reported suggestive linkage to urine ACR on chromosome 8 with a multipoint logarithm of odds (LOD) score of 2.22 after full adjustment. However, the genetic architecture of urine ACR in a population-based sample of Mexican Americans has not been well studied. This paper presents the results of the first genome scan for urine ACR among low-income Mexican American families enrolled in the San Antonio Family Heart Study (SAFHS).

MATERIALS AND METHODS

The San Antonio Family Heart Study

The SAFHS is the first large, population-based genetic study of risk of heart disease in Mexican Americans. It was established in 1991 to detect, characterize, map, and identify polymorphic genes that influence variation in susceptibility to CVD. Probands for the SAFHS were selected randomly from a

census tract in San Antonio of low-income Mexican Americans regardless of any preexisting medical conditions. Probands who were 40 to 60 years of age, had a spouse who was willing to participate, and had at least six offspring who were 16 years of age or older were recruited. Family members including all first-, second-, and third-degree relatives of probands 16 years of age or older and his or her spouse were invited to participate.²² In this study, we analyzed data collected from 486 individuals participating in the third phase of the SAFHS. These individuals were taken from the 26 largest pedigrees for whom phenotypic and genotypic information was available. A 10-cM map was used for the multipoint linkage analysis. The Institutional Review Board of the University of Texas Health Science Center at San Antonio approved all procedures, and all subjects gave written informed consent.

Phenotypic data sets in the San Antonio Family Heart Study

A rich set of phenotypes have been collected on study participants during the three phases of this study. Data include measurements of phenotypes related to obesity, diabetes, and CVD. Anthropometrics and blood pressure were measured according to standardized methods. Weight was measured to the nearest tenth pound and then to the nearest tenth kilogram using an ISO-9001 certified Scale-Tronix (White Plains, NY) electronic scale with a capacity of 880 lb (400 kg). Standing height was measured twice to the nearest 0.1 cm using a SECA wall-mounted stadiometer. BMI was calculated as weight (in kilograms) divided by height squared (in meters). Blood pressure was measured three times, with an appropriate arm cuff, using a Random-Zero sphygmomanometer (Gelman-Hawksley Ltd., Sussex, England). The first measurement of the SBP and DBP was discarded, and the mean of the second and third readings was used in the analyses.

Blood samples were collected from all participants after an overnight fast, and plasma was prepared and stored at -80°C until analyzed. Other phenotypes that were measured were fasting levels of circulating total plasma cholesterol, high-density lipoprotein cholesterol (HDL-C), triglycerides (TGs), HbA1c, glucose, and glucose 2 hours after a standardized oral glucose load.²³ Type 2 diabetes was diagnosed according to the World Health Organization (WHO) plasma glucose criteria.²⁴ Individuals who reported a history of diabetes and stated that they were receiving insulin or oral antidiabetic agents were also considered to have diabetes. For additional information regarding these variables, refer to MacCluer et al.²² and Mitchell et al.²³

In the third phase of the study, a total of 486 individuals from 26 families were phenotyped for serum levels of creatinine. A single-void morning urine sample was collected from each participant, and urinary albumin and creatinine levels were measured. Albumin in the urine was quantitatively determined by turbidimetric method (Beckman Synchron LX[®] 20, Beckman Coulter, Inc., Fullerton, CA). Creatinine in the urine was measured using the modified kinetic Jaffé method.²⁵ Urinary albumin excretion was indexed to urinary creatinine as the ACR to account for differences in urine concentration.

Urine ACR is a validated, reliable, single-sample measure of urinary albumin excretion that is highly correlated ($r = 0.82$, $P < 0.001$) with albumin excretion rates assessed by 24-hour urine collection.^{1,26,27} Because of the skewness of the urine ACR, it was log transformed and the log-transformed value was used in the analysis.

