The Relationship Between the −344C>T Polymorphism of Gene Encoding Aldosterone Synthase and the Prothrombin Time in Patients With Intracerebral Hemorrhage

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Summary

Objective: Gene encoding aldosterone synthase gene (CYP11B2), has been suggested to contribute to stroke. The present study was designed to investigate whether common functional polymorphism −344C>T (rs179998) of the CYP11B2 gene is associated with ischemic stroke (IS) and intracerebral hemorrhage (ICH) in a Russian population.
Methods: A total 936 unrelated subjects (446 ischemic stroke, 57 hemorrhagic stroke patients and 433 healthy controls) from Central Russia were recruited for this study. DNA samples from all study participants were genotyped for the CYP11B2 gene polymorphism through TaqMan assay.
Results: No differences in both CYP11B2 allele and genotype frequencies were found between entire patients and control groups. The analysis stratified by stroke subtype and gender was also failed to reveal the association between −344C>T polymorphism and stroke susceptibility. However, the −344TT genotype showed an association with increased prothrombin time in ICH patients (p=0.01).
Conclusion: Increased aldosterone levels in carriers of the -344TT genotype directly affect the coagulation system in ICH patients, however, the mechanisms, by which aldosterone is associated with an increased prothrombin time are not understood.

Key words: Intracerebral hemorrhage, aldosterone synthase gene, prothrombin time

Özet

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INTRODUCTION

Stroke is a complex multifactorial disorder with high prevalence worldwide[8]. Renin–angiotensin aldosterone system (RAAS) plays a major role in vascular homeostasis and genes of the RAAS are shown to be attractive candidates of stroke susceptibility[4]. Aldosterone, a key player of this system, is involved in the regulation of water and electrolyte homeostasis. The biosynthesis of aldosterone is determined by enzyme aldosterone synthase (CYP11B2)[11]. It has been found that a common polymorphism −344C>T in the promoter region of the CYP11B2 gene is associated with higher aldosterone synthase activity and enhanced aldosterone production[3]. Several studies have been done so far to investigate the role of CYP11B2 −344C>T polymorphism in the pathogenesis of the pathogenesis of ischemic stroke (IS), but their results were inconsistent[2,5,11], thereby requiring further investigation in ethnically distinct populations of the world. In the meantime, no studies have investigated the contribution of the CYP11B2 gene polymorphism to the risk of intracerebral hemorrhage (ICH). The present study was designed to investigate the relationship between the −344C>T polymorphism of the CYP11B2 gene with the risk of IS and ICH in Russian population and to evaluate the impact of the polymorphism on the blood coagulation and lipid parameters.

MATERIAL AND METHODS

The study was approved by Ethical Review Committee of Kursk State Medical University. A total 936 Russian unrelated subjects from Central Russia (predominantly from Kursk region) were included in the study. The study population included 433 healthy subjects with normal blood pressure and 503 stroke patients (446 ischemic stroke and 57 patients with intracerebral hemorrhage) recruited at the Regional Vascular Center of Kursk Regional Clinical Hospital and Neurology Clinics of Kursk Emergency Medicine Hospital over two periods: between 2007 and 2010[14], and between 2012 and 2013. The mean age of stroke patients (266 males, 237 females) was 61.07±10.09 years; the mean age of the healthy controls (236 mails, 197 females) was 61.72±8.02 years. The control subjects were enrolled based on having no history of any cardiovascular diseases and normal blood pressure. Diagnosis of cerebral stroke was verified by computed tomography (CT) or magnetic resonance imaging (MRI) of the brain. Patients with subarachnoid hemorrhages were not included in the study. Genomic DNA was isolated from peripheral blood samples using a standard phenol/chloroform procedure. The polymorphism C-344T (rs179998) was genotyped through a TaqMan SNP Genotyping Assay (Applied Biosystems, USA) on the CFX96™ s case/control status and re-genotyping of about 5% of randomly selected samples yielded 100% reproducibility. The total cholesterol (TC) and triglycerides (TG) levels were measured by enzymatic method on the automatic biochemical analyzer «Flexor XL» (Vital Scientific, the Netherlands). The prothrombin time (PT), activated partial thromboplastin time (APTT) and international normalized ratio (INR) were analyzed by the clotting test on the automatic coagulation analyzer «STA Compact» (Roche Diagnostics, Switzerland). The association between the polymorphism and hypertension risk was estimated by odds ratio (OR) with 95% confidence interval (CI) using unconditional logistic regression. The biochemical parameters were tested for normality using the Kolmogorov-Smirnov test. The Kruskal-Wallis test was applied to assess the relationship between the CYP11B2 gene polymorphism and biochemical parameters. The statistical significance was established at $P \leq 0.05$. Statistical calculations were performed with STATISTICA for Windows 8.0

(StatSoft Inc; Tulsa, OK, USA).'Real-Time PCR Detection System (Bio-Rad Laboratories, USA). The genotyping results were scored by two independent investigators blindly to the patient

