Similar Frequencies of Renin Gene Restriction Fragment Length Polymorphisms in Hypertensive and Normotensive Subjects

Florent Soubrier, Xavier Jeunemaitre, Brigitte Rigat, Anne-Marie Houot, François Cambien, and Pierre Corvol

A prospective study was conducted to compare the frequency of renin gene polymorphisms in normotensive and hypertensive subjects. Hypertensive (n=102, blood pressure 168±17/103±9 mm Hg) and normotensive (n=120, blood pressure 122±10/75±9 mm Hg) subjects were white, had similar age and sex distributions (hypertensive group, 45±10 years old and 52% female; normotensive group, 44±9 years old and 55% female) and similar body mass index (hypertensive group, 23.2±2.6; normotensive group, 22.5±2.4 kg/m², p=0.048). The familial susceptibility to hypertension was defined as at least one parent and one sibling who were hypertensive before age 65; subjects in the normotensive group had no familial history of hypertension. Renin gene polymorphisms located throughout the renin gene were identified by using three restriction enzymes (Taq I, HinfI, HindII). For each polymorphic restriction site, allele frequencies were similar in the hypertensive and the normotensive groups. In the absence of parental genotypes, the haplotype frequencies combining the three restriction fragment length polymorphisms were estimated by using maximum likelihood techniques and were similar in both groups (hypertensive group, 0.429, 0.277, and 0.177; normotensive group, 0.453, 0.245, and 0.195 for the three most common haplotypes). A rare haplotype detected by Taq I/HindIII was apparently more frequent in the hypertensive than in the normotensive group (hypertensive group, tH 0.086, th 0.022; normotensive group, tH 0.038, th 0.050), but the difference was not statistically significant. In conclusion, no association between renin gene polymorphisms and essential hypertension was demonstrated in the present study. (Hypertension 1990;16:712–717)

Human essential hypertension is a heterogeneous disorder of multifactorial origin. Genetic susceptibility plays an important role in the development of the disease, and it has been estimated that about 30% of the blood pressure variance is genetically determined. The unimodal distribution in the general population as well as in the offspring of hypertensive parents suggests that several genes are likely involved in this genetic determination. In such a disorder, the role of a single gene can be investigated by case-control studies designed to look for an association between the disease and a marker genotype at the selected gene locus. Using this approach, linkage disequilibriums have been found for hypercholesterolemia with the apolipoprotein genes and for non-insulin-dependent diabetes mellitus with candidate genes such as the insulin or the glucose transporter genes. Results from such association studies need confirmation by finding a linkage in affected families and by identification of the deleterious gene defect.

Among several candidate genes in essential hypertension, the genetic analysis of the human renin gene appears particularly relevant for several reasons: 1) renin is the limiting enzyme of the biosynthesis cascade leading to the potent vasoactive hormone angiotensin II, 2) increase in renin production can generate a major increase in blood pressure as illustrated by renin secretory tumors and renal artery stenosis, 3) the blockade of the renin-angiotensin system is highly efficient in the treatment of human hypertension as illustrated by the recent development of angiotensin I converting enzyme inhibitors, 4) genetic studies have shown that renin is associated with the development of hypertension in Dahl rats, and 5) transgenic rats bearing the mouse Ren-2 gene develop a fulminant hypertension.

Two studies concerning the human renin gene have been published so far, showing no evidence for its implication in essential hypertension. To further explore the hypothesis of a potential role of
this gene in the genetic determinism of hypertension, we compared several restriction fragment length polymorphism (RFLP) frequencies of the human renin gene in two prospectively selected populations matched for age, sex, and body weight but differing in blood pressure levels and contrasted for familial history of hypertension.

Methods

Patient Selection

A group of 102 hypertensive case patients was selected from the Hypertension Clinic at the Brous- sais Hospital in Paris, according to the following criteria: 1) age between 20 and 60 years, 2) established hypertension defined either by chronically treated hypertension \((n=21)\) or by a diastolic blood pressure greater than 95 mm Hg at two consecutive visits for those without antihypertensive treatment \((n=81)\), 3) essential hypertension (any secondary form of hypertension was eliminated, if necessary, after an extensive inpatient work-up), 4) familial history of early onset of hypertension defined as occurring before age 65 years, in at least one parent and one sibling. This was established by a questionnaire on familial medical history using the computerized Artemis system.\(^9\)

The normotensive control patient group included 120 healthy subjects who were recruited from blood donors at the Broussais transfusion center \((n=30)\) and from patients examined in a preventive medicine center in Paris \((n=90)\). They were selected if they were 1) between 20 and 60 years old and had a systolic and diastolic blood pressure less than 140 and 90 mm Hg, respectively, without antihypertensive treatment and 2) had no personal history of elevated blood pressure and no familial history of hypertension.

