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ORIGINAL ARTICLE

Angiotensin II Type-1 Receptor A1166C Polymorphism is Associated With Increased Risk of Ischemic Stroke in Hypertensive Smokers

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Abstract

Recent observations revealed a novel role of angiotensin-converting enzyme 2 and the angiotensin II type-1 receptor (AT1R) in lung injury, thereby extending knowledge about the functions of the angiotensin system. Angiotensin II, whose target is the AT1R, is a potent vasoconstrictor. Accordingly, an imbalance leading to enhanced activity of the angiotensin II–AT1R axis is postulated to contribute to both circulatory disturbances and lung injury. In this context, a functional single-nucleotide polymorphism, AT1R A1166C, which leads to enhanced responsiveness of the AT1R, has been postulated as a candidate susceptibility factor for ischemic stroke. The aim of our study was to investigate its occurrence in ischemic stroke and to analyze its possible synergistic associations with clinical risk factors. Genetic and clinical data on 308 consecutive patients with acutely developing ischemic stroke were analyzed. A total of 272 stroke and neuroimaging alteration-free subjects served as a control group. Univariate and logistic regression statistical approaches were used. Alone, the AT1R 1166C allele did not pose a risk of stroke. In hypertensive smokers, however, it was associated with an increased risk of ischemic stroke (OR 22.3, 95% CI 5.8–110.2, $p < 0.001$). Further subgroup analysis revealed the same association for both small-vessel (OR 24.3, 95% CI 6.1–121.1, $p < 0.001$) and large-vessel (OR 21.3, 95% CI 4.6–81.1, $p < 0.001$) infarction. On a pathophysiological basis, our results suggest the possibility that the AT1R A1166C polymorphism might give rise to ischemic stroke indirectly via an unfavorable effect on the cardiorespiratory function.
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Index Entries: AT1R A1166C polymorphism; stroke risk; genetic interaction.

Introduction

The renin-angiotensin system (RAS) plays an important role in the maintenance of blood pressure hemostasis (Agachan et al., 2003; Henderson et al., 2004; Miller et al., 2004). The angiotensin-converting enzyme (ACE) converts angiotensin I

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into angiotensin II. Angiotensin II, which binds the angiotensin II type-1 receptor (AT1R), is a potent vasoconstrictor and a stimulator of cardiac growth (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). However, ACE2 converts angiotensin II into angiotensin$_{1–7}$ (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005), which has functions opposite those of angiotensin II, acting as a potent vasodilator and a repressor of cardiac growth (Ferreira and Santos, 2005; Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). Recent papers have raised the possibility that an elevated level of angiotensin II and an enhanced activity of AT1R might be associated with lung injury (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). Hence, an imbalance leading to enhanced activity of the angiotensin II–AT1R axis might contribute to both circulatory disturbances and lung damage. The ACE D/D polymorphism associated with an elevated angiotensin II level has been demonstrated to play an important role in the development of ischemic stroke, indicating genetic involvement in the affected function of the RAS (Szolnoki et al., 2002, 2003; Um et al., 2003). In this context, a functional single-nucleotide polymorphism, AT1R A1166C, which leads to enhanced responsiveness of the AT1R, has been postulated as a candidate susceptibility factor for ischemic stroke, cardiovascular events, and hypertension (Hindorff et al., 2002; Treszl et al., 2003; Fukazawa et al., 2004; Kobashi et al., 2004; Rubattu et al., 2004; Sugimoto et al., 2004). A direct role of the AT1R A1166C polymorphism in ischemic stroke has not been proved, although its unfavorable effect is strongly suggested (Rubattu et al., 2004). The aims of the present study were to investigate the occurrence of the AT1R A1166C polymorphism in ischemic stroke and to analyze its possible synergistic association with clinical risk factors (smoking and hypertension), which might also be associated indirectly with lung disorders.