RESULTS

The CYP11B2 genotype frequencies were in agreement with Hardy–Weinberg equilibrium (HWE) in both patients and control groups (p>0.05). The genotype and allele frequencies are shown in Table 1. As can be seen from Table 1, no significant difference in allele and genotype frequencies was observed between the study groups. The analysis stratified by the type of stroke (i.e. ischemic stroke, intracerebral hemorrhage) has not revealed the association between the CYP11B2 gene and susceptibility to these pathogenetic variants of stroke (Table 1). Moreover, the analysis stratified by gender was also failed to reveal the association of the CYP11B2 gene polymorphism and stroke subtypes (data not shown). The distribution of blood coagulation and lipid metabolism parameters in the carriers of the CYP11B2 genotypes is presented in Table 2. The Kolmogorov-Smirnov test was done to test biochemical parameters for normality. All biochemical parameters showed a screw from the normal distribution (P<0.01), excepting TC level. For this reason, we preferred the Kruskal-Wallis test over ANOVA to indicate the association between the CYP11B2 genotypes and biochemical parameters. The distribution of biochemical parameters of lipid metabolism and coagulation in stroke persons stratified by the CYP11B2 genotypes is shown in Table 2. Patients with intracerebral hemorrhage possessing -344TT genotypes have higher PT levels (5.2) than those in carriers of genotypes -344CT (11.77) and -344CC (10.53). Figure 1 shows a significant association (p=0.01) between the -344C>T polymorphism of the CYP11B2 gene and prothrombin time in patients with intracerebral hemorrhage. As can be seen from Table 2, CS patients with the -344CC genotype have slightly higher TC concentration (5.5) in comparison with heterozygous -344CT (5.2) and homozygous -344TT genotypes (5.1). We also observed that CS patients with variant genotype -344TT have a higher INR (1.0) in comparison with patients with genotypes -344CT and -344CC (P=0.08).

Table 1: Allele and genotype frequencies of polymorphism -344C/T of the *CYP11B2* gene in patients and controls

CYP11B2 allele and genotype frequencies		CS patients (n=503) n (%)[1]	Controls (n=433) n (%)[1]	χ^2 (p)[2]	OR (95% CI)[3]
Entire group					
Allele frequencies					
	-344C	0,516	0,514	0,01	0,99
	-344T	0,484	0,486	(0,93)	(0,83-1,19)
Genotype frequencies					
	-344CC	134 (26,6)	121 (27,9)	0,20 (0,65)	1,07 (0,80-1,42)
	-344CT	251 (49,9)	203 (46,9)	0,85 (0,36)	1,13 (0,87-1,46)
	-344TT	118 (23,5)	109 (25,2)	0,37 (0,54)	0,91 (0,68-1,23)
Ischemic Stroke Patients (n=446) and Controls (n=433)					
Allele frequencies					
	-344C	0,506	0,514	0,12	1,03
	-344T	0,494	0,486	(0,73)	(0,86-1,25)

Genotype frequencies					
	-344CC	113 (25,3)	121 (27,9)	0,77 (0,38)	1,14 (0,85-1,54)
	-344CT	225 (50,4)	203 (46,9)	1,12 (0,29)	1,15 (0,89-1,50)
	-344TT	108 (24,2)	109 (25,2)	0,11 (0,74)	0,95 (0,70-1,29)
		Hemorrhagic Stroke Patients (n=57) and Controls (n=433)			
Allele frequencies					
	allele -344C	0,596	0,514	2,76	0,72
	allele -344T	0,404	0,486	(0,10)	(0,48-1,06)
Genotype frequencies					
	-344CC	21 (36,8)	121 (27,9)	1,94 (0,16)	0,66 (0,37-1,18)
	-344CT	26 (45,6)	203 (46,9)	0,03 (0,86)	0,95 (0,55-1,65)
	-344TT	10 (7,5)	109 (25,2)	1,59 (0,21)	0,63 (0,31-1,29)

[1] Absolute number and percentage of individuals with particular genotype;

[2] Chi-square statistics with Yates' correction and (df=1);

