Exclusion of Angiotensinogen Gene in Molecular Basis of Human Hypertension: Sibpair Linkage and Association Analyses in Australian Anglo-Caucasians

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Linkage with essential hypertension has been claimed for a microsatellite marker near the angiotensinogen gene (AGT; chromosome 1q42), as has association for the AGT variants M235T, G(−6)A and A(−20)C. To more rigorously evaluate AGT as a candidate gene for hypertension we performed sibpair analysis with multiple microsatellite markers surrounding this locus and using more sophisticated analysis programs. We also performed an association study of the AGT variants in unrelated subjects with a strong family history (two affected parents). For the linkage study, single and multiplex polymerase chain reaction (PCRs) and automated genescan analysis were conducted on DNA from 175 Australian Anglo-Celtic Caucasian hypertensives for the following markers: D1S2880−(2.1 cM)−D1S213−(2.8 cM)−D1S251−(6.5 cM)−AGT−(2.0 cM)−D1S235. Statistical evaluation of genotype data by nonparametric methods resulted in the following scores: Single-point analysis − SPLINK, P > 0.18; APM method, P > 0.25; ASPEX, MLOD < 0.28; SIB-PAIR, P > 0.24; Multipoint analysis − MAPMAKER/SIBS, MLOD < 0.24; GENEHUNTER, P > 0.35. Exclusion scores of Lod −4.1 to −5.1 were obtained for these markers using MAPMAKER/SIBS for a λs of 1.6. The association study of G(−6)A, A(−20)C and M235T variants in 111 hypertensives with strong family history and 190 normotensives with no family history showed significant linkage disequilibrium between particular haplotypes, but we could find no association with hypertension. The present study therefore excludes AGT in the etiology of hypertension, at least in the population of Australian Anglo-Celtic Caucasians studied. Am. J. Med. Genet. 87:53–60, 1999.

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KEY WORDS: angiotensinogen gene; linkage; association; caucasian; chromosome 1; hypertension, essential; microsatellite markers; sibpair analysis

INTRODUCTION

Essential hypertension is a common, complex, polygenic disease that affects one in five adults in Western society. The renin-angiotensin system is a key regulator of salt and fluid homeostasis and blood pressure. Such control could involve not just increased secretion of renin, which is rate limiting in the reaction, but also angiotensinogen, whose concentration in plasma is close to the $K_m$ [Gould and Green, 1971; Reid et al., 1978]. Since angiotensinogen is ~20% higher in patients with essential hypertension and shows a positive correlation with blood pressure [Walker et al., 1979; Bennett et al., 1993; Schrader et al., 1996] the angiotensinogen gene (AGT; chromosome 1q42 [Isa et al., 1990]) has attracted attention as a candidate gene for this condition. In support of such a possibility, a Utah/Paris study of affected sibpairs using a microsatellite marker located < 2.5 kilobase-pairs (kb) [Kotelevtsev et al., 1991] 3′ of AGT has demonstrated linkage with essential hypertension [Jeunemaitre et al., 1992]. Apparent confirmation was provided subsequently in a pedigree study by others [Caulfield et al., 1994]. However, a recent sibpair study of the same AGT marker failed to demonstrate linkage [Brand et al., 1998]. Case-control studies have also produced conflicting results. The initial report showed association of a

Grant sponsor: National Health and Medical Research Council of Australia.
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Received 29 March 1999; Accepted 13 July 1999

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Met235Thr (M235T) variant with hypertension [Jeunemaitre et al., 1992] and this has been confirmed in some [Jeunemaitre et al., 1993; Schmidt et al., 1995; Johnson et al., 1996; Krizanova et al., 1997; Schunkert et al., 1997], but not in other [Bennett et al., 1993; Caulfield et al., 1994; Fornage et al., 1995; Hingorani et al., 1995; Tiret et al., 1995; Hegele et al., 1996; Hingorani et al., 1996; Kiema et al., 1996; Beige et al., 1997] studies of Caucasian populations. A further study found no relationship with ambulatory blood pressure [Beige et al., 1997]. Another saw an association with nonmodulation in hypertensive men and postmenopausal women [Hopkins et al., 1996], which they claimed might explain the original linkage finding for male–male, but not female–female hypertensive sibpairs [Jeunemaitre et al., 1992].