Genotypic data sets in the San Antonio Family Heart Study

Genomic DNA was prepared from lymphocytes from blood collected from all enrolled subjects and amplified for polymerase chain reaction (PCR) with fluorescently labeled primers from the MapPairs 6 and 8 Linkage Screening Sets (Research Genetics, Inc., Huntsville, AL). PCR for each primer set was performed separately, but aliquots of reaction products were pooled according to the multiplexed panels of MapPairs Sets 6 and 8 for typing with an automated DNA sequencer (Applied Biosystems model 377 with Genescan and Genotyper programs, Foster City, CA). In addition, polymorphic markers in candidate genes were also included in the map as described previously by Comuzzie et al.²⁸ All family members were genotyped for 417 markers. Markers were spaced at an average interval of 10-cM. The screening set and genotyping protocols are available at the website of the Center for Medical Genetics, Marshfield Medical Research Foundation (<http://research-marshfieldclinic.org/genetics>). After completion of the genotyping for the 10-cM marker map, the data were searched for spurious double recombination and those that were reflected by a high posterior probability of genotyping error were corrected. This correction significantly reduced the map expansion and has led to the calculation of more accurate multipoint identity-by-descent (IBD) probabilities.

Genetic statistical analyses

We used a pedigree-based multipoint variance-component approach to test for linkage between marker loci and the urine ACR phenotype, using a maximum-likelihood method.^{29,30} This method is implemented in the software program Sequential Oligogenic Linkage Analysis Routines (SOLAR).³⁰

As a first step in the genetic analyses, heritability (h^2) of ACR was estimated. Heritability of any phenotype is the proportion of the total phenotypic variance (σ^2_p) that is attributable to additive genetic effects (σ^2_G) and is denoted by $h^2 = \sigma^2_G / \sigma^2_p$.³¹ It denotes the extent to which a phenotype is influenced by genes that are transmitted from the parents. Once the heritability is significant, the next step is to investigate the chromosomal locations of the gene/genes that might be affecting the variation in the particular phenotype (in this case, it is urine ACR).

A multipoint variance-component linkage method was used to test for linkage between marker loci and the ACR under study. This method assumes that the genetic covariances between relative pairs in a pedigree are expected to be a function of the IBD relation at a marker.²⁹ If a locus on a chromosome is linked to the quantitative trait (e.g., ACR), then the expected genetic covariances between family members can be expressed as a function of the IBD relationships at that locus. The overall expected IBD relationship between relative pairs is twice the

kinship (Φ). Kinship is defined as the probability that two homologous genes drawn at random, one from each individual will be IBD. IBD at the specific quantitative trait loci (QTL) locus (Π) is estimated using genetic marker data. The covariance matrix takes the following form: $\Omega = \Pi \sigma^2_q + 2\Phi \sigma^2_a + I \sigma^2_e$ where Ω is the covariance matrix of the entire family. Π is a matrix of the proportions of the specific QTL that the relative pairs share as IBD. σ^2_q is the additive genetic effect of the specific QTL, Φ is the kinship matrix, σ^2_a is the residual (non-QTL) genetic effect, I is an identity matrix, and σ^2_e represents the random environmental effect.

The null hypothesis is that the additive genetic variance of the specific QTL (σ^2_q) for the trait equals zero. The likelihood of the null hypothesis (H_0), where σ^2_q is constrained to zero, was compared with the likelihood of the alternative hypothesis (H_a), where the σ^2_q is estimated. Twice the difference between the log likelihood of the two models yields a test statistic. This test statistic is asymptotically distributed as a 1/2:1/2 mixture of a χ^2 distribution with 1 df (degree of freedom).³² The LOD score is used to demonstrate the significance of the test. It is calculated as the following: $\text{LOD} = \log 10(\text{likelihood of } H_a) - \log 10(\text{likelihood of } H_0)$. The calculations of locus-specific IBDs for relative pairs and the multipoint linkage analyses were performed using the SOLAR program.³⁰ LOD scores were interpreted using common thresholds to define genome-wide significance.³³

Urine ACR is influenced by other covariates such as age, sex, BMI, diabetes, hypertension, and serum TGs.^{11,34} Therefore, we conducted several types of analyses using different number of covariates in a given model. Thus, the age, sex, and age² and their interactions were included in the analyses as covariates. The analysis was done first with AGE, SEX, AGE*SEX, AGE², AGE²*SEX terms as covariates (M1 analysis), and then with BMI, TGs, and hypertension status as covariates (M7 analysis) and with BMI, TGs, and diabetic status as covariates (M8 analysis) using an approximately 10-cM map and a threshold model as implemented in the SOLAR program. For meeting the assumption of normal distribution of the variance-component linkage method, the distributions of all phenotypes were examined and the ACR was log transformed. Data ± 4 SDs or more from the means were blanked out. The kurtosis of all phenotypes was reexamined, and additional outliers were removed so that the kurtosis for each of the traits was ≤ 1.9 , thereby avoiding an inflation of type I error.³⁵