Materials and Methods

Study Population

The data on 308 consecutive patients with acutely developing ischemic stroke who had never suffered a previous stroke event were analyzed. These subjects had been admitted to our Department of Neurology and Neurophysiology between January 1998 and January 2004 after being examined by an internist in the local emergency unit or by a family physician at their homes. All 308 subjects underwent detailed clinical scrutiny, including medical history, family history, an evaluation of vascular risk factors, general physical and neurological examinations, urine analysis, extensive laboratory examinations, electrocardiography, extracranial and transcranial Doppler sonography of the brain-supplying arteries, transthoracic and/or transesophageal echocardiography, where appropriate, and MRI examinations within 2 d after the onset of symptoms. All scans were read by an experienced investigator without knowledge of the clinical and laboratory data. The patients were enrolled immediately after the clinical neurological and MRI examinations. Subjects on whom MRIs could not be recorded or for whom the examined clinical parameters and risk factors could not be obtained with certainty in consequence of some technical cause or death were excluded from the study groups. Patients with atrial fibrillation were also excluded to make the study groups more homogenous.

Following evaluation of the clinical and radiological features, the patients were enrolled into three subgroups: Group 1 corresponded to large-vessel infarction (cortical or cerebellar lesions and/or brain stem infarcts or subcortical hemispheric infarcts >1.5 cm in diam. on the MRIs, with a cerebral cortical impairment, or brain stem or cerebellar dysfunction); group 2 corresponded to small-vessel occlusion (one or more subcortical hemispheric or brain stem infarcts <1.5 cm in diam. on the MRIs, with one of the features of the traditional clinical lacunar syndrome and without cerebral cortical dysfunction); and group 3 corresponded to a mixed vascular pathology (one or more lacunar and large-vessel infarcts on the MRIs). This classification, based on the clinical and radiological features, was considered to be the most exact and quantifiable method with regard to the requirement that the subgroups reflect the main well-defined vascular pathologies and their overlapping, which might possibly be affected by the mutations examined.

As a control group, 272 stroke and neuroimaging alteration-free Caucasian Hungarian subjects were examined. The controls were randomly selected by using a sex-matched technique from general practice registers from the same locality as the stroke cases, with the requirement that they had negative brain MRI or CT findings to avoid silent brain infarctions. They were healthy and believed to be free of cerebrovascular disease. Subjects with any previous clinical data suggesting a cerebrovascular or cardiovascular event (such as TIA or angina pectoris) were

excluded from the control group. Both controls and patients gave their informed consent to the clinical workup and the DNA analysis. The study was approved by the local ethics committee.

Assessment of Clinical Data

Smoking and drinking habits and the presence of hypertension or diabetes mellitus were recorded in all groups. Serum cholesterol level, serum triglyceride level, platelet count, and hematocrit were also measured and analyzed as important clinical parameters. Hypertension was diagnosed when blood pressure repeatedly exceeded 140 mmHg systolic and/or 85 mmHg diastolic, or when the patient was taking antihypertensive medication. Diabetes mellitus was diagnosed when the glucose level was at least 7.78 mmol/L in a fasting state and/or at least 11.11 mmol/L 2 h after a meal or 75 g oral glucose loading, according to World Health Organization criteria (WHO 1985). Ischemic heart disease was diagnosed when a history of angina pectoris or acute myocardial infarction was present or if there was ECG evidence of coronary heart disease.

Patients were classified as smokers if they had ever smoked more than five cigarettes per day for at least a year. Patients were considered to be moderately heavy drinkers if they drank 40 g or more per day. The body mass index (BMI) was calculated as weight in kilograms, divided by the square of the height in meters.

DNA Analysis

Genomic DNA was extracted from 200 μL of peripheral blood anticoagulated with EDTA by the desalting method (Miller et al., 1988). All blood samples were stored at –20°C until DNA isolation.

