Association Analyses of $S_A$ Gene Variant in Essential Hypertensives

Robert Y.L. Zee, Amanda L. Stephen, Naoharu Iwai, and Brian J. Morris

A gene designated $S_A$ has been implicated in hypertension (HT) in rat genetic models and Japanese HT patients. However, a linkage study in whites was negative. Because of the limitations of genetic analyses, confirmation in different settings is imperative. Therefore, we conducted a cross-sectional case-control study involving 106 HT and 96 normotensive (NT) white subjects. A polymerase chain reaction technique was developed for PstI restriction fragment length polymorphism (RFLP) determination. We could find no association of this RFLP with HT (frequency of minor allele, $A_2 = 0.11$ in HTs v 0.07 in NTs). However, $A_2$ displayed an association with increase in body mass index in HTs: for a body mass index mean of 26 kg/m$^2$ or more, $A_2 = 0.17$ compared to 0.06 for body mass index of less than 26 kg/m$^2$ ($\chi^2 = 6.4, P = .01;$ odd ratio 3.4, 95% confidence interval 1.2 to 10.0); for a body mass index of 28 kg/m$^2$ or more, $A_2 = 0.20$ ($\chi^2 = 10.4, P = .001; $ odds ratio 4.0, 95% confidence interval 1.5 to 10.5). Furthermore, $A_2$ tracked significantly with elevation in body mass index in the HTs ($F = 4.8, P = .01$ by one-way ANOVA).

In conclusion, we could find no association of $S_A$ genotype with HT, but obtained preliminary evidence for a possible association with variation in body mass index in a severely affected HT group with a strong family history of HT.

The $S_A$ gene has been implicated in hypertension (HT), although its function is not known. This is because its identification as a candidate HT gene involved a novel approach, based on the premise that the kidney may be responsible for HT, in which mRNAs overexpressed in the spontaneously HT rat (SHR) kidney were cloned. The $S_A$ gene locus cosegregates with elevation in blood pressure (BP), accounting for up to 25% of the genetic variance in systolic and diastolic BP in F$_2$ populations involving SHR, stroke-prone SHR, and Dahl salt-sensitive genetically HT rat strains. The effect on BP appears to result from its proximal tubular expression in the kidney, where increased renal vascular resistance in young rats in the F$_2$ generation of a SHR x normotensive (NT) Wistar-Kyoto cross had been shown previously to cosegregate with increased BP. In addition, longitudinal analysis suggested a pressor mechanism that is either slow or indirect.

To extend these findings to human HT, the rat $S_A$ cDNA was used as a probe to isolate human $S_A$
cDNA,\textsuperscript{9,10} which was shown to encode a 578-amino-acid (\(M_r\) 65.4, \(pI\) 9.3) signal-peptide-containing protein having 84\% homology to putative rat S\(_A\). The human S\(_A\) gene is on chromosome 16,\textsuperscript{9,12} in the vicinity of the renal epithelial sodium channel \(\beta\)-subunit gene recently linked to Liddle’s syndrome,\textsuperscript{13} and displays several restriction fragment length polymorphisms (RFLPs), one of which, involving PstI, has shown a highly significant \((P < .001)\) association with essential HT in a group of 89 Japanese patients.\textsuperscript{9}

Subsequent sibling-pair studies in France have failed to confirm linkage of the S\(_A\) locus to HT in whites. Although association analyses of several RFLPs also gave negative results,\textsuperscript{10} as seen for other RFLPs in Japanese subjects,\textsuperscript{9} the critical PstI RFLP was only tested in 13 HTs, a number too low to draw a conclusion. The discrepancy between the Japanese association and French linkage findings could involve racial differences. However, the demonstration of significant association in the absence of linkage in racially similar populations has been noted in other studies, such as of the angiotensin II type 1 receptor gene and HT,\textsuperscript{14} a minisatellite in the 5’-flanking region of the insulin gene and insulin-dependent diabetes mellitus,\textsuperscript{15,16} and the dopamine D2 receptor and alcoholism.\textsuperscript{17} This can be a consequence of methodologic difficulties in testing a highly polymorphic marker whose different alleles carry the same susceptibility variant. In the diabetes mellitus studies, the problem was overcome by testing just those with parents heterozygous for the disease-associated allele.\textsuperscript{18}

