Familial Aggregation of Low-Renin Hypertension


Abstract—Low-renin hypertension, representing roughly one quarter of all essential hypertension, is widely recognized by distinct physiological features, including salt-sensitivity, diuretic-responsiveness, and a favorable natural history. Although certain demographic features including age, ethnicity, and diabetes mellitus predispose to low-renin hypertension, these factors account for only a minority of cases. We examined familial concordance for renin status in 119 families with 257 hypertensive members. Low-renin was defined rigorously by plasma renin activity $ \leq 0.69$ ng angiotensin I/L per second, drawn when subjects had achieved balance after 5 to 7 days on a 10 mmol sodium diet and had stood upright for at least 1 hour. Given the prevalence of low-renin hypertension in our general population, low-renin hypertension was significantly more concordant among siblings than expected by chance ($P=0.01$). There were twice as many low-renin families as expected (10.9% versus 5.5%), in sharp contrast to the normal-renin state, in which the observed and expected were similar (61.0% versus 58.6%). These results were independent of age, race, and gender. Variance in renin status attributable to family membership was 35%. Association studies were performed on 8 polymorphisms in 5 candidate genes, and significant association was confirmed with the G460W polymorphism of the adducin gene. Familial determinants, which are probably but not definitely genetic, contribute to the low-renin hypertension state. (Hypertension. 2002;39:914-918.)

Key Words: renin • sodium • siblings • genetics

Low-renin hypertension describes a widely recognized subset of essential hypertension, marked by distinct physiological features, including salt-sensitivity, diuretic-responsiveness, and a favorable natural history. Increased age, diabetes mellitus, and ethnicity are widely held to be important contributors to the development of low-renin hypertension. During a recent analysis of $\approx 100$ low-renin hypertensives, we found that these factors accounted for only a minority of cases. Despite the intense interest in the genetics of hypertension, the scientific community has addressed questions of heritability that go beyond the simple distant phenotype of high blood pressure in only a limited way.

Our clinical research studies performed in sibling pairs have enabled us to examine the potential role of heritability underlying low-renin hypertension. Significant heritability of plasma renin activity (PRA) has been reported among black sibling pairs and in 1 twin study, but was not confirmed in 2 large studies. In this study, we examined concordance for renin status itself under controlled conditions in 119 families with hypertensive siblings. An association study using 5 candidate genes in the renin-angiotensin system was also performed. Our results demonstrate convincingly the contribution of familial influences to the low-renin state, which are probably genetic but not proven to be genetic.

Methods

We studied 257 hypertensives, 34 black and 223 white, from 119 families, in Boston, Paris, and Salt Lake City. Some characteristics of a subset of this population have been reported previously. However, the present analyses are original.

Overall, there were 101 sibling pairs, 17 sets of 3 siblings, and 1 family with 4 members. In addition, 67 hypertensives with no hypertensive relatives were classified and contributed to the estimate for low-renin hypertension in the overall population. Race was determined by self-identification and supported by physical appearance.

Hypertension was defined by seated blood pressures $>90$ mm Hg diastolic and $>140$ mm Hg systolic, measured manually with a standard mercury sphygmomanometer on $\geq 2$ visits. All antihypertensive medications were discontinued at least 2 weeks before study.

Protocol

On admission to the metabolic ward, each subject was placed on a constant 10 mmol sodium/100 mmol potassium isocaloric diet. After 5 to 7 days, when external sodium balance had been achieved, upright posture studies were performed. Measurement of PRA was assessed in each subject after 1 or 2 hours in the standing position. Low-renin hypertension was defined by a PRA of $<0.69$ ng angiotensin (Ang) I/L per second ($<2.5$ ng Ang I/mL per hour) at the end of the posture study, according to conventional criteria used by us for $\approx 30$ years. After the low-salt protocol, subjects were placed on a high Na$^+$ (200 mmol) intake for 3 days. Blood pressure was measured in the supine position, once patients were in balance on this intake.
Laboratory Procedures/Genotyping
PRA was assayed by a radioimmunoassay technique for generation of Ang I, as previously described.\(^8,9\) DNA was obtained from stored leukocytes. Genotyping was performed on the following 8 polymorphisms of 5 candidate genes: aldosterone synthase promoter region CYP11B2 C-344T; angiotensinogen (AGT) M235T, AGT A-20C, AGT A-6G; the Ang II type 1 receptor (AT1 R) A1166C and morphisms of 5 candidate genes: aldosterone synthase promoter T573C; ACE insertion/deletion ACE I/D; and \(-\)adducin G460T.