To date, linkage studies have tested only the AGT marker. In view of the controversy surrounding AGT in hypertension, we decided to test additional microsatellite polymorphisms near the AGT locus and to use more sophisticated programs for data analysis. We also performed a case-control study of several AGT polymorphisms that have shown association with hypertension, viz. the G(−6)A, A(−20)C and M235T bi-allelic variants [Jeunemaitre et al., 1992, 1997]. Such an analysis might explain the original linkage finding for male–male, but not female–female hypertensive sibpairs [Jeunemaitre et al., 1992].

<table>
<thead>
<tr>
<th>TABLE I. Characteristics of Patients and Controls*</th>
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<tbody>
<tr>
<td>Linkage study</td>
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<tr>
<td>----------------</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Age (y)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
</tr>
<tr>
<td>Systolic pressure</td>
</tr>
<tr>
<td>Pretreatment (mmHg)</td>
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<tr>
<td>Diastolic pressure</td>
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</table>

*Values are mean ± S.D.

Genotyping

Microsatellite markers. DNA was isolated from whole blood by a modified salting out method [Miller et al., 1988] or using a kit (Qiagen, cat no. 29304, Germany). Five markers were tested. Fluorescently labeled polymerase chain reaction (PCR) primers for D1S213 and D1S235 were from panel 1 of the Applied Biosystems Inc (ABI; Foster City, CA) PRISM® Linkage Mapping Set, for another two (D1S251 and D1S2880) synthesis was performed by Bresatec (Adelaide, South Australia) and primers for the AGT 3′ microsatellite were made by Life Technologies (Melbourne, Australia). D1S213 was labeled with TET, D1S235 was labeled with FAM, and AGT, D1S251, and D1S2880 were labeled with HEX. The sequences of the ABI primers were as described by the supplier. In the case of the others, primer sequences were: AGT – (forward) 5′-TAG ATC TCT CAG CTA TTA CAA GG-3′ (HEX-labeled), (reverse) 5′-GGT TCA GAA CTG ACC TGT GG-3′; D1S251 – (forward) 5′-GTC TCC AGC CTG CCA-3′ (HEX-labeled), (reverse) 5′-GAC CAA GCA ACT TCA CTC-3′; D1S2880 – (forward) 5′-CGT CTT TCT GCT AGG CAG G-3′ (HEX-labeled), (reverse) 5′-CAT CAT ATG AAT CTT TGT GCT GC-3′. Genotypes were determined by either PCR for an individual marker or by multiplex PCR in which markers from the same ABI panel and labeled with the same dye were used in a common PCR mixture. Since PCR products for these differed in size they could be distinguished subsequently by electrophoresis. Each 15 µl PCR mix consisted of 50 ng of genomic DNA, each primer (83 mmol/l for the ABI primers, 40 mmol/l for AGT primers, and 450 mmol/l for D1S251 and D1S2880 primers), 200 µmol/l each dNTP, 2 mmol/l MgCl₂, 50 mmol/l KCl, 10 mmol/l Tris-HCl, pH 8.3, and 0.75 Units of AmpliTaq Gold® (Perkin-Elmer, Norwalk, CT). Composition was the same for single and multiplex mixtures, with the example of primers in the
multiplex mix, which were as specified for each marker previously [Veenstra-WanderWeele, 1997]. Amplification was carried out on a PTC-200 Peltier Thermal Cycler (MJ Research, Watertown, MA). For single marker PCRs, this involved an initial denaturation step at 94°C for 5 min, followed by 35 cycles of 94, 55 and 72°C for 1 min each, and then a final extension step at 72°C for 10 min. For the multiplex reactions, a "hot-start" protocol was employed, involving 94°C for 5 min, followed by 10 cycles of 94, 60 and 72°C for 30 s each, 15 cycles of 94, 58 and 72°C for 30 s each, and then 20 cycles of 94, 55 and 72°C for 30 s each, finishing with a step at 72°C for 30 min. PCR products were electrophoresed on an ABI 377 automated sequencer and genotypes were assigned using ABI Genotyper® software. To confirm accuracy of genotype assignment, for the AGT marker, genotyping was performed twice and was also determined by a different Laboratory (Griffith University, Gold Coast), using an ABI 310 capillary electrophoresis system.

