PAPER

Polymorphisms of the renin–angiotensin system are associated with blood pressure, atherosclerosis and cerebral white matter pathology

M J E van Rijn, M J Bos, A Isaacs, M Yazdanpanah, A Arias-Vásquez, B H Ch Stricker, O H Klungel, B A Oostra, P J Koudstaal, J C Witteman, A Hofman, M M B Breteler, C M van Duijn

J Neural Neurosurg Psychiatry 2007;**78**:1083–1087. doi: 10.1136/jnnp.2006.109819

See end of article for authors' affiliations

Correspondence to:
Professor C M van Duijn,
Department of Epidemiology
and Biostatistics, Erasmus
Medical Centre, PO Box
1738, 3000 DR Rotterdam,
the Netherlands;
c.vanduijn@
erasmusmc.nl

Received 25 October 2006
Revised 14 December 2006
Accepted 27 December 2006
Published Online First
12 January 2007

Background: The renin–angiotensin system is involved in the development of hypertension, atherosclerosis and cardiovascular disease. We studied the association between the M235T polymorphism of the angiotensinogen gene (*AGT*) and the C573T polymorphism of the angiotensin II type 1 receptor (*AT1R*) and blood pressure, carotid atherosclerosis and cerebrovascular disease.

Methods: We genotyped over 6000 subjects from the Rotterdam Study and more than 1000 subjects from the Rotterdam Scan Study. We used logistic regression and univariate analyses, adjusting for age and sex with, for *AGT*, the MM and, for *AT1R*, the TT genotype as reference.

Results: We found that *AGT*–235T increased systolic (p for trend=0.03) and diastolic blood pressure (p for trend=0.04). The prevalence of carotid plaques was increased 1.25-fold (95% CI 1.02–1.52) in *AGT*–TT carriers. There was a significant increase in mean volume deep subcortical white matter lesions (WML) for *AGT*–TT carriers (1.78 ml vs 1.09 ml in the reference group; p=0.008). A significant interaction was found between *AGT* and *AT1R*, further increasing the effect on periventricular and subtotal WML (p for interaction=0.02). We found a non-significant increased risk of silent brain infarction for *AGT*–TT carriers and *AT1R*–CC carriers, but no effect on stroke.

Conclusion: We found an association between *AGT* and blood pressure, atherosclerosis and WML. Also, we found synergistic effects between *AGT* and *AT1R* on the development of WML. These findings raise the question of whether the renin–angiotensin system may be a therapeutic target for the prevention of cerebral white matter pathology.

The renin–angiotensin system (RAS) regulates blood pressure, cardiovascular homeostasis and vascular tone.¹ Polymorphisms in genes that encode for the proteins of the RAS are candidate genes for hypertension, cardiovascular and cerebrovascular diseases. The angiotensin (*AGT*) and the angiotensin II type I receptor (*AT1R*) genes are two key players in *AGT* protein metabolism.

The *AGT*–M235T polymorphism encodes the substitution of methionine by threonine at residue 235 of the *AGT* protein, increasing plasma *AGT* levels in 235T homozygotes.² A haplotype at the *AGT* promoter, which was in complete linkage disequilibrium with the M235T polymorphism, was found to increase transcriptional activity in astrocytes.³ *AGT* has been associated with hypertension, carotid atherosclerosis, and cardiovascular and cerebrovascular disease, although findings have been inconsistent.^{4–7} *AGT* has been associated consistently with cerebral small vessel disease.^{8–10}

The *AT1R* gene has been associated with hypertension, and cardiovascular and cerebrovascular disease.^{11–15} The *AT1R*–C573T polymorphism, which is in linkage disequilibrium with the frequently studied A1166C polymorphism,^{14 15} has been associated with blood pressure and vascular complications in hypertensive patients.^{16 17}

Hypertension, atherosclerosis and cerebrovascular diseases are all complex diseases. For these traits, a network of interactions between genetic factors can be supposed.¹⁸ Both the *AGT* and *AT1R* gene products are part of the RAS. To date, an interaction has not been reported. We studied the *AGT*–M235T and the *AGT*–C573T polymorphisms in relation to blood pressure, carotid atherosclerosis and small and large vessel cerebral pathology. Also, we studied the interaction

between *AGT* and *AT1R* with respect to all of the above mentioned outcomes.

