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 been previously 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 Hertfordshire, 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) in men (p = 0.03), 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.

ORIGINAL ARTICLE

One of the most important physiological pathways affecting the cardiovascular system and fluid and electrolyte balance is the renin angiotensin system (RAS) which, in parallel with kinins, has diverse regulatory roles in vasoconstriction, cell proliferation, and secretion of aldosterone from the adrenal gland (fig 1). Angiotensin II (AGT II) is the central component of the RAS pathway. It acts through two main receptors: the angiotensin II type I receptor (AGTR1) or AGTR1 and the angiotensin II type II receptor (AGTR2). It is generally believed that AGTR1 is the dominant receptor in the cardiovascular system. AGTR1 is located on 3q23–25 and spans about 60 kb including five exons and four introns. Exon sizes range from 59 to 2014 bp. Exon 5 is the largest and the only coding exon, while the first four exons encode a 5’ untranslated region (UTR).

AGTR1 is expressed in different organs including the heart, skeletal muscle, brain, human liver, lung, and adrenal gland. This receptor is included in the guanyl nucleotide binding protein (G-protein) coupled receptor superfamily for which the intracellular messengers are phospholipase, calcium, and protein kinase. It has also been shown that angiotensin converting enzyme (ACE) inhibitors or AGTR1 antagonists are effective in the treatment of hypertension, chronic heart failure, and diabetic nephropathy (DN). Many polymorphisms in genes of the RAS pathway have been identified. In AGTR1 (GenBank accession no. AF245699), A1166C (single nucleotide polymorphism [SNP] ID: rs5186) represented in the 3’ UTR of the mRNA has been widely studied. It has been associated with phenotypic effects including essential hypertension,12–14 systolic blood pressure,15 myocardial infarction, left ventricular hypertrophy,16 coronary artery disease (CAD),17–18 pre-eclampsia,19 pulse wave velocity in Caucasian subjects,20–22 and also stroke in Japanese subjects.23 Moczulski et al24 in a linkage study of discordant siblings identified a 20 cM region on the long arm of chromosome 3 containing AGTR1 which harbours a major locus for susceptibility to DN.

The effect of the insertion/deletion (I/D) polymorphism in intron 16 of the ACE gene on metabolism has also been studied. The ACE D allele is associated with higher plasma ACE.25 Cambien et al26 showed that ACE I/D modulates the consequences of small for gestational age for insulin resistance in young adults: D allele attenuated the adverse effects of low birth weight and short gestational age. In addition, ACE I/D is associated with metabolic syndrome27 and ACE inhibitors lower the risk of diabetes development.28,29 Furthermore, Montgomery et al30,31 reported that the insertion allele was associated with improved endurance performance, and it was concluded that the I/I genotype might maintain a positive energy balance during rigorous training suggesting enhanced metabolic efficiency in insertion carriers. Moreover, interaction between AGT II and insulin receptor signalling in the vasculature has been reported, in which AGT II inhibits insulin stimulated production of nitric oxide; this effect is mediated through AGTR1.32 There seems also to be a synergistic effect of A1166C and ACE I/D on CAD.31 Interestingly, it has been reported that in patients with
Angiotensinogen (AGT)

Renin

Angiotensin I

ACE

Angiotensin II

AGTR1 (3q21-q25)

AGTR2

- 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 analysis of metabolic syndrome traits in relation to AGTR1 SNPs and haplotypes. These subjects were selected from among all births in the county of Hertfordshire, UK during 1911–1930, who were followed forward and found to be alive and still resident there in 1990–1995. The subset selected for detailed analyses in males and females.

Statistical analysis

Hardy-Weinberg (HW) equilibrium was tested, and phenotypic association analysis for genotypes was by ANOVA and transformed to normal distributions as appropriate, and unadjusted and adjusted analyses were undertaken, as specified in table 3.

RESULTS

Genotype frequencies for AGTR1 A1166C are presented in table 2, and 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 with 1.7 units lower body mass index (BMI; p = 0.03), a lower waist-hip ratio (p = 0.01), 8% lower waist circumferences was also measured. Birth weight and 1 year weight were available from historical records.