Bi-allelic AGT polymorphisms. In the association study, genotypes for the AGT A(−6)G and A(−20)C polymorphisms were determined by PCR RFLP analysis. Primers for detection of each were: (upstream) 5’-AGA GGT CCC AGC GTG AGT GTC–3’ and (downstream) 5’-ATC 3’. Each reaction mixture contained 120 ng genomic DNA, 0.2 μmol/l each primer, 0.2 mmol/l each dNTP, 10 mmol/l Tris-HCl, pH 9.0, 50 mmol/l KCl, 2 mmol/l MgCl₂, and 1 U AmpliTaq® DNA polymerase (PerkinElmer). PCR involved 95°C for 3 min, followed by 36 cycles of 94, 60 and 72°C for 1 min each, and finally 72°C for 15 min. For determination of A(−6)G genotype, an aliquot of 4 μl of PCR product was then digested for 10 h at 50°C with 5 U BstZI (Promega, Madison, WI) made up to 12 μl with buffer and bovine serum albumin, according to the directions of the supplier. After electrophoresis on a 4% agarose gel, a 267 bp ethidium bromide stained band was indicative of the A(−) allele (designated A) and bands of 154 and 113 bp showed the G(−) allele (designated G) was present. The procedure for determination of the A(−20)C genotype was the same, except that PCR products were incubated at 37°C with 5 U EcoO109I (New England BioLabs, Beverly, MA), which yielded bands of 205 and 62 bp (A(−20) allele; designated a) or 141, 64 and 62 bp (C(−20) allele; designated C). In the case of the M235T polymorphism (alleles M and T), genotypes were determined as described previously [Bennett et al., 1993]. All genotype determinations were performed on two separate occasions to confirm genotype assignment.

Statistical analysis

Allele sharing by descent (IBD) or by state (IBS) was determined using 7 linkage programs suitable for complex traits in order to test whether there was concordant sibpair sharing of alleles more often than expected under random Mendelian segregation. Since each program has relative advantages and disadvantages, the use of several is deemed desirable [Davis and Weeks, 1997]. These included SPLINK [Holmans and Clayton, 1995], which uses allele shared IBD estimates for all possible pairs in a sibship and computes probabilities for each marker genotype when parents are not available, ASPEX [Hauster et al., 1996], which uses an alternate restriction to SPLINK when performing maximum likelihood calculations, MAPMAKER/SIBS [Kruglyak and Lander, 1995], the Affected Pedigree Member (APM) method [Weeks and Lange, 1988; Brown et al., 1994; Schroeder et al., 1994], which uses a nonrandom cosegregation statistic to test distortions in alleles shared IBS at a marker locus, SIB-PAIR [Duffy, 1997], and GENEHUNTER [Kruglyak et al., 1996], which can be used for multipoint analysis of pedigree data and can be applied to sibpair data. The significance thresholds we set for acceptance of linkage were as recommended [Lander and Kruglyak, 1995]. In the case–control study, differences were tested by χ² analysis (df = 1 for alleles; df = 2 for genotypes). Determination of linkage disequilibrium between AGT polymorphisms involved analysis of haplotype frequencies in the largest group as described by Hill [1974].