METHODS

Study populations

The Rotterdam Study is an ongoing prospective population based cohort study on chronic and disabling diseases in the elderly.¹⁹ Baseline examinations were performed between 1990 and 1993. A total of 7983 subjects (age ≥55 years) participated in this study. In 6444 (80.7%) and 6367 (79.8%) participants, the M235T polymorphism of *AGT* and the C573T polymorphism of *AT1R*, respectively, were successfully genotyped. No DNA was available for 1455 subjects and there was genotyping failure in 84 (*AGT*) and 161 (*AT1R*) subjects.

The Rotterdam Scan Study was designed to study the aetiology and natural history of age related brain changes in the elderly, using a similar protocol (baseline between 1995). A total of 1077 non-demented elderly persons (age ≥60 years) participated in this study. In 1995/1996, subjects aged between 60 and 90 years were selected randomly in strata of age (5 years) and sex, from the Zoetermeer Study²⁰ and the Rotterdam Study. In 1048 (97.3%) participants, the M235T polymorphism of *AGT* and in 1011 (93.9%) participants, the C573T polymorphism of *AT1R*, were successfully genotyped. There was a genotyping failure in 29 (*AGT*) and 66 (*AT1R*)

Abbreviations: ACE, angiotensin converting enzyme; *AGT*, angiotensinogen gene; *AT1R*, angiotensin II type I receptor gene; BMI, body mass index; DBP, diastolic blood pressure; HR, hazard ratio; RAS, renin–angiotensin system; SBI, silent brain infarctions; SBP, systolic blood pressure; WML, white matter lesions

subjects. The medical ethics committee of the Erasmus Medical Centre, Rotterdam, the Netherlands approved both studies and all participants gave written informed consent and permission to retrieve information from treating physicians.

Measurements

Height and weight were measured and body mass index (BMI in kg/m²) was calculated. Blood pressure was based on the average of two measurements with a random zero sphygmomanometer. Hypertension was defined as a systolic blood pressure (SBP) ≥ 140 mmHg and/or a diastolic blood pressure (DBP) ≥ 90 mmHg and/or use of antihypertensive drugs. For 1184 (14.8%) patients, blood pressure measurements were not available. Information on smoking habits was obtained.

We collected non-fasting blood samples from all participants. We defined diabetes mellitus as a random glucose level ≥ 11.1 mmol/l and/or use of oral antidiabetics or insulin. Total serum cholesterol and high density lipoprotein cholesterol were determined using an automated enzymatic method.²¹

Measurements of atherosclerosis and stroke

As part of the Rotterdam Study, the total number of plaques was assessed by duplex scan ultrasonography.²² Plaques were defined as focal widening of the vessel wall with protrusion into the lumen. The total plaque score reflected the total number of sites with plaques ranging from 0 to 6 (left and right sided, common carotid arteries, bifurcation and internal carotid arteries). This score was dichotomised (0, 1 or 2 vs >2). For 2372 (29.7%) participants, the number of plaques could not be assessed.

Incident stroke was also assessed as part of the Rotterdam Study. A prevalent stroke was determined during the baseline interview. Research physicians reviewed information on all

possible strokes with an experienced stroke neurologist to verify all diagnoses. Subarachnoid haemorrhages and retinal strokes were excluded. A stroke was classified as ischaemic when a patient had typical symptoms and a CT or MRI ruled out other diagnoses, or when indirect evidence (deficit limited to one limb or completely resolved within 72 h or atrial fibrillation in the absence of anticoagulants) pointed to an ischaemic nature of the stroke. A stroke was classified as haemorrhagic when a relevant haemorrhage was shown on CT or MRI scan, or the subject lost consciousness permanently or died within hours after the onset of focal signs.

Cerebral infarcts and white matter lesions

As part of the Rotterdam Scan Study, we obtained axial T1, T2 and proton density MRI scans of the brain. Infarcts were defined as focal hyperintensities on T2 weighted images, 3–20 mm in size. Silent brain infarctions (SBI) were defined as evidence of infarcts on MRI, without a history of a (corresponding) stroke or transient ischaemic attack.²³

White matter lesions (WML) were scored as hyperintense on proton density and T2 weighted images, without prominent hypointensity on T1 weighted scans and, according to their location, as periventricular or subcortical.²⁴ Periventricular WML were rated semiquantitatively (range 0–9). A total volume of subcortical WML was approximated based on the number and size of lesions (volume range 0–29.5 ml). In four participants (0.004%), subcortical WML could not be measured because of the quality of the MRI scans.