In HT, however, this presents considerably difficulty. Because HT is a late onset, quantitative trait parents are generally not available for genotyping. Also, the sibling-pair approach may not have enough power to show linkage, especially when the cause involves common susceptibility alleles that confer only a small increase in risk, ie, a causative S\(_A\) allele may not be necessary or sufficient for HT onset. This is a particular problem for the S\(_A\) gene, as an absence of knowledge about the encoded protein obviates examination of intermediate phenotypes that may confer increased potential for HT onset. Finally, the possibility of a spurious result, despite the high level of significance obtained, means that genetic findings always require confirmation in separate settings.

For these reasons we deemed it imperative to conduct a cross-sectional study of the PstI RFLP of the S\(_A\) gene in a separate white population. The one used has been well characterized and involves patients with severe, familial HT.

<table>
<thead>
<tr>
<th>TABLE 1. CHARACTERISTICS OF HT AND NT GROUPS</th>
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</thead>
<tbody>
<tr>
<td></td>
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<tr>
<td>(n)</td>
</tr>
<tr>
<td>Age</td>
</tr>
<tr>
<td>Male/female</td>
</tr>
<tr>
<td>Systolic BP (mm Hg)</td>
</tr>
<tr>
<td>Diastolic BP (mm Hg)</td>
</tr>
<tr>
<td>BMI (kg/m(^2))</td>
</tr>
<tr>
<td>Plasma cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
</tr>
<tr>
<td>HDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>LDL cholesterol (mmol/L)</td>
</tr>
<tr>
<td>Renin (pmol ANG I(\cdot h^{-1} \cdot mL^{-1}))</td>
</tr>
<tr>
<td>((n = 56))</td>
</tr>
<tr>
<td>Angiotensinogen (pmol/mL)</td>
</tr>
<tr>
<td>((n = 58))</td>
</tr>
<tr>
<td>ACE (nmol Gly-Gly(\cdot min^{-1} \cdot mL^{-1}))</td>
</tr>
<tr>
<td>((n = 19))</td>
</tr>
</tbody>
</table>

\(\textit{Values shown are mean } \pm \text{ SD, except for renin, angiotensinogen, and ACE, which are mean } \pm \text{ SE.}\)

\(\textsuperscript{*} P < .0005.\)

\(\text{METHODS}\)

Subjects Two groups of subjects were used: treated HT patients who were the offspring of two HT parents (representing 2\% of adults or 10\% of all HTs\textsuperscript{19}), and NT subjects who were selected on the basis of both parents being NT at age \(\geq 50\) years. All were unrelated whites of British descent and were mainly contacted by news media appeals and public notices. Further details of ascertainment, together with genotype data for more than 15 other polymorphisms in these subjects have been described previously.\textsuperscript{19–28} Demographic parameters are shown in Table 1. The patients displayed severe HT of early onset,\textsuperscript{19} possibly contributed by their strong family history. A clear separation of BPs was evident between the HT and NT groups (Figure 1), possibly arising from selection bias or the strong family history of HT and NT in the subjects used. As commonly noted,\textsuperscript{30} plasma lipids were elevated in the HTs, as was plasma angiotensinogen.\textsuperscript{28} In some of the analyses subjects were stratified according to mean body mass index (BMI). For HTs, 50 of 102 had a BMI \(\geq 26 \text{ kg/m}^2\) and for NTs 46 of 96 had a BMI \(\geq 25 \text{ kg/m}^2\). A sample of 20 mL of blood was collected from the antecubital fossa of each subject and placed in a heparinized tube. The peripheral leukocytes obtained by centrifugation were then stored at \(-70^\circ\text{C}.\)