RESULTS

Sibpair Linkage Study

The markers used spanned a recombination fraction (θ) of 0.335 of the q arm of chromosome 1 and were highly informative, having an average heterozygosity of 0.79 ± 0.05 SD. Allele frequencies for the marker closest to AGT were also determined in the normotensive group and resembled the frequencies seen in the hypertensive sibs (Fig. 1). Table II shows the outcome of single point analyses of genotype data by SPLINK and APM, as well as multipoint analysis by MAPMAKER/SIBS. Values obtained using ASPEX were MLOD = 0.00, SIBPAIR were P = 0.24–0.50, and GENEHUNTER were nonparametric lod (NPL) = −2.2–0.39 and P = 0.35–0.99. For each marker, all of the statistical parameters generated consistently indicated an absence of excess allele sharing in the hypertensive sibpairs. Results for pairs with age of onset < 45 y (n = 87), those with pre-treatment diastolic pressure > 100 mmHg (n = 86), or male–male pairs (n = 100) were also not significant (e.g., P = 0.54, 0.55 and 0.51, respectively, by SPLINK). In addition we calculated exclusion values using MAPMAKER/SIBS under the

![Fig. 1. Allele frequencies for AGT allele in the hypertensive sibs and the unrelated normotensive control group.](image-url)
Furthermore, other parameters in Table I across genotypes did not (data not shown). Comparison of blood pressure and ants also did not show association with hypertension undertaken. Haplotype analysis involving these variants counted on the basis of the multiple comparisons being undertaken. Hardy-Weinberg equilibrium was observed. No significant differences in frequencies were seen between the hypertensive and normotensive groups, with the exception of a weak (P = 0.04) association of the A(−20)C variant with hypertension. This can, however, be discounted on the basis of the multiple comparisons being undertaken. Haplotype analysis involving these variants also did not show association with hypertension (data not shown). Comparison of blood pressure and other parameters in Table I across genotypes did not demonstrate any significant tracking (data not shown). Furthermore, χ² analysis after stratification of genotype data according to mean plasma angiotensinogen, blood pressure, BMI, age of onset, sex, and other parameters still did not reveal any significant association with hypertension. We noted strong linkage disequilibrium between the G(−6)A, A(−20)C and M235T variants of AGT, the A(−6), C(−20) and T235 alleles being co-inherited (Table IV).

### Association Study

Genotype and allele frequencies for the M235T, G(−6)A and A(−20)C variants are shown in Table III. Hardy-Weinberg equilibrium was observed. No significant differences in frequencies were seen between the hypertensive and normotensive groups, with the exception of a weak (P = 0.04) association of the A(−20)C variant with hypertension. This can, however, be discounted on the basis of the multiple comparisons being undertaken. Haplotype analysis involving these variants also did not show association with hypertension (data not shown). Comparison of blood pressure and other parameters in Table I across genotypes did not demonstrate any significant tracking (data not shown). Furthermore, χ² analysis after stratification of genotype data according to mean plasma angiotensinogen,