Genotyping

Genotyping of the *AGT*-M235T polymorphism and the *AT1R*-C573T polymorphism was performed using TaqMan allelic discrimination Assays-By-Design (Applied Biosystems,

Table 1 Baseline characteristics stratified by *AGT* and *AT1R* genotype (MM, MT or TT)

	MM	MT	TT
<i>AGT</i> -M235T			
Rotterdam Study (n)	2341 (36.3)	3084 (47.9)	1019 (15.8)
Age (y)	69.4 (9.1)	69.7 (9.2)	69.0 (9.1)
Sex (% men)	39.6	41.0	40.7
BMI (kg/m ²)	26.4 (3.8)	26.3 (3.7)	26.2 (3.6)
Current smoking (%)	21.9	23.1	23.2
Total cholesterol (mmol)	6.6 (1.2)	6.6 (1.3)	6.6 (1.2)
Diabetes mellitus (%)	10.1	10.2	8.9
Rotterdam Scan Study (n)	386 (36.8)	503 (48.0)	159 (15.1)
Age (y)	72.2 (7.3)	72.2 (7.4)	72.4 (7.6)
Sex (% men)	46.4	50.3	42.8
BMI (kg/m ²)	26.7 (3.6)	26.7 (3.6)	26.5 (3.6)
Current smoking (%)	18.1	16.1	17.7
Total cholesterol (mmol)	5.9 (1.1)	5.9 (1.0)	5.9 (1.1)
Diabetes mellitus (%)	5.2	8.3	7.5
	TT	CT	CC
<i>AT1R</i> C573T			
Rotterdam Study (n)	1484 (23.3)	3126 (49.1)	1755 (27.6)
Age (y)	69.5 (9.3)	69.4 (8.9)	69.5 (9.4)
Sex (% men)	38.8	41.3	40.6
BMI (kg/m ²)	26.2 (3.7)	26.3 (3.7)	26.4 (3.8)
Current smoking (%)	21.6	23.9	21.4
Total cholesterol (mmol)	6.6 (1.2)	6.6 (1.2)	6.6 (1.2)
Diabetes mellitus (%)	9.4	10.0	10.8
Rotterdam Scan Study (n)	219 (21.7)	504 (49.9)	288 (28.5)
Age (y)	72.6 (7.5)	72.1 (7.1)	71.8 (7.2)
Sex (% men)	46.1	49.8	48.3
BMI (kg/m ²)	26.7 (4.2)	26.8 (3.6)	26.4 (3.3)
Current smoking (%)	17.4	15.7	19.9
Total cholesterol (mmol)	5.9 (1.0)	5.9 (1.0)	5.9 (1.1)
Diabetes mellitus (%)	5.5	7.3	7.3

AGT, angiotensin gene; *AT1R*, angiotensin II type I receptor gene; BMI, body mass index. All values are percentages or mean (SD).

Table 2 The Rotterdam Study: association between the M235T and the C573T polymorphism with SBP, DBP and hypertension									
	n	SBP (mm Hg)		n	DBP (mm Hg)		n	Hypertension (OR (95% CI))	
		Model 1	Model 2		Model 1	Model 2		Model 1	Model 2
AGT–M235T									
MM	2198	138.2 (0.5)	138.2 (0.5)	2197	73.4 (0.2)	73.4 (0.2)	2206	1.0 (reference)	1.0 (reference)
MT	2910	139.5 (0.4)*	139.4 (0.4)*	2910	73.7 (0.2)	73.7 (0.2)	2927	1.13 (1.01 to 1.27)*	1.15 (1.02 to 1.30)*
TT	955	139.6 (0.7)	139.6 (0.7)	955	74.2 (0.4)*	74.2 (0.4)*	964	1.17 (1.00 to 1.36)	1.20 (1.02 to 1.41)*
p for trend		0.03	0.03		0.04	0.04			
AT1R–C573T									
TT	1397	139.4 (0.6)	139.6 (0.6)	1396	73.7 (0.3)	73.8 (0.3)	1409	1.0 (reference)	1.0 (reference)
CT	2942	139.3 (0.4)	139.2 (0.4)	2942	73.9 (0.2)	73.9 (0.2)	2952	1.04 (0.91 to 1.18)	1.02 (0.89 to 1.17)
CC	1657	138.6 (0.5)	138.4 (0.5)	1657	73.4 (0.3)	73.4 (0.3)	1669	0.95 (0.82 to 1.10)	0.92 (0.79 to 1.07)
p for trend		0.3	0.1		0.4	0.3			