DNA Sequencing In the previous report Southern blotting was used for S\(_A\) PstI genotype determination.\textsuperscript{9} This requires microgram quantities of DNA, and has the advantage of not requiring knowledge of the DNA
 sequence. On the other hand, the PCR method can be performed much more quickly and with very much less DNA, but requires sequence information to design oligonucleotide primers. Therefore, to develop a polymerase chain reaction (PCR) method we first had to derive sequence information in the vicinity of the *Pst*I polymorphic site. To do this genomic DNA was amplified by PCR using sense primer 5'-CTT GCA AAT GTG GCC TGT CTG CGA AC-3', corresponding to nucleotides 379 to 404 in the *Sₐ* cDNA, and antisense primer 5'-CAC ATC TGA GGG TGT CAA ATC TAG CCA G-3' (nucleotides 789 to 762) and *Taq* polymerase extender (Stratagene, La Jolla, CA). That the 5-kb PCR fragment so amplified contained the polymorphic site was confirmed by a combination of Southern analysis and PCR-RFLP analysis in 10 *A₁A₁*, 10 *A₁A₂*, and 10 *A₂A₂* subjects as described below. The 5-kb fragment was subcloned and partially sequenced from either side of the *Pst*I site.

**Detection of PstI RFLP of Sₐ Gene** Leukocyte DNA was obtained from each subject in the HT and NT groups by conventional phenol/chloroform extraction as described previously and 100 ng was used for genotype determination by PCR. The RFLP was caused by a single base difference (Figure 2). Suitable primers flanking the *Pst*I site were chosen as follows: sense primer, 5'-GTC ACA CAT TAG GCC AGC TCA C-3' and antisense primer, 5'-GCC AGG CAT GGT GAT GCA ATC CTG-3'. The reaction mixture included 100 pmol of each primer, 0.5 U *Taq* polymerase (Perkin-Elmer, Norwalk, CT), 10 mmol/L Tris-HCl, pH 8.4, 5 mmol/L MgCl₂, 50 mol/L KCl, 0.1% gelatin, and 0.5 mmol/L each dNTP in a total volume of 50 μL. Amplification was carried out in a Perkin-Elmer DNA Thermal Cycler, in which, after an initial denaturation step at 95°C for 5 min, there were 30 cycles of 94°C, 62°C, and 72°C for 1 min each. During manipulations, all necessary precautions and controls were used to prevent PCR-product carryover contamination. An aliquot of 10 μL of the post-PCR mixture was incubated at 37°C for 2 to 3 h with 8 U of *Pst*I (Promega, Madison, WI) in a total volume of 20 μL with 90 mmol/L Tris-HCl, pH 7.5, 50 mmol/L NaCl, and 10 mmol/L MgCl₂. A 20-μL portion was then subjected to electrophoresis at 100 V for 40 min in a 3% agarose gel and DNA was visualized by ethidium bromide staining. The polymorphism was characterized by two bands, one of 315 bp (designated allele *A₁*) and the other of 230 bp (allele *A₂*). To confirm genotype assignment the PCR procedure was performed on all samples on two separate occasions.

**Plasma Assays** Methods described previously were used for determination of plasma lipid profile, plasma renin, plasma angiotensinogen, and plasma angiotensin converting enzyme (ACE). Because of limitations in plasma available from HTs, renin and angiotensinogen were determined in only 56 and 58 patients, respectively, and the need to exclude HTs receiving ACE inhibitor therapy restricted ACE data in the HT group to 19.

**Statistical Analyses** Genotypes were assigned to each subject and from these values allele frequencies were calculated. The significance of the difference between total observed alleles on all chromosomes for the HT and NT groups was tested by *χ²* analysis with one degree of freedom. The data were analyzed using the Statistical Analysis System (SAS) program for personal computers (SAS Institute, Cary, NC). The significance level was set at 0.05.

**FIGURE 1.** Distributions of systolic BP and diastolic BP in NT and HT groups in which parental BP status was the same as the subjects themselves. Data are expressed as percentage of total for the whole of each respective group.