Genotyping

DNA was extracted from 5 ml K-EDTA (ethylenediaminetetraacetic acid potassium salt) venous blood, and quantitation was done by picogreen assay. Long term aliquots were stored at −80°C and 7 ng/μl working dilutions in water were prepared. In the next step, a long PCR (3 kb) spanning exon 5 was prepared and this was followed by a nested four primer

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long PCR</td>
<td></td>
</tr>
<tr>
<td>Forward</td>
<td>5'-TCTCTAAAGTGCGACGCTCCTTCTGCAGC-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-GATTITTTGCCGGGGGAGCTAAACATGAA-3'</td>
</tr>
<tr>
<td>ARMS</td>
<td></td>
</tr>
<tr>
<td>Allele specific A</td>
<td>5'-TCTCGAACCTTACCTACAAATGACAC-3'</td>
</tr>
<tr>
<td>Allele specific C</td>
<td>5'-TCTCCCTTACCTGAAAAGATGTGAGC-3'</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-GCAATCCATTCACACTTACAAATGACAC-3'</td>
</tr>
<tr>
<td>Reverse</td>
<td>5'-AACAGCGCTAGGGAGATGCAATCCCTTG-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 = 0.06), fasting glucose (p = 0.08), height (p = 0.07), and glucose at 120 min (p = 0.08), and height (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 results. A significant difference by genotype in women; other RAS genotypes (AGTR1 C573T and ACE I/D) have previously been associated.

**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 v 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. 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.

**Table 2** Genotype frequencies for AGTR1 A1166C

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Men</th>
<th>Women</th>
<th>Observed</th>
<th>Expected</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>122</td>
<td>61</td>
<td>183</td>
<td>190</td>
</tr>
<tr>
<td>AC</td>
<td>50.83%</td>
<td>44.2%</td>
<td>48.41%</td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>101</td>
<td>69</td>
<td>170</td>
<td>156</td>
</tr>
<tr>
<td>7.08%</td>
<td>5.8%</td>
<td>6.61%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>240</td>
<td>138</td>
<td>378</td>
<td>378</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>%</th>
<th>Men</th>
<th>Women</th>
<th>Combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA</td>
<td>0.03</td>
<td>0.61</td>
<td>0.25</td>
</tr>
<tr>
<td>AC</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
</tr>
<tr>
<td>CC</td>
<td>0.05</td>
<td>0.05</td>
<td>0.002</td>
</tr>
<tr>
<td>Total</td>
<td>0.008</td>
<td>0.008</td>
<td>0.01</td>
</tr>
</tbody>
</table>

**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>p Value</th>
<th>Women</th>
<th>p Value</th>
<th>Combined analysis</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>BMI (kg/m²)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.99</td>
<td>0.99</td>
<td></td>
</tr>
<tr>
<td>(mm)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Waist to hip</td>
<td>0.001</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Ratio</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Adult weight (kg)</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Height</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(m)</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td>0.008</td>
<td></td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(mg/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Glucose at 30 min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Glucose at 120 min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(mmol/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Insulin at 0 min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(ug/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Insulin at 30 min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
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<tr>
<td>(ug/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Insulin at 120 min</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(ug/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Systolic BP</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Pulse pressure</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>(mmHg)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fasting cholesterol (mmol/l)</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Fasting TG</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
</tbody>
</table>

Mean values of each genotype group 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.
AGTR1 A1166C and metabolic traits

Figure 2  AGTR1 transcript (Ensembl Genome Browser: ENST00000326871). A1166C (M), two putative zip codes55 responsible for localisation of mRNA in β-actin (underlined), and 3′-UTR motifs (italics), capable of reacting with some trans-acting elements, are represented. An RNA-binding protein (RNAbp) interacts with the 3′ UTR of the AGTR1.55

with height.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 and influences specific receptor function, 52 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.13 Giachetti et al40 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.16

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.

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

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www.jmedgenet.com
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doi: 10.1136/jmg.2004.026716

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