RESULTS

The relative pairs used in the quantitative genetic and linkage analyses are shown in Table 1. Data on a total of 486 subjects from 26 pedigrees were available for this analysis (Table 2). The mean age of the study participants was 47.7 ± 14.6 years, with a range of 24 to 66 years and 36% were male. About 24% of the subjects had type 2 diabetes and 15% were hypertensive. The mean SBP was 123.84 ± 18.42 (mm Hg), whereas the mean DBP was 70.2 ± 10.5 mm Hg. The mean BMI was 31.0 ± 7.3 kg/m². The mean serum creatinine was 0.83 ± 0.45

Table 1
Relative pairs in this study

Relative relationship	Size
Parent-offspring	322
Siblings	367
Grandparent-grandchild	82
Avuncular	762
Half-siblings	73
Grand avuncular	161
Half avuncular	96
First cousins	803
Half grand avuncular	5
First cousins, once removed	777
Half first cousins	65
First cousins, twice removed	32
Half first cousins, once removed	7
Second cousins	314
Double first cousins	6
Double first cousins, once removed	13
Double second cousins	6
Half first cousins and second cousins	4
Total	3895

Table 2
Characteristics of ACR study subjects

Characteristic	Mean \pm SD or %
Total no. of subjects	486
Male (%)	36
Hypertension (%)	15
Diabetes (%)	24
ACR \geq 0.03 (%)	20
Age (yr)	47.66 \pm 14.6
Body mass index (kg/m ²)	31.91 \pm 7.12
Systolic blood pressure (mm Hg)	123.84 \pm 18.42
Diastolic blood pressure (mm Hg)	70.2 \pm 10.5
Serum triglycerides (mg/dL)	128.34 \pm 89.41
Serum albumin (mg/dL)	3.9 \pm 0.34
Serum creatinine (mg/dL)	0.83 \pm 0.45
Urinary ACR (mg/mg)	0.07 \pm 0.43

Data listed as mean \pm SD for continuous measures and n (%) for dichotomous measures.

ACR, albumin-to-creatinine ratio.

mg/dL and the mean urine ACR was 0.07 \pm 0.43 mg/mg (log ACR was -0.05 ± 0.9 mg/mg).

Before conducting the linkage analysis for ACR, we determined its heritability (h^2). Age and sex were first included as

Table 3

Heritability (h^2) of log-urinary albumin-to-creatinine ratio in the study participants		
Covariates	$h^2 \pm$ SE	<i>P</i>
None	0.15 \pm 0.08	0.01
Age	0.17 \pm 0.08	0.007
Sex	0.15 \pm 0.08	0.02
Age, sex	0.17 \pm 0.08	0.008
Age, age ² , sex	0.17 \pm 0.08	0.008
Age, sex, BMI, SBP, TGs	0.24 \pm 0.10	0.003
Age, sex, age ² , age ² *sex, age*sex	0.17 \pm 0.09	0.008
Age, sex, age ² , age ² *sex, age*sex, SBP status	0.18 \pm 0.09	0.014
Age, sex, age ² , age ² *sex, age*sex, hypertension status	0.19 \pm 0.09	0.005
Age, sex, age ² , age ² *sex, age*sex, BMI	0.16 \pm 0.08	0.015
Age, sex, age ² , age ² *sex, age*sex, TGs	0.19 \pm 0.09	0.009
Age, sex, age ² , age ² *sex, age*sex, BMI, SBP, TGs	0.23 \pm 0.10	0.003
Age, sex, age ² , age ² *sex, age*sex, BMI, TGs, hypertension status	0.18 \pm 0.09	0.009
Age, sex, age*sex, age ² , age ² *sex, BMI, TGs, diabetic status	0.13 \pm 0.05	0.04

BMI, body mass index; SBP, systolic blood pressure; TGs, triglycerides; BP, blood pressure; SBP status = systolic blood pressure >120 mm Hg versus systolic blood pressure <120 mm Hg; hypertension status = SBP >120 and diastolic blood pressure >80 mm Hg versus SBP <120 and diastolic blood pressure <80 mm Hg.

covariates while calculating heritability. Subsequently, age, sex, and age² were used as covariates in estimating the heritability of ACR. On both accounts, heritability was 0.17 ($P < 0.008$) (Table 3). After accounting for the covariate effects of age, sex, BMI, TGs, and SBP, UACR exhibited higher heritability ($h^2 = 24\%$).

