Angiotensin II Type 1 Receptor Gene Polymorphisms in Human Essential Hypertension

Alain Bonnardeaux, Eleanor Davies, Xavier Jeunemaitre, Isabelle Féry, Anne Charru, Eric Clauser, Laurence Tiret, François Cambien, Pierre Corvol, Florent Soubrier

Abstract  We conducted the present study to determine whether the angiotensin II type 1 receptor (AT1) gene might be implicated in human essential hypertension by using case-control and linkage studies. The entire coding and 3' untranslated regions of the AT1 receptor gene (2.2 kb) were amplified by polymerase chain reaction and submitted to single-strand conformation polymorphism in 60 hypertensive subjects with a familial susceptibility. We identified five polymorphisms (T573-»C, A1062-*G, A1878-»G, G1587-»T, and A1687-»G). However, no mutations that alter the encoded amino acid sequence were detected. A case-control study performed on white hypertensive (n=298; blood pressure, 168±16/103±9 mm Hg) and normotensive (n=298; blood pressure, 122±10/75±9 mm Hg) subjects using three of five polymorphisms showed a significant increase in allelic frequency of C1587 in hypertensive subjects (0.36 versus 0.28 for normotensive subjects, χ²=6.8, P<.01). Frequencies for the alleles of the other two polymorphisms (T573-»C, A1878-»G) were similar in both groups. We performed a linkage study using the affected sib pair method and a highly polymorphic marker of the AT1 receptor gene. There was no evidence for linkage in 267 sib pairs analyzed from 138 pedigrees. These findings would be compatible with a common variant of the AT1 receptor imparting a small effect on blood pressure; further studies will be needed to address this possibility.

Key Words  • receptors, angiotensin • hypertension, essential • genetics • renin-angiotensin system • 3q22 • angiotensin II

Human essential hypertension is thought to result from the interaction of environmental and genetic factors, with approximately 30% of the interindividual variability in blood pressure being genetically determined.¹ The renin-angiotensin system is an important component of blood pressure regulation, playing roles in saltwater homeostasis and vascular tone,² and has been suspected to be involved in hypertension. Indeed, evidence for a genetic linkage of human essential hypertension to the angiotensinogen locus was recently obtained in an extensive collaborative study.³ However, linkage and association studies of the human renin and angiotensin I (Ang I)-converting enzyme loci have given negative results.⁴⁻⁹

Ang II receptors, which mediate the vasoconstrictive and salt-conserving actions of the renin-angiotensin system, also represent interesting candidate genes for essential hypertension. Two subtypes of cell surface receptors have been identified (AT1 and AT2) using ligand binding studies.¹⁰ In humans, the AT1 receptor is present predominantly in vascular smooth muscle cells, and the AT2 receptor is present in the uterus, brain, and adrenal medulla. Both subtypes are also expressed in the adrenal cortex and kidney. The AT1 receptor, through which are exerted most of the actions of Ang II, is a G protein-coupled receptor spanning seven transmembrane domains, and the cDNA and gene encoding human AT1 have been cloned.¹¹⁻¹³ The mouse and rat AT1 receptor cDNAs¹³⁻¹⁴ have been recently cloned and also represent G protein-coupled seven-transmembrane domain receptors whose functions have not been clearly established.

Several studies suggest that the AT1 receptor might be involved in hypertension: (1) Spontaneously hypertensive rats have an enhanced AT1 receptor-mediated renal vascular responsiveness to Ang II infusion but not to periarterial renal nerve stimulation compared with Wistar-Kyoto rats;¹⁵ (2) some patients with hypertension present with an enhanced pressor responsiveness to infused Ang II;¹⁶ (3) in humans, altered AT1 receptor function/regulation could play a role in non-modulators, a subgroup of hypertensive individuals who fail to appropriately modulate renal vascular and adrenal reactivity in response to Ang II infusion with changes in dietary sodium;¹⁷ and (4) although probably a distinct hypertensive phenotype, pregnancy-induced hypertensive patients have an enhanced pressor response to Ang II and increased platelet AT1 receptor number.¹⁸

In the present study we assessed whether the AT1 receptor gene might be involved in human essential hypertension by directly studying AT1 receptor genotypes in hypertensive patients, because all potential intermediate phenotypes of the AT1 receptor are difficult to quantify in a large-scale study. We looked for sequence variations within the AT1 receptor gene by screening the entire coding and 3' untranslated regions and conducted case-control and linkage studies on a large group of subjects with familial essential hypertension.
Methods