All subjects were white. Subjects with exogenous factors that could influence blood pressure were eliminated, in particular those with an alcohol intake of more than three drinks per day or oral contracep-

tive therapy. Other exclusion criteria were a body mass index (BMI) \((weight/height^2)\) greater than 27 kg/m\(^2\), the presence of diabetes mellitus or renal insufficiency. In both groups, blood pressure was measured with a standard sphygmomanometer with the subject in the supine position, with the diastolic measurement corresponding to the fifth phase of the Korotkoff sounds.

Normotensive control patients were chosen to match the hypertensive case patients for age and sex, and a similar distribution of these two parameters was obtained (Table 1). However, mean BMI was slightly greater in hypertensive case patients \((23.2 \pm 2.6 \text{ versus } 22.5 \pm 2.4 \text{ kg/m}^2, p=0.048)\).

Experimental Protocols

DNA extraction. DNA was extracted from blood leukocytes using standardized techniques.\(^10\)

Probes. The HindIII polymorphism\(^11\) was detected with the purified and labeled insert of clone pHr22, corresponding to a 1.1 kb human renin complementary DNA fragment.\(^12\) The Taq I and HinI I polymorphisms\(^3,14\) were detected using a 307 base pair genomic DNA fragment corresponding to the Pvu II-Pvu II fragment located between position -502 and -195 from the transcription start, in the 5' region of the renin gene, clone Phrp.\(^15\) Probes were labeled at high specific activity \((4-8 \times 10^9 \text{ cpm/} \mu \text{g})\) with the random primer labeling method,\(^16\) using a commercial kit (Amersham Corp., Buckinghamshire, UK).

DNA gel-blot hybridization. Human genomic DNA (10 \(\mu\)g) was digested by Taq I, or HinI I, or HindIII (New England Biolabs, Beverly, Mass.). Each enzyme detects a two-allele RFLP at the human renin gene locus. Digested DNAs were electrophoresed on a 0.7% (Taq I and HindIII digests) or a 1.2% agarose gel (HinI I digests), denatured, and transferred to a nylon membrane (Hybond-N\(^+\), Amersham). Filters were prehybridized, hybridized to the labeled probe, and then washed under high stringency conditions according to previously described

### Table 1. Main Clinical Characteristics of the Selected Populations

<table>
<thead>
<tr>
<th></th>
<th>Normotensives</th>
<th>Hypertensives</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(n)</td>
<td>Age (yr)</td>
</tr>
<tr>
<td><strong>20–40 years old</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>17</td>
<td>34.2±3.7</td>
</tr>
<tr>
<td>Women</td>
<td>22</td>
<td>33.7±5.9</td>
</tr>
<tr>
<td><strong>40–60 years old</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Men</td>
<td>37</td>
<td>48.9±5.6</td>
</tr>
<tr>
<td>Women</td>
<td>44</td>
<td>50.1±5.4</td>
</tr>
<tr>
<td>Total</td>
<td>120</td>
<td>44.6±9.0</td>
</tr>
</tbody>
</table>

*Results are expressed as mean±1 SD. BMI, body mass index (weight/height\(^2\)); SBP/DBP, systolic/diastolic blood pressure. *\(p=0.048\) between normotensive and hypertensive subjects.
protocols. Filters were exposed for about 48 hours on XAR-5 film (Kodak, Rochester, N.Y.) with an intensifying screen at -70°C.

Statistical Analysis
The clinical characteristics of the two groups were expressed as mean±1 SD and were compared using the unpaired Student's \( t \) test. For each biallelic RFLP, allele frequencies were deduced from genotype frequencies, and deviation from Hardy-Weinberg equilibrium was tested by the \( \chi^2 \) test with 1 df. Haplotype frequencies involving two or three biallelic RFLPs were estimated according to Hill's method. For example, the three biallelic loci \( a, \alpha, \beta, \gamma \), the log likelihood of a particular sample is

\[
\ell(\alpha \beta \gamma) = \sum n(x) \cdot \ln g(x)
\]

where \( n(x) \) is the observed frequency of genotype \( x \) and \( g(x) \) is its expected frequency written as a function of expected haplotype frequencies. The summation is over all possible genotypes. This model is valid only if the observed frequencies of the genotypes defined by the three RFLPs are in Hardy-Weinberg equilibrium. This can be tested by comparing the likelihood of the model where \( g(x) \) is written as a function of haplotype frequencies to the likelihood of the model where

\[
g(x) = n(x)/N
\]

where \( N \) is the total number of subjects in the sample. For three biallelic loci, these models have a maximum of eight \( (2^3) \) and 27 \( (3^3) \) parameters, respectively.