[3] Odds ratio with 95% confidence intervals

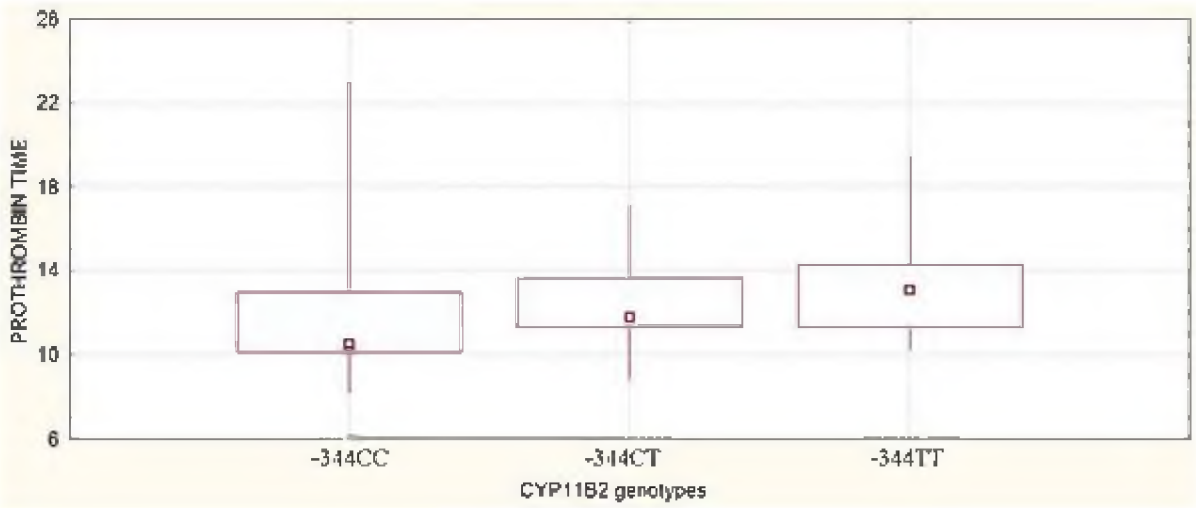

Figure 1: *The distribution of the prothrombin time (PT) in patients with intracerebral hemorrhage stratified by the CYP11B2 genotypes*

Table 2: The distribution of biochemical parameters of lipid metabolism and coagulation in stroke persons stratified by the *CYP11B2* genotypes

	Median, Lower and Upper Quartiles														
	TC, mmol/L			TG, mmol/L			PT, seconds			APTT, seconds			INR		
	CS	IS	HS	CS	IS	HS	CS	IS	HS	CS	IS	HS	CS	IS	HS
-344CC	5.5 (4.3-6.2)	5.5 (4.3-6.3)	5.1 (4.5-5.8)	1.3 (1.1-1.8)	1.4 (1.2-1.8)	1.2 (1.0-1.2)	10.79 (10.14-13.13)	10.79 (10.14-13.13)	10.53 (10.14-13.0)	34,75 (29.0-37.80)	34.95 (28.90-38.05)	30.9 (29.0-35.0)	0.97 (0.9-1.2)	0.97 (0.9-1.03)	0.96 (0.90-1.0)
-344CT	5.2 (4.4-5.8)	5.2 (4.4-5.9)	5.3 (4.5-5.4)	1.3 (1.0-2.1)	1.4 (1.1-2.2)	1.2 (1.0-1.5)	11.31 (10.4-13.39)	11.31 (10.40-13.13)	11.77 (11.31-13.65)	32.65 (29.65-37.0)	32.60 (29.40-36.8)	34.2 (30.1-37.9)	0.98 (0.92-1.04)	0.96 (0.92-1.04)	0.98 (0.95-1.09)
-344TT	5.1 (4.5-5.9)	5.2 (4.5-6.0)	4.5 (3.5-5.3)	1.2 (1.0-1.7)	1.2 (1.0-1.8)	1.2 (0.7-1.4)	11.31 (10.27-13.13)	11.31 (10.27-13.0)	13.07 (11.31-14.3)	31.80 (28.10-34.90)	31.50 (28.25-35.0)	33.2 (26.0-34.0)	1.0 (0.94-1.09)	1.0 (0.95-1.09)	1.0 (0.91-1.08)
p^1	0.67	0.84	0.50	0.48	0.49	0.77	0.13	0.43	**0.01***	0.17	0.21	0.33	0.08	0.10	0. 47

1- *P* – values for the Kruskal-Wallis test
* means significant association
NS means not significant

DISCUSSION

The present study found for the first time that the -344C/T polymorphism of the CYP11B2 gene is associated with increased prothrombin time in patients with intracerebral hemorrhage. It is known a polymorphism that the −344C/T is located in the promoter region of the CYP11B2 gene and influences the transcription of aldosterone synthase gene and, thus increasing aldosterone biosynthesis[3,10]. Although the literature data on the relationship between aldosterone and the coagulation system are extremely limited, this regulatory peptide may be related to the risk of intracerebral hemorrhage through several intermediate phenotypes of the disease. In particular, increased aldosterone levels in carriers of the -344TT genotype may increase the risk of hemorrhagic stroke through a well-recognized mechanism of elevating of blood pressure. Moreover, aldosterone was found to inhibit the activity of endothelial nitric oxide synthase[12] and increase vascular tone via the upregulation of angiotensin II receptors[16] . Since aldosterone plays a crucial role in remodeling of small and large arteries it may increase the odds of stroke development through enhanced collagen synthesis and increased arterial stiffness[7,13,15]. It is also known that aldosterone in cooperation with angiotensin II may cause thrombosis through an increased expression of plasminogen activator inhibitor-1 in endothelial and smooth muscle cells of the vessels[6]. This means, that increased aldosterone levels in carriers of the -344TT genotype directly affect the coagulation system in these patients, however, the mechanisms, by which aldosterone is associated with an increased prothrombin time are not understood. We cannot exclude the possibility that high thrombin levels in the patients may be responsible for cerebral edema[9] and brain injury[1] after intracerebral hemorrhage. In conclusion, the present study provides an evidence that the −344C>T polymorphism of the CYP11B2 may play a role in both pathogenesis and course of intracerebral hemorrhage, however, further studies are required to substantiate such relationships.

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