A Lack of Genetic Linkage of Renin Gene Restriction Fragment Length Polymorphisms With Human Hypertension

Allen J. Naftilan, Roger Williams, David Burt, Martin Paul, Richard E. Pratt, Peter Hobart, John Chirgwin, and Victor J. Dzau

Because renin is an important enzyme in blood pressure regulation, we studied the possibility that an alteration in the structure of the human renin gene is genetically linked to human essential hypertension or associated with levels of plasma renin activity or blood pressure. By using specific DNA probes, we have identified four polymorphisms in the human renin gene with the restriction enzymes Taq I, HindIII, Bgl I, and Bgl II. The gene location of all of these polymorphisms except for the Bgl II polymorphism has been determined, and their frequencies were initially estimated in a population of 50 random subjects. To test the clinical significance of these polymorphisms, we studied 68 persons from a large Utah pedigree with a high incidence of hypertension. Among nine relatives with hypertension, genetic linkage without recombination was ruled out by observing several obligate recombinants. We also found no significant association of the restriction fragment length polymorphisms with quantitative measurements of sitting or standing, systolic or diastolic blood pressures, or plasma renin activity in 59 untreated members of this pedigree. Although we found no genetic linkage in this set of study subjects, the characterization of the restriction fragment length polymorphisms for the renin gene may be useful in future studies of other selected pedigrees for the presence of one or more of these to be a genetic marker in hypertension. (Hypertension 1989;14:614-618)

Data from epidemiological and twin studies suggest that a genetic component to essential hypertension exists. Because renin is an important enzyme in blood pressure regulation, it is reasonable to explore the possibility that an alteration of the renin gene is in some respect linked to the pathophysiology of human essential hypertension.

In recent years, the use of restriction fragment length polymorphism (RFLP) has been a useful tool to explore alterations in the genes encoding insulin,1-3 α- and β-globin,4 and numerous others.5 In some diseases such as diabetes it has been difficult to link the RFLP to the phenotypic expression of the disease,6-7 but in others such as sickle cell anemia,8 Huntington’s Chorea,9 and Duchenes muscular dystrophy10 such linkages have led to the development of important screening tests. Recently the gene for human renin has been cloned and sequenced.11 Using specific DNA probes, we first screened a random population of subjects within 24 restriction enzymes. We report here the identification of four RFLPs in the human renin gene, an estimation of the gene frequencies, and a preliminary analysis of a selective population chosen because of a high incidence of coronary artery disease and hypertension.

Methods

Isolation and Restriction Analysis of Human DNA

DNA was prepared from human lymphocytes by a slight modification of the procedure of Bell et al.1
Naftilan et al  Renin Polymorphisms 615

UTAH KINDRED 500

Key:  
- Hypertension
- Renin Gene Haplotypes
- Deceased
- Inferred haplotype

FIGURE 1. Schematic diagram of kindred 500 showing only founding sibship and a few offspring with hypertension and known renin phenotype. Obligate recombinants are observed in generations II (e.g., 2,2 and 5,5) and III (e.g., 5,8 and 2,2).

Before digestion, all of the DNAs were run on 0.4% agarose gels to assure they were of high molecular weights. Restriction enzymes were obtained from New England Biolabs (Beverly, Massachusetts) and used according to the manufacturer’s directions. Restriction digests of human DNA were carried out at a final concentration of 100 μg/ml with parallel digestions of human DNA and A phage DNA to monitor for complete digestion. After electrophoresis in 0.8% agarose gels and transfer to nylon membrane (Micron Separations Inc., Honeoye Falls, New York), the fragments of the renin gene were detected by hybridization with nick-translated probes. The probes used were three fragments of the human renin gene including all exons except exon Va (see Figure 3). Autoradiography was performed for 1–5 days at −80°C with Kodak XR-5 film backed with a lightening-plus intensifying screen (DuPont, Wilmington, Delaware).

Patient Study

The initial population of patients who were screened for the presence of the polymorphisms by using 24 restriction enzymes were 20 random subjects from both Boston and Utah. To approximate the gene frequencies of three selective RFLPs, an additional 30 random subjects were studied from Boston, Utah, and Connecticut. To test the possible significance of these polymorphisms, haplotype was determined on nine treated hypertensive relatives and 59 untreated subjects from a large Utah family with approximately a 20% incidence of hypertension (Figure 1). This was done by restriction digestion and Southern hybridization of the patient’s DNA with HindIII, Taq I, and Bgl I as described above.

