Angiotensin II type I receptor gene polymorphism: anthropometric and metabolic syndrome traits


Background: The renin angiotensin system is important in the regulation of vascular tone and fluid and electrolyte balance. The angiotensin converting enzyme (ACE) genotype has been shown to affect exercise response and glucose load response dependent on birth weight. Angiotensin II type I receptor (AGTR1) A1166C has previously been associated with the development of hypertension and coronary disease, but its metabolic effects have not been investigated.

Method: AGTR1 A1166C was genotyped by allele specific PCR in 378 individuals from Herfordshire, UK, who had been characterised for metabolic syndrome traits.

Results: Genotype counts were: AA, 183; AC, 170; CC, 25, consistent with Hardy-Weinberg equilibrium. The CC genotype was associated with significantly lower body mass index (by 1.7 units) and the same magnitude effect in women with significant lower weight in both genders (p = 0.01), also lower waist circumference and waist-hip ratio (p = 0.01) in men, with a trend for lower waist circumference in women also. Additionally, the CC genotype and/or C allele was associated with lower fasting glucose and insulin, and 30 and 120 min glucose in men (respectively, p = 0.08, 0.04, 0.01, 0.06). Lower means of systolic blood pressure, pulse pressure, cholesterol, and fasting triglyceride were also observed for the CC genotype in both genders though these were not statistically significant.

Conclusions: The AGTR1 1166 CC genotype appears to predispose to favourable anthropometric and metabolic traits, relative to cardiovascular risk.
Angiotensinogen (AGT)

Renin

Angiotensin I

ACE

AGTR2

Angiotensin II

AGTR1 (3q21-q25)

– Vasoconstriction
– Cell proliferation
– Sodium/water reabsorption (homeostasis)

Cardiovascular and circulation

CAD carrying the CC genotype of AGTR1 A1166C, the response to AGT II is increased. In addition, pharmacological blockade of AGTR1 induces peroxisome proliferator activated receptor-γ activity which promotes differentiation in adipocytes. These reports encouraged us to study the possible associations of AGTR1 A1166C with metabolic traits since the ACE findings suggest that the genetic diversity of the RAS pathway may impact not only on vascular but also on metabolic traits.

METHODS

Subjects

Caucasian subjects aged 59–72 years (mean age 64.4 years, SD 3.0) from East Hertfordshire, UK were enrolled for studies of late life traits in relation to early life anthropometric measures, subject to ethical approval (North and East Hertfordshire Ethical Committee) and subject anonymity. A total of 215 men and 123 women were included in the A1166C genotyping

RESULTS

Genotype frequencies for AGTR1 A1166C are presented in Table 1. These results are consistent with HW equilibrium ($\chi^2 = 3.1$, p = 0.08). Initial validations, using control genomic DNAs, of the approach of nested allele specific PCR following AGTR1 long PCR confirmed identical results irrespective of whether diluted (1/100) long PCR or genomic DNA was used as the template for allele specific assays. Allele frequencies were 0.71 for allele A and 0.29 for allele C, consistent with previous reports. Table 3 shows the results of genotype-phenotype analyses in males and females. In ANOVA tests, the CC genotype in males was associated

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
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<tbody>
<tr>
<td>Long PCR Forward</td>
<td>5'-CTCTCAAGTCGACCCCTCCTCCAGC'3'</td>
</tr>
<tr>
<td>Reverse ARM5</td>
<td>5'-GAATTTTGACCGGGGAGACTTATGAAGA'3'</td>
</tr>
<tr>
<td>Allele specific A Forward</td>
<td>5'-TCTGCGACCTTTATGCATCTAAATGACACG'3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GCAATAATCTTTCATTTCGTAACG'3'</td>
</tr>
<tr>
<td>Allele specific C Forward</td>
<td>5'-TCTCTTCCATGCCAAATAGTTTG'3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-AAGGAGCTGAGGAGTGACCTTCTT'3'</td>
</tr>
</tbody>
</table>
lower baseline insulin (p = 0.04), and trends of associations with lower adult weight (p = 0.06), fasting glucose (p = 0.08), height (p = 0.07), and glucose at 120 min (p = 0.06). The same genotype (CC) in women was significantly associated with lower fasting triglyceride (p = 0.04) and fibrinogen (p = 0.01), and also with trends of associations with lower waist circumference (p = 0.09), adult weight (p = 0.07), and fasting cholesterol (p = 0.07). The magnitudes of glucose effects, although not statistically significant, were similar to those in men.