### DISCUSSION

The present study found no evidence for linkage of markers in the vicinity of AGT with essential hypertension. A negative sibpair linkage result in a complex polygenic trait may indicate either that the loci tested are not linked to the condition or the size of the study group was insufficient to provide sufficient power to demonstrate a small genetic contribution of loci in this region to the trait. It has been estimated that in hypertension with strong family history and disease onset before age 55 years, relative risk to a sibling (λc) would be approximately 3.8 [Williams et al., 1991]. Analyses by others have suggested that for a complex disease ~100 sibpairs would be sufficient to provide 90% power to show significant linkage at the Lod = 3 (P = 0.0001 [Lander and Kruglyak, 1995]) level if its λc exceeds ~3.5 [Weeks and Lathrop, 1995]. For a disease with multiple weak contributing loci (e.g., λc values of ~1.6), the 175 sibs we tested should have 90% power to provide evidence at the Lod = 2 (P = 0.001) level [Weeks and Lathrop, 1995]. That our sibs do indeed have sufficient power to reveal a locus for hypertension is evident from recent findings in the same cohort [Rutherford et al., 1997] and in a study by others [Julier et al., 1997] of linkage on chromosome 17 with hypertension. Our sib number was, moreover, comparable to that used in the original work that claimed hypertension linkage after testing the same AGT microsatellite marker, viz. 135 sibpairs after weighting [Hodge, 1984] for the Paris sibs and 244 for the Utah sibs, significance being obtained at the P < 0.05 level for the former (8% excess of shared alleles), but not for the latter (4% excess) [Jeunemaitre et al., 1992]. For early-onset (<45 y), the excess was 12% and 7% in Paris and Utah, respectively (P = 0.02 and 0.07) and for severe hypertension (diastolic = 100 mmHg or = 2 drugs), excess was 15% and 18% (P = 0.02 and 0.01). For our sibs, age of onset of hypertension was 43 ± 10 SD years and when we confined our analyses to those with onset < 45 y we still did not find significance. Similarly, significance was not achieved for our severely affected sibs using the same criteria as the Utah/Paris study. In the Utah/

### TABLE II. Results of Statistical Analyses of Genotype Data for Microsatellite Markers Near Angiotensinogen Locus*

<table>
<thead>
<tr>
<th>Locus</th>
<th>Distance (θ)</th>
<th>Locus het.</th>
<th>No. of alleles</th>
<th>No. of genotypes</th>
<th>P-value</th>
<th>Associated Lod</th>
<th>T-statistic</th>
<th>Theoretical P-value</th>
<th>MLS</th>
<th>Exclusion Lod</th>
</tr>
</thead>
<tbody>
<tr>
<td>D1S2880</td>
<td>0.029</td>
<td>0.87</td>
<td>14</td>
<td>213</td>
<td>0.64</td>
<td>0.23</td>
<td>-2.71</td>
<td>0.01</td>
<td>-4.60</td>
<td>-4.40</td>
</tr>
<tr>
<td>D1S213</td>
<td>0.028</td>
<td>0.77</td>
<td>11</td>
<td>227</td>
<td>0.57</td>
<td>0.00</td>
<td>-1.70</td>
<td>0.96</td>
<td>-5.07</td>
<td>-4.05</td>
</tr>
<tr>
<td>D1S251</td>
<td>0.007</td>
<td>0.96</td>
<td>14</td>
<td>213</td>
<td>0.58</td>
<td>0.00</td>
<td>-1.14</td>
<td>0.87</td>
<td>-4.05</td>
<td>-3.50</td>
</tr>
<tr>
<td>D1S235</td>
<td>0.23</td>
<td>0.73</td>
<td>13</td>
<td>206</td>
<td>0.58</td>
<td>0.00</td>
<td>-1.70</td>
<td>0.96</td>
<td>-4.05</td>
<td>-4.05</td>
</tr>
</tbody>
</table>

*Values in brackets are fraction.

### TABLE III. Association Analysis of AGT Variants in Hypertension*

<table>
<thead>
<tr>
<th>Genotype frequencies</th>
<th>Allele frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>M235T variant</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>MM</td>
</tr>
<tr>
<td>HT</td>
<td>111</td>
</tr>
<tr>
<td>NT</td>
<td>190</td>
</tr>
<tr>
<td>χ²</td>
<td>1.8</td>
</tr>
<tr>
<td>P</td>
<td>0.4</td>
</tr>
</tbody>
</table>

| G(−6)A variant       |                   |
| Group                | GG | GA | AA | G  | A  |       |
| HT                   | 111 | 33 | 63 | 15 | 129 | 93    |
| NT                   | 190 | 65 | 55 | 61 | 206 | 174   |
| χ²                   | 0.3 | 0.1 |
| P                    | 0.9 | 0.8 |

| A(−20)C variant      |                   |
| Group                | aa | aC | CC | a  | C  |       |
| HT                   | 111 | 20 | 52 | 2  | 193 | 29    |
| NT                   | 190 | 65 | 55 | 61 | 206 | 174   |
| χ²                   | 5.0 | 4.4 |
| P                    | 0.03 | 0.037 |
Paris work significance was confined to male–male pairs (13% excess overall, $P < 0.01$; and 33%, $P < 0.0001$, for those with severe hypertension), but our male–male pairs did not show significant linkage.