The quantitative phenotypic variables analyzed were sitting blood pressures (systolic and fourth phase diastolic, average of three morning measurements each), standing blood pressures (systolic and fourth phase diastolic, average of two measurements each), and plasma renin activity.

The details of these phenotypic determinations are as follows: the blood pressures were measured in the morning and adjusted for the effects of age and sex by expressing them as standardized deviates from the mean blood pressures for age- and sex-specific subgroups (age subgroups in 10-year intervals). For example, the diastolic blood pressure for subject i in age and sex subgroup j would be expressed as

\[ D_i - \text{mean} \frac{D_j}{sD_j}, \]

where \( D_i \) is the diastolic blood pressure of person i in age and sex subgroup j, \( D_j \) is the mean diastolic blood pressure of persons in subgroup j, and \( sD_j \) is the standard deviation of diastolic blood pressures in subgroup j. The mean and standard deviation of blood pressures were determined from a total clinic population of over 2,500 subjects not on medication for hypertension.

Blood was usually drawn in the morning from the antecubital vein into chilled Na2 EDTA tubes from subjects who had been sitting for 15–30 minutes. Plasma renin activity was measured by the method of Haber et al. Analysis of the data on plasma renin activity was adjusted to account for the potentially confounding effects of age, sex, body size, and salt intake as reflected by urinary sodium excretion in the previous 12 hours. Regression analysis of the effects of these variables on plasma renin activity revealed that the best mathematical model of the relation between plasma renin activity and these variables was one in which the natural logarithm of plasma renin activity was predicted by age and urinary sodium excretion. This relation was established on the same study population as described above. The phenotypic trait chosen for the association study was the difference between the observed plasma renin activity (log transformed) and the plasma renin activity as predicted by age and sodium excretion. The effect of these manipulations was to express the plasma renin activity as that part of the measurement not due to age, sex, size, and sodium intake.

Once the phenotypic measurements were obtained and adjusted as described, analysis of variance was performed to determine if any of the three genotypic groups with seven or more subjects were associated with a detectably different phenotype. Twelve of the 59 subjects were not eligible for analysis of variance because they were found in genotypic groups with too few subjects to produce reliable variance estimates for this analysis. This included three diploid genotypes with one subject...
Hypertension Vol 14, No 6, December 1989

By screening an initial population of 20 random subjects with 24 restriction enzymes, we have identified four RFLPs. Representative blots are shown in Figure 2. The polymorphisms with their respective gene frequencies are described in Table 1. We then determined in 50 subjects the gene frequencies for the Taq I, the Bgl I, and the HindIII. The frequency of the Bgl II polymorphism was determined in only 20 subjects. The Taq I polymorphism demonstrated the appearance of an 11 kb allele in a minority of subjects, the Bgl I polymorphism the appearance of a 6 kb allele, the HindIII polymorphism the appearance of a 6.2 kb allele, and the Bgl II polymorphism the appearance of a 25 kb allele. Table 1 also lists the 20 restriction enzymes for which no polymorphisms were detected.

By using restriction enzyme mapping, the precise locations for the Taq I, the Bgl I, and the HindIII polymorphisms on the renin gene were determined. The exact location for the Bgl II polymorphism could not be determined. The localization of the polymorphisms on the renin gene is shown in Figure 3.

Among the nine related pedigree members with treated hypertension, the renin marker genotypes showed no evidence of cosegregation (Figure 1). Several obligate recombinants are observed in the second and third generations. These observations rule out genetic linkage of this DNA marker with hypertension in those subjects.

