ORIGINAL ARTICLE

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

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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[1 2] (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 *AT1R*) and the angiotensin II type II receptor (*AGTR2*). It is generally believed that *AGTR1* is the dominant receptor in the cardiovascular system.[1–3] *AGTR1* is located on 3q23–25[4] 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).[5–7] *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.[8 9] 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).[10 11] 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 al*[24] 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 al*[26] 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 syndrome[27] and *ACE* inhibitors lower the risk of diabetes development.[28] Furthermore, Montgomery *et al*[29 30] 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*.[31 32] There seems also to be a synergistic effect of A1166C and *ACE* I/D on CAD.[33 34] Interestingly, it has been reported that in patients with

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

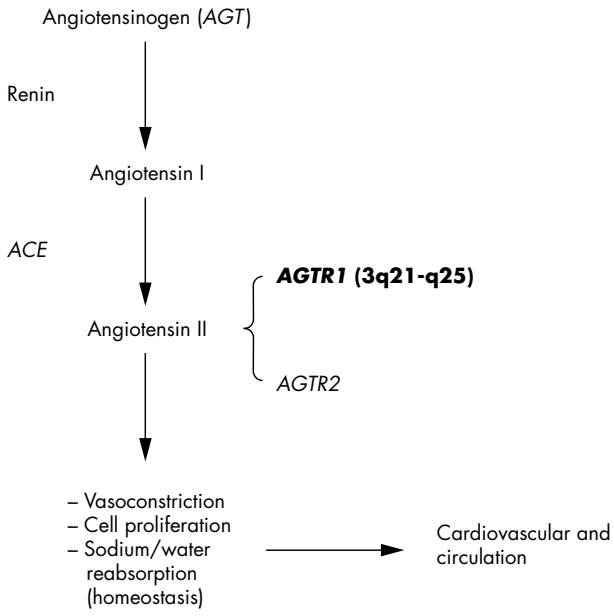

Figure 1 Renin angiotensin pathway.

CAD carrying the CC genotype of *AGTR1* A1166C, the response to *AGT II* is increased.[35] In addition, pharmacological blockade of *AGTR1* induces peroxisome proliferator activated receptor-γ activity which promotes differentiation in adipocytes.[36]

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.[37–39] 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,[40] and quantitation was done by picogreen assay. Long term aliquots were stored at −80℃ 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.[41] 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 $MgCl_2$, 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℃ for 2 min; 94℃ for 20 s, 65℃ for 30 s, 68℃ for 3 min, repeated for 35 cycles; then 68℃ 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 Fluorimager 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 $MgCl_2$, 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℃ for 2 min; then 94℃ for 1 min, 58℃ for 1 min, 72℃ for 1 min, repeated for 25 cycles; and a final extension step at 72℃ for 2 min. Bufferless electrophoresis was for 15 min at 150 V in 5% polyacrylamide MADGE gels prestained with ethidium bromide, as described previously.[42]

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 $\log_e$ 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 circumference ($p = 0.008$), lower glucose at 30 min ($p = 0.01$), 30%

Table 1 PCR primers for the ARMS assay and long PCR

Primer	Sequence
Long PCR	
Forward	5′- TCCTCAAAGTCGAGCCCTACCTCCTACG-3′
Reverse	5′- TGATTTTTGACCGGGGAAGCTAAACATGA-3′
ARMS	
Allele specific A	5′- TCTGCAGCACTTCACTACCAAATGAACA-3′
Allele specific C	5′- TCTCCTTCAATTCTGAAAAGTAGCTGAG-3′
Forward	5′- GCCAAATCCCACTCAAACCTTTCAACAA-3′
Reverse	5′- AAGCAGGCTAGGGAGATTGCATTTCTGT-3′

Table 2 Genotype frequencies for *AGTR1* A1166C

	Sex			
Genotype	Men	Women	Observed	Expected
AA	122 50.83%	61 44.2%	183 48.41%	190
AC	101 42.08%	69 50%	170 44.97%	156
CC	17 7.08%	8 5.8%	25 6.61%	32
Total	240 100%	138 100%	378 100%	378

