Association Analysis of Restriction Fragment Length Polymorphism for \( \alpha_2 \)-Adrenergic Receptor Genes in Essential Hypertension in Japan

Satoshi Umemura, Nobuhito Hirawa, Tamio Iwamoto, Satoshi Yamaguchi, Yoshiyuki Toya, Shunichi Kobayashi, Izumi Takasaki, Gen Yasuda, Kouichi Tamura, Masao Ishii, Lu Sun, William A. Pettinger

Abstract

Recently, restriction fragment length polymorphism (RFLP) of \( \alpha_2 \)-adrenergic receptor gene (\( \alpha_2 \)-C10) digested with \( Bsu361 \) restriction enzyme has been reported in US populations. Therefore, we examined the association of this RFLP with essential hypertension by comparing the frequency of specific alleles for this gene in Japanese populations. The distribution of this RFLP was compared with that in US populations. Subjects were hypertensive patients with a family history of essential hypertension (n=56) and normotensive subjects whose parents had no history of essential hypertension (n=46). DNA was prepared from leukocytes. RFLP was determined by use of Southern blot analysis with an \( \alpha_2 \)-C10 probe and \( Bsu361 \). The frequencies of the major (12-kb) and minor (5.8-kb) alleles were 0.30 and 0.70 in hypertensive patients and 0.38 and 0.62 in normotensive subjects, respectively. The difference between observed alleles in all subjects in each group was not significant (*) 2=1.33, \( P > .1 \). The difference between the overall allelic frequency in Japan and that reported in US populations was significant. This study found no evidence for an association between \( \alpha_2 \)-adrenergic receptor gene/\( Bsu361 \) RFLP and essential hypertension in Japan. However, the findings showed that the allele frequency in Japan differed from that reported in US populations. (Hypertension. 1994;23 [suppl I]:I-203-I-206.)

Key Words
- \( \alpha_2 \)-adrenergic receptors
- hypertension, primary
- restriction fragment length polymorphism
- ethnicity

Several lines of evidence suggest that the \( \alpha_2 \)-adrenergic receptor may play a pathogenic role in primary hypertension in rats as well as humans. The \( \alpha_2 \)-adrenergic receptors in the kidney and central nervous system have been suggested to play important roles in the regulation of blood pressure. The density of cerebral and renal \( \alpha_2 \)-adrenergic receptors is increased in genetically hypertensive rats even before their blood pressure becomes elevated, but not in animal models of acquired hypertension. In humans, platelet \( \alpha_2 \)-adrenergic receptor density is better correlated in monozygotic twins than in dizygotic twins (in whom receptor number correlates better than in age-matched random pairs), suggesting that the density of \( \alpha_2 \)-adrenergic receptors can be inherited. This platelet \( \alpha_2 \)-adrenergic receptor may be altered in patients with essential hypertension as well as in young normotensive subjects who have a family history of essential hypertension.

Recently, at least three \( \alpha_2 \)-adrenergic receptor genes have been cloned in humans. The molecular classification of \( \alpha_2 \)-C10, \( \alpha_2 \)-C4, and \( \alpha_2 \)-C2, which localized in human chromosomes 10, 4, and 2, has been suggested to correspond to the pharmacologic classification of \( \alpha_2 \)-A, \( \alpha_2 \)-C, and \( \alpha_2 \)-B adrenergic receptors, respectively. Using cDNA, two restriction fragment length polymorphisms (RFLPs) of the \( \alpha_2 \)-adrenergic receptor gene in humans have been reported. Furthermore, Sun et al. using an \( \alpha_2 \)-C10 probe representing the human platelet \( \alpha_2 \)-adrenergic receptors, reported the frequency of \( \alpha_2 \)-adrenergic receptor RFLPs in normotensive subjects and hypertensive patients in US populations.

Therefore, we examined the association of these RFLPs with essential hypertension by comparing the frequency of specific alleles for this gene in Japanese populations. The distribution of these RFLPs was compared with that reported in US populations.

Methods

Subjects
All subjects were adult Japanese of either sex. The diagnosis of hypertension was based on the criteria of a systolic blood pressure greater than 140 mm Hg and a diastolic pressure greater than 90 mm Hg on at least three readings on at least two visits. To diagnose essential hypertension and exclude secondary hypertension, all patients were admitted to the Yokohama City University Hospital for evaluation.

Fifty-six patients with essential hypertension, 58.5±11.3 years old (range, 27 to 77 years), and 46 normotensive subjects, 47.7±15.9 years old (range, 20 to 72 years), were invited to participate in the study. A detailed history was taken for each...
subject. Only patients with essential hypertension and a family history of hypertension were enrolled in this study. Normotensive subjects were selected on the basis of both parents also being normotensive. Patients with an uncertain family history of hypertension were excluded from the study. All subjects were informed about the study protocol and subsequently provided informed consent.