To identify chromosomal regions that influence susceptibility to variation in UACR, we subsequently performed a genome-wide scan for this trait. We found significant evidence of linkage of ACR to a region on chromosome 20q12 (LOD score of 3.5) near marker *D20S481* for M7 analysis (Table 4). As can be seen from Table 4, this region also exhibited a LOD score of 2.8 with diabetes status as a covariate (M8 analysis) and 3.0 with SBP as covariate (M6 analysis), suggesting that the effect of this locus on urine ACR is largely independent of diabetes, hypertension, TGs, and BMI (Fig. 1).

Given the strong evidence of linkage of urine ACR at the marker *D20S481*, the 1-LOD support interval (between *D20S478* and *D20S197* markers) around our linkage peak spans an approximately 9.0-Mb region on 20q12 (Fig. 2). A search of current genome databases indicated that there are several expressed sequence tagged sites (ESTs) and approximately 53 genes with known functions that have been mapped to this region (www.ucsc.org). Of these positional genes, *HNF4A* (hepatocyte nuclear factor 4 α) was found to be localized just 216 kb away from the linkage marker *D20S481* (Fig. 2).

Table 4
Linkage of ACR to genetic regions with LOD scores ≥ 1.175 ($P = 0.001$)

Chromosome	Location (cM)	Nearest marker	Multipoint LOD score								
			M1 analysis	M2 analysis	M3 analysis	M4 analysis	M5 analysis	M6 analysis	M7 analysis	M8 analysis	M9 analysis
1	14	D1S1612	1.46		1.63	1.46	1.29	1.53	1.41		
1	15	D1S1612		1.2							
2	173	D2S1328						1.29			1.33
2	258	D2S434	1.51		1.3	1.67		1.2			
2	210	D2S1776									1.3
8	10	D8S1099–D8S1130							1.3		
10	100	D10S1432							1.2		1.40
10	167	D10S1213									1.6
10	168	D10S1213						1.53	1.42	1.89	
12	41	D12S373								1.28	
12	130	D12S330									1.21
20	77	D20S481–D20S107	2.2		2.77	2.3					
20	79	D20S481									2.8
20	80	D20S481						3.05		3.50	3.1
20	81	D20S481							3.0		
20	82	D20S197		2.2							

M1 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX.

M2 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, SBP status.

M3 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, hypertension status.

M4 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, BMI.

M5 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, TGs.

M6 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, BMI, TGs, SBP.

M7 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, BMI, TGs, hypertension status.

M8 analysis: AGE, SEX, AGE*SEX, AGE², AGE²*SEX, BMI, TGs, diabetes status.

M9 analysis: AGE*SEX, BMI, TG, SBP.

SBP status, systolic blood pressure >120 versus systolic blood pressure <120 ; hypertension status, systolic blood pressure >120 and diastolic blood pressure >80 versus systolic blood pressure <120 and diastolic blood pressure <80 ; BMI, body mass index; TGs, triglycerides.

DISCUSSION

Findings from several population-based studies consistently show that microalbuminuria is an important independent risk factor for CVD in the general population. Microalbuminuria, regardless of diabetes status, clusters in families of different ethnic origins suggesting that chromosomal regions harboring the genes may control variation in ACR. We examined the genetic basis of ACR in a population-based sample of low income Mexican Americans enrolled in the SAFHS. We found moderate heritability (0.24) for urine ACR and significant evidence for linkage on chromosome 20q12 close to the marker *D20S481* after controlling for all known covariates. Our findings concerning the heritability of urinary albumin excretion corresponded with the FHS findings that demonstrated heritability of 0.16 for urine ACR in a community-based sample of predominately whites. However, our linkage results did not replicate the suggestive linkage of urine ACR to chromosome 8 (LOD score = 2.22) reported in the FHS. This difference in the findings may be due to the fact that a significant number (N =