Patient Population

Hypertensive Index Cases and Sibships With Multiple Hypertensive Subjects

This study was approved by an institutional review committee, and the subjects recruited gave informed consent. A total of 206 white index cases (101 men, 105 women) were selected at the Hypertension Clinic of Broussais Hospital in Paris (n=173) and at several centers in the Bordeaux and Toulouse areas (n=33) according to the following criteria: (1) age more than 20 years, (2) onset of hypertension less than 60 years, (3) absence of antihypertensive treatment, and (4) history of hypertension as defined by long-term treatment or diastolic blood pressure greater than 95 mm Hg on two consecutive visits for those untreated, (5) family history of hypertension (occurring before 60 years of age) with at least one parent and one sibling being affected. Subjects with a history of alcohol intake (more than three drinks per day), oral contraceptive therapy, diabetes mellitus, or renal failure were excluded. Blood pressure was measured with subjects in the supine position using a sphygmomanometer. Fifth phase Korotkoff sounds were taken as diastolic blood pressure.

Affected siblings of hypertensive index cases were screened according to the criteria mentioned above; the study sample comprised 344 hypertensive sibs (167 men, 177 women), yielding a total of 138 sibships composed of 97 pairs, 40 trios, 5 quartets, and 2 quintets representing 267 sib pairs.

Control Subjects

A group of 298 white normotensive control subjects was selected from the Broussais transfusion center (n=30) and from patients examined in preventive medicine centers in Paris (n=76) and in the Nancy region (n=192). Selection was made on the following criteria: (1) age more than 20 years, (2) systolic and diastolic blood pressures less than 140 and 90 mm Hg, respectively, and (3) absence of antihypertensive treatment.

Identification and Detection of Polymorphisms of the AT1 Receptor Gene

Enzymatic Amplification of Segments of the AT1 Receptor Gene

From the known genomic structure of the AT1 receptor gene,12 eight overlapping fragments of approximately 300 bp were enzymatically amplified to cover the entire coding and 3′ untranslated regions (see Table 1 for primers) using 50 ng DNA in a total volume of 5 μL containing 50 mmol/L KCl, 5 mmol/L Tris-HCl (pH 8.3), 1.5 mmol/L MgCl2, 50 μmol/L dNTPs, 10 pmol of each primer, and 0.5 U Taq polymerase (Boehringer). For single-strand conformation polymorphism, each primer was end labeled with [γ-32P]ATP.

Detection of Polymorphisms by Single-Strand Conformation Polymorphism

Polymerase chain reaction (PCR) products were enzyme restricted overnight by addition of 5 U of an appropriate enzyme to yield fragments of approximately 150 bp. Thereafter, products were diluted twofold in a solution containing 95% formamide, 10 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. After denaturation at 94°C for 4 minutes, the samples were placed on ice, and 4 μL was loaded onto nondenaturing 5% acrylamide gels (polyacrylamide-to-bisacrylamide ratio of 49:1). Three different conditions were used for electrophoresis: gels containing 5% formamide, 10 mmol/L EDTA, 0.05% bromophenol blue, and 0.05% xylene cyanol. After denaturation at 94°C for 4 minutes, the samples were placed on ice, and 4 μL was loaded onto nondenaturing 5% acrylamide gels. After electrophoresis at 4°C for 6 hours, the gels were stained with ethidium bromide and visualized under UV light. The gels were dried and autoradiographed overnight.

Direct Sequencing of Electrophoretic Variants

DNA from patients presenting with variant electrophoretic patterns was reamplified by PCR using the above conditions (unlabeled primers). PCR products were purified by 2% agarose gel electrophoresis and eluted using Geneclean (Bio 101). Asymmetric PCR (45 cycles) was performed with each.

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primer (sense and antisense) using one tenth of the original double-stranded template. The single-stranded template was purified with a Centricon 30 column (Amicon). Sequencing was performed in five rounds of PCR with [γ-32P]dATP end-labeled primers using a direct sequencing kit (Circumvent, New England Biolabs).