For AT1R (GenBank accession no. NT 005612) genotyping, a new primer set was constructed. The forward primer was 5′-AAAAGCCAAATC CCACTCAA-3′, and the reverse primer was 5′-CAGGACAAAAGCAGGCTAGG-3′. PCR was performed with an MJ Research PTC-200 thermal cycler. The reaction volume was 50 μL, containing 1 μL of DNA (40–80 ng), 0.2 μmol/L each of the primers, 5 μL of reaction buffer (100 m*M* Tris-HCl at pH = 9.0, containing 500 m*M* KCl, 15 m*M* $MgCl_2$), 200 μ*M* dNTP, and 2 U of *Taq* polymerase. The PCR conditions were as follows: initial denaturation at 96°C for 120 s, followed by 35 cycles of denaturation (96°C for 30 s), annealing (53°C for 30 s), and extension (72°C for 60 s). PCR products were digested with 1.5 U of DdeI (New England Biolabs) at 37°C overnight. The restriction fragments were separated by electrophoresis on 3% agarose gels containing ethidium bromide and visualized by UV illumination. The AT1R 1166A allele results in 58- and 374-bp fragments, and the AT1R 1166C allele results in 58-, 143-, and 231-bp fragments.

Statistics

The clinical data were expressed as means ± S.D., where appropriate. The differences between the clinical parameters in the stroke group and the controls were assessed by using the χ^2 test or the Mann-Whitney test, where appropriate. The stroke groups were tested versus the control group for the frequencies of the different genotypes and their combinations with the clinical risk factors by the χ^2 test. Logistic regression models were evolved to evaluate the importance of the co-occurrences of the AT1R A1166C polymorphism and the significant clinical risk factors in the development of ischemic stroke. The AT1R A1166C polymorphism was coded in the following way: score 1 for both the homozygous and heterozygous status, and 0 for lack of the 1166C allele. For all odds ratios (ORs), 95% confidence intervals (95% CIs) were calculated. Logistic regression analyses were performed with the statistical package Systat 10 (Chicago, IL) for Windows.

Results

Clinical data are listed in Table 1. The AT1R 1166C allele did not occur more frequently in the stroke groups (large vessel, 51.3%; small vessel, 51.7%; mixed type, 50.7%; overall, 51.3%) than in the controls (52.9%) (Table 2). Co-occurrence of the AT1R 1166C allele and hypertension and smoking was significantly more frequent in the stroke groups (large vessel, 12%; small-vessel, 16.1%; mixed type, 14.1%; overall stroke group, 13.6%) than in the controls (0.7%, $p < 0.01$) (Table 2).

The co-occurrence of the AT1R 1166C allele and hypertension and smoking also enhanced the risk of ischemic stroke (large vessel, adjusted OR 21.3; small vessel, adjusted OR 24.3; mixed vascular type, adjusted OR 20.5; overall, adjusted OR 22.3), as compared with risk caused by the combination of hypertension and smoking (large vessel, adjusted OR 8.1; small vessel, adjusted OR 10.2; mixed vascular type, adjusted OR 10.1; overall, adjusted OR 9.1) (Table 3). No synergistic effects were found either between the AT1R A1166C polymorphism and hypertension or between the AT1R A1166C polymorphism and smoking inasmuch as the

Table 1
Characteristics of Patients and Control Subjects

Clinical features	Stroke group (n = 308)	Control group (n = 272)
Sex (females/males)	148/160	129/143
Age (yr)	63.2 ± 11.5[a]	53.7 ± 14.8
BMI (kg/m^2)	26.9 ± 2.0[a]	23.1 ± 3.1
Cholesterol (mmol/L)	6.9 ± 1.5[a]	5.2 ± 1.5
Triglycerides (mmol/L)	1.92 ± 0.9[a]	1.2 6 ± 0.8
Hematocrit (%)	45 ± 9	45 ± 6
Platelet count (¥ 10^9)	250 ± 53	251 ± 48
Hypertension	50%[b]	18%
Diabetes mellitus	30.8%[b]	4.8%
Smokers	31.2%[b]	9.9%
Drinkers	11.7%[b]	3.7%
Ischemic heart disease	15.9%[b]	6.6%

The stroke group was compared with the control group by the χ^2 test or the Mann-Whitney test, where appropriate.
[a] $p < 0.001$.
[b] $p < 0.0005$.

presence of the AT1R 1166C allele did not lead to an additional increase in the ORs of hypertension or smoking for ischemic stroke (Table 3). Likewise, we did not find an association between the AT1R 1166C allele and any other clinical risk factor (data not shown).

