A Genome-Wide Scan for Urinary Albumin Excretion in Hypertensive Families


Abstract—Albuminuria increases the risk of cardiovascular events in patients with essential hypertension and diabetic subjects. The heritability (h²) of albuminuria in multiplex hypertensive families is unknown. We calculated the familial aggregation of urine albumin:creatinine ratio (ACR) and performed a genome-wide scan to assess for loci contributing to ACR in participants enrolled in the Hypertension Genetic Epidemiology Network (HyperGEN). To perform the genome scan, we analyzed genotype results from 2589 individuals from 805 families in the Family Blood Pressure Program. ACR and covariates were available in 1727 individuals (mean age, 57.1 years). Estimates of h² were obtained by using variance component methodology as implemented in the SOLAR software package. Linkage was tested between 387 markers spanning the genome at an average interval of 9.32 cM, using SOLAR multipoint analysis. The h² of log urine ACR was 0.49 (P<1×10⁻⁷) after controlling for significant main and interactive effects of age, gender, race, body mass index, blood pressure, and use of ACE inhibitors or angiotensin-2 receptor blockers. The genome-wide scan revealed a maximum LOD score of 2.73 on chromosome 19 (robust corrected LOD, 2.40; P=0.009) at marker D19S591 and a LOD score of 2.0 on chromosome 12 (robust corrected LOD, 1.75; P=0.005) at marker PAH. These analyses demonstrate the marked heritability of urine ACR in families enriched for the presence of members with essential hypertension. They suggest that a gene(s) associated with urinary ACR may be present on human chromosomes 19 and 12. (Hypertension. 2003;42:291-296.)

Key Words: albuminuria ■ nephrosclerosis ■ blacks ■ race ■ hypertension, essential

The heritability and role of inherited factors in the causation of elevated urinary albumin excretion (UAE) among hypertensive subjects remain unknown. Several reports reveal that albuminuria clusters tightly in the diabetic and nondiabetic members of multiplex families with type 2 diabetes mellitus. The presence of microalbuminuria in diabetic individuals portends an increased risk for development of progressive renal failure and subsequent end-stage renal disease (ESRD). In and of itself, ESRD has a strong familial component in diabetes and hypertension. Elevated levels of UAE in diabetic individuals are associated with increased rates of cardiovascular morbidity and mortality.

In hypertensive subjects, microalbuminuria is a risk factor for premature cardiovascular morbidity and mortality. The most recent Joint National Commission on Hypertension (JNC VII) report includes microalbuminuria as evidence for the presence of target organ damage. Target organ damage indicates the need for more aggressive control of blood pressure.
(NHLBI)-sponsored Family Blood Pressure Program (FBPP), were evaluated. HyperGEN study methods have previously been reported.\(^1,^3\) In brief, family members were recruited from 5 clinical centers (Framingham, Mass; Minneapolis, Minn; Salt Lake City, Utah; Forsyth County, NC; and Birmingham Ala). Probands had essential hypertension with an age at onset <60 years and at least one additional participating hypertensive sibling.

**Phenotyping**

Morning urine samples from study participants were collected in a resting state and run in duplicate for albumin, total protein, and creatinine concentration. Results were entered directly into an electronic file that also contained the results of quality control samples run that day. Every month, the cumulative laboratory database was sent electronically to the Data Coordinating Center. The SPO Test System (Diasorin, Inc; Stillwater, Minn) for microalbumin permitted the quantitative determination of human albumin through the use of an automated immunoprecipitin analysis on the Roche/Hitachi 911 (Roche Diagnostics Corp). Albumin was measured by immunonuturbidimetry, with the use of antibody to human albumin in an automated immunoprecipitin analysis system (Diasorin, Inc). To prevent antigen excess errors, total protein was assayed for each sample. Urine values for albumin were released if the total protein value was <100 mg/dL. If the value was >100 mg/dL, the urine was diluted and the albumin measurement repeated.

Colorimetric dye binding on the Roche/Hitachi 911 was used to measure total protein concentration. Pyrogallol red dye was combined with molybdenum acid, forming a red complex with maximum absorption at 470 nm. When this complex is combined with protein under acidic conditions, its maximum absorption is shifted to a longer wavelength, and a red-purple color develops at 604 nm. The concentration of protein in the specimen is equivalent to the absorbance of the dye urine mixture measured at 600 nm.

Urine creatinine was also measured with the use of a colorimetric dye-binding technique on the Roche/Hitachi 911. In an alkaline medium, creatinine reacts with picric acid to form a yellow-orange-colored complex. The rate of color formation is proportional to the concentration of creatinine present and is measured photometrically at 505 nm (BMC Technical Application Booklet 450019, Roche Diagnostics Corp).