Case patients and control patients were compared using the following procedure. Let \( L_1(k), L_2(k), \) and \( L(k) \) be the maximum likelihood obtained in cases, controls, and cases plus controls, respectively, for a model including the same \( k \) parameters. The statistic test used to compare case patients and control patients is

\[
2(L(k) - L_1(k) - L_2(k))
\]

that is distributed as a \( \chi^2 \) variant with \( k \) degrees of freedom. This test has low power, but particular subhypotheses may be investigated. Nonrandom associations of alleles were measured by the standardized linkage disequilibrium coefficient \( \rho \) and the ratio \( \rho^2 \) of the value of the linkage disequilibrium coefficient to its maximal absolute value taking in account the allelic frequencies.

Results
Restriction Fragment Length Polymorphism Alleles and Genotype Frequencies (Table 2)
The following RFLP alleles were observed: 11 and 9.8 kb alleles (Taq I), 1.4 and 1.3 kb alleles (HinfI), and 9.0 and 6.2 kb alleles (HinIII). For each restriction enzyme, the genotype frequencies satisfied the Hardy-Weinberg proportions and were similar in the normotensive and hypertensive groups. Comparable results were obtained when the allele frequencies were analyzed according to sex and age.

Linkage Disequilibrium
A pronounced linkage disequilibrium (Table 3) was found between the HinfI/HinIII \( (R = -0.243, \chi^2 = 26.2, p < 0.0001) \) and HinfI/Taq I \( (R = -0.160, \chi^2 = 11.3, p < 0.0001) \) polymorphisms, as a consequence of their physical proximity at the renin gene locus. In contrast, no significant disequilibrium was found between the two most distant polymorphic sites.

Haplotype Frequencies
Because parental genotypes were not determined and double and triple heterozygotes were observed,
that the haplotype frequencies (Table 4) were estimated by the maximum likelihood method.

Combining two RFLP alleles, four haplotypes could be defined for each pair of restriction enzymes (Ti, Ti, Ti, and Ti for Taq I/HinIII sites, for example). Similar frequencies of these 12 haplotypes were observed in the normotensive and hypertensive groups. However, it is interesting to note that the frequencies of the rare haplotype determined by the Taq I/HindIII polymorphisms were different in the two groups: (HIT) 0.806 (17.5/204 haplotypes) in hypertensive case patients versus 0.038 (9.1/240 haplotypes) in normotensive control patients and (hIT) 0.022 (4.5/204 haplotypes) in hypertensive case patients versus 0.050 (12.0/240 haplotypes) in normotensive control patients, although it did not reach statistical significance: \( \chi^2 = 4.3 \) NS. By combining the three RFLP alleles, it was possible to define eight different haplotypes. The informativeness of the three combined RFLPs, estimated by the polymorphism information content, was 0.651. In the two groups, three of the haplotypes (HIT, HIT, HIT) comprised 88% of the total. No significant difference was found \( (\chi^2 = 7.8 \) NS) on the overall frequencies between normotensive case patients and hypertensive control patients.

**Discussion**

The aim of the present study was to compare the renin gene RFLP frequencies in two populations with contrasted blood pressure. Using previously cloned renin gene DNA probes,12,15 we studied, as marker genotypes, three RFLPs located throughout the renin gene: the Taq I polymorphism located in the 5' region, the HindIII located in the 3' region,19 and the HinfI located in the first intron (unpublished result from our laboratory).

The opportunity to detect population associations between a disease and marker genotypes depends on at least four factors: the number of the subjects studied, the clinical and genetic heterogeneity of the disease, the linkage disequilibrium between the marker locus and the disease locus, and the marker's informativeness, which depends on the number and the frequency of alleles in the population. Taking these points into consideration, we carefully designed the present study with respect to the selection of subjects and analyzed several renin RFLPs. Two hundred and twenty two subjects were selected according to contrasted blood pressure levels and familial history of hypertension but matched for sex, age, and race (all subjects were white). The hypertensive case patients were chosen if they had a strong familial predisposition to hypertension, defined by at least one parent and one sibling who were hypertensive. Indeed, we have previously shown that such familial conditions are present in around 13% of the hypertensive subjects recruited in our center and are associated with higher levels of diastolic blood pressure and an earlier onset of the disease.20 Perusse et al21 recently reported that the higher frequency of hypertension in adults is probably more the consequence of environmental influences than of a genetic susceptibility. Therefore, taking into consideration the young age of our hypertensive patients (45.3±10 years), the familial history of hypertension, and the exclusion of two other main interacting factors (obesity and diabetes mellitus), it is likely that most of the selected hypertensive case patients had a strong genetic predisposition to the disease.