**Allele-Specific Oligonucleotide Hybridization**

To determine the genotypes for each polymorphism identified by single-strand conformation polymorphism and direct sequencing, we performed allele-specific oligonucleotide hybridization. After enzymatic amplification of genomic DNA using primers U3 and L3, PCR products were denatured in 0.4 mol/L NaOH and 25 mmol/L EDTA, blotted in duplicate onto nylon membranes (Hybond N+, Amersham), neutralized in 3 mol/L sodium acetate (pH 5.5), and cross-linked with ultraviolet light. Each membrane was then hybridized for 3 to 16 hours in 7% polyethylene glycol and 10% sodium dodecyl sulfate with [γ-32P]ATP end-labeled 15-mer oligonucleotide probes. These probes were TGG-CAACCCCTCGATA and CAG-CAACCCTCCCGATAG for polymorphism T573→C, A1166-AATTGAGGATTCAGTCA and C1156-ATGAGGCTTAGCTA for polymorphism A1156→C, and A1156-CAACATATATATGATTATGAT and G1158-ATACATATACATATCTG (reverse strand) for polymorphism A1158→G, corresponding to the sequences of each allele of the polymorphism. The membranes were washed twice at room temperature in 2x SSC and 0.1% sodium dodecyl sulfate, and for 10 minutes in 1x SSC at the following temperatures (°C): 48 and 50 for T573 and C573, 42 and 46 for A1156 and C1156, and 36 and 45 for A1158 and G1158, respectively.

**Genotyping of CA Alleles at the AT1 Receptor Locus**

AT1 receptor genotypes were established by enzymatically amplifying a 120-bp fragment comprising a highly informative dinucleotide repeat in the 3' flanking region of the AT1 receptor gene.11 The primers were designed as follows: 5'-AGGAGAATGTTCCAAGGACAA-3', 5'-GTTATTC-CATTGGAACAGCTTCA-3'; PCR was performed with 50 ng genomic DNA in a total volume of 25 μL containing 50 mmol/L KCl, 5 mmol/L Tris-HCl (pH 8.3), 0.01% gelatin, 1.5 mmol MgCl2, 50 μmol/L dNTPs, 10 μmol of each primer (with one primer end-labeled with [γ-32P]ATP), and 0.5 U Taq polymerase. After an initial denaturation step of 4 minutes at 94°C, 30 cycles of PCR consisting of 94°C for 20 seconds and 56°C for 30 seconds were carried out. Subsequently, 50 μL formamide was added to each reaction, and after denaturation at 94°C for 4 minutes, 5 μL was loaded on a 6% polyacrylamide gel containing 7 mol/L urea. Electrophoresis was carried out at 60 W for 4 hours, and gels were autoradiographed overnight. Allele frequencies were determined by genotyping the hypertensive index cases. A total of 10 alleles were detected, yielding a heterozygosity of 69% (polymorphism information content of 66%).

<table>
<thead>
<tr>
<th>Variant</th>
<th>Location</th>
<th>Position</th>
<th>Substitution</th>
<th>Allele Frequency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Coding</td>
<td>573</td>
<td>T→C</td>
<td>0.56</td>
</tr>
<tr>
<td>2</td>
<td>Coding</td>
<td>1062</td>
<td>A→G</td>
<td>0.03</td>
</tr>
<tr>
<td>3</td>
<td>3'</td>
<td>1166</td>
<td>A→C</td>
<td>0.36</td>
</tr>
<tr>
<td>4</td>
<td>3'</td>
<td>1517</td>
<td>G→T</td>
<td>1 ind</td>
</tr>
<tr>
<td>5</td>
<td>3'</td>
<td>1878</td>
<td>A→G</td>
<td>0.13</td>
</tr>
</tbody>
</table>

SSCP indicates single-strand conformation polymorphism; 1 ind, one individual presenting with the variant. Position of polymorphism given as position from start of coding exon. Allele frequencies given as fraction (variant from published sequence only).

**Statistical Analysis**

**Analysis of Genotype and Allele Frequencies for AT1 Receptor Variants**

For each biallelic marker, allele frequencies were calculated from genotype frequencies in the hypertensive and normotensive groups. Deviation from Hardy-Weinberg equilibrium was assessed by a χ2 test with 1 df. We used maximum likelihood methods23 to estimate haplotype frequencies. Differences in genotype distributions between hypertensive and normotensive control subjects were tested by a χ2 test with 2 df. Codominance was tested by the analysis of linear trends in proportions. No deviation from codominance was observed, so allele effect was tested assuming a codominant model (χ2 with 1 df).