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 *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

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

	Men			p Value		Women			p Value		Combined analysis			p Value	
	AA	AC	CC	ANOVA	Reg.	AA	AC	CC	ANOVA	Reg.	AA	AC	CC	ANOVA	Reg.
BMI (kg/m^2)	26.9 (3.3)	27.4 (3.4)	25.2 (3.0)	0.03	0.61	26.3 (3.6)	27.3 (4.7)	25.5 (1.9)	0.25	0.56	26.7 (3.4)	27.4 (4.0)	25.3 (2.6)	0.01	0.99
Waist to hip Ratio	0.94 0.05	0.94 0.05	0.90 0.05	0.01	0.01	0.80 0.06	0.79 0.05	0.77 0.04	0.48	0.40	0.89 0.09	0.88 0.09	0.86 0.08	0.004	0.013
Waist circumference (cm)	99.2 (10.2)	98.4 (9.0)	91.5 (8.2)	0.008	0.02	82.6 (9.0)	83.9 (9.6)	76.5 (2.9)	0.09	0.61	93.7 (12.6)	92.5 (11.7)	86.7 (9.9)	0.001	0.02
Adult weight (kg)	80.4 (11.5)	80.3 (10.8)	73.6 (11.0)	0.06	0.12	67.7 (9.8)	70.5 (12.0)	62.1 (5.3)	0.07	0.86	76.2 (12.5)	76.3 (12.3)	69.9 (10.9)	0.008	0.24
Height (m)	1.73 (0.07)	1.71 (0.05)	1.71 (0.07)	0.07	0.03	1.60 (0.06)	1.61 (0.05)	1.56 (0.06)	0.11	0.42	1.67 (0.09)	1.67 (0.07)	1.67 (0.1)	0.07	0.02
Fasting glucose (mmol/l)	6.1 (1.2)	5.9 (1.2)	5.6 (1.1)	0.08	0.03	5.7 (1.1)	5.7 (1.2)	5.8 (1.1)	0.89	0.82	6.0 (1.2)	5.8 (1.2)	5.6 (1.1)	0.18	0.07
Glucose at 30 min (mmol/l)	9.7 (1.3)	9.3 (1.2)	8.2 (1.2)	0.01	0.008	8.8 (1.2)	8.7 (1.2)	8.1 (1.2)	0.58	0.50	9.4 (1.3)	9.1 (1.2)	8.2 (1.2)	0.01	0.01
Glucose at 120 min (mmol/l)	6.8 (1.4)	6.3 (1.4)	5.7 (1.2)	0.06	0.02	7.1 (1.3)	7.1 (1.3)	6.6 (1.4)	0.73	0.73	6.9 (1.3)	6.6 (1.4)	6.0 (1.3)	0.08	0.03
Insulin at 0 min (pm/l)	42.7 (1.2)	40.8 (1.8)	27.4 (2.0)	0.04	0.05	48.4 (1.7)	42.3 (1.8)	50.1 (1.2)	0.34	0.41	44.6 (1.9)	41.4 (1.8)	33.5 (1.9)	0.08	0.04
Insulin at 30 min (pm/l)	271.3 (1.9)	266.7 (1.9)	238.2 (1.6)	0.74	0.53	248.3 (1.7)	249.9 (1.8)	353.0 (1.6)	0.24	0.30	263.3 (1.8)	259.6 (1.9)	271.6 (1.6)	0.95	0.96
Insulin at 120 min (pm/l)	161.9 (2.3)	135.7 (2.4)	121.4 (1.8)	0.21	0.08	231.0 (1.9)	238.1 (1.9)	223.8 (1.7)	0.94	0.92	183.1 (2.2)	172.6 (2.3)	151.7 (1.9)	0.35	0.15
Systolic BP (mmHg)	165.0 (21.8)	162.9 (20.8)	156.9 (16.8)	0.30	0.15	156.4 (21.2)	156.0 (23.6)	161.3 (17.1)	0.82	0.78	162.1 (21.9)	160.1 (22.2)	158.3 (16.7)	0.61	0.39
Pulse pressure (mmHg)	74.8 (16.1)	72.7 (14.6)	69.2 (13.4)	0.29	0.12	75.1 (14.6)	72.8 (15.8)	76.9 (17.6)	0.61	0.70	74.9 (15.5)	72.7 (15.0)	71.7 (15.0)	0.32	0.14
Fasting cholesterol (mmol/l)	6.7 (1.2)	6.5 (1.2)	6.2 (1.2)	0.32	0.16	7.0 (1.2)	7.2 (1.2)	6.2 (1.2)	0.07	0.72	6.8 (1.2)	6.8 (1.2)	6.2 (1.2)	0.08	0.18
Fasting TG (mmol/l)	1.5 (1.7)	1.4 (1.7)	1.3 (1.5)	0.41	0.19	1.2 (1.6)	1.4 (1.5)	1.0 (1.4)	0.04	0.72	1.4 (1.7)	1.4 (1.6)	1.2 (1.5)	0.19	0.28
Fasting fibrinogen (g/l)	309.5 (1.2)	295.7 (1.3)	302.9 (1.2)	0.28	0.22	302.9 (1.2)	283.8 (1.1)	267.1 (1.1)	0.01	0.002	307.4 (1.2)	290.9 (1.2)	290.5 (1.2)	0.03	0.01

