Symposium

Lack of Correlation between Mbo I Restriction Fragment Length Polymorphism of Renin Gene and Essential Hypertension in Japanese

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Predisposition to essential hypertension is associated with gene polymorphisms of the renin angiotensin system (RAS). Gene polymorphisms of the angiotensinogen and angiotensin converting enzyme genes are known to be risk factors for hypertension, while few studies concerning the renin gene polymorphism have been published. In the present investigation, we carried out a case control study using a Japanese population to examine the genetic influence of the renin gene on the predisposition to hypertension. Patients (n=235) recruited from outpatients at Osaka University Hospital and diagnosed with essential hypertension or receiving long-term antihypertensive medication participated in the study. Normotensive control subjects (n=510) without a history of hypertension and without diabetes mellitus were recruited from the same population, and were sex-matched with experimental subjects. A polymorphism in intron 9 of the human renin gene was determined as the Mbo I restriction fragment length polymorphism (Mbo I-RFLP). There was no significant association between Mbo I-RFLP of the renin gene and predisposition to essential hypertension in Japanese (p>0.05, \( \chi^2 = 2.1 \)). These results suggest that the Mbo I (+) allele of the renin gene does not increase the risk for hypertension in Japanese. (Hypertens Res 2001; 24: 295-298)

Key Words: hypertension, renin gene, Mbo I-RFLP, case-control study

Introduction

Essential hypertension is a very common disease. Genetic susceptibility plays an important role in the development of essential hypertension, and it has been estimated that about 30% of blood pressure variance is genetically determined (1). The reverse genetic approach has revealed that some genetic variants, such as angiotensinogen, lipoprotein lipase, and \( \alpha \)-adducin gene polymorphisms, increase the risk for hypertension. In recent years, molecular-based genetic studies of the renin gene have been carried out in animals and humans (2-9). Both in rats and humans, the genetic predisposition to hypertension was confirmed mainly for gene polymorphisms in the renin angiotensin system (RAS) and \( \alpha \)-adducin gene variants (10-12), but has not been confirmed for a number of candidate genes (13). Renin is a key enzyme of RAS, and plays an important role in the regulation of blood pressure (BP) and circulating volume homeostasis. Although studies using rat hypertension models have indicated that the renin gene locus cosegregates with BP (3, 5-7), definite findings have not been obtained in human essential hypertension.

The human renin gene is an attractive candidate in the etiology of essential hypertension, because (a) renin is a limiting enzyme of the biosynthesis cascade leading to a strong vasoconstrictor, angiotensin II, (b) genetic studies

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have shown that renin is associated with the development of hypertension in some rat strains (5-7), and (c) blockade of RAS is highly effective in the treatment of most hypertensive patients, as illustrated by angiotensin I-converting enzyme inhibitors.

We have carried out a case-control study using the Mbo I RFLP of the renin gene, to examine its association with hypertension in the Japanese population.

**Methods**

**Population**

Patients with essential hypertension and control subjects were recruited from outpatients of the Department of Geriatric Medicine, Osaka University Medical School. All patients and control subjects were Japanese and gave informed consent for genetic analysis before participating in the research protocol, which was approved by the Hospital Ethics Committee. All patients (n=235) had a family history of hypertension in first-degree relatives and were diagnosed as having primary hypertension. Patients with secondary hypertension, diabetes mellitus, or apparent ischemic heart disease were excluded. The criteria for hypertension were defined as systolic blood pressure (SBP) higher than 140 mmHg, diastolic blood pressure (DBP) higher than 90 mmHg, or receipt of long-term antihypertensive therapy. Control subjects (n=510) without a history of hypertension or diabetes mellitus were recruited from the same population and were sex-matched with the patient population. We also excluded from the control group those subjects whose first-degree relatives had hypertension. Participants completed a standard questionnaire on personal medical history and family history of hypertension. BP was measured twice, after the subject had been sitting for 5 min.

**Determination of Genotypes**

DNA was extracted from 200 µl buffy coat using a QIAamp Kit (Qiagen, Valencia, USA). We designed a set of primers to amplify the flanking region of Mbo I-RFLP of the renin gene (14). Polymerase chain reaction (PCR) was carried out with 100 ng genomic DNA as a template using an Omni Gene DNA thermal cycler (Hybaid Ltd., Middlesex, UK). The PCR protocol was as follows: initial denaturation at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 68°C for 30 s, and 72°C for 30 s. PCR products were digested with 1 U Mbo I (New England Biolabs, Beverly, USA) at 37°C for a period between 3 h and overnight. All digested products (20 µl) were separated on 3.0% MetaPhor agarose gel (FMC BioProducts, Rockland, USA) and visualized with ethidium bromide staining. The Mbo I (+) and Mbo I (−) alleles were detected as 79 and 171 bps fragments or a 250 bps fragment, respectively (14).

**Statistical Analysis**

All statistical analyses were conducted using StatView 4.5J (Abacus Concepts, Berkeley, USA) and JMP 3.1.5 (SAS Inst., Cary, USA). The difference in genotype distribution between the patients and control subjects was examined by χ² analysis. The association between polymorphism and each value of classical risk factors for hypertension was examined by one-way ANOVA. To assess the quantitative effects of the covariates (sex, age, body mass index and triglyceride concentration), multiple logistic regression analysis was performed using JMP.