**Analysis of Linkage in Hypertensive Sib Pairs**

Linkage analysis was performed with the affected sib pair method, a nonparametric test based on the analysis of affected members of a pedigree. This approach does not require any a priori assumptions on the model of inheritance and can accommodate genetic heterogeneity. In addition, entire pedigrees are not necessary for testing. In the present study, we calculated the frequency of each allele in the hypertensive group and used identity-by-state methods to calculate the expected proportions of alleles shared.23 The comparison between the observed and expected mean number of alleles shared by the siblings of each sibship was performed through a one-sided Student's t test. The weighting of each sibship size was performed according to Hodge.24

**Results**

**Association Between Hypertension and Polymorphisms of the AT1 Receptor Gene**

Sixty hypertensive subjects were selected in the initial search for molecular variants. Five polymorphisms were identified within the coding and 3' untranslated regions of the human AT1 receptor gene. Table 2 shows the positions of base substitutions. No mutations that alter the encoded amino acid sequence were detected. Genotypes for the three most frequent variants (T573→C, A1156→C, A1158→G) were then determined in the probands of the hypertensive families and in the control population. Because our control population was recruited from two distinct geographical areas (separated by 300 km), we verified that there were no differences in allele frequencies for the markers presented in this study (biallelic and CA repeat) as well as for polymorphisms M235T of the angiotensinogen gene and the insertion/deletion (I/D) of the Ang I-converting enzyme gene to exclude the possibility of a genetic heterogeneity between the normotensive subgroups that might provide spurious results. The χ2 tests per-
formed on allele frequencies yielded nonsignificant probability values of ≥0.46 for these markers.

Clinical parameters from both groups are presented in Table 3 and genotypes in Table 4. For each polymorphism, the genotype frequencies in the hypertensive and normotensive groups satisfied the Hardy-Weinberg equilibrium law. The C\textsuperscript{1166} was significantly more frequent in the hypertensive group compared with the normotensive control group (0.36 versus 0.28, \( \chi^2 = 6.8, P < 0.01 \)), with a further increase among the cases with an earlier age of onset of hypertension and more severe cases (Table 5). However, there were no significant differences in allele frequencies for polymorphisms T\textsuperscript{573} –> C and A\textsuperscript{1878} –> G or for the CA repeat alleles (\( \chi^2 = 5.7, P > 0.5, 1 \) df).

To determine whether the presence of a particular haplotype was associated with an increased odds ratio, we estimated AT\textsubscript{1} receptor haplotypes using maximum likelihood methods by combining polymorphism A\textsuperscript{1166} –> C on the one hand and polymorphisms T\textsuperscript{573} –> C, A\textsuperscript{1878} –> G, or the frequent \((q \geq 0.1)\) CA repeat alleles (A5, A6, and A7) on the other hand. C\textsuperscript{1166} was in complete linkage disequilibrium with C\textsuperscript{573}, with haplotype T\textsuperscript{573}-C\textsuperscript{1166} not detected in either population. Consequently, the frequency of haplotype C\textsuperscript{573}-A\textsuperscript{1166} was identical to the frequency of T\textsuperscript{573}. The frequency of haplotype C\textsuperscript{573}-A\textsuperscript{1166} could then be deduced from the frequencies of C\textsuperscript{573}-C\textsuperscript{1166} and T\textsuperscript{573}-A\textsuperscript{1166}.

TABLE 4. Comparison of Genotype and Allele Frequencies for Variants T\textsuperscript{573} –> C, A\textsuperscript{1166} –> C, and A\textsuperscript{1878} –> G in Normotensive and Hypertensive Subjects