Statistical Analyses
The hypothesis that low-renin hypertension clustered significantly within families was tested by 2 approaches. First, maximum marginal likelihood estimation was used to perform mixed models logistic regressions (Cytel Software, Egret for Windows, 1999).\(^13\) Low-renin status was predicted with family membership as a random effect. The intraclass correlation was the proportion of total variance attributable to familial aggregation, with the logistic density function assumed for the underlying response strength, and the normal for the random effect. The 1-sided test of the random effect variance term for family membership was performed and retested after individual fixed effects of race, gender, and age group were added to the model. Generalized logistic regression also allowed the testing of individual proposed genotypes in predicting low-renin status in the unrestricted database of 324 individuals.

The second approach was to test whether the observed familial aggregation for renin status exceeded the aggregation that would occur by chance. The expected proportions of sibships concordant for normal-renin, and discordant for renin status were estimated using the proportions of low-renin and normal/high-renin individuals observed in the unrestricted database of 324 individuals. The hypothesis that low-renin hypertension clustered significantly over some factor unique to the family such as heredity or immediate environment. The significant intraclass correlation of 0.35 in the random effects models indicated that the probability of low-renin status was not constant but depended on the family, and thus the excess aggregation where each family received equal weight.

An expanded Methods section can be found in an online data supplement available at http://www.hypertensionaha.org.

Results
PRA was markedly lower among low-renin hypertensives, by definition (Table 1). As anticipated, low-renin hypertensives were older than their normal-renin counterparts. Blacks were slightly over-represented among the low-renin group. Females made up three fifths of all low-renin hypertensives; moreover, 29% of all hypertensive women compared with 18% of men had low-renin hypertension. The groups were well matched for body mass index. Although the resting blood pressure did not differ between the groups on a low-salt diet, the low-renin hypertensives had significantly higher blood pressures on the high-salt diet.

The proportion of low-renin hypertension in our general hypertensive population was 23.5%. The observed proportions of multi-sibling families concordant for low renin, concordant for normal renin, and discordant for renin status differed significantly from the proportions expected by chance (P=0.01). The observed proportion of sibships concordant for low-renin status was 10.9%, double the expected (5.5%; Figure). In sharp contrast, the observed proportion of sibships discordant for normal-renin status was almost equal to the expected (61.9% versus 58.6%). The observed proportion of families discordant for renin status was less than expected (27.2% versus 35.0%). Thus, the excess aggregation of renin status within families was attributable to the low-renin status category.

This general pattern of excess aggregation for the concordant low-renin category was seen for all demographic strata, whether divided by race (black or white), gender, or age group. The ratio of observed to expected frequency of low-renin concordance was, in fact, higher among subjects age <49 than >49 (2.4 versus 1.92). The excess familial aggregation was statistically significant for the white race subgroup (P=0.03), the largest subgroup with 105 families. Variance in renin status attributable to family aggregation was 35% by mixed models logistic regression (P=0.01). This result was unchanged when the random effect of family was combined with the fixed effects of age and gender. Table 2 presents logistic regression analyses for individual predictors with and without explicitly including the heterogeneity of the family clusters in the model. When familial aggregation was ignored, the raw probability of low-renin status in 257 subjects from multiple sibling families was a constant 0.25. The significant intraclass correlation of 0.35 in the random effects models indicated that the probability of low-renin status was not constant but depended on the family, and thus on some factor unique to the family such as heredity or immediate environment. The significant intraclass correlations in the random effects models with individual covariates indicated that the effect of each individual covariate on low-renin status was not constant across families. For example, low-renin status was more likely for female gender, but the potentiating effect of female gender for low-renin status was not homogeneous across families.