AGT, angiotensin gene; AT1R, angiotensin II type I receptor gene; DBP, diastolic blood pressure; n, absolute number of subjects; SBP, systolic blood pressure. Values are mean (SEM) unless otherwise indicated.

All means and ORs are adjusted in model 1 for: age and sex. All means and ORs are adjusted in model 2 for: age, sex, body mass index, total cholesterol, diabetes mellitus, smoking (and use of antihypertensive medication in SBP and DBP analyses).

*p<0.05 compared with the MM genotype.

Foster City, California, USA). Based on the analysis of blind duplicates (326 control pairs), there was 99.4% concordance in genotyping *AGT* and 100% concordance in genotyping *AT1R*. The two discordant pairs were set to missing.

Statistical analyses

Hardy–Weinberg equilibrium proportions of the M235T and the C573T polymorphisms were tested using the GENEPOP package. Baseline characteristics were compared using univariate ANOVA or χ^2 statistics. Analysis of variance was used to assess the relation between *AGT* and *AT1R* and SBP and DBP, as well as WML. In order to obtain a normal distribution of WML, we used the natural logarithm transformation. Cox proportional hazards regression analysis was used to assess the relative risk of stroke. For this analysis, we excluded prevalent stroke. We used logistic regression to assess the odds ratio for carotid artery plaques and SBI for *AGT* and *AT1R* (SPSS version 11.0). All analyses were adjusted for age and sex (model 1) and additionally adjusted for SBP, DBP, BMI, total cholesterol, diabetes mellitus and smoking (model 2). The analyses on blood pressure levels were also adjusted for use of antihypertensives. A p value <0.05 was considered statistically significant.

RESULTS

Genotype frequencies were in Hardy–Weinberg-Equilibrium for both study populations. Table 1 shows the baseline characteristics stratified by *AGT* and *AT1R* genotype. No significant

differences were observed between genotype groups and baseline characteristics.

In table 2, we show that for *AGT*, SBP (p for trend = 0.03) and DBP (p for trend = 0.04) increased with the number of *AGT*–235T alleles. In the fully adjusted model, including antihypertensive medication use, these findings remained significant. Also, a (borderline) significant increase in the prevalence of hypertension was found. *AT1R* was not associated with blood pressure or hypertension.

Figure 1 shows that carriers of the *AGT*–235T allele had an increased risk of plaques. The OR for the *AGT*-MT genotype was 1.16 (95% CI 1.00 to 1.34; p = 0.05) and for the TT genotype 1.25 (95% CI 1.02 to 1.52; p = 0.03). In the fully adjusted model, the OR for the *AGT*-MT genotype was 1.17 (95% CI 1.01 to 1.37; p = 0.04) and for the TT genotype 1.27 (95% CI 1.03 to 1.57; p = 0.03). We did not find an association between *AT1R* and carotid artery plaques.

Periventricular WML were present in 219 (20.3%) and deep subcortical WML in 84 (7.8%) participants. No association was found between *AGT* or *AT1R* and periventricular WML. Participants with the TT genotype of *AGT* had an increased volume of deep subcortical WML (1.78 ml vs 1.09 ml for the reference group, p = 0.008) (fig 2). Participants with the CT genotype of the *AT1R* genotype also showed an increased volume (1.45 ml vs 0.99 ml for the reference group; p = 0.03). Findings remained significant in the fully adjusted model (*AGT*-TT 1.81 ml vs 1.08 ml for the reference group (p = 0.004) and *AT1R*-CT 1.39 ml vs 0.98 ml for the reference group (p = 0.05)).