<table>
<thead>
<tr>
<th>Variant</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
<th>Hypertensive</th>
<th>Normotensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Genotypes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>34 (16.5)</td>
<td>61 (20.5)</td>
<td>84 (40.8)</td>
<td>153 (51.3)</td>
<td>156 (75.1)</td>
<td>221 (74.2)</td>
</tr>
<tr>
<td>Aa</td>
<td>113 (54.9)</td>
<td>151 (50.7)</td>
<td>95 (46.1)</td>
<td>121 (40.6)</td>
<td>48 (23.5)</td>
<td>72 (24.2)</td>
</tr>
<tr>
<td>aa</td>
<td>59 (28.6)</td>
<td>86 (28.9)</td>
<td>27 (13.1)</td>
<td>24 (8.1)</td>
<td>2 (1.4)</td>
<td>5 (1.7)</td>
</tr>
<tr>
<td>q</td>
<td>0.56</td>
<td>0.54</td>
<td>0.36</td>
<td>0.28</td>
<td>0.13</td>
<td>0.14</td>
</tr>
<tr>
<td>Significance*</td>
<td>( \chi^2 = 1.42, P = \text{NS} )</td>
<td>( \chi^2 = 6.83, P &lt; .05 )</td>
<td>( \chi^2 = 0.52, P = \text{NS} )</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Odds ratios (vs AA)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>1.43</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>aa</td>
<td>2.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Significance†</td>
<td>( \chi^2 = 6.8, P &lt; .01 )</td>
<td></td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

Genotype frequencies are indicated in absolute values (percentages). Allele frequencies are indicated in fraction. "A" and "a" represent the two alleles of a polymorphism ("a" is the variant detected in this study).

*Test of comparison of genotype distributions (\( \chi^2 \) with 2 df).
†Test of allele effect assuming a codominant model (\( \chi^2 \) with 1 df).
### Table 5. Genotype and Allele Frequencies of Polymorphism A11M→C in Different Subsets of Hypertensive Subjects

<table>
<thead>
<tr>
<th>Genotypes</th>
<th>Age of Onset ≤40</th>
<th>BMI ≤25 kg/m²</th>
<th>Gender</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(≥Two Drugs or DBP ≥105)</td>
<td>Male</td>
<td>Female</td>
</tr>
<tr>
<td>AA</td>
<td>38 (35.2)</td>
<td>38 (38.2)</td>
<td>44 (43.5)</td>
</tr>
<tr>
<td>Aa</td>
<td>56 (51.9)</td>
<td>49 (50.0)</td>
<td>47 (46.5)</td>
</tr>
<tr>
<td>aa</td>
<td>14 (13.0)</td>
<td>11 (11.2)</td>
<td>10 (9.9)</td>
</tr>
<tr>
<td>q</td>
<td>0.39</td>
<td>0.36</td>
<td>0.33</td>
</tr>
</tbody>
</table>

Significance:
- $\chi^2=8.7\dagger$
- $\chi^2=10.4\dagger$
- $\chi^2=4.8\ddagger$
- $\chi^2=3.3\ddagger$
- $\chi^2=4.1\ddagger$

### Analysis of Linkage of AT1 Receptor in Hypertensive Sibships

A total of 138 sibships representing 344 hypertensive subjects (267 sib pairs) were collected. Clinical characteristics are shown in Table 3. A highly informative genetic marker based on a variable number of tandem repeats of the (CA) motif located in the 3' flanking region of the AT1 receptor gene was characterized in all study subjects.21 As shown in Table 6, there was no excess of alleles shared in the total group of hypertensive sib pairs. We also carried out the analysis in different subsets of hypertensive sib pairs, as defined in a previous study:6 As demonstrated in Table 7, there was no evidence of linkage in any of the subgroups analyzed (excess of alleles shared of 1.1%, $P=NS$). The upper limit of the 95% confidence interval of the present study suggests that at most 3% of alleles are shared in excess of expected in the total group and 10% in the subgroups detailed in Table 7.

### Discussion

In this study we used the candidate gene approach to determine whether the gene encoding the AT1 receptor, an important effector of the renin-angiotensin system, is involved in human essential hypertension. Because the coding region of the AT1 receptor gene is included within a single exon spanning 1 kb, we initially sought to identify molecular variants that might also be functional, using a sensitive mutation detection technique. Such functional variants of human G protein-coupled seven-transmembrane domain receptors have been described for the human thyrotropin and luteinizing hormone receptors26,27 as well as for the murine melanocyte-stimulating hormone receptor28 and result in a constitutive activation or hyperresponsiveness of the receptor. In the case of the AT1 receptor gene, a constitutive activation, even mildly effective, could lead to increased Ang II actions on target cells. Although we screened 60 probands of hypertensive families, we could not identify any functional variants in the coding region of the AT1 receptor gene. The sensitivity of single-strand conformation polymorphism for detecting polymorphisms in DNA fragments of approximately 150 bp ($≥95\%$), as was done here, would not suggest that such variants are frequent, although we cannot formally exclude the possibility that one or more variants were not detected.