676) of FHS subjects were on the basis hypertension. Our genetic analyses were performed using data enriched with normotensive subjects (Table 2), whereas genetic analyses in the FHS were performed with data collected from subjects enriched for hypertension. Another important difference is the value of urine ACR between subjects enrolled in the FHS and our study. The median urine ACR was 5.8 mg/mg i.e., the proteinuria range, in subjects enrolled in FHS and it was 0.07 mg/mg, i.e., the microalbuminuria range, in subjects enrolled in our study. Nevertheless, the difference in the findings between the two studies may also suggest possible different susceptibility genes for the expression of low albumin excretion vs. proteinuria in a population-based sample. It is possible that microalbuminuria and proteinuria are two major traits that aggregate (and are heritable) in families but are not correlated genetically.³⁶ Future research evaluating the genetic basis of microalbuminuria and proteinuria in a population-based sample is needed.

Additionally, linkage findings from our study did not replicate or corroborate other genome-wide linkage analyses per-

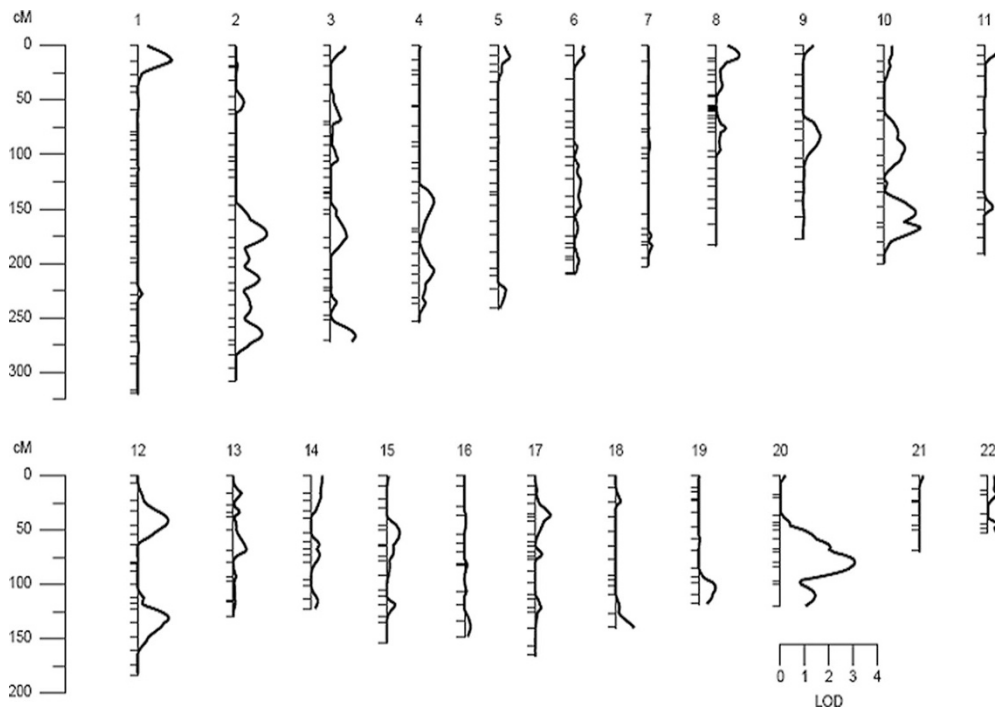


Fig. 1. Summary of the multipoint linkage analysis of urine albumin-to-creatinine ratio (ACR). Plot of the genome scan for urine ACR as a continuous trait. For each autosome (1–22), the genetic distance along the chromosome is plotted on the *x*-axis, starting at the left (corresponding to the p-arm of the chromosome). The strength of the linkage signal is plotted on the *y*-axis of each autosome in two ways.