Discussion

In our population, we did not find a direct association between the AT1R A1166C polymorphism and ischemic stroke. The AT1R 1166C allele did not increase the risk of stroke in the presence of either hypertension or smoking separately, as compared with the risk of ischemic stroke caused by hypertension and smoking alone. However, the co-occurrence of the AT1R 1166C allele, hypertension, and smoking enhanced the risk of ischemic stroke as compared both with the control group and the risk of ischemic stroke caused by the combination of hypertension and smoking. The subgroup analysis revealed that the interaction between the two clinical risk factors and the AT1R 1166C allele reached a similar extent in all subgroups of stroke. The crude and adjusted ORs demonstrated that the presence of the AT1R 1166C allele increased the risk of ischemic stroke only if the stroke patient was both a smoker and suffered from hypertension. Because no association was found between the AT1R 1166C allele and hypertension or smoking alone, an additive interaction might be presumed between the two clinical risk factors and the AT1R 1166C allele.

Table 2
Distribution of Different Genotypes Among Stroke Subgroups and Control Group and Co-Occurrences of the AT1R 1166C Allele, Hypertension, and Smoking

Genotypes	Large vessel n = 150	Small vessel n = 87	Mixed type n = 71	Overall n = 308	Controls n = 272
AT1R 1166AA	73 48.7%	42 48.3%	35 49.3%	150 48.7%	128 47.1%
AT1R 1166AC	64 42.7%	38 43.7%	30 42.3%	132 42.9%	119 43.8%
AT1R 1166CC	13 8.7%	7 8%	6 8.5%	26 8.4%	25 9.2%
Patient carrying the AT1R 1166C allele	77 51.3%	45 51.7%	36 50.7%	158 51.3%	144 52.9%
AT1R 1166C allele + hypertension	37[a] 24.7%	21[a] 24.1%	18[a] 25.4%	76[a] 24.7%	25 9.2%
AT1R 1166C allele + smoking	23[a] 15.3%	16[a] 18.3%	12[a] 17%	51[a] 16.6%	14 5.1%
Hypertension + smoking	22[a] 14.7%	16[a] 18.4%	12[a] 16.9%	50[a] 16.2%	5 1.8%
AT1R 1166C allele + hypertension + smoking	18[a] 12%	14[a] 16.1%	10[a] 14.1%	42[a] 13.6%	2 0.7%

The stroke group was compared with the control group by the χ^2 test.
[a] $p < 0.01$.

There is no exact explanation for this additive effect. On a pathophysiological basis, however, both smoking and hypertension lead to endothelial dysfunction and vasoregulation disturbances (Sainani and Maru, 2004; Cheng et al., 2005; Munzel et al., 2005; Schmieder, 2005; Schram and Stehouwer, 2005). The presence of the AT1R 1166C allele might make the endothelial cell more susceptible to dysfunction. This finding was consistent with the result of Rubattu et al. (2004), in that the association between the AT1R 1166C allele and ischemic stroke was stronger in the presence of hypertension. Data show that the AT1R 1166C allele is associated with increased angiotensin II responsiveness (van Geel et al., 2000; Jones et al., 2003). This feature of the AT1R A1166C polymorphism might be the molecular basis of its enhancing effect on the endothelial dysfunction caused by hypertension and smoking. The evidence concerning a direct role of the AT1R C1166A

Table 3
Interactions Between the Clinical Risk Factors and the AT1R 1166C Allele in the Different Stroke Groups

