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 gene (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 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.

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. 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 AT1R) 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 first AGTR1 is included in the guanyl nucleotide binding protein.

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Abbreviations: ACE, angiotensin converting enzyme; AGT II, angiotensin II; AGTR1, angiotensin II type I receptor; AGTR2, angiotensin II type II receptor; BMI, body mass index; CAD, coronary artery disease; DN, diabetic nephropathy; HW, Hardy-Weinberg; I/D, insertion/deletion; K-EDTA, ethylenediaminetetra-acetic acid potassium salt; LD, linkage disequilibrium; OGTT, oral glucose tolerance test; RAS, renin angiotensin system; RNAbp, RNA-binding protein; SNP, single nucleotide polymorphism; UTR, untranslated region

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Angiotensinogen (AGT) → Renin → Angiotensin I → ACE → Angiotensin II

\[
AGTR1 \text{(3q21-q25)}
\]

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

Cardiovascular and circulation

Figure 1 Renin angiotensin pathway.

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 association 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 early life anthropometric measures, subject to ethical approval (North and East Hertfordshire Ethical Committee) and subject anonymity.

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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 ARMS assay of the A1166C site. Primer sequences are represented in table 1.

Long PCR

Templates were 3 μl (6–7 ng/μl) of genomic DNA. Reaction mix for 20 μl was: 2 μl of 10× long PCR buffer (140 mM ammonium acetate and 500 mM Tris-HCL pH 8.9), 0.25 mM dNTPs, 0.4 pmol primers (MWG-Biotech, Ebersberg, Germany), 2 mM MgCl₂, 1.3 M betaine, 0.05 U/μl Gibco Taq DNA polymerase (Promega, Madison, WI, USA), 0.1 U/μl 1/250 Pwo (Roche Diagnostics, Lewes, UK), and water to 20 μl. Thermal cycling was on an MJ Tetrad (Bio-Rad, Hercules, CA): 94°C for 2 min; 94°C for 20 s, 65°C for 30 s, 68°C for 3 min, repeated for 35 cycles; then 68°C for 20 min. Checking electrophoresis for long PCR products was performed in submerged 1×TBE, 0.7% agarose gels at 100 V for 15 min. Detection was by ethidium bromide staining and scanning was on Fluorimag 595 (Molecular Dynamics, Sunnyvale, CA).

A1166C genotyping

Samples (2 μl) of 1/100 dilution in water of long PCR products were taken as templates for AGTR1 tetraprimer ARMS reaction. Reaction mix was: 10×PCR buffer, 1% (v/v) w1, 2.0 mM MgCl₂, 0.2 mM dNTPs, 2.2 pmol/μl oligos, and 0.05 U/μl Gibco Taq DNA polymerase. Thermal cycling was on an MJ Tetrad: 94°C for 2 min; then 94°C for 1 min, 58°C for 1 min, 72°C for 1 min, 72°C for 1 min, repeated for 25 cycles; and a final extension step at 72°C for 2 min. Bufferless electrophoresis was for 15 min at 150 V in 5% polyacrylamide MADGE gels prestained with ethidium bromide, as described previously.

Statistical analysis

Hardy-Weinberg (HW) equilibrium was tested, and phenotype association analysis for genotypes was by ANOVA and for alleles by regression in STATA 7.0. Variables were log 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 a 1.7 units lower body mass index (BMI; p = 0.03), a lower waist-hip ratio (p = 0.01), 8% lower waist circumference (p = 0.03), 0.71 for allele A and 0.29 for allele C, consistent with previous 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.

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Table 1 PCR primers for the ARMS assay and long PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long PCR Forward</td>
<td>5'-TCCTCAAAAGTGGAGCCCTACCTCCATCA'3'</td>
</tr>
<tr>
<td>Long PCR Reverse</td>
<td>5'-TGGTITTGGAGCCCGGAGGACAATGATG'3'</td>
</tr>
<tr>
<td>ARMS Allele specific A</td>
<td>5'-TCTGCCACCTTACCTCAAGAAATGACG'3'</td>
</tr>
<tr>
<td>ARMS Allele specific C</td>
<td>5'-GCCAATCCTACCTCAACCTTGACAAAG'3'</td>
</tr>
<tr>
<td>Forward</td>
<td>5'-AACGAGCTAGGAGGATGCACTTCCTG'3'</td>
</tr>
</tbody>
</table>

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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 (p = 0.07), and height (p = 0.07), and glucose at 120 min (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 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.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 cardiovascular disease.

### 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>101</td>
<td>69</td>
<td>170</td>
<td>156</td>
</tr>
<tr>
<td>CC</td>
<td>17</td>
<td>8</td>
<td>25</td>
<td>32</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>Trait</th>
<th>Men</th>
<th>Women</th>
<th>Combined analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AA</td>
<td>AC</td>
<td>CC</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>26.9 (3.3)</td>
<td>27.4 (3.4)</td>
<td>25.2 (3.0)</td>
</tr>
<tr>
<td>Glucose at 30 min (mmol/l)</td>
<td>9.7 (0.07)</td>
<td>9.3 (0.05)</td>
<td>8.2 (0.07)</td>
</tr>
<tr>
<td>Glucose at 120 min (mmol/l)</td>
<td>6.8 (1.2)</td>
<td>6.3 (1.2)</td>
<td>5.7 (1.2)</td>
</tr>
<tr>
<td>Fasting glucose (mmol/l)</td>
<td>6.1 (1.2)</td>
<td>5.9 (1.2)</td>
<td>5.6 (1.2)</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>165 (21.8)</td>
<td>162 (20.8)</td>
<td>156 (16.8)</td>
</tr>
<tr>
<td>Fasting TG (g/l)</td>
<td>1.5 (1.2)</td>
<td>1.4 (1.2)</td>
<td>1.3 (1.2)</td>
</tr>
</tbody>
</table>
The lack of significant ACE polyclonal effects for metabolic traits. A number of studies have observed metabolic pathways more generally in influencing anthropometric and polymorphism, and implicate the diversity of the RAS ascertainment, and environment and genetic background all remains obscure although the age range, method of overweight and abdominal obesity and blood pressure but DD was associated with 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 over a wide age range of men working at the Olivetti factory in southern Italy is unknown. While the mechanism of AGTR1 A1166C genotypes clearly differentiate, 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. Giachetti et al 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.

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/](http://www.hapmap.org/).

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Figure 2  AGTR1 transcript (Ensembl Genome Browser: ENS960000326871). A1166C (M), two putative zip codes<sup>55</sup> responsible for localisation of mRNA in β actin (underlined), and A-U 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.<sup>21</sup>
We thank the UK MRC and BHF for support. MRA is an Iranian Ministry of Health PhD Scholar.

Competing interests: none declared

REFERENCES


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