**FIGURE 2.** Nucleotide sequence determined for 512 bp of human *Sₐ* gene in the vicinity of the *Pst*I polymorphic site (which is shown in bold, with underlining). Primers chosen for PCR corresponded to the underlined sequences on either side of the *Pst*I site.
Weinberg equilibrium. Although the HT group displayed higher \( A_2 \) frequency than the NT group, this difference was not significant. Values for men and women were also similar and there was no change in genotype frequency with age.

Only one HT patient was homozygous for the \( A_2 \) allele and had higher BP (180/150 mm Hg, systolic/diastolic), BMI (29 kg/m\(^2\)), plasma triglycerides (5.7 mmol/L), and a younger age (35 years) than did the other genotypic groups. Differences between genotypes in relation to the various parameters in Table 1 were tested by one-way ANOVA and showed significant tracking of the \( A_2 \) allele with BMI (Table 3).

FIGURE 3. Detection of \( PstI \) RFLP of human \( S_A \) gene by PCR. Shown is an example of an ethidium bromide-stained gel after running a \( PstI \) digest of PCR-amplified samples from HT subjects homozygous for the \( A_1 \) allele (single 315-bp band: lanes 1, 2, 5, 6, 7, 8, and 13), homozygous for the \( A_2 \) allele (230-bp band: lane 12), or \( A_1A_2 \) heterozygotes (315-bp and 230-bp bands: lanes 3, 4, 9, and 10). Lane 14: DNA size marker (pUC19 cut with \( HpaII \)).

degree of freedom (df) as described previously. Comparison of two sets of data involved Student’s t test, and determination of a difference for a given parameter between the three genotypes for the HT and NT groups involved analysis of variance using the program StatView version 1.03 (Abacus Concepts, Berkeley, CA). The correlation coefficient of linear regression was calculated for relevant sets of parameters and significance was assessed by determination of F values using the same statistical package. Odds ratios (OR) and 95% confidence intervals (CI) were determined using Epi Info 6 (Centers for Disease Control & Prevention, World Health Organization, Geneva, Switzerland).

RESULTS

An example of PCR products for the three possible genotypes (\( A1A1 \), \( A1A2 \), and \( A2A2 \)) is shown in Figure 3. Genotype and derived allele frequencies for the HT and NT groups are shown in Table 2. In the NTs, genotype frequencies were consistent with Hardy-Weinberg equilibrium. Although the HT group displayed higher \( A_2 \) frequency than the NT group, this difference was not significant. Values for men and women were also similar and there was no change in genotype frequency with age.

Only one HT patient was homozygous for the \( S_A \) \( A_2 \) allele and had higher BP (180/150 mm Hg, systolic/diastolic), BMI (29 kg/m\(^2\)), plasma triglycerides (5.7 mmol/L), and a younger age (35 years) than did the other genotypic groups. Differences between genotypes in relation to the various parameters in Table 1 were tested by one-way ANOVA and showed significant tracking of the \( A_2 \) allele with BMI (Table 3).

Bonferroni correction for multiple comparisons was not applied because of the risk of excluding a true relation. We used an alternative method of analysis by stratifying according to mean BMI and performing \( \chi^2 \) analysis. This showed a significant difference in allele frequencies between HTs with a BMI of \( \geq 26 \) kg/m\(^2\) and HTs with BMI less than this mean value (\( P = .01 \)) (Table 4). More severely obese patients were also tested using an arbitrarily chosen demarcation value of 28 kg/m\(^2\). As can be seen in Table 4, comparison of HTs having a BMI of \( \geq 28 \) kg/m\(^2\) with HTs with a BMI of \( < 28 \) kg/m\(^2\) yielded differences in genotype (\( P = .005 \)) and allele (\( P = .003 \)) frequencies that were even more significant. For NTs, although \( A_2 \) frequency in obese subjects (0.10) exceeded that in lean (0.05), the difference was not significant by \( \chi^2 \) analysis. Differences between the HT and NT groups in relation to genotype and BMI were tested by two-way ANOVA, giving an F value of 3.1 (\( P = .082 \)). We also noted that \( A_2 \) frequency of the more severely obese HTs (0.20) was higher than in obese NTs (0.10) (\( \chi^2 = 3.9, P = .049 \); OR = 2.3 [95% CI = 0.9 to 6.1]).