A noteworthy result of our analyses was the finding of exclusion scores of $\lambda_\nu$ to $-5.1$ for a pairs did not show significant linkage. for those with severe hypertension), but our male–male pairs (13% excess overall, $P = 0.02$) and the excess was similar (25%; $P < 0.001$) for 31 families with 70 severely affected (diastolic > 100 mmHg) patients as for the whole group (26%; $P < 0.001$).

Thus closer examination of the various data to date, especially in the light of more recent findings, such as described herein, provide little reassurance that $AGT$ is linked to hypertension.

Our case-control study of the G(−6)A and A(−20)C variants, like M235T [Bennett et al., 1993], also failed to support association of these $AGT$ variants with hypertension. The G(−6)A and M235T variants [Jeunemaitre et al., 1997] and the A(−20)C and M235T variants [Sato et al., 1997] have been reported by others to be in linkage disequilibrium and our data confirm this for both variants in a Caucasian population. The number of subjects we used should have provided sufficient power, as judged from previous estimates [Cox and Bell, 1989] and our positive results for variants of other genes in the same subjects, where our use of only hypertensives with two hypertensive parents, and normotensives with two normotensive parents, would have increased the genetic input and power of our study, so increasing the likelihood of revealing an association [Chambers and Morris, 1996; Morris et al., 1997; Benjafeld et al., 1998]. The present findings thus add to the disparity between reports for $AGT$ variants in various Caucasian populations, as well as in other racial groups [reviewed in Morris, 1999]. Interestingly, one study of French Caucasians found association with hypertension only in patients who were not overweight [Tiret et al., 1995]. However, a study of a UK Caucasian group in East Anglia failed to find association even after subdivision according to BMI, sex or age [Hingorani et al., 1996] and we too could not see differences by subgroup analyses. Curiously, the initial M235T association was much stronger in females, whereas the linkage data were only significant for males [Jeunemaitre et al., 1992]. T235 allele frequency is quite variable, ranging from 0.35 [Jeunemaitre et al., 1992] to 0.49 [Caulfield et al., 1994] in different Caucasian groups, suggesting that cryptic ethnic or other differences between various populations of the same race may be contributing to the disparate findings.

### TABLE IV. Estimate of Pairwise Haplotype Frequency and Disequilibrium Statistic for G(−6)A, A(−20)C and M235T Variants of $AGT$ (n = 190)

<table>
<thead>
<tr>
<th>Variants</th>
<th>Estimated haplotype frequency</th>
<th>$D$</th>
<th>$P^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>M235T/G(−6)A</td>
<td>M/G 0.534 0.035</td>
<td>T/G 0.008 0.423</td>
<td>96.5% &lt;0.001</td>
</tr>
<tr>
<td></td>
<td>M/a 0.538 0.004</td>
<td>A/C 0.264 0.194</td>
<td>96.4% &lt;0.001</td>
</tr>
<tr>
<td>M235T/A(−20)C</td>
<td>G/a 0.568 0.234</td>
<td>G/C 0.000 0.197</td>
<td>100% &lt;0.001</td>
</tr>
</tbody>
</table>

$^*$Significance of the standardized disequilibrium whose square (under the null hypothesis, $D = 0$) is approximately distributed as $\chi^2_{1, d.f.}$, as described in Hill [1974].
Thus the M235T variant may not be an ideal marker for a causative variant, if there is one. Other polymorphisms in disequilibrium with T235, such as allele (GT)$_{16}$ of the dinucleotide repeat and the A(−6) or C(−20) alleles of the promoter polymorphisms, may have similar problems. Potential problems may be exacerbated when using APM, due to its inherent sensitivity to variations in allele frequency [Weeks and Lange, 1988; Morgan et al., 1996]. Thus at least some of the positive findings to date for AGT have the potential to be spurious.

