Symposium

Association Study between the Variants of the Human ANP Gene and Essential Hypertension

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Variants of atrial natriuretic peptide (ANP) are reported to be more common in blacks with hypertension than in normotensive controls and constitute an independent risk factor for cerebral infarction. The purpose of the present study was to investigate the role of ANP in the pathogenesis of essential hypertension (EH) in the Japanese. We investigated 2 previously reported ANP gene markers, G1837A and T2238C, for their possible associations with EH. A total of 233 individuals with EH and 213 age-matched normotensive (NT) control subjects were studied. The frequencies of the G and A alleles were 0.09 (42/466) and 0.91 (424/468), respectively, for the NT group and 0.11 (47/426) and 0.89 (379/426), respectively, for the EH group. These frequencies did not differ significantly between the two groups. The frequencies of the T and C alleles were 0.024 (11/466) and 0.97 (455/468), respectively, for the NT group and 0.03 (13/426) and 0.97 (413/426), respectively, for the EH group. These frequencies also did not differ significantly between the two groups. Neither G1837A nor the T2238C polymorphism of the ANP gene was associated with EH. Our findings do not support the hypothesis that the G1837A and T2238C polymorphisms of the ANP gene are markers for EH in the Japanese. (Hypertens Res 2001; 24: 291-294)

Key Words: atrial natriuretic peptide, polymorphism, association, essential hypertension

Introduction

Atrial natriuretic peptide (ANP) is an endogenous vasodilator produced mainly in the cardiac atria and released into the circulation in response to volume expansion (1). This peptide controls sodium-water balance (1) and exerts inhibitory (or antagonistic) effects on the renin-angiotensin-aldosterone system (2-5) and other vasoactive components, including vasopressin (6, 7) and catecholamines (8, 9), thereby acting as an anti-hypertensive hormone. Investigators have shown that reducing the production of ANP in mice leads to salt-sensitive hypertension (10). Homozygous mutants had no circulating ANP, and their blood pressures were elevated when the mice were fed standard and intermediate salt diets. In contrast, heterozygotes were normotensive with normal levels of ANP and became hypertensive only when fed a high-salt diet. These findings suggest that ANP is involved in the development of salt-sensitive hypertension and support further investigation of genetic variants within the human ANP system. Several biallelic variants of the ANP gene have been identified in humans (11, 12). Association of a restriction fragment length polymorphism in intron 2 of ANP has recently been reported in African-Americans with high blood pressure (13). Mutations in the stop codon appear to occur at equal frequencies in persons with and without hypertension. These findings are of clinical importance because human hypertension is a multifactorial disease, and patients cannot be treated as if it is a single disease entity. We studied two polymorphic regions within the ANP gene in a Japanese population with
Subjects and Methods

Subjects

Study subjects were 233 patients, aged 50 ± 8 years (mean ± SD), with EH (grade 2 and 3) diagnosed according to the WHO/ISH criteria. Sitting systolic blood pressure (SBP) was greater than 160 mm Hg and/or diastolic blood pressure (DBP) was greater than 100 mm Hg. Individuals diagnosed with secondary hypertension were excluded. The NT group consisted of 213 healthy subjects aged 49 ± 10 years. NT subjects had no family history of hypertension; SBP was less than 130 mm Hg, and DBP less than 85 mm Hg. A positive family history was defined as hypertension diagnosed in a grandparent, parent, or sibling. All subjects were recruited from the northern part of Tokyo, and informed consent was obtained from each individual according to a protocol approved by the Ethics Committee of Nihon University School of Medicine (12).

Determination of the Sma I and Sca I ANP Genotypes

Genomic DNA was extracted from peripheral white blood cells by standard methods. For the characterization of the ANP genotype, we used 2 previously described markers, G1837A and T2238C. G1837A is located in the second intron of the ANP gene at position 1837 of the published sequence (13). The presence of either a guanine or an adenine results in the presence or absence, respectively, of a Sma I site. The T2238C polymorphism is located within the third exon at position 2238. The C variant instead of the wild-type T changes the sequence of tga, the stop codon, to the cga resulting in a peptide with 2 supernumerary arginine residues at its carboxy terminal. This C allele also lacks a wild-type recognition site for Sca I. For the G1837A marker, we amplified a 446-bp product from genomic DNA that contained either 4 (1837G) or 3 (1837A) Sma I restriction sites, respectively, using 2 flanking oligonucleotides (ANP-Sma I: 5'-CCCCCAACCCAGGCCTGATGACCCTCTG-3' and ANP-R Sma I: 5'-GGAAATCCAGGCCCCGGCCAGAGAT-3'). For the T2238C marker, we followed a previously reported procedure (13) using 2 flanking oligonucleotides (ANP-Sca I: 5'-GGCACACTCATACATGAACGTGAC-3' and ANP-A Sca I: 5'-GCAGCTGTCCTCTCTA-GGCCCA-3'). All polymerase chain reactions (PCR) were performed with an initial denaturation at 94°C for 3 min followed by 30 cycles of 94°C for 30 s, 63°C for 1 min, 72°C for 1 min, and a final extension of 72°C for 10 min. Digestion with the appropriate enzyme was carried out as recommended by the manufacturer (Gibico BRL, Tokyo, Japan). PCR products were loaded onto 1% agarose and 13% polyacrylamide gels for the G1837A and T2238C markers, respectively, and then visualized by ethidium bromide staining and UV illumination.