Other analyses of data for the subjects studied indicated anticipated correlations, including that between plasma angiotensinogen and both systolic BP (\( r = 0.28, P = .0015 \)) and diastolic BP (\( r = 0.32, P = .0002 \)) (for men: \( r = 0.20, P = .10 \) [systolic] and \( r = 0.28, P = .023 \) [diastolic] and for women: \( r = 0.33, P = .008 \) [systolic] and \( r = 0.37, P = .003 \) [diastolic], indicating

### TABLE 2. GENOTYPE AND DERIVED ALLELE FREQUENCIES OF \( PstI \) RFLP OF \( S_A \) GENE IN WHITE HT AND NT GROUPS

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequencies</th>
<th>Alleles</th>
<th>Total Alleles</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>( A1A1 )</td>
<td>84</td>
<td>21</td>
<td>1</td>
<td>0.89</td>
<td>0.11</td>
</tr>
<tr>
<td>( A1A2 )</td>
<td>84</td>
<td>10</td>
<td>2</td>
<td>0.93</td>
<td>0.07</td>
</tr>
<tr>
<td>( A2A2 )</td>
<td></td>
<td>315 bp</td>
<td>230 bp</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

\( \chi^2 = 4.0, P = .13 \)

OR, Odds ratio; CI, confidence intervals.
that the effect was contributed mainly by the women). In NTs, renin showed a weak correlation with systolic (r = 0.21, P = 0.043) and diastolic (r = 0.21, P = 0.038) BP and was higher in men than in women (7.3 ± 0.7 vs 5.2 ± 0.4; P = .01 by t test). No other sex differences were detected.

The S_A PstI RFLP was in Hardy-Weinberg equilibrium with other cardiovascular disease-associated polymorphisms, that is, the ACE insertion / deletion (I/D) variant ($\chi^2$ 8 df = 8.5, $P = .4$) and angiotensinogen gene (AGT) Met235→Thr variant ($\chi^2_{dark} = 4.0, P = .9$). Moreover, for all parameters in Table 1 compared across ACE and AGT genotypes, the only association was of the D allele with elevation in plasma ACE, as is well established, and, in the HTs, lower plasma renin (9.6 ± 2.0 SE, 7.3 ± 1.2, and 4.6 ± 1.5 for II, ID, and DD, respectively; $P = .05$ by one-way ANOVA).

**DISCUSSION**

The present study has not found an association of the PstI RFLP of the S_A gene with HT in a well-studied group of severely affected HT patients. If this polymorphism, or any other variant in linkage disequilibrium with it, was making a major genetic contribution to the disease, then it should have been apparent in the subjects studied, as evidenced by the strong associations seen for markers at other genetic loci in relation to HT, obesity, and death rate in the present patient group and the positive ($P < .0001$) findings in an association study of the same S_A gene variant in 89 Japanese HTs and 81 NTs. Nevertheless, it should be emphasized that negative results in association studies are not considered conclusive because there could have been insufficient power to detect the contribution of the locus to disease susceptibility. Although cross-sectional approaches are reasonably powerful and require no assumptions about mode of transmission, the latter, as well as quantitative contribution of the locus to disease susceptibility and degree of linkage disequilibrium between susceptibility and marker loci, can never be known when an association study is designed, thus obviating specification of sample sizes needed to show significance. Nevertheless, in complex polygenic diseases, estimates can, however, be made for the number of subjects needed to demonstrate an existing association under a range of situations. Such analyses have yielded values of the same order of magnitude as the number of subjects used in the present study, that is, about 10^2 per group, suggesting that there should have been a good chance of detection of any strong contribution of the S_A gene variant to HT in our study.