We observed 217 (20.2%) participants with a SBI (Rotterdam Scan Study) and 637 (8.0%) with incident stroke (Rotterdam Study). The prevalence of SBI was increased in *AGT*-TT carriers (OR 1.44 (95% CI 0.89 to 2.33); p = 0.14) and *AT1R*-CC carriers (OR 1.43 (95% CI 0.89 to 2.30); p = 0.14, model 2) but the findings were not significant. No significant association was found between *AGT* or *AT1R* and overall stroke (*AGT*-TT hazard ratio (HR) 0.99 (95% CI 0.75 to 1.30), *AT1R*-CC HR 1.19 (95% CI 0.91 to 1.56), model 2), ischaemic stroke (*AGT*-TT HR 1.05 (95% CI 0.73 to 1.50), *AT1R*-CC HR 1.34 (95% CI 0.94 to 1.92), model 2) or haemorrhagic stroke (*AGT*-TT HR 1.19 (95% CI 0.50 to 2.80), *AT1R*-CC HR 1.14 (95% CI 0.52 to 2.52), model 2).

Finally, we studied the interaction between *AGT* and *AT1R*. Within the Rotterdam Study, SBP and DBP were highest in participants carrying both the TT genotype of *AGT* and the CC genotype of *AT1R* (n = 234) compared with the reference group of participants with the MM genotype of *AGT* and the CC genotype of *AT1R* (n = 593) (model 1: SBP 141.9 mm Hg vs

Figure 1 The Rotterdam Study: association between the angiotensin (*AGT*) and angiotensin II type I receptor (*AT1R*) genotypes and carotid artery plaques. From a total plaque score of 0–6, plaques were scored low if the total plaque score was 0, 1 or 2, and high if the total plaque score was greater than 2. *p<0.05 compared with the TT genotype, adjusted for age and sex.

Figure 2 The Rotterdam Scan Study: association of the angiotensin (*AGT*) and angiotensin II type I receptor (*AT1R*) genotypes with subcortical white matter lesions (WML). ** $p < 0.01$ compared with the MM genotype for *AGT* and the TT genotype for *AT1R*, adjusted for age and sex.

137.3 mm Hg, $p = 0.007$; model 2: SBP 141.7 mm Hg vs 137.2 mm Hg, $p = 0.007$; model 1: DBP 74.8 mm Hg vs 72.7 mm Hg, $p = 0.02$; model 2: DBP 75.0 mm Hg vs 72.6 mm Hg, $p = 0.005$).

For periventricular WML, we found the highest degree in participants with both the TT genotype of *AGT* and the CC genotype of *AT1R* (model 1: 3.06 ($n = 37$) vs 2.06 ($n = 105$), in the reference group, $p = 0.008$; model 2: 2.93 vs 2.09, $p = 0.03$), as well as for deep subcortical WML (model 1: 2.60 ml ($n = 37$) vs 0.93 ml ($n = 105$) in the reference group, $p = 0.001$; model 2: 2.67 ml vs 0.98 ml, $p = 0.001$). The p for interaction was significant for both periventricular ($p = 0.02$, both models) and deep subcortical WML ($p = 0.02$, both models). No interaction was found for carotid artery plaques or SBI.

DISCUSSION

Within the Rotterdam Study, we found that mean systolic and diastolic blood pressure levels increased with the number of T alleles of the M235T polymorphism of *AGT*, as did the risk of the prevalence of carotid artery plaques. Within the Rotterdam Scan Study, we found that subjects with the TT genotype of *AGT* and the CT genotype of *AT1R* had an increase in mean volume deep subcortical WML. A significant interaction between *AGT* and *AT1R* was found for periventricular and deep subcortical WML.

We believe the strength of our study lies in the size of our study populations and the follow-up of patients over time. Also, we were able to study the effect of *AGT* and *AT1R* in two study populations, which made it possible to study both clinical and subclinical stroke (SBI), as well as WML of the brain and atherosclerosis. Results on plaques and incident stroke were obtained from the Rotterdam Study, while results on SBI and WML were obtained from the Rotterdam Scan Study. None of these outcomes was available in either cohort. Participants of the Rotterdam Scan Study were randomly selected from the Rotterdam Study and the Zoetermeer Study in age (>60 years) and sex strata. Participants in the Rotterdam Study and the Rotterdam Scan Study therefore partly overlapped. Both cohorts consisted of elderly participants, living in the Netherlands, who were participants of two large prospective population based studies, and were therefore comparable.