In considering the findings from linkage and association studies it should be noted that what is tested differs for each approach and that each has different strengths and weaknesses. Linkage studies prefer multi-allelic markers to better track inheritance, whereas in association studies bi-allelic markers are preferred. Linkage studies test whether a disease and an allele show a correlated transmission (concordant inheritance) within a pedigree, whereas association studies test for correlated occurrence in a population [Lander and Schork, 1994]. Linkage may be seen without association, as can happen when there are many independent trait-causing loci in a population, so that association with any particular allele is weak, and association may be detected without linkage, such as when an allele explains only a minor proportion of the variance for a trait, meaning that even though the allele is more frequent in affected individuals, it does not predict disease status within a pedigree very well [Lander and Schork, 1994]. This situation could apply to hypertension considering its relatively small relative risk value and complexity and the expectation that many hypertension susceptibility loci may each contribute to a relatively minor proportion of the variance. A potential confounding factor in association studies is selection bias (all of our subjects in every group were respondents to requests for volunteers). However, the possibility of population stratification artefact does not apply to linkage analysis, since this is insensitive to population structures. Thus a negative result for AGT from both association and linkage analysis helps provide some assurance about the validity of the conclusion reached, but does not completely rule out a minor contribution to the disease tested.

There may be an effect of genotype on plasma angiotensinogen concentration [Jeunemaitre et al., 1992; Yanai et al., 1996; Inoue et al., 1997; Zhao et al., 1999]. The elevated plasma angiotensinogen in hypertension could be at least partly a result of stimulation of hepatic synthesis by hypertension-associated hyperinsulinemia [Schrader et al., 1996] or of adipose synthesis in the insulin resistant state of obese hypertensives [Aubert et al., 1998]. Moreover, AGT genotype is associated with insulin resistance [Sheu et al., 1998]. Any rise in plasma angiotensin that ensues would, by normal negative feedback mechanism, be quickly offset by a corresponding suppression in renin secretion. Indeed, as long as renin secretion can feed back normally, blood pressure is not angiotensinogen dependent [Gahinem et al., 1994; Sealey and Laragh, 1995; Catanzaro et al., 1999]. The net effect would be the absence of any change in the biologically active effector, angiotensin II. Thus there is no logical reason why a genetically determined elevation in plasma angiotensinogen in hypertension might play a role in the etiology of this condition. Finally, recent congenic experiments involving a spontaneously hypertensive rat chromosomal segment containing the angiotensinogen gene have shown that even though there is a quantitative locus for blood pressure in this region, this is not explained by differences in angiotensinogen expression or plasma concentrations [St Lezin et al., 1999].

ACKNOWLEDGMENTS

We thank the Australian Red Cross Blood Bank, Sydney, for normotensive subjects and Nicholas Martin, NHMRC Twin Registry, for facilitating contact with 20 dizygotic twin sibships in which each twin had hypertension and 20 in which one twin and nontwin sib(s) were affected. Assistance in recruitment of the majority of sibships (for the linkage study) and unrelated hypertensives (for the association study), as well as blood collection and DNA extraction was provided by Judith O'Neill, Andrew Schrader, Robert Zez, Susan Chambers, and Kazuo Suzuki in the Sydney Laboratory. Some assistance was also given by Sue Rutherford, Sharon Quinlan, Robert Curtian, Sharon Boywright, and Monique Salzmann in the Laboratory of Lyn Griffiths, Griffith University, who provided 14 of the 104 sibships and 16 of the 111 unrelated hypertensives. Partial salary support was provided to D.R.N. by Gemini Research Ltd, UK.

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