**Results**

The clinical data of the patients and control subjects are summarized in Table 1. The hypertensive group had higher BP (SBP and DBP), BMI (body mass index) and triglyceride concentration, but was younger than the control group. However, no significant difference was observed in the values of other risk factors for hypertension, such as total cholesterol or fasting plasma glucose concentration, between the hypertensives and normotensives.

The genotypes of all subjects were clearly determined by PCR-RFLP. The genotype distribution did not significantly deviate from Hardy-Weinberg’s expectation (p>0.05, χ²=2.1) in either the hypertensive or control group.
Table 2. Distribution of Genotype and Allele for Mbo I-RFLP of Renin Gene in Hypertensive and Normotensive Subjects

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>+/+</td>
</tr>
<tr>
<td>Hypertensives (n=235)</td>
<td>5 (2.0%)</td>
</tr>
<tr>
<td>Normotensives (n=510)</td>
<td>20 (3.9%)</td>
</tr>
</tbody>
</table>

Odds ratio = 0.53 (95% CI: 0.2 - 1.4).

Table 3. Association between Renin Gene Mbo I-RFLP Genotype and Clinical Characteristics

<table>
<thead>
<tr>
<th>Genotype</th>
<th>+/-</th>
<th>+/-</th>
<th>-/-</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensives</td>
<td>64.6 ± 4.5</td>
<td>62.0 ± 1.2</td>
<td>60.5 ± 0.8</td>
<td>64.6 ± 1.2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>166.2 ± 8.0</td>
<td>180.2 ± 2.2</td>
<td>179.2 ± 1.4</td>
<td>198.2 ± 2.4</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>101.6 ± 5.2</td>
<td>104.9 ± 1.5</td>
<td>104.1 ± 0.9</td>
<td>104.9 ± 1.5</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>65.8 ± 2.2</td>
<td>65.1 ± 0.8</td>
<td>64.5 ± 4.5</td>
<td>65.1 ± 0.8</td>
</tr>
<tr>
<td>Normotensives</td>
<td>112.3 ± 2.3</td>
<td>112.5 ± 0.8</td>
<td>112.5 ± 0.6</td>
<td>112.5 ± 0.6</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.7 ± 1.7</td>
<td>70.6 ± 0.6</td>
<td>71.2 ± 0.4</td>
<td>70.6 ± 0.6</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>112.3 ± 2.3</td>
<td>112.5 ± 0.8</td>
<td>112.5 ± 0.6</td>
<td>112.5 ± 0.6</td>
</tr>
<tr>
<td>DBP (mmHg)</td>
<td>70.7 ± 1.7</td>
<td>70.6 ± 0.6</td>
<td>71.2 ± 0.4</td>
<td>70.6 ± 0.6</td>
</tr>
</tbody>
</table>

(Table 2).

The Mbo I (+) allele frequency in Japanese hypertensive patients (17%) was significantly lower than that in United Arab Emirates (UAE) hypertensive patients (51%). In the normotensives, the allele frequency of Mbo I (+) in Japanese (19%) was also lower than that in UAE (36%) (14), but was similar to that in North Americans (20%), as shown in Fig. 1 (15).

At the same time, we analyzed the association between the Mbo I-RFLP genotype (+/-, +/-, -/-) and clinical characteristics (age, SBP and DBP). However, there were no significant associations between them (Table 3). In addition, multiple logistic regression analysis did not show any significant association between renin gene polymorphism and hypertension after adjusting for the effect of confounding factors (data not shown).

Discussion

Many epidemiological studies have suggested that a genetic component strongly contributes to essential hypertension. Renin is a key enzyme of the renin-angiotensin-aldosterone system, and the renin gene locus has long been examined as a candidate gene for salt-sensitive hypertension using genetically hypertensive rat models. In addition, recent studies have shown the development of severe hypertension in transgenic rats harboring the mouse Ren-2 gene (16).

On the other hand, there have been only a few reports on the association between the renin gene RFLP and genetic predisposition to essential hypertension. Morris and Griffiths (17) failed to find an association between hypertension and the renin alleles in 29 hypertensive patients and 202 normotensive subjects. Similar negative results were reported by Soubrier et al. (18) and Berge et al. (19). And several studies using the sib-pair method have reported a lack of association between the renin gene locus and hypertension (20-22). However, the statistical power of the sib-pair method is weaker than that of association study, and the latter has a greater chance of detecting relationships between the slight effects of genetically predisposing factors and such multifactorial diseases as hypertension. In addition, it is known that the genotype distribution of a given polymorphism is heterogeneous among different races. We therefore considered it worthwhile to perform an association study comparing the genetic involvement of a single polymorphism among different races.

In a study using only 25 hypertensives and 20 normotensives, Okura et al. first reported that Mbo I-RFLP of the renin gene was a risk factor for family history of hypertension (23). Frossard et al. (14) used 331 hypertensive and 279 normotensive subjects, and found an association between hypertension and the renin Mbo I (+) allele in UAE. In contrast, no association between hypertension and the renin Mbo I (+) allele was detected in the current study. The reason for this might be that the methods of RFLP were different, and/or that renin RFLP shows significant racial difference (24). Indeed, the Mbo I (+) allele frequency is significantly different among races (Fig.
1. Another report by Okura et al. also showed no association between hypertension and the Mbo I-RFLP renin allele in a Japanese family with a high incidence with essential hypertension (25). Even if we cannot rule out the possibility that the renin gene polymorphism played a minor role, we conclude that the Mbo I (+) allele of the renin gene is not a major genetic predisposing factor for essential hypertension in Japanese.

References