For genotyping measurements, as well as measurements of blood pressure and plaques, there were missing data. We did not find differences between participants with and without a risk genotype with regard to demographic or cardiovascular characteristics. Participants with missing data on blood pressure and plaques were significantly older and more often male. As there were no genotype differences between these participants, this most likely did not bias our results.

Carriers of the 235T allele of the *AGT* gene have been reported to have increased plasma angiotensinogen levels,² hypertension

and atherosclerosis.^{5,6} In line with these findings, we found an increase in SBP and DBP in *AGT*-235T allele carriers in the Rotterdam Study and a (borderline) significant increased risk of hypertension. Also, we observed a significant association between *AGT* and carotid artery plaques. Previously, an association between *AGT*-M235T and carotid intima-media thickness was reported.^{5,25} Even though we found an association between *AGT*-M235T and SBP and DBP, the observed association with carotid artery plaques may not be solely attributable to the effect on blood pressure. After adjusting for blood pressure, the association between the M235T polymorphism and carotid artery plaque remained significant, suggesting that blood pressure levels may not be part of the intermediate pathway.

Within the Rotterdam Scan Study, we found that the risk of SBI was increased in *AGT*-TT and *AT1R*-CC carriers, although this was not statistically significant. *AGT* was significantly associated with deep subcortical WML. We did not find an association with periventricular WML. We used two different scales to define periventricular and deep subcortical WML, categorical and volumetric, respectively. As the scale to define subcortical WML was quantitative and the scale to define periventricular WML semiquantitative, the power to detect an effect is most likely higher for subcortical WML. This may explain why a significant association was only observed for subcortical WML in this study.

To date, three studies found an association between *AGT* and small vessel disease and periventricular hyperintensity grade.⁸⁻¹⁰ This finding has been explained by an increase in plasma AGT levels, which may lead to increased formation of angiotensin II, which has several proatherogenic effects,²⁶ and may also explain the effect on carotid atherosclerosis. However, the lack of a convincing association with SBI or stroke does not support this pathway. Another mechanism explaining our findings and those of others may be related to the fact that an independent renin-angiotensin system exists in the brain, which might amplify cerebrovascular pathology, in particular WML.²⁷ Also, a haplotype at the *AGT* promoter has been found to increase transcriptional activity in astrocytes.³

As plaques and WML are precursors of stroke,^{28,29} they are less heterogeneous compared with (sub) clinical stroke. This may also explain why we did not find a significant association for stroke and SBI, not even after subtyping stroke in order to increase homogeneity. As genes involved in so-called complex diseases, such as stroke, usually have small effects, they may be difficult to detect, even in large study populations. Studying intermediate phenotypes increases homogeneity as they focus on a specific pathophysiological pathway.

An interaction was observed between *AT1R* and *AGT*. Cross talk between genes is plausible as both the *AGT* and *AT1R* gene products are part of the RAS. This is the first study addressing the interaction between *AGT* and *AT1R*. In complex traits, such as WML, one may expect joint effects of multiple genes. Other genes of the RAS may also be of interest in relation to small vessel pathology.

There is increasing interest in the association between cognitive decline and depression, and severe WML.^{30,31} The consistent association between *AGT* and WML, and the interaction with *AT1R* reported here, raises the question of whether the RAS may be a therapeutic target for the prevention of cerebral small vessel pathology. Previously, subjects with hypertension and the DD genotype of the insertion/deletion polymorphisms of the angiotensin converting enzyme (ACE) gene, also part of the RAS, showed a relative resistance to ACE inhibitor therapy and therefore an increase in cardiovascular mortality.³² In addition, several other studies found that this polymorphism may influence antihypertensive response,

particularly when using ACE inhibitors.³³ Also, *AGT* was found to be an independent predictor of blood pressure response to ACE inhibitors³⁴ and a protective association between ACE inhibitor use and non-fatal stroke was found among 235T allele carriers of *AGT*.⁴

In conclusion, we found that the *AGT*-235T allele was associated with increased blood pressure levels and carotid artery plaques. With respect to WML, we found evidence of an interaction between *AGT* and *AT1R*. As no significant evidence was found for an association with SBI or stroke, the effect of *AGT* and *AT1R* may be specific for small vessel pathology, perhaps related to blood pressure early in life.