To pursue this implication that genetic underpinnings were responsible for a large fraction of the low-renin state, we performed association studies with a limited number of polymorphisms in 5 candidate genes. Four of them encode for...
genes belonging to the renin-angiotensin-aldosterone system (AGT, ACE, AT,R, and CYP11B2), whereas adducin encodes for a gene with a product that has been associated with renin status. We found an association between low-renin status and the adducin G460W polymorphism. Genotype frequencies revealed 23% 460GG and 17% of the GT heterozygotes to be low-renin hypertensives, contrasted with 67% of the 460TT homozygotes \((P=0.004)\). Genotype frequencies within low renin were 39 for GG, 13 for GT, and 6 for TT; for normal renin the corresponding frequencies were 133, 62, and 3. Allele frequencies among normal-renin hypertensives were 0.83 for the 460Gly allele and 0.17 for the 460Trp; among low-renin hypertensives, the respective allele frequencies were 0.78 and 0.22. The association was significant with whites alone, \(P=0.01\). Too few blacks have yet been genotyped for this polymorphism to allow separate analysis.

Genotype frequencies were consistent with Hardy-Weinberg equilibrium (HWE) in the overall hypertensive population, although not within the low-renin subset.

No significant associations were seen with the following polymorphisms: aldosterone synthase promoter region C-344T, AGT M235T, AGT A-20C, and AGT A-6G; the AT,R A1166C and T573C; and ACE I/D (Table 3).

Because of the apparent influence of gender on renin status and because of our interest in the effects of gender on sexual dimorphisms in hypertension, we examined these same associations separately by gender. The adducin G460W association was highly significant among females \((P=0.003)\) but not among men \((P=0.8)\). Of all the other genotypes, a trend toward significant association was seen among men only for the aldosterone synthase gene CYPC344T \((P=0.08)\). We further explored the interaction between the 2 suspect genes.

**TABLE 2. Mixed Models Logistic Regression Predicting Low-Renin Status by Individual Covariates**

<table>
<thead>
<tr>
<th>Model</th>
<th>Without Family Clustering</th>
<th>With Family Clustering*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Odds Ratio</td>
<td>95% CI</td>
</tr>
<tr>
<td>Female</td>
<td>1.85</td>
<td>(1.05, 3.30)</td>
</tr>
<tr>
<td>Age ≥49 years</td>
<td>1.86</td>
<td>(1.05, 3.31)</td>
</tr>
<tr>
<td>Black</td>
<td>1.79</td>
<td>(0.83, 3.87)</td>
</tr>
</tbody>
</table>

95\% CI indicates 95\% confidence interval.

*Intracluster correlation = 0.35, \(P=0.006\)
by examining the subset of adducin460 heterozygotes according to aldosterone synthase genotype (CY344T homozygotes versus CYP344CT and CC), and observed a significant association among males (P=0.03) but not among females (P=0.2). Of 58 low-renin hypertensives genotyped at both the adducin 460 and CYP 344, 10% are homozygous at adducin 460; taking into account the heterozygotes for adducin, those who are also homozygous for the CYP344T represent an additional 30% of low-renin males.

### Discussion

We report a clear familial aggregation of low-renin hypertension, with twice as many families concordant than expected. In contrast, the observed number of families with siblings concordant for normal-renin hypertension was similar to that expected.

One of the main obstacles confounding elucidation of the primary defects behind hypertension is the complex interplay of various genetic and environmental factors, resulting in a widely heterogeneous distant phenotype. This heterogeneity could easily obscure any positive relationship between a gene and hypertension. Therefore, intermediate phenotypes are sought that separate the essential hypertensive population into more homogeneous subgroups in which the role of a particular candidate gene might be more reliably assessed. In general, the most useful intermediate phenotypes are derived from pathophysiologic characteristics of the hypertensive population that show (1) greater frequency in the hypertensive than in the normotensive population, (2) bimodality of the trait in the hypertensive population, and (3) association of the trait with hypertension in affected sibling pair studies. This study has strengthened the position of low-renin hypertension as an intermediate phenotype.