**DNA Extraction and RFLP Analysis**

Blood samples of approximately 10 mL were drawn into heparinized tubes and used for the separation of white blood cells. Genomic DNA was extracted from peripheral leukocytes of the hypertensive patients and normotensive subjects as previously described. Human genomic DNA (10 μg) was digested overnight with 40 U Bsu36I restriction endonuclease at 37°C. The products were separated by electrophoresis on a 1% agarose gel and transferred to nylon membranes. The filter membrane was baked for 2 hours at 80°C. Prehybridization was performed in a solution containing 6x saline/sodium phosphate/EDTA buffer (SSPE), 10× Denhardt’s solution, 1% sodium dodecyl sulfate (SDS), and 50 μg/mL salmon sperm DNA at 42°C for 4 hours. After prehybridization, the solution was changed to the hybridization solution containing 6x SSPE, 5x Denhardt’s solution, 0.5% SDS, 100 μg/mL salmon sperm DNA, 50% deionized formamide, 10% dextran sulfate, and [32P]deoxy-adenosine triphosphate-labeled DNA probes and incubated for 18 to 24 hours at 42°C. The 950-bp PstI restriction fragment of the human platelet α2-adrenergic receptor gene (α2-C10) was used as a probe as previously reported in US populations. This PstI restriction fragment is from the coding region of α2-C10 and runs from base 151 to base 1099. The filters were washed twice in 3x standard saline citrate (SSC) and 0.1% SDS at 55°C for 30 minutes followed by once in 0.1x SSC/0.1% SDS at 55°C for 10 minutes. The filters were exposed to Kodak XAR-5 film with a Lightning Plus intensifying screen for several days at -70°C.

**Statistical Analysis**

Data were compiled according to genotype, and allele frequencies were calculated. Statistical analysis required calculation of the observed number of alleles from genotype data in each group. Differences between groups were tested by χ² analysis with one degree of freedom. χ² analysis was also used for the comparison between Japanese and US populations. A value of P<.05 was considered significant.

**Results**

The three possible patterns of hybridization of the α2-C10 to a Southern blot of human genomic DNA digested with Bsu36I restriction endonuclease are shown in the Figure. Homozygotes for the Bsu36I RFLP have either a 12-kb or a 5.8-kb band, and heterozygotes have both the 12-kb and 5.8-kb bands. Nondifferentiating additional bands were also seen in the Southern blots.

Genotype and derived allele frequencies are shown in the Table. In examining data for normotensive and hypertensive groups, the correct data for analysis were the observed numbers of alleles, ie, the sum of the number of alleles on each chromosome of all of the subjects in each group (eg, for the hypertensive group, the 12-kb allele was [2×7]+20=34). By χ² analysis of observed alleles, no significant difference was apparent between the hypertensive and normotensive groups in Japanese populations. Because blood pressure elevation usually starts after the age of 40 years in patients with essential hypertension, we also analyzed the data only from subjects more than 40 years old. However, we again found no significant difference in the frequency of observed alleles between the hypertensive and normotensive groups (data not shown).

The allele frequencies were compared between the US and Japanese populations. The overall allele frequencies in Japanese differ from those reported in US populations (0.34 versus 0.66 in Japanese and 0.49 versus 0.51 in the United States, 12-kb versus 5.8-kb, respectively).

<table>
<thead>
<tr>
<th>Genotype (kb)</th>
<th>Allele Frequencies (kb)</th>
<th>Total Alleles on All Chromosomes (kb)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>12</td>
<td>12+5.8</td>
</tr>
<tr>
<td>Hypertensive (n=56)</td>
<td>7</td>
<td>20</td>
</tr>
<tr>
<td></td>
<td>0.30</td>
<td>0.70</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normotensive (n=46)</td>
<td>8</td>
<td>19</td>
</tr>
<tr>
<td></td>
<td>0.38</td>
<td>0.62</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

χ²=1.33 (P>.1)

RFLP indicates restriction fragment length polymorphism.
respectively; $\chi^2=9.40, P<.01$). The same probe and restriction enzyme were used to examine the frequency of distribution of allelic polymorphisms of the $\alpha_2$-adrenergic receptor gene in normotensive ($n=47$) and hypertensive ($n=60$) humans in the US study.\textsuperscript{11}

**Discussion**

In the present study, we found no association of an $\alpha_2$-adrenergic receptor RFLP with essential hypertension in Japan using an $\alpha_2$-C10 probe and Bsu36I digestion. However, we found that the allele frequency in Japanese differed from that reported in US populations.