In an attempt to test for a possible relation of these polymorphisms with plasma renin activity or blood pressure, we analyzed the untreated 59 related subjects from this large family (hypertensive patients receiving treatment were presumed to have medication effects on these two quantitative traits and were excluded from this analysis). Nine different haplotypic combinations were identified, three of

![Figure 2. Representative blots showing examples of the Bgl I, HindIII, and Taq I polymorphisms found by restriction enzyme analysis performed on random subjects. Arrows identify the polymorphic bands.](image)

![Figure 3. Simplified schematic diagram of the human renin gene demonstrating location of the Taq I, Bgl I, and HindIII restriction sites and polymorphic sites of each enzyme. Third line demonstrates fragments of renin gene DNA that were used as probes. Fourth line is a schematic diagram of renin gene showing position of the 10 exons and nine introns. Bottom lines further emphasize position and size of polymorphisms.](image)

### TABLE 1. Polymorphisms and Their Respective Gene Frequencies

<table>
<thead>
<tr>
<th>Polymorphism</th>
<th>Allele (kb)</th>
<th>Frequency</th>
<th>Allele (kb)</th>
<th>Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>Taq I</td>
<td>10.0</td>
<td>0.88</td>
<td>11.0</td>
<td>0.12</td>
</tr>
<tr>
<td>Bgl I</td>
<td>11.0</td>
<td>0.84</td>
<td>6.0</td>
<td>0.16</td>
</tr>
<tr>
<td>HindIII</td>
<td>8.7</td>
<td>0.69</td>
<td>6.2</td>
<td>0.31</td>
</tr>
<tr>
<td>Bgl II</td>
<td>22.0</td>
<td>0.90</td>
<td>25.0</td>
<td>0.10</td>
</tr>
</tbody>
</table>

these diploid genotypes were represented by only one subject, two by two subjects each, and one by one subject. Analysis of variance was performed on the three genotypes represented by more than seven subjects to determine if any of the three genotypes were associated with a distinguishable phenotype. Table 2 demonstrates the probabilities of the null hypothesis (no statistically significant phenotype difference among the genotypic groups) being true for the five phenotypic traits measured. As is evident, no significant association between any of the phenotypic traits and the genotypic groups could be identified.

### Discussion

These common renin gene polymorphisms we studied have been independently found by Frossard et al., unlike their report, we have found the size of the more common BglI allele to be 11 kb rather than 9 kb. In addition, Morris and Griffiths have recently reported the HindIII polymorphism. The allele frequencies we observed in 50 random subjects are compatible with those reported earlier.

Recently, renin gene RFLP has been reported in hypertensive rat strains. Lindpaintner et al. reported a polymorphism that was found in the genetic hypertensive rat strain, the stroke-prone spontaneously hypertensive rat, but not in the normotensive Wistar-Kyoto control. More interesting is the observation of Rapp et al. that an RFLP in the first intron of the renin gene between Dahl salt-sensitive (DS) and Dahl salt-resistant (DR) rats cosegregates with blood pressure increment in the F2 population derived from crossing DS and DR rats. These studies would suggest renin RFLP to be a potentially useful marker for certain forms of genetic hypertension.

In a preliminary report on random subjects, Morris and Griffiths failed to observe a difference in the frequency of the HindIII polymorphism between hypertensive and normotensive individuals. Our more extensive data of several RFLPs on a pedigree do not support a genetic linkage of the renin gene with hypertension. Hypertension is common and likely heterogeneous. If two or more etiologically distinct forms of hypertension occurred among studied relatives, it would not be detectable by a simple study like this, which assumes etiologic homogeneity of all affected persons. It is clear that if we are to establish a linkage between essential hypertension and one or more of the polymorphisms, extended studies in additional pedigrees with hypertension will be necessary. In this way linkage analysis as described by White and his colleagues could be properly done. Nevertheless, the identification and localization of these RFLPs coupled with the preliminary linkage studies provide the basis for such linkage studies in the future.

### Acknowledgments

We thank Ms. Donna MacDonald for expert secretarial assistance.

### References

12. Haywood GS, Smith MA: The chromosomal site of Bacterio
15. Wahl A, Stern M, Stark AR: Efficient transfer of large DNA fragments from agarose gels to diazobenzyloxymethyl
papaper. Proc Natl Acad Sci USA 1979;76:3083–3087
16. Feinleib M, Garrison RJ: The contribution of family studies to the pertitioning of population variation of blood pressure,

KEY WORDS • renin • polymorphisms • renin gene
A lack of genetic linkage of renin gene restriction fragment length polymorphisms with human hypertension.
A J Naftilan, R Williams, D Burt, M Paul, R E Pratt, P Hobart, J Chirgwin and V J Dzau

Hypertension. 1989;14:614-618
doi: 10.1161/01.HYP.14.6.614

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1989 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/14/6/614

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Hypertension can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Hypertension is online at:
http://hyper.ahajournals.org//subscriptions/