**Genotyping**

Genotyping was performed by the NHLBI-funded Mammalian Genotyping Service. For additional information regarding the genotyping methods, see the web site of the Center for Medical Genetics at the Marshfield Medical Research Foundation. The genome screen was performed by means of an automated technique with the SCAnning FLUorescence Detector (SCAFUD). The Cooperative Human Linkage Center screening set 8, which includes 387 microsatellite markers approximately equally spaced every 9.32 cM throughout the genome, was used. The average marker heterozygosity was 0.76. Analyses and assignment of the marker alleles were performed with the use of computerized algorithms.

**Statistical Analysis**

Urine ACR (mg/g) was calculated as 100×urine microalbumin (mg/dL) averaged across duplicates, divided by urine creatinine (g/dL) averaged across duplicates. The distribution of urine ACR was positively skewed; thus, the natural logarithm (log urine ACR) was used for all analyses. Pedigree and genotype data were screened for possible errors through the use of ASPEX software, version 2.2; MAPMAKER/SIBS, version 2.11;\(^1,^2\) PedCheck, version 1.11;\(^1,^2\) and PREST, version 2.01.\(^1,^2\) One family was dropped from all analyses because of unresolvable potential errors in the genetic data. Potential errors in 9 other families were resolved by correcting or dropping individual family member data.

Heritability of log urine ACR was estimated by means of variance component modeling as implemented in SOLAR software, version 1.7.3.\(^1,^7\) Covariates in the model were age, gender, race, body mass index (BMI), medications (ACE inhibitor or angiotensin-2 receptor blocker), mean arterial pressure (MAP), age\(^2\), MAP\(^2\), gender×MAP, age×race, gender×race, gender×BMI, race×BMI, medications×age, medications×race, medications×age\(^2\), and race×age\(^2\). These were selected by using a backward elimination approach allowing for reentry of eliminated covariates at each step (significance level=0.10 for both backward and forward steps). Covariates were selected among age, gender, race, BMI, medications, MAP, age\(^2\), MAP\(^2\), medications×MAP, gender×MAP, age×MAP, BMI×MAP, race×MAP, age×gender, age×race, age×BMI, gender×race, gender×BMI, race×BMI, medications×gender, medications×age, medications×race, medications×age\(^2\), race×age\(^2\), gender×age\(^2\), MAP×age\(^2\), and BMI×age\(^2\). This allowed for adjustments for both main and interactive effects among demographic and physical variables, together with variables that can affect ACR more directly. The observed significant interactive effects support age and race as modifiers of the effect of the medications and support gender as a modifier of the effect of MAP in this population. Centered values were used to model the effects of continuous covariates and indicator variables (0/1) were used for discrete covariates. The heritability estimate adjusted for the effects of covariates is reported together with corresponding estimates of standard error, probability value, and proportion of variance due to covariates.

Multipoint linkage analysis as implemented in SOLAR software, version 1.7.3,\(^17\) was performed to detect and localize quantitative trait loci (QTLs) influencing variation in log urine ACR. This approach has been described in detail.\(^18,^19\) LOD scores are reported both with and without correction for possible model misspecification, and empirical probability values are reported for the robust corrected LODs.\(^20\)

**Results**

Genotype data were available from 2589 individuals in 805 families who participated in the FBPP. Of these, 1727 individuals were recruited into HyperGEN and had measurement of urine ACR. The mean age (±SD) of these individuals was 57.1±10.9 years, they had been hypertensive for a mean of 17.12±11.59 years, and they had mean ACR of 45.26±360.90 mg/g (median ACR was 4.9 mg/g). Among these 1727 individuals, there were 1164 sibling pairs, 22 parent-offspring pairs, 61 avuncular pairs, 2 half-sibling pairs, 4 first cousins, 5 identical sibling pairs, and 37 unrelated pairs. The mean family size with available demographic information.

### Demographic Characteristics of HyperGEN Study Population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
</tr>
<tr>
<td>Female</td>
<td>1042 (60.34)</td>
</tr>
<tr>
<td>Male</td>
<td>685 (39.66)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>834 (48.29)</td>
</tr>
<tr>
<td>White</td>
<td>893 (51.71)</td>
</tr>
<tr>
<td>Age, y</td>
<td>57.14±10.89</td>
</tr>
<tr>
<td>Body mass index, kg/m²</td>
<td>31.77±6.96</td>
</tr>
<tr>
<td>Mean arterial pressure, mm Hg</td>
<td>91.65±13.20</td>
</tr>
<tr>
<td>Use of medications affecting ACR</td>
<td>699 (40.42)</td>
</tr>
<tr>
<td>ACE inhibitor</td>
<td>672 (36.77)</td>
</tr>
<tr>
<td>Angiotensin receptor blocker</td>
<td>27 (3.76)</td>
</tr>
<tr>
<td>Duration of hypertension, y</td>
<td>17.12±11.59</td>
</tr>
<tr>
<td>Urine ACR mg/g</td>
<td>64.25±360.90</td>
</tr>
</tbody>
</table>

Data listed as mean±SD for continuous measures and n (%) for dichotomous measures. ACR indicates albumin:creatinine ratio.
The heritability ($h^2$) of log urine ACR was 0.49 ($P<1 \times 10^{-7}$) after controlling for significant main and interactive effects of age, gender, race, BMI, blood pressure, and use of ACE inhibitor (ACEi) and angiotensin receptor blocker (ARB) medications.