Because essential hypertension is an inherited disease, many investigators are trying to find a gene responsible for this disease. The angiotensinogen,\textsuperscript{13} insulin receptor,\textsuperscript{14} and angiotensin converting enzyme\textsuperscript{15} genes have been reported to be candidates using techniques similar to those used in the present study. However, results are not yet consistent.\textsuperscript{16}

Altered $\alpha_2$-adrenergic receptors have also been suggested as a possible pathogenic factor in the genetic predisposition to hypertension.\textsuperscript{1} In humans, the density of $\alpha_2$-adrenergic receptors in platelets can be inherited,\textsuperscript{4} and this density has been reported to be altered in patients with essential hypertension as well as in young normotensive subjects with a family history of essential hypertension.\textsuperscript{1-2,7} Thus, we compared the $\alpha_2$-adrenergic receptor genes in patients with essential hypertension and normotensive control subjects by use of RFLP. Recently, the RFLPs of human $\alpha_2$-adrenergic receptors have been reported.\textsuperscript{9,10} With these RFLPs, association studies have been performed by others.\textsuperscript{11,17} However, both prior studies demonstrated no difference in the frequencies of $\alpha_2$-adrenergic receptor RFLPs between patients with essential hypertension and normotensive subjects. One study\textsuperscript{11} used RFLPs shown by Bsu36I digestion and an $\alpha_2$-C10 probe (950 bp) in a US population, and the other\textsuperscript{17} used DruI digestion and the platelet $\alpha_2$-adrenergic receptor gene (1500 bp) in an Australian population. Our results also revealed no association between $\alpha_2$-adrenergic receptor RFLPs and essential hypertension in a Japanese population.

This lack of association indicates that the particular DNA changes causing these polymorphisms are not themselves responsible for hypertension. However, we cannot rule out the possibility that polymorphism of this gene might be associated with essential hypertension if other detection methods could be used. The distribution of $\alpha_2$-adrenergic receptor alleles did not differ between the hypertensive patients and the normotensive subjects. We calculated that approximately 270 participants would be needed to see significant differences in gene frequency based on this study.

There are additional nondifferentiating bands in the Southern blots (Figure). The actual reason for the existence of these bands is not known. One possibility is the cross-hybridization between $\alpha_2$-C4 and $\alpha_2$-C10, because a previous report showed the same bands and further demonstrated that the 3' end probes (corresponding to the third cytoplasmic loop, which shares less sequence homology) from both $\alpha_2$-C10 and $\alpha_2$-C4 genes identified the RFLP.\textsuperscript{11} Additionally, after hybridization of the $\alpha_2$-C2 probe, they failed to identify the RFLP as seen with the other two probes.\textsuperscript{11} Therefore, these results might suggest that the observed polymorphism could exist in $\alpha_2$-C10 or $\alpha_2$-C4 adrenergic receptor genes.

There was a significant association between allelic frequencies and ethnic group for these RFLPs. Similar ethnic differences have been reported in renin gene RFLP between Afro-Caribbeans and Europeans.\textsuperscript{18} The actual meaning of these ethnic differences is unknown. However, these differences are interesting because the reports from the United States\textsuperscript{1} show increased $\alpha_2$-adrenergic receptor density compared with normotensive control subjects with no patient manipulation, which is different from reports from Japan\textsuperscript{5-7,19} showing that an alteration of $\alpha_2$-adrenergic receptor density in patients with essential hypertension can be demonstrated by manipulations such as salt restriction\textsuperscript{4,6} and standing stress.\textsuperscript{7}

In summary, the present study could find no evidence for an association of essential hypertension with $\alpha_2$-adrenergic receptor RFLP in Japanese. However, the allele frequency in Japan was different from that reported in US populations.

**Acknowledgments**

This study was supported in part by Grants-in-Aid 1004412 and 02670404 for Scientific Research from the Ministry of Education, Science and Culture, Japan. We thank Dr John Regan for kindly providing the $\alpha_2$-C10 probe and C. Hayashi for secretarial help.

**References**

10. Hoehe MR, Berrettini WH, Regan JW. A biallelic RFLP of the human $\alpha_2$-C4 adrenergic receptor gene (ADR2R2L2) localized on the short arm of chromosome 4 and encoding the putative $\alpha_2$ receptor is identified with Bsu 36 I using a 1.5 kb probe (pADRA2R2L2). *Nucleic Acids Res.* 1989;17:10148.


Association analysis of restriction fragment length polymorphism for alpha 2-adrenergic receptor genes in essential hypertension in Japan.
S Umemura, N Hirawa, T Iwamoto, S Yamaguchi, Y Toya, S Kobayashi, I Takasaki, G Yasuda, K Tamura and M Ishii

Hypertension. 1994;23:I203
doi: 10.1161/01.HYP.23.1_Suppl.I203

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1994 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/23/1_Suppl/I203