Allelic frequencies of each RFLP as well as the haplotype frequencies estimated from the combination of these three RFLPs in the hypertensive subjects were compared with the frequencies calculated in the normotensive subjects. No statistical difference for the allelic and haplotype frequencies were found between the two groups. Two other studies concerning renin gene RFLPs in human hypertensive subjects have been reported previously. Morris et al7 retrospectively compared 29 subjects receiving hypertensive drugs to 202 adult white subjects and did not find an association between hypertension and the renin gene alleles. However, no familial data and no other clinical precisions were available and renin

### Table 4. Estimated Haplotype Frequencies Defined by the Combination of the Three Restriction Fragment Length Polymorphism Sites

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>Estimated frequency (n=204)</th>
<th>Estimated frequency (n=240)</th>
<th>Estimated frequency (n=444)</th>
<th>N*</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIT</td>
<td>0.429</td>
<td>87.5</td>
<td>0.453</td>
<td>108.7</td>
</tr>
<tr>
<td>Hit</td>
<td>0.085</td>
<td>17.3</td>
<td>0.035</td>
<td>8.4</td>
</tr>
<tr>
<td>HiT</td>
<td>0.177</td>
<td>36.1</td>
<td>0.195</td>
<td>46.8</td>
</tr>
<tr>
<td>Hi</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>hiT</td>
<td>0.277</td>
<td>56.5</td>
<td>0.245</td>
<td>58.8</td>
</tr>
<tr>
<td>hit</td>
<td>0.022</td>
<td>4.5</td>
<td>0.042</td>
<td>10.1</td>
</tr>
<tr>
<td>hi</td>
<td>0.009</td>
<td>1.8</td>
<td>0.020</td>
<td>4.8</td>
</tr>
<tr>
<td>hut</td>
<td>0.000</td>
<td>0.0</td>
<td>0.010</td>
<td>2.4</td>
</tr>
<tr>
<td>Loglik</td>
<td>222.4</td>
<td>269.8</td>
<td></td>
<td>495.6</td>
</tr>
</tbody>
</table>

For haplotypes, capital letters designate the frequent allele. HIT, Hit, HiT for the HindIII, Taq I, and HinfI polymorphisms, respectively. N*, expected number under the hypothesis of independence between markers. Comparison between normotensive and hypertensive subjects: \( \chi^2 = 2 \times (495.6 - 222.4 - 269.4) = 7.8 \), NS.
alleles were defined by a single RFLP (HindIII). Another study was performed on a single large Utah pedigree with high prevalence of hypertension.8 The authors found neither an association between renin genotypes and blood pressure level nor a relation between these polymorphisms and plasma renin activity. However, the interest of the study of a single large family was counterbalanced by the limited number of subjects.

Our results are strengthened by a high polymorphism information content (0.65) obtained by the combined analysis of the three RFLPs, much higher than that which could be obtained from isolated RFLP analysis (0.164, 0.338, and 0.273 for the Taq I, HindIII, and HindIII RFLPs, respectively). Using three biallelic RFLPs, eight different haplotypes were expected. However, because of the linkage disequilibrium existing between the RFLPs, we only observed seven different haplotypes, and three of those accounted for 88% of all haplotypes (n=444). We found two haplotypes, combining HindIII and Taq I RFLPs, in different proportions between hypertensive case patients and normotensive control patients. However, this difference did not reach a statistical significance. Thus, the present study cannot exclude an effect of a rare allele of the renin gene present in a small subpopulation. The potency of such genetic studies would probably be increased with more polymorphic alleles. Indeed, a highly polymorphic genetic marker consisting of a variable number of tandem repeat has been described in the first intron of the rat renin gene,22 which also displays polymorphic microsatellite sequences of the (CA)(TG) type.23 Finally, recent progress in DNA amplification by the polymerase chain reaction and direct sequencing of amplified DNA make possible the design of studies looking directly for deleterious mutations in the renin gene responsible for a coding sequence modification or a modified gene expression. Such work should be performed in subjects for whom strong arguments suggest renin gene responsibility for hypertension development.

In conclusion, the present study suggests that the renin gene is not strongly implicated in the majority of hypertensive subjects. However, knowing the heterogeneity of human hypertension, we cannot rule out a strong effect of the renin gene in a few patients or a slight effect in many patients, both hypotheses that could only be demonstrated in a very large sample of hypertensive subjects. Future studies should lead to the detection of new genetic markers at the renin gene locus and should also test for association with other candidate genes involved in the pathogenesis of hypertension.

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References


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