formed to identify a chromosomal region for urine ACR. Linkage analysis was performed among sibpairs with diabetic nephropathy, and suggestive evidence of linkage was found on chromosome 10p.³⁷ In a large African American family (18 of 23 family members affected) with hypertensive nephropathy, a LOD score of 5.4 was found on chromosome 9q31-32.³⁸ The Family Investigation of Nephropathy and Diabetes (FIND) study collected large numbers of diabetic sibpairs concordant and discordant for diabetic nephropathy. Genome-wide scans for diabetic nephropathy and albuminuria was performed in multiethnic populations.³⁹ Linkage analysis detected evidence of linkage to diabetic nephropathy on chromosomes 7q21.3, 10p15.3, 14q23.1, and 18q22.3 and to albuminuria on 2q14.1, 7q21.1, and 15q26.3 (unpublished data). Several genome-wide linkage analyses were performed to identify chromosomal regions for CVD risk in the SAFHS⁴⁰. These include leptin concentrations (chromosome 2),²⁸ cholesterol concentrations in low-density lipoprotein cholesterol size fractions (chromosomes 3 and 4),⁴¹ BMI (chromosome 8),⁴² and fasting serum insulin concentrations (chromosome 3).⁴³ All these studies used data (i.e., baseline) from 10 large pedigrees (479 individuals) for which both phenotypic and genotypic data were available. Our peak LOD score for urine ACR of 3.5 on chromosome 20q did not overlap with any of the SAFHS linkage findings. However, this is not surprising because the above-mentioned traits represent different phenotypes. Interestingly, our linkage region has previously been reported in the literature for traits that affect CVD. For instance, there is substantial evidence of a susceptibility locus for type 2 diabetes on chro-

mosome 20q13.1-13.2 and surrounding regions.^{44–46} The Finland-U.S. Investigation of Non-Insulin-Dependent Diabetes Mellitus Genetics (FUSION) study revealed that the weighted multipoint maximum LOD score for type 2 diabetes was 2.06 on map locus 20q12-13.1.⁴⁵ Likewise, evidence of linkage of type 2 diabetes was reported on chromosome 20q13.1-13.2 between the markers *D20S119* and *D20S428* in large white families with type 2 diabetes.⁴⁴

Candidate gene(s) for urine ACR

Several genome-wide scans for type 2 diabetes susceptibility loci have identified linkage on chromosome 20q12-13 in a region that encompasses the *HNF4A* locus. *HNF4A* is a transcription factor expressed in many tissues including liver and pancreas.⁴⁷ Mutations in both the coding and regulatory regions of *HNF4A* have been associated with maturity-onset diabetes of young (MODY type 1), a dominantly inherited, early-onset form of type 2 diabetes.⁴⁸

Recently, Shike et al.⁴⁹ mapped a QTL for the development of albuminuria in a diabetic mouse model to a chromosomal region that is syntenic with the region on human chromosome 20q12 that harbors *HNF4A*. A genome-wide analysis of susceptibility loci for albuminuria with microsatellite-based chromosomal maps showed a contributing KK/Ta locus, provisionally designated *UA-1*, with a significant linkage with the interval on chromosome 2 at 83.0 cM close to the microsatellite marker *D2Mit311* with a maximum LOD of 3.5 ($\chi^2 = 13.2$, $P = 0.0003$).⁴⁹ Furthermore, in a cohort of white type 2 diabetic nephropathic patients, a 7 base pair deletion allele in the P1