$A2$ allele frequency was similar in the present NT white study group (0.07) as seen in NT Japanese (0.09). However, in Japanese HTs $A2$ frequency (0.27) was significantly higher than the value in the Japanese NTs ($P < .0001$). In contrast we could find no significant difference between $A2$ frequency in our white HTs (0.11) and NTs. The Japanese HTs were from an outpatient clinic, whereas our HTs were volunteers obtained from public appeals. In each study population onset of HT was before age 60 years and in the Japanese study pretreatment systolic/diastolic BP of HTs was $>160/95$ mm Hg. Recruitment of HTs into the latter study was on the basis of them having one or more HT first-degree relatives.

**TABLE 3. BMI (kg/m^2; MEAN ± SE) AS A FUNCTION OF S_A GENOTYPE, TESTED BY ONE-WAY ANOVA IN THE HT GROUP**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>BMI &gt; 26 kg/m^2</th>
<th>BMI &lt; 26 kg/m^2</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A1A1</td>
<td>26 ± 0.5</td>
<td>26 ± 0.5</td>
<td>29</td>
<td>4.8</td>
</tr>
<tr>
<td>A1A2</td>
<td>28 ± 0.8</td>
<td>28 ± 0.8</td>
<td>46</td>
<td>0.06</td>
</tr>
<tr>
<td>A2A2</td>
<td>29 ± 0.8</td>
<td>29 ± 0.8</td>
<td>58</td>
<td>4.0</td>
</tr>
</tbody>
</table>

**TABLE 4. GENOTYPE AND DERIVED ALLELE FREQUENCIES OF PstI RFLP OF S_A GENE IN HTs WITH BMI > OR < MEAN OF 26 kg/m^2 AND IN THOSE WITH BMI > OR < 28 kg/m^2**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Frequencies</th>
<th>Allele</th>
<th>Total Alleles on all Chromosomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI &gt; 26 kg/m^2</td>
<td>A1A1, 34</td>
<td>A1, 0.83</td>
<td>83, 3.4, 1.2–10.0</td>
</tr>
<tr>
<td>BMI &lt; 26 kg/m^2</td>
<td>A1A2, 15</td>
<td>A2, 0.17</td>
<td>17, 6</td>
</tr>
<tr>
<td>BMI &gt; 28 kg/m^2</td>
<td>A2A2, 1</td>
<td>A1, 0.79</td>
<td>57, 15, 4.0, 1.5–10.5</td>
</tr>
<tr>
<td>BMI &lt; 28 kg/m^2</td>
<td>A1A1, 22</td>
<td>A2, 0.21</td>
<td>122, 6</td>
</tr>
<tr>
<td>BMI &gt; 26 kg/m^2</td>
<td>A1A2, 58</td>
<td>A1, 0.94</td>
<td>4.0, 1.5–10.5</td>
</tr>
<tr>
<td>BMI &gt; 28 kg/m^2</td>
<td>A2A2, 0</td>
<td>A2, 0.06</td>
<td>122, 6</td>
</tr>
</tbody>
</table>

*BMI data was available for 102 of the 106 HTs.
whereas our HTs had to have two HT parents. One possibility is that there could be a racial difference in the contribution of the $S_A$ gene locus to HT, although even in the same race, association studies have given different results for particular polymorphisms in different settings (e.g., the angiotensinogen M235T variant).\(^{38,40-45}\) Thus, further studies in both Japanese and whites, as well as other racial groups, would further assist in the process of verification of findings to date. In possible support of the present findings, a quite different study, not of HTs, but of young, NT white adults with contrasting family history of HT, was unable to find a difference in $PstI$ RFLP frequency, or renal function, for each $S_A$ genotype.\(^{46}\) In the only other association study of the $PstI$ RFLP in white HTs, not only were there insufficient HTs and NTs (ie, 13 and 18, respectively) to give meaningful data, but genotype frequencies of various other RFLPs tested in the NT group deviated significantly from Hardy-Weinberg equilibrium.\(^{10}\) The HTs were also leaner than ours.