For BMI (p = 0.01), waist-hip ratio (p = 0.004), waist circumference (p = 0.001), adult weight (p = 0.008), glucose at 30 min, and fasting fibrinogen, the associations were significant in combined analysis adjusted for gender.

Regression tests on the C allele gave broadly similar significances and these tests are also presented in table 3. A stronger statistical significance of effects was observed particularly for all glucose time points in the tolerance test.

## DISCUSSION

We have examined anthropometric traits and the principal traits of metabolic syndrome in relation to AGTR1 A1166C, which has been extensively studied with regard to hypertension and CAD. Our analyses suggest that AGTR1 A1166C affects BMI, weight, waist circumference, and waist-hip ratio, CC homozygotes showing lower values. Baseline, 30 min, and 120 min glucose levels are also generally lower in CC homozygotes, being particularly significant in men.

Given known gender differences for anthropometric and metabolic traits, males were examined separately from females under a prior hypothesis. The lower significance in women may reflect the smaller number studied (138 vs 240). Furthermore, differences of a similar magnitude are seen for CC genotype women for BMI and glucose values at OGTT time points; a post hoc combined analysis is also shown in table 3. It is possible that the effects are stronger in men, or are male specific, since the statistical signals do not strengthen in the combined analysis. It is notable that association and linkage of the ACE gene with hypertension was observed to be male specific in the Framingham Heart Study.43 The CC genotype seems to be associated with lower BMI by 1.7 units and lower waist circumference by about 7 cm. Most of the BMI association is due to weight, although there is a trend on height (p = 0.07) in men and in combined analysis (the AA genotype is 2 cm taller) and non-significant difference by genotype in women; other RAS genotypes (AGTR1 C573T and ACE I/D) have previously been associated with these traits.

## Table 3

The result of ANOVA and regression analysis (Reg.) of anthropometric and metabolic traits for the AGTR1 A1166C polymorphism in 240 men and 138 women.

<table>
<thead>
<tr>
<th>Men</th>
<th>Women</th>
<th>p Value</th>
<th>Combined analysis</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>AC</td>
<td>CC</td>
<td>ANOVA Reg.</td>
<td>AA</td>
</tr>
<tr>
<td>BMI</td>
<td>26.9</td>
<td>27.4</td>
<td>25.2</td>
<td>0.03</td>
</tr>
<tr>
<td>(kg/m²)</td>
<td>(3.3)</td>
<td>(3.4)</td>
<td>(3.0)</td>
<td>(3.6)</td>
</tr>
<tr>
<td>Waist to hip</td>
<td>0.94</td>
<td>0.94</td>
<td>0.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Ratio</td>
<td>0.05</td>
<td>0.05</td>
<td>0.05</td>
<td>0.01</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>99.2</td>
<td>98.4</td>
<td>91.9</td>
<td>0.008</td>
</tr>
<tr>
<td>Height (m)</td>
<td>1.73</td>
<td>1.71</td>
<td>1.71</td>
<td>0.07</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6.1</td>
<td>5.9</td>
<td>5.6</td>
<td>0.08</td>
</tr>
<tr>
<td>Glucose at 30 min</td>
<td>9.7</td>
<td>9.3</td>
<td>8.2</td>
<td>0.01</td>
</tr>
<tr>
<td>Glucose at 120 min</td>
<td>6.8</td>
<td>6.3</td>
<td>5.7</td>
<td>0.06</td>
</tr>
<tr>
<td>Fasting TG (mmol/l)</td>
<td>1.5</td>
<td>1.4</td>
<td>1.3</td>
<td>0.41</td>
</tr>
<tr>
<td>Fasting fibrinogen (g/l)</td>
<td>309.5</td>
<td>297.3</td>
<td>302.9</td>
<td>0.28</td>
</tr>
</tbody>
</table>