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.

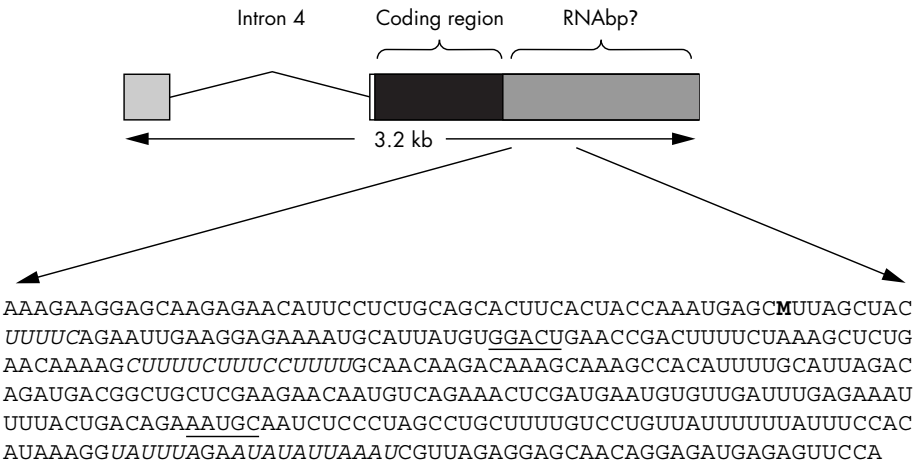

Figure 2 *AGTR1* transcript (Ensembl Genome Browser: ENSt00000326871). A1166C (M), two putative zip codes[55] responsible for localisation of mRNA in β actin (underlined), and A+U motifs (italics), capable of reacting with some trans-acting elements, are represented. An RNA-binding protein (RNAbp) interacts with the 3′ UTR of the *AGTR1*.[52]

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* I/D.[26 29 30] A study by Strazzullo *et al*[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[46] of a wide age range of both sexes of hypertensive subjects and controls under 60 years old may not have had the power to detect the effects of CC genotype (six normotensives) of *AGTR1* A1166C.

It is possible but uninvestigated that *AGTR1* A1166C in the 3′ UTR (fig 2) might affect mRNA stability or polyadenylation. 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, T-810A, T-713G, C-521T, A-214C, G-213C, and A-153G) and T55C in exon 4 of the *AGTR1* gene.[47] However, Lajemi *et al*[48] found an additive effect of 1166C and -153G on aortic stiffness, and Jin *et al*[49] reported the possible association of *AGTR1* A-810T with essential hypertension in Chinese subjects, although Zhang *et al*[50] 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.[51] 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, glycogenin, 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,[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.[53] Giacchetti *et al*[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.[36]

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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