Genotypes	Large-vessel (group 1)	Small-vessel (group 2)	Mixed type (group 3)	Overall
		Crude ORs		
AT1R 1166C allele	0.9 (0.6–1.4)	0.9 (0.6–1.5)	0.9 (0.5–1.5)	0.9 (0.7–1.3)
Hypertension	4.3[a] (2.6–6.9)	4.8[a] (2.4–7.8)	4.2[a] (2.3–7.1)	4.3[a] (2.9–6.3)
Hypertension + AT1R 1166C allele	3.2[a] (1.9–5.6)	3.1[a] (1.7–6)	3.4[a] (1.7–6.6)	3.2[a] (2–5.2)
Smoking	4.6[b] (2.5–7.9)	4.4[b] (2.1–7.6)	4.5[a] (2.4–8.2)	4.6[b] (2.8–7.5)
Smoking + AT1R 1166C allele	3.3[a] (1.7–6.7)	4.1[a] (1.9–8.9)	3.7[a] (1.6–8.5)	3.7[a] (2–6.8)
Smoking + hypertension	9.2[a] (3.4–24.8)	12[a] (4.3–34)	10.9[a] (3.7–32)	10.3[a] (4–26.4)
AT1R 1166C allele + hypertension + smoking	18.4[b] (4.2–80.5)	25.9[b] (5.8–116.5)	22.1[a] (4.7–103.6)	21.3[b] (5.1–88.9)
		Adjusted ORs[c]		
AT1R 1166C allele	1.1 (0.5–1.7)	1.2 (0.5–1.8)	0.9 (0.4–1.5)	0.9 (0.7–1.4)
Hypertension	4.4[a] (2.3–7.0)	4.1[a] (1.9–6.5)	4.4[a] (2.2–7.1)	4.8[a] (2.9–7.9)
Hypertension + AT1R 1166C allele	3.4[a] (2.0–6.1)	2.9[a] (1.5–7.1)	3.1[a] (1.6–7.3)	3.3[a] (2.1–6.2)
Smoking	4.5[a] (2.3–8.9)	4.1[a] (2.2–8.2)	4.4[a] (2.5–8.8)	4.1[a] (2.4–7.8)
Smoking + AT1R 1166C allele	3.5[a] (1.6–7.1)	4.0[a] (1.5–7.9)	3.8[a] (1.4–8.1)	3.2[a] (1.7–7.1)
Hypertension + smoking	8.1[a] (3.1–23.6)	10.2[a] (4.1–34.1)	10.1[a] (4.0–33.3)	9.1[a] (3.9–26.6)
AT1R 1166C allele + hypertension + smoking	21.3[b] (4.6–81.1)	24.3[b] (6.1–121.1)	20.5[a] (4.1–80.8)	22.3[b] (5.8–110.2)

[a] $p < 0.025$.

[b] $p < 0.001$.

[c] Adjusted ORs of the AT1R 1166C allele and clinical risk factors from the logistic regression models after adjustment for differences in age, BMI, serum cholesterol, serum triglycerides, diabetes mellitus, drinking habits, and ischemic heart disease.

mutation in ischemic stroke and hypertension, however, seems to be inconclusive (Hindorff et al., 2002; Agachan et al., 2003; Jones et al., 2003; Sugimoto et al., 2004). This might be caused by differences in geographic regions of ethnicity. Recent data underline the importance of the enhanced activity of the AT1R in the development of acute lung injury (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). This raises the possibility that a complex cardiorespiratory interplay is involved in the interactions between smoking and hypertension and the AT1R A1166C mutation. The enhanced activity of the AT1R caused by the A1166C mutation might lead to a respiratory malfunction in different distress states (Imai et al., 2005; Kuba et al., 2005; Nicholls and Peiris, 2005). Hypertension and smoking might rive rise to such a chronic distress state. Therefore, an imbalance of the cardiorespiratory function might indirectly increase the probability of the evolution of a stroke attack.

In conclusion, the AT1R A1166C polymorphism might lead to ischemic stroke via an imbalance of the complex function of the RAS causing a cardiorespiratory malfunction.

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