Mean values of each genotype groups are shown; standard deviations (SD) are given in parentheses. Geometric means and SDs were used for glucose, insulin, cholesterol, triglyceride, and fibrinogen values. p Values are on 2 df from ANOVA and 1 df from regression on allele unadjusted unless mentioned. The mentioned analysis was adjusted for gender.
with height.\textsuperscript{44} The CC genotype also associates with a lower glucose level at all points in OGTT by about 0.5 mmol/l, and (non-significant) triglyceride and cholesterol by about 0.2–0.3 mM each. However, the pattern for insulin levels in OGTT differs between males and females. These findings add to the observations of metabolic associations for the ACE I/D polymorphism, and implicate the diversity of the RAS pathway more generally in influencing anthropometric and metabolic traits. A number of studies have observed metabolic effects for ACE I/D.\textsuperscript{26,29,30} A study by Strazzullo et al.\textsuperscript{45} of a wide age range of men working at the Olivetti factory in southern Italy observed that ACE DD was associated with overweight and abdominal obesity and blood pressure but did not find similar associations for A1166C. The basis of negative finding for A1166C in their study compared with ours remains obscure although the age range, method of ascertainment, and environment and genetic background all differ. A small study\textsuperscript{46} of a wide age range of both sexes of the body. The expression of AGTR1 also has an effect on cardiovascular risk traits.

AGTR1 A1166C.A recent study\textsuperscript{37} found that the A1166C SNP is associated with aortic stiffness, and Jin et al.\textsuperscript{48} reported the possible association of this SNP with cardiovascular and metabolic traits. Alternatively, it may be in linkage disequilibrium (LD) with some other functional marker(s) located elsewhere in the AGTR1 gene or within a nearby gene that could explain the observed associations of this SNP with cardiovascular and metabolic phenotypes.

No LD was detected between A1166C and SNPs in the 5′ UTR and promoter region (G-2228A, C-1424G, A-214C, G-213C, and A-153G) and T55C in the promoter region (G-2228A, C-1424G, T-810A, T-713G, G-521T, A-214C, G-213C, and A-153G) and T55C in exon 4 of the AGTR1 gene.\textsuperscript{49} However, Lajemi et al.\textsuperscript{50} found an additive effect of 1166C and -153G on aortic stiffness, and Jin et al.\textsuperscript{48} reported the possible association of AGTR1 A1166C with essential hypertension in Chinese subjects, although Zhang et al.\textsuperscript{51} found no hypertension association with any of nine newly characterised AGTR1 promoter SNPs. A recent study showed that there are two main haplotype blocks in American white and black subjects.\textsuperscript{52} One of these haplotypes spans the 5′ UTR and the other spans exon 5. However, the extent of these blocks outside AGTR1 remains unknown. Syntenic genes include pancreatic carboxypeptidase B1 precursor, mast cell carboxypeptidase A3 precursor, glyco-genin, and transmembrane 4 superfamily number 1 and 4 are located 0.1–0.8 cM from AGTR1 (International HapMap Project: http://www.hapmap.org/). The lack of significant LD between SNPs in the promoter region and A1166C suggests a functional effect arising from the 3′ block.

Effects of the 3′ UTR on cell signalling, translation, and cell proliferation have been reported. Studies on Chinese hamster ovary cells (CHO-K1) have revealed the effect of AGTR1 3′ UTR on the angiotensin II receptor mediated cell signalling pathway and have shown the presence of a 55 kDa RNA-binding protein (RNAbp) which interacts with AGTR1 3′ UTR and influences specific receptor function,\textsuperscript{53} but the exact position of the reaction is not yet known (fig 2). While the mechanism of AGTR1 A1166C genotype-phenotype associations remain uncertain, this study suggests that in addition to effects on vascular function, AGTR1 A1166C can influence anthropometric and metabolic traits, providing further evidence of the integral effects of this gene and genotype on cardiovascular risk traits.

Angiotensin II has widespread effects on different organs of the body. The expression of AGTR1 and AGTR2 in different tissues such as the adrenal cortex, kidney, and rat uterus has been reported. The former is the predominant form in vascular smooth muscle and the human uterus, whereas the latter is expressed more predominantly in the adrenal medulla and brain.\textsuperscript{37} Giaclietti et al.\textsuperscript{54} reported the expression of angiotensin, and ACE and AGTR1 genes in visceral and subcutaneous adipose tissue. The effect of haplotype(s) distinguished by A1166C at the mRNA level and splicing and receptor quantity or quality are as yet unknown. AGTR1 pharmacological blockade lowers the risk of type 2 diabetes and is also known to promote adipocyte differentiation and insulin sensitivity.\textsuperscript{37}

Our study suggests that, like the ACE genotype, the AGTR1 genotype may also influence metabolic as well as vascular phenotypes and invites investigation of both AGTR1 and the whole RAS pathway with respect to metabolic traits.

\textbf{ELECTRONIC-DATABASE INFORMATION}

Details of the International HapMap Project can be found at http://www.hapmap.org/.

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