Statistical Analysis

Data are presented as the mean ± SD. Allele frequencies were calculated from the genotypes of all subjects. Hardy-Weinberg equilibrium was assessed by chi-square (χ²) analysis. Significant differences between the total number of alleles on all chromosomes for the EH and NT groups were assessed by χ² analysis with one degree of freedom. Differences in the clinical data between the EH and NT groups and between genotypes were assessed by analysis of variance (ANOVA) followed by Bonferroni's test. A value of less than 0.05 was considered to indicate statistical significance.

Results

Frequencies of Two Polymorphisms in the Human ANP Gene

The genotypes and allele frequencies of the G1837A and T2238C polymorphisms are shown in Tables 1 and 2. The heterozygosity indices of the G1837A and T2238C polymorphisms in the healthy subjects were 43.7% and 33.3%, respectively. The genotype distribution was in Hardy-Weinberg equilibrium and was not significantly different between the NT and EH groups (NT: χ²=0.21; EH: χ²=0.19).
Table 3. Subject Characteristics

<table>
<thead>
<tr>
<th></th>
<th>EH group</th>
<th>NT group</th>
<th>p</th>
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<tbody>
<tr>
<td></td>
<td>(n=233)</td>
<td>(n=213)</td>
<td></td>
</tr>
<tr>
<td>Age (years)</td>
<td>50±8</td>
<td>49±10</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>153/80</td>
<td>149/64</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mmHg)</td>
<td>170±20</td>
<td>115±13</td>
<td>&lt;0.0001</td>
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<tr>
<td>DBP (mmHg)</td>
<td>105±12</td>
<td>70±10</td>
<td>&lt;0.0001</td>
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<tr>
<td>Heart rate (beats/min)</td>
<td>78±13</td>
<td>72±11</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dl)</td>
<td>0.85±0.25</td>
<td>0.83±0.2</td>
<td>NS</td>
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<tr>
<td>Total cholesterol (mg/dl)</td>
<td>207±42</td>
<td>201±41</td>
<td>NS</td>
</tr>
<tr>
<td>HDL cholesterol (mg/dl)</td>
<td>56±24</td>
<td>56±17</td>
<td>NS</td>
</tr>
<tr>
<td>Uric Acid (mg/dl)</td>
<td>5.7±1.6</td>
<td>5.9±4.7</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>24.7±3.7</td>
<td>23.3±1.0</td>
<td>&lt;0.0001</td>
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</tbody>
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EH, essential hypertension; NT, normotensive control; SBP, systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index.

Association Study

Clinical characteristics of the EH and NT subjects are shown in Table 3. Systolic and diastolic blood pressures and body mass index (BMI) were significantly higher in the EH group than in the NT group. Age, pulse rate, plasma concentration of total cholesterol, serum concentrations of creatinine and uric acid were not different between the two groups. The distribution of the genotypes and the frequencies of the alleles of the G1837A and T2238C polymorphisms did not differ significantly between the NT and EH groups (Tables 1, 2).

Discussion

We did not find a significant association between the G1837A or T2238C polymorphisms of the ANP gene and hypertension in Japanese. This contrasts with the positive results for the Hpa II RFLP in black Africans (14) and the G664A and 1837A alleles for stroke victims (15). Further investigations will be needed to determine whether these findings are simply due to uncommon DNA polymorphisms in a specific population or are applicable to other ethnic groups. Halushka et al. (16) identified single-nucleotide polymorphisms in a series of candidate genes for BP homeostasis. For the ANP gene, they reported a total of six polymorphisms in European Americans and in black Africans. Although two of these alleles appeared to be specific for European Americans, two polymorphisms, G-191A and T1766C, were detected across the three ethnic groups. Given these findings, it will be of interest to investigate whether some ANP polymorphisms reported arose after human population differentiation. Our data do not support an association of the ANP gene with essential hypertension in the Japanese, but it is possible that gene variants may predispose a subgroup of the population to hypertension, influenced by some "intermediate" phenotype, such as salt sensitivity. The functional significance of any of the identified polymorphisms has not yet been determined. It should be noted that each of the uncommon variants has an allele frequency of less than 10% in Japanese subjects. Given the likely ethnic variation, there may be other yet undiscovered candidate mutations that are too rare to be detected in the Japanese but may be relatively common in other ethnic groups. Recent data from the offspring of patients with EH suggest that individuals genetically predisposed to hypertension may fail to increase plasma levels of ANP adequately under a high-salt diet (17, 18). Similarly, the increase in plasma ANP levels after salt loading was lower in salt-sensitive patients with EH than it was in controls (19), although this has not been confirmed by other investigators (20). Abnormal regulation of ANP release could not be proved in this study, since ANP levels were not examined. The localization of the Smo I variant in an intron makes it unlikely that it is of functional significance. Should future studies in other ethnic groups confirm the relationship between this variant and hypertension, further investigations will need to address whether this variant is of biological significance, influencing the release or activity of ANP.

References