ACKNOWLEDGEMENTS

The Rotterdam Study is supported by: the Erasmus Medical Centre and Erasmus University Rotterdam, the Netherlands Organisation for Scientific Research (NWO), the Netherlands Organisation for Health Research and Development (ZonMW), the Research Institute for Diseases in the Elderly (RIDE), the Ministry of Education, Culture and Science, the Ministry of Health, Welfare and Sports, the European Commission (DG XII) and the Municipality of Rotterdam.

Authors' affiliations

M J E van Rijn, M J Bos, A Isaacs, M Yazdanpanah, A Arias-Vásquez, B H Ch Stricker, B A Oostra, J C Witteman, A Hofman, M M B Breteler, C M van Duijn, Departments of Epidemiology and Biostatistics and Clinical Genetics, Erasmus Medical Centre, Rotterdam, the Netherlands

M J Bos, P J Koudstaal, Department of Neurology, Erasmus Medical Centre, Rotterdam, the Netherlands

O H Klungel, Department of Pharmacoepidemiology and Pharmacotherapy, Utrecht Institute of Pharmaceutical Sciences, Utrecht University, the Netherlands

Competing interests: None.

REFERENCES

- MacGregor GA, Markandu ND, Roulston JE, *et al.* Maintenance of blood pressure by the renin-angiotensin system in normal man. *Nature* 1981;**291**:329–31.
- Paillard F, Chansel D, Brand E, *et al.* Genotype-phenotype relationships for the renin-angiotensin-aldosterone system in a normal population. *Hypertension* 1999;**34**:423–9.
- Schmidt H, Aulchenko YS, Schweighofer N, *et al.* Angiotensinogen promoter B-haplotype associated with cerebral small vessel disease enhances basal transcriptional activity. *Stroke* 2004;**35**:2592–7.
- Bis JC, Smith NL, Psaty BM, *et al.* Angiotensinogen Met235Thr polymorphism, angiotensin-converting enzyme inhibitor therapy, and the risk of nonfatal stroke or myocardial infarction in hypertensive patients. *Am J Hypertens* 2003;**16**:1011–17.
- Bozec E, Fassot C, Tropeano AI, *et al.* Angiotensinogen gene M235T polymorphism and reduction in wall thickness in response to antihypertensive treatment. *Clin Sci (Lond)* 2003;**105**:637–44.
- Sethi AA, Nordestgaard BG, Tybjaerg-Hansen A. Angiotensinogen gene polymorphism, plasma angiotensinogen, and risk of hypertension and ischemic heart disease: a meta-analysis. *Arterioscler Thromb Vasc Biol* 2003;**23**:1269–75.
- Um JY, Moon KS, Lee KM, *et al.* Polymorphism of angiotensin-converting enzyme, angiotensinogen, and apolipoprotein E genes in Korean patients with cerebral infarction. *J Mol Neurosci* 2003;**21**:23–8.
- Schmidt R, Schmidt H, Fazekas F, *et al.* Angiotensinogen polymorphism M235T, carotid atherosclerosis, and small-vessel disease-related cerebral abnormalities. *Hypertension* 2001;**38**:110–15.
- Takami S, Imai Y, Katsuya T, *et al.* Gene polymorphism of the renin-angiotensin system associates with risk for lacunar infarction. The Ohasama study. *Am J Hypertens* 2000;**13**:121–7.
- Schmidt H, Fazekas F, Kostner GM, *et al.* Angiotensinogen gene promoter haplotype and microangiopathy-related cerebral damage: results of the Austrian Stroke Prevention Study. *Stroke* 2001;**32**:405–12.
- Kainulainen K, Perola M, Terwilliger J, *et al.* Evidence for involvement of the type 1 angiotensin II receptor locus in essential hypertension. *Hypertension* 1999;**33**:844–9.
- Bonnardeaux A, Davies E, Jeunemaitre X, *et al.* Angiotensin II type 1 receptor gene polymorphisms in human essential hypertension. *Hypertension* 1994;**24**:63–9.
- Tiret L, Bonnardeaux A, Poirier O, *et al.* Synergistic effects of angiotensin-converting enzyme and angiotensin-II type 1 receptor gene polymorphisms on risk of myocardial infarction. *Lancet* 1994;**344**:910–13.