The apparent lack of association of the $PstI$ RFLP with HT in the present group of white HTs does not, however, by itself, exclude the $S_A$ gene locus in HT etiology. In general, cross-sectional case-control studies provide information only for the particular polymorphism tested, and any polymorphisms that may be in linkage disequilibrium with that variant, and do not say anything about the locus as a whole. Moreover, although linkage studies are able to test the whole locus, there can be significant shortcomings in interpretation of data arising from these, as discussed in the Introduction. Nevertheless, a linkage study in Japanese HTs would be of interest in view of the positive $PstI$ RFLP findings.\(^{9}\)

An unanticipated outcome of the present study was the finding of a possible association of the $S_A$ allele elevated in Japanese HTs, with increase in BMI in our white HTs. This was demonstrated by two alternative methods of data analysis, showing 1) Significantly higher $A2$ allele frequency in HTs with BMI $\approx 26$ kg/m$^2$ ($P = .01$) and an even more significant excess in those with BMI $\approx 28$ kg/m$^2$ ($P = .003$). 2) Tracking of the $A2$ allele with elevation in BMI in the HT group ($P = .01$). No significant association or tracking was seen in NTs. This is consistent with a possible influence of other factors peculiar to HTs, in whom such disorders tend to cluster.\(^{30}\) Indeed, association with obesity in HTs, but not NTs, has been demonstrated previously in similar studies by us of RFLPs at the low density lipoprotein receptor locus.\(^{23,27}\) However, the difference between the HT and NT groups in relation to BMI did not quite reach significance by two-way ANOVA, so that the question of an effect confined just to the HTs needs to be explored more fully before drawing firm conclusions.

A physiological explanation for the $S_A$ association finding is not immediately apparent, especially in view of the absence of information on the function of the putative $S_A$ protein. In the study of HT Japanese, a race that tends to be leaner than whites, including those we used, no significant relationship was evident for $S_A$ genotype and BMI.\(^{3}\) In this regard it is notable that the present association, although significant for those with a BMI $\approx 26$ kg/m$^2$, became much more significant when subdividing the group according to a higher BMI ($\approx 28$ kg/m$^2$), ie, the study of Japanese HTs may have not reached significance because of the lower number of patients with BMIs as high as seen in our obese HTs. Moreover, unlike our white group, it would appear that any effect of $S_A$ on BP in Japanese is not secondary to an effect on body weight. Alternatively, it remains possible that $A2$ represents a susceptibility allele, where the actual association could be with another condition that tends to occur more frequently in obese HT patients. In the SHR, not only kidney, but also liver, displays increased $S_A$ mRNA with reductions in brain and testis and none in heart, lung, adrenal, or spleen.\(^{1,47}\) Such tissue-specific differences were shown to be determined primarily by $S_A$ genotype.\(^{47}\) Thus, any action of $S_A$ on BMI in humans could involve hepatic, renal, or other effects. Moreover, it will be of interest to measure additional physiologic parameters in relation to $S_A$ genotype in the SHR for clues to the actions of the yet-to-be identified $S_A$ protein.

In conclusion, the present study of a group of severely HT whites with a strong family history of HT provides preliminary evidence for a possible association of $S_A$ genotype with variation in BMI and does not support a strong association of $S_A$ genotype with HT. This suggests that in whites either $S_A$ is not involved in HT (consistent with linkage findings), that any association that might exist is weaker than in Japanese HTs, and could involve a much smaller proportion of the white HT population, or that the marker tested might not be in complete linkage disequilibrium with a causative variant at the $S_A$ gene locus.

**NOTE ADDED IN PROOF**

Since submission of this work a paper has appeared reporting that the $S_A$ gene region of chromosome 1 also influences body weight in the F$_2$ generation obtained by crossing normotensive Lewis rats $\times$ Dahl salt-sensitive hypertensive rats.\(^{48}\)

**ACKNOWLEDGMENTS**

We thank the Australian Red Cross Blood Bank, Sydney, for providing NT subjects, Drs. A.P. Schrader and L.R. Griffiths for help in obtaining blood samples from HTs, Drs. S.M. Chambers and A.P. Schrader for performing some of the data analysis, and Drs. L. Irwig and R.A.L. Dampney for advice with statistical tests.
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