However, these negative results do not exclude the possibility that molecular variants involving the regulation of the expression of the AT1 receptor gene could exist in hypertensive or normotensive populations. To test this hypothesis, we identified polymorphisms within the coding and 3' flanking regions and compared the genotypes between hypertensive and normotensive subjects for these polymorphisms. We found a significant increase in the allele frequency of the variant for a polymorphism located in the 3' flanking region (1166) of...
the AT₁ receptor gene in hypertensive subjects with a positive family history. There was a further increase in the frequency of this variant in subsets of hypertensive subjects with a more severe form or with an earlier age of onset, using the same criteria shown to characterize angiotensinogen as a gene involved in hypertension.3 This suggests that there is an association between the AT₁ receptor gene and that the severity increases the probability of finding the susceptibility polymorphism. No significant preferential distribution of this susceptibility polymorphism according to gender could be noted, however. The A₁₁⁶⁶→C transversion is located at the 5' end of the 3' untranslated region on the same exon as the open reading frame and does not alter a potential mRNA polyadenylation or destabilization signal. This polymorphism does not appear to be functional, so we postulate that it might be in linkage disequilibrium with an unidentified functional variant.

To support the data of the association study, we also performed a linkage analysis in hypertensive families using the affected sib pair method and a highly informative marker located in the 3' flanking region of the gene.2¹ This method is particularly well adapted for studying predisposing genes as it accommodates genetic heterogeneity.2³ Although we analyzed a large number of affected sibs, we could not demonstrate the presence of linkage. Several reasons could explain this observation.

First, it could be a methodological problem of testing linkage with a highly polymorphic marker whose different alleles bear the same common susceptibility variant (ie, for which both parents can be heterozygous or homozygous). For example, association between a minisatellite in the 5' flanking region of the insulin gene and diabetes mellitus was found,2⁰ but initial linkage studies were negative.2¹ By restricting linkage analysis to families in which one parent was heterozygous for the disease-associated allele, linkage was demonstrated.2² Family-based association tests can also be performed to resolve a situation in which association is suggested but linkage is not.3³,3⁴ However, such analyses require complete pedigrees and parental genotypes. As hypertension is a continuous trait appearing relatively late in life, the parents and entire pedigree frequently are not available for genotyping, and it is therefore not possible to perform these tests.

A second reason for finding the absence of linkage despite suggested association could be due to the lack of power of the affected sib pair method. This can be the case particularly when the susceptibility alleles are frequent and associated with a weak increase in the relative risk of disease. The AT₁ receptor gene could be a relatively weak susceptibility and not a necessary or sufficient locus for hypertension, in which case linkage studies can be negative despite association. This has been noted for the dopamine D2 receptor in alcoholism.3⁴ Furthermore, in our study the association was stronger in the severe hypertensive group for which there was a relatively small number of pairs in which both siblings fell into this category. In this respect, the upper limit of the 95% confidence interval of 10% of alleles shared in excess of expected for the sib pairs with severe or early-onset hypertension in our study does not allow us to exclude an effect on allele sharing in these subgroups but can be reasonably excluded on the total group. Thus, increasing the power of this study and potentially detecting linkage could have been done with more sib pairs with severe hypertension or, alternatively, by performing a sib pair analysis on a different subset of pedigrees in which a defined intermediate phenotype (density of platelet AT₁ receptors, response to AT₁ receptor antagonists, non-modulators) would be correlated to AT₁ receptor variants. Finally, as with any positive case-control study, a spurious association cannot be eliminated, and the absence of linkage is relevant only to the population studied as no inference can be drawn regarding the relevance of variants of this gene in other populations. Independent confirmation of the present findings will be of critical value.

In conclusion, this extensive study does not suggest that functional variants of the coding sequence of the human AT₁ receptor gene are a common cause of hypertension. Second, we noted the existence of an association between an AT₁ receptor gene polymorphism and human essential hypertension. This could be due to an unidentified functional variant involving the regulation of the expression of the gene in linkage.
disequilibrium with the marker used. Third, our sib pair study failed to demonstrate linkage, suggesting that this AT, receptor gene variant might be a weak genetic risk factor. Further studies are required to confirm these results using characterized individuals for an intermediate phenotype of the AT, receptor.

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References


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