Linkage of ACR on 20q12 and the selected genes

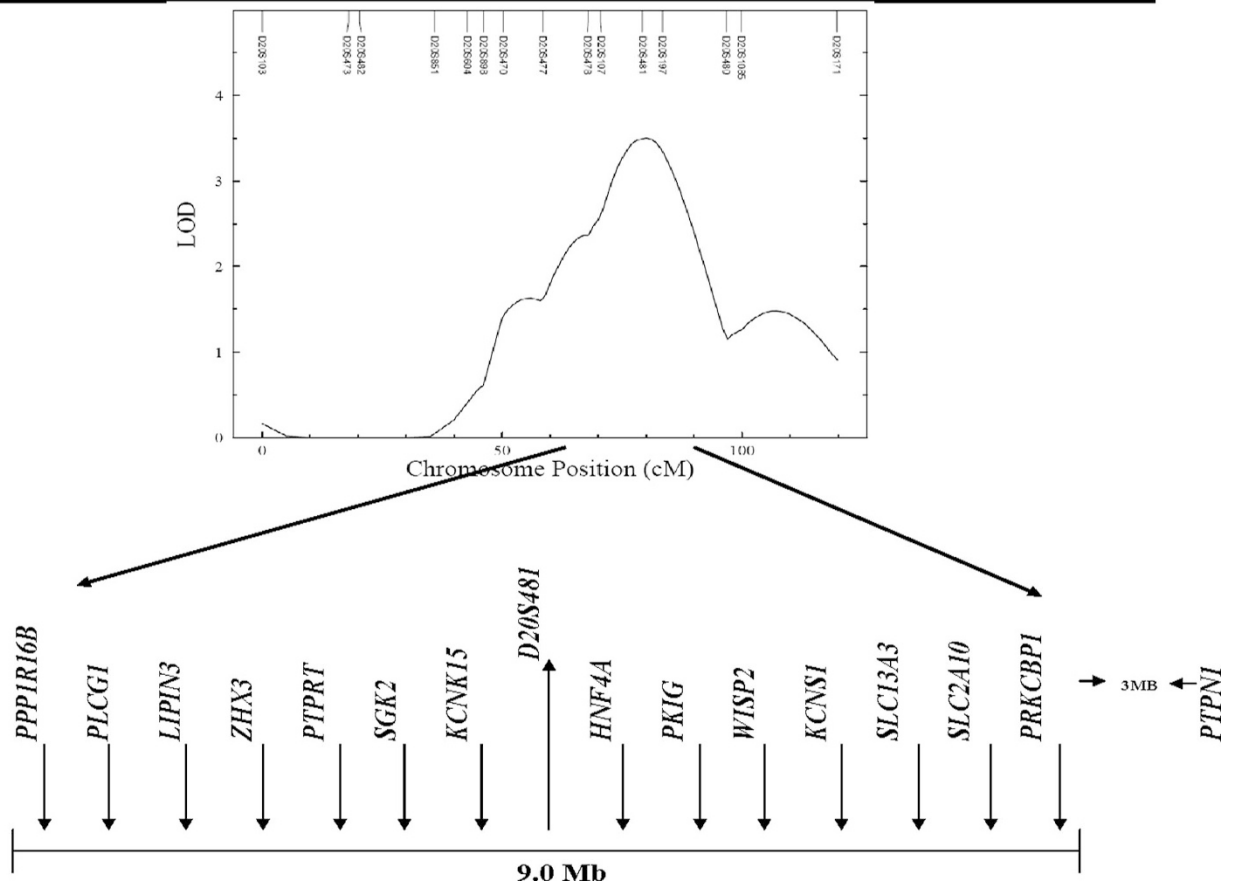


Fig. 2. Linkage analysis of urine ACR on 20q12 and the selected genes located within the linkage. Chromosome 20q12 linkage results for the urine ACR continues the trait displayed by selected genes located within the linkage. The genetic distance along the chromosome is plotted on the x-axis, starting at the left (corresponding to the p-arm of the chromosome). The strength of the linkage signal is plotted on the y-axis in two ways.

promoter of *HNF4A* was identified to cosegregate with diabetes and renal target organ damage in a single family.⁵⁰

Additionally, the protein tyrosine phosphatase-1B gene (*PTP-1B*), a ubiquitously expressed intracellular human protein, maps to chromosome 20q13.1.⁵¹ *PTP-1B* is involved in the negative regulation of insulin signaling. Another candidate gene for protein kinase inhibitor γ (*PKIG*), a member of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase inhibitor family, also resides on chromosome 20 at the location 20q12-13.1.⁵² Early work shows that *PKIG* is widely expressed in several mammalian adult tissues including kidney.⁵²

The striking variation in phenotypes and the diverse linkage results in relation to urine ACR highlight that phenotypic heterogeneity in CVD risk factors and renal disease are likely to be associated with substantial genetic heterogeneity. Our findings suggest that there are genes located on human chromosome 20q12 that have functional relevance to albuminuria in a Mexican American cohort. Plans are under way to identify and characterize the genetic factors that influence urine ACR, especially in nondiabetic and normotensive individuals. Identification of gene(s) influencing microalbuminuria would increase the knowl-

edge about the risk for a given patient and help identify those for whom intensive preventive measures may be most beneficial. An understanding of the role of susceptibility gene(s) will ultimately allow the development of novel therapeutic strategies.

Our analyses were based on data from a population-based sample of low-income Mexican Americans, eliminating possible ascertainment bias. However, the predominantly Mexican population that comprises the majority of the SAFHS may limit the generalizability of our findings to a broader population. Nevertheless, the importance of microalbuminuria as an independent cardiovascular risk factor has been validated in ethnically diverse cohorts reinforcing the representativeness of our data. Additional analyses with inclusion of other ethnic groups are needed to replicate current findings.

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