Since Helmer et al. reported in 1964 that some patients with essential hypertension had PRA concentrations less than those observed in normotensives, there has been extensive investigation of the pathophysiologic, therapeutic, and prognostic implications of this state. Both age and race have been documented to be determinants of PRA response to sodium restriction and to upright posture, but much less information on inheritance or familial aggregation has previously been reported. The heritability of PRA under varied conditions of sodium intake and posture was reported in normotensive twin volunteers by Grim et al. >20 years ago. In a more recent study of normotensive twins, however, no statistically significant heritability of PRA was found. Rossi et al. studied 69 pairs of white twins under random conditions, and found a statistically significant heritability of plasma ACE levels but not of PRA. However, without controlling environmental factors, particularly sodium intake and posture, the “noise” in determining PRA can be so great as to override any genetic signal. Similarly, Williams et al. also reported an extremely low genetic heritability of PRA, contrasted with a greater influence from shared environment in a 2500 person database from 98 Utah pedigrees. These samples were also drawn under random conditions. In contrast, Kotchen et al. examined hypertensive black sibling pairs and found PRA to have a heritability (h²) of 0.44 in the supine position and 0.69 after 10 minutes upright posture, at a fixed moderate salt intake (140 mmol Na⁺) for 2 days. In our protocol, rigorous study conditions enabled us to type patients by renin class, and to perform statistical analyses on the familial aggregation of renin status itself. The results reported here represent an expansion of a preliminary finding in our earlier study focusing on the familial aggregation of aldosterone response to Ang II infusion.

Although no genotype has been convincingly demonstrated to be associated with low-renin hypertension, the clinical and biochemical features of low-renin hypertension make genes of the renin-angiotensin system automatic candidates. Specific direction also stems from our report of altered adrenal responsiveness to Ang II among patients with low-renin hypertension, which depended on sodium balance. In approaching these association studies, we faced the same problem that all investigators in current clinical research face: given a pool of DNA and a large roster of physiological measurements, there are infinite ways to examine the data. In this context, given the current sample sizes and the large number of questions being asked, hypothesis generation is valuable, but genetic causality is not proven.

Adducin is a cytoskeletal protein that may be involved in ion transport across the cell membrane. In humans, many research groups have attempted to identify an association between the α-adducin gene and salt sensitivity and blood pressure; results have been conflicting, and if real, its effect is probably mild at the population level. We report an association between adducin genotype and low-renin hypertension. Although the overall population fit HWE, the low-renin subset did not. There are several possible explanations for deviation from HWE, which holds only for large random samples. One highly relevant factor is sample size; 58 subjects is a small number for application of this law. Second, any association between a sub or intermediate phenotype and a particular genotype, such as we propose, would necessitate subset deviation from HWE; indeed, it would be unexpected to fit a small population selected for a trait (like low-renin hypertension) that might bias the representation of the genotypes. This result stands in contrast to the total population of hypertensives in whom HWE was present. Finally, our data set may contain errors of mistyping, although the likelihood is low because the departure is selective among the low-renin subjects.
The CYP11B2 encoding aldosterone synthase (P450c11AS) has been implicated in low-renin hypertension in both Chilean and Japanese populations. The present data set showed no significant association with low-renin hypertension in the group as a whole, but a trend toward significance emerged among the men only. Population differences or population sizes may contribute to the varied results. An association has been demonstrated between the aldosterone synthase CYP-344T gene polymorphisms and higher urinary aldosterone excretion among Finnish men. In our study, low-renin hypertension was found to be associated with men heterozygous for adducin460 and homozygous for the CYP-344C polymorphism, entirely consistent with our earlier report of lower aldosterone concentrations in patients with low-renin essential hypertension. The influence of gender on expression of genetic traits related to blood pressure is complex and has been discussed earlier, but it is clear that association studies examining these candidate genes must be examined separately in men and women.

We have shown that AGT is probably not related to renin status. The association analysis with 2 polymorphisms of the AT,R was also negative, but it must be stressed that these are only 2 isolated polymorphisms out of a much greater possible number, and that our negative results are preliminary.

Like other complex human traits, essential hypertension is multifactorial, probably caused by the impact of several different “risk” and “protection” genes, physiological and environmental factors. Familial aggregation of low-renin hypertension, although suggestive, does not necessarily represent its heritability. Low-renin hypertension is known to increase with increasing age, so it was important to eliminate the possibility that concordance of low-renin hypertension in families was simply owing to older siblings. Confirmation can be inferred from the observation that the excess present in low-renin siblings was even greater among younger siblings than older.

The current study has established that familial determinants independent of age, race and gender contribute to the low-renin hypertension state. Genetic causes are probable but are not definite. Polymorphisms in the adducin gene, either alone or in combination with polymorphisms in the aldosterone synthase gene, are among the genetic factors involved. It is our hope that larger sample sizes and expanded genotyping will allow for a more complete genetic understanding of the heritability of low-renin hypertension.

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**References**


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