- Rubattu S, Di Angelantonio E, Stanzione R, *et al.* Gene polymorphisms of the renin-angiotensin-aldosterone system and the risk of ischemic stroke: A role of the $\alpha 1166c/\alpha 11$ gene variant. *J Hypertens* 2004;**22**:2129–34.
- Takami S, Imai Y, Katsuya T, *et al.* Gene polymorphism of the renin-angiotensin system associates with risk for lacunar infarction. The Ohasama study. *Am J Hypertens* 2000;**13**:121–7.
- Poirier O, Georges JL, Ricard S, *et al.* New polymorphisms of the angiotensin II type 1 receptor gene and their associations with myocardial infarction and blood pressure: the ECTIM study. Etude Cas-Témoin de l'Infarctus du Myocarde. *J Hypertens* 1998;**16**:1443–7.
- Chaves FJ, Pascual JM, Rovira E, *et al.* Angiotensin II AT1 receptor gene polymorphism and microalbuminuria in essential hypertension. *Am J Hypertens* 2001;**14**:364–70.
- Szolnoki Z, Melegh B. Gene-gene and gene-environment interplay represent specific susceptibility for different types of ischaemic stroke and leukoaraiosis. *Curr Med Chem* 2006;**13**:1627–34.
- Hofman A, Grobbee DE, de Jong PT, *et al.* Determinants of disease and disability in the elderly: the Rotterdam Elderly Study. *Eur J Epidemiol* 1991;**7**:403–22.
- Hofman A, van Laar A, Klein F, *et al.* Coffee and cholesterol. *N Engl J Med* 1983;**309**:1248–50.
- van Gent CM, van der Voort HA, de Bruyn AM, *et al.* Cholesterol determinations. A comparative study of methods with special reference to enzymatic procedures. *Clin Chim Acta* 1977;**75**:243–51.
- Bots ML, Hoes AW, Koudstaal PJ, *et al.* Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. *Circulation* 1997;**96**:1432–7.
- Vermeer SE, Hollander M, van Dijk EJ, *et al.* Silent brain infarcts and white matter lesions increase stroke risk in the general population: the Rotterdam Scan Study. *Stroke* 2003;**34**:1126–9.
- de Leeuw FE, de Groot JC, Oudkerk M, *et al.* A follow-up study of blood pressure and cerebral white matter lesions. *Ann Neurol* 1999;**46**:827–33.
- Tabara Y, Kohara K, Nakura J, *et al.* Risk factor-gene interaction in carotid atherosclerosis: effect of gene polymorphisms of renin-angiotensin system. *J Hum Genet* 2001;**46**:278–84.
- Burnier M, Brunner HR. Angiotensin II receptor antagonists. *Lancet* 2000;**355**:637–45.
- Bunnenmann B, Fuxe K, Ganten D. The brain renin-angiotensin system: localization and general significance. *J Cardiovasc Pharmacol* 1992;**19**(Suppl 6):S51–62.
- Vermeer SE, Hollander M, van Dijk EJ, *et al.* Silent brain infarcts and white matter lesions increase stroke risk in the general population: The Rotterdam Scan Study. *Stroke* 2003;**34**:1126–9.
- Hollander M, Hak AE, Koudstaal PJ, *et al.* Comparison between measures of atherosclerosis and risk of stroke: The Rotterdam Study. *Stroke* 2003;**34**:2367–72.
- Steffens DC, Krishnan KR, Crump C, *et al.* Cerebrovascular disease and evolution of depressive symptoms in the cardiovascular health study. *Stroke* 2002;**33**:1636–44.
- Minett TS, Dean JL, Firbank M, *et al.* Subjective memory complaints, white-matter lesions, depressive symptoms, and cognition in elderly patients. *Am J Geriatr Psychiatry* 2005;**13**:665–71.
- Bleumink GS, Schut AF, Sturkenboom MC, *et al.* Mortality in patients with hypertension on angiotensin-I converting enzyme (ACE)-inhibitor treatment is influenced by the ACE insertion/deletion polymorphism. *Pharmacogenet Genomics* 2005;**15**:75–81.
- Baudin B. Angiotensin I-converting enzyme gene polymorphism and drug response. *Clin Chem Lab Med* 2000;**38**:853–6.
- Hingorani AD, Jia H, Stevens PA, *et al.* Renin-angiotensin system gene polymorphisms influence blood pressure and the response to angiotensin converting enzyme inhibition. *J Hypertens* 1995;**13**:1602–9.