The genome-wide scan results are depicted in Figure 1. A maximum LOD score of 2.73 was observed on chromosome 19 at 9.0 cM (marker D19S591, $P=0.0004$). A lesser peak with a LOD score of 2.0 was observed on chromosome 12 at 112.0 cM (marker PAH, $P=0.002$). Ten thousand simulations were performed to determine the robust corrected LOD scores and corresponding empirical probability values for the peaks observed on chromosomes 19 and 12.

For chromosome 19 at position 9 cM, the robust corrected LOD score was 2.40 (empirical $P=0.0009$) (Figure 2). For chromosome 12 at position 112 cM, the robust corrected LOD score was 1.75 (empirical $P=0.005$) (Figure 3).

**Discussion**

This report reveals that inherited factors appear to play a major role in the regulation of UAE in individuals with essential hypertension. The heritability of urine ACR remained highly significant in these analyses after controlling for the effects of age, race, blood pressure, BMI, and the use of medications known to reduce proteinuria (ACEi and ARBs). Additionally, the genome scan provided suggestive evidence that genes regulating urine ACR are present on chromosomes 19 and 12.
The marked heritability of urine ACR appears consistent with the findings from two reports in multiplex type 2 diabetic families. A segregation analysis of urine ACR in 1269 white subjects from the Joslin Diabetes Clinic (630 type 2 diabetics and 639 nondiabetic relatives) revealed a significant correlation between median ACR in diabetic and nondiabetic members of the same family. A Mendelian model with evidence for a major gene was most strongly supported in all study subjects. Evidence for Mendelian inheritance was improved when only the diabetic subjects were evaluated, although a single major locus with multifactorial effects was more strongly supported. A segregation analysis of overt proteinuria in 2107 Pima Indians from 715 families revealed that the existence of a major gene effect with Mendelian inheritance as most likely. A dominant model provided the best fit. Taken together, these two reports suggest that urine ACR is regulated by a major gene in type 2 diabetic families.

There are potential limitations in the present analyses. It is now clear that microalbuminuria may be transient in individuals with type 1 diabetes mellitus. Less is known about the natural history of albuminuria in treated and untreated hypertensive patients. Additionally, controversy exists regarding the selection of appropriately sensitive assays for measuring albuminuria. The assay used in this study was extremely sensitive, having a lower limit of detection of 1.3 mg albumin per liter of urine. Although an assay might underestimate the

Figure 2. Genome scan plot for urine ACR on chromosome 19.

Figure 3. Genome scan plot for urine ACR on chromosome 12.
true amount of albuminuria, this would tend to bias the results toward the null and probably would reduce the heritability estimates.

A genome scan for renal function (creatinine clearance) has previously been reported in members of the HyperGEN study.11,12 In these reports, the heritability of creatinine clearance was 0.17 and 0.18 among black and white subjects, respectively. The best evidence for linkage in black subjects was found on chromosome 3 (LOD = 3.61 at 214.6 cM) and in white subjects at chromosome 3 (LOD = 3.36 at 115.1 cM). In this genome scan for urine ACR, we did not identify any evidence for linkage in these regions on chromosome 3. The linkage peaks for urine ACR (chromosomes 19: LOD = 2.73 at 9.0 cM, robust corrected LOD 2.40, P = 0.0009; and chromosome 12: LOD = 2.00 at 112.0 cM, robust corrected LOD 1.75, P = 0.005) do not overlap with those that regulate renal function in these individuals.

The LDL receptor (LDLR) locus regulating atherosclerosis susceptibility is located on 19p13.3 to 13.2,22 within our broad region of linkage. Polymorphisms in the LDLR gene could conceivably result in altered urinary ACR. Recent reports reveal that elevated urinary LDL and excess cardiovascular morbidity and mortality rates are strongly associated.5,6 Type 2 diabetic individuals with microalbuminuria are at far greater risk for cardiovascular death than of progression to renal replacement therapy.4 The Heart Outcomes Prevention Evaluation (HOPE) study demonstrated the impact of microalbuminuria on cardiovascular event rates in nondiabetic individuals.6 Elevated urinary ACR can be reduced by intake of lipid-lowering drugs (particularly the LDL-lowering statin class).24 Reductions in serum lipids may also slow progression of renal disease.24 Therefore, elevated urinary albumin excretion could result from generalized endothelial disease with concomitant large and small vessel atherosclerosis. It is more probable that another gene on chromosome 19 or 12 directly affects urinary protein excretion, since a previous report in HyperGEN families failed to demonstrate linkage between markers on chromosome 19 and serum LDL levels.25

Perspectives

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