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) 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 gland12 (fig 1). Angiotensin II (RAS) which, in parallel with kinins, has diverse regulatory roles in vasoconstriction, cell proliferation, and secretion of aldosterone from the adrenal gland12 (fig 1). 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) 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\(^1\) (fig 1). Angiotensin II (RAS) which, in parallel with kinins, has diverse regulatory roles in vasoconstriction, cell proliferation, and secretion of aldosterone from the adrenal gland\(^1\) (fig 1). 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) 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\(^1\) (fig 1). Angiotensin II (RAS) which, in parallel with kinins, has diverse regulatory roles in vasoconstriction, cell proliferation, and secretion of aldosterone from the adrenal gland\(^1\) (fig 1). 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) 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\(^1\) (fig 1). Angiotensin II (RAS) which, in parallel with kinins, has diverse regulatory roles in vasoconstriction, cell proliferation, and secretion of aldosterone from the adrenal gland\(^1\) (fig 1). 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) 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.
AGTR1 A1166C and metabolic traits

Angiotensinogen (AGT)

Renin

Angiotensin I

ACE

Angiotensin II

AGTR1 (3q21-q25)

AGTR2

- Vasoconstriction
- 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 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 evaluation of metabolic syndrome comprised those willing to undergo an oral glucose tolerance test (OGTT) and did not differ significantly from the larger group with regard to birth weight or socioeconomic status. They underwent metabolic characterisation including measurements of blood pressure, pulse rate, and 0, 30, and 120 min glucose and insulin responses to 75 g OGTT. Their heights, weights, waist, and hip circumferences were 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 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-Biotec, 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 a 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, 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 3% polyacrylamide MADGE gels prestained with ethidium bromide, as described previously.

Statistical analysis

Hardy-Weinberg (HW) equilibrium was tested, and phenotypic 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 (χ² = 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 circumference (p = 0.008), lower glucose at 30 min (p = 0.01), 30%
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 differences by genotype in women; other RAS genotypes (AGTR1 C573T and ACE I/D) have previously been associated with lower 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 A1166C and ACE I/D) have previously been associated with lower BMI 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.

### 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.

### 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>50.83%</td>
<td>44.2%</td>
<td>48.41%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AC</td>
<td>101</td>
<td>69</td>
<td>170</td>
<td>156</td>
</tr>
<tr>
<td>42.08%</td>
<td>50%</td>
<td>44.97%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>17</td>
<td>8</td>
<td>25</td>
<td>32</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>
<tr>
<td>100%</td>
<td>100%</td>
<td>100%</td>
<td></td>
<td></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 analysis (Reg.) of anthropometric and metabolic traits for the AGTR1 A1166C polymorphism in 240 men and 138 women. A stronger statistical significance of effects was observed particularly for all glucose time points in the tolerance test.
The lack of significant bolic effects for polymorphism, and implicate the diversity of the RAS differ. A small study of a wide age range of both sexes of ascertainment, and environment and genetic background all remains obscure although the age range, method of overweight and abdominal obesity and blood pressure but 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 the observed associations of this SNP with cardiovascular and metabolic traits remains unknown.

No LD was detected between A1166C and SNPs in the 5' UTR and promoter region (G-2228A, T-1424G, T-710A, T-713G, C-521T, A-214C, G-213C, and A-153G) and T55C in the 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, 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. Giacchetti 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.
We thank the UK MRC and BHF for support. MRA is an Iranian Ministry of Health PhD Scholar.

Competing interests: none declared

REFERENCES


24 Fall OH, Osmond C, Barker DJ, Clark PM, Hales CN, Stirling Y, Meade TW. Fetal and infant growth and impaired glucose tolerance at age 64. BMJ 1999;313(7068):1019–22.


33 Jin W, Liu Y, Zheng HH, Jin J, Shen YY, Hua Q, Li Y, Xu JD, Huang W. Single nucleotide polymorphisms in promoter of angiotensin II type 1 receptor gene

34 Abdollahi, Gaunt, Syddall, et al.
Clinical Evidence—Call for contributors

Clinical Evidence is a regularly updated evidence-based journal available worldwide both as a paper version and on the internet. Clinical Evidence needs to recruit a number of new contributors. Contributors are healthcare professionals or epidemiologists with experience in evidence-based medicine and the ability to write in a concise and structured way.

Areas for which we are currently seeking authors:
- Child health: nocturnal enuresis
- Eye disorders: bacterial conjunctivitis
- Male health: prostate cancer (metastatic)
- Women’s health: pre-menstrual syndrome; pyelonephritis in non-pregnant women

However, we are always looking for others, so do not let this list discourage you.

Being a contributor involves:
- Selecting from a validated, screened search (performed by in-house Information Specialists) epidemiologically sound studies for inclusion.
- Documenting your decisions about which studies to include on an inclusion and exclusion form, which we keep on file.
- Writing the text to a highly structured template (about 1500–3000 words), using evidence from the final studies chosen, within 8–10 weeks of receiving the literature search.
- Working with Clinical Evidence editors to ensure that the final text meets epidemiological and style standards.
- Updating the text every six months using any new, sound evidence that becomes available. The Clinical Evidence in-house team will conduct the searches for contributors; your task is simply to filter out high quality studies and incorporate them in the existing text.
- To expand the topic to include a new question about once every 12–18 months.

If you would like to become a contributor for Clinical Evidence or require more information about what this involves please send your contact details and a copy of your CV, clearly stating the clinical area you are interested in, to Klara Brunnhuber (kbrunnhuber@bmjgroup.com).

Call for peer reviewers

Clinical Evidence also needs to recruit a number of new peer reviewers specifically with an interest in the clinical areas stated above, and also others related to general practice. Peer reviewers are healthcare professionals or epidemiologists with experience in evidence-based medicine. As a peer reviewer you would be asked for your views on the clinical relevance, validity, and accessibility of specific topics within the journal, and their usefulness to the intended audience (international generalists and healthcare professionals, possibly with limited statistical knowledge). Topics are usually 1500–3000 words in length and we would ask you to review between 2–5 topics per year. The peer review process takes place throughout the year, and our turnaround time for each review is ideally 10–14 days.

If you are interested in becoming a peer reviewer for Clinical Evidence, please complete the peer review questionnaire at www.clinicalevidence.com or contact Klara Brunnhuber (kbrunnhuber@bmjgroup.com).
Angiotensin II type I receptor gene polymorphism: anthropometric and metabolic syndrome traits
M R Abdollahi, T R Gaunt, H E Syddall, C Cooper, D I W Phillips, S Ye and I N M Day

doi: 10.1136/jmg.2004.026716

Updated information and services can be found at:
http://jmg.bmj.com/content/42/5/396

These include:

References
This article cites 46 articles, 23 of which you can access for free at:
http://jmg.bmj.com/content/42/5/396#BIBL

Email alerting service
Receive free email alerts when new articles cite this article. Sign up in the box at the top right corner of the online article.

Topic Collections
Articles on similar topics can be found in the following collections
- Hypertension (60)
- Molecular genetics (1254)
- Ischaemic heart disease (43)

Notes

To request permissions go to:
http://group.bmj.com/group/rights-licensing/permissions

To order reprints go to:
http://journals.bmj.com/cgi/reprintform

To subscribe to BMJ go to:
http://group.bmj.com/subscribe/