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Influence of *COL1A2* gene variants on the incidence of hypertensive intracerebral hemorrhage in a Chinese population

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ABSTRACT. Type I collagen (transcribed by *COL1A1* and *COL1A2* genes) is important for maintaining vessel wall elasticity and is a critical part of the extracellular matrix. We conducted a case-control study to investigate the role of the *COL1A2* rs42524 polymorphism in the development of hypertensive intracerebral hemorrhage. Between January 2012 and December 2014, a total of 227 patients with hypertensive intracerebral hemorrhage and 227 controls were selected from the Affiliated Hospital of Yanan University (China). Genotyping of the *COL1A2* rs42524 polymorphism was performed using polymerase chain reaction coupled with restriction fragment length polymorphism. By logistic regression analysis, we found that the CC genotype was associated with increased risk of hypertensive intracerebral hemorrhage as compared to the GG genotype (OR = 12.67, 95%CI = 3.03-112.11). In a dominant model, the GC + CC genotype of the *COL1A2* rs42524 polymorphism was associated with a 2.57-fold increased risk of hypertensive intracerebral hemorrhage as compared to the GG genotype. In a recessive model, the CC genotype of the *COL1A2* rs42524 polymorphism was correlated with a higher risk of hypertensive intracerebral hemorrhage as compared to the GG + GC

genotype (OR = 12.07, 95%CI = 2.89-106.75). The GC and CC genotypes of the *COL1A2* rs42524 polymorphism were associated with a substantial risk of hypertensive intracerebral hemorrhage among patients who consumed alcohol and used tobacco. In conclusion, our study suggests that the *COL1A2* rs42524 polymorphism is associated with the development of hypertensive intracerebral hemorrhage, particularly in conjunction with tobacco use and alcohol consumption.

Key words: Type I collagen; *COL1A2*; Polymorphism; Hypertensive intracerebral hemorrhage

INTRODUCTION

Stroke is a leading cause of death and disability in China and many other developed countries worldwide. Approximately 10 and 55% of strokes worldwide and in China, respectively, are due to intracerebral hemorrhage (Fernando, 2005). The intracerebral hemorrhage is caused by complex multistep processes that involve multiple factors, and many environmental and genetic factors are involved in its development, such as hypertension (Maslehaty et al., 2012). Hypertension is the most prominent cause of intracerebral hemorrhage, but not all individuals who are diagnosed with hypertension develop intracerebral hemorrhage, which suggests that many genetic factors are involved in the development of hypertensive intracerebral hemorrhage. It is well known that collagen destruction can induce a decrease in vessel integrity and an increase in vessel permeability (Kazi et al., 2003). Type I collagen is important for maintaining vessel wall elasticity and is a critical part of the extracellular matrix (Ikonomidis et al., 2006). Type I collagen is a heterotrimer consisting of two a1 and one a2 chains, which are encoded by the *COL1A1* and *COL1A2* genes, respectively. Previous study reported that the *COL1A2* rs42524 polymorphism alters the integrity of type I collagen, reducing vessel wall rigidity and inducing the destruction of blood vessel walls, and thus influence the process of cardiovascular disease (Yoneyama et al., 2004). Only one previous study has reported an association between the *COL1A2* rs42524 polymorphism and development of intracerebral hemorrhage (Liu et al., 2012). Therefore, we conducted a case-control study to investigate the role of the *COL1A2* rs42524 polymorphism in the development of hypertensive intracerebral hemorrhage.

MATERIAL AND METHODS

Subjects

Between January 2012 and December 2014, a total of 240 patients with hypertensive intracerebral hemorrhage were selected from the Affiliated Hospital of Yanan University (China), and all incidents of hypertensive intracerebral hemorrhage were confirmed by brain computed tomography (CT) and/or brain magnetic resonance imaging scans (MRI). The patients with hypertensive intracerebral hemorrhage were diagnosed by the criteria determined at the fourth National Cerebrovascular Academic Conference, which was held in 1995. The exclusion criteria for our study were patients with tumor, cerebral vascular malformation, or aneurysm. Finally, 227 patients fulfilled the inclusion criteria and agreed to participate in our study, resulting in a participation rate of 94.58%.

The control group consisted of 227 individuals who had experienced intracerebral hemorrhage and were randomly recruited from individuals who had come for regular medical examinations at our hospital during the same period. One control was matched with each case by gender and age (±5 years). All the control subjects were confirmed to not have intracerebral hemorrhage by brain CT and/or brain MRI. All patients with hypertensive intracerebral hemorrhage and control subjects provided written informed consent before enrolling in our study. The protocol of this study was approved by the Ethics Committee of the Affiliated Hospital of Yanan University (China).

The demographic and clinical information of patients with hypertensive intracerebral hemorrhage and control subjects were collected from a self-designed questionnaire and medical records, including data regarding gender, age, body mass index (BMI), diabetes mellitus, tobacco use, alcohol consumption, diastolic blood pressure (DBP), systolic blood pressure (SBP), triglycerides, total cholesterol, high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C).

Genotyping

A 5-mL peripheral blood sample was drawn from each patient with hypertensive intracerebral hemorrhage and each control subject, and the peripheral blood samples were kept at -80°C until use. Genotyping of the *COL1A2* rs42524 polymorphism was performed using polymerase chain reaction (PCR) coupled with restriction fragment length polymorphism (RFLP). Primer sequences for the *COL1A2* rs42524 polymorphism were 5'-TAGGTGACCGTTTGAGAC-3' (forward) and 5'-ATGGGGAGGTGTTGTTAT-3' (reverse). The PCR fragments of the investigated polymorphisms were subsequently digested with their specific restriction enzyme. The reaction conditions for PCR were an initial denaturation step of 8 min at 94°C, followed by 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 60 s. The resulting DNA fragments were electrophoresed on a 3.5% agarose gel and visualized under UV light after ethidium bromide staining.

Statistical analysis

The means of quantitative variables were compared between groups using the Student *t*-test, while distributions of categorical variables were compared by the Pearson χ^2 test. Hardy-Weinberg equilibrium (HWE) was examined using the χ^2 test with one degree of freedom. Multiple-conditional logistic regression models were established to estimate the association between the *COL1A2* rs42524 polymorphism and risk of hypertensive intracerebral hemorrhage after adjusting for potential confounding factors. The results are reported as odds ratios (ORs) and their 95% confidence intervals (95%CIs) were calculated. A P value was considered significant at a level of <0.05. The SPSS 21.0 package (SPSS Inc., Chicago, IL, USA) was used for statistical analysis.

RESULTS

The distributions of baseline characteristics of patients with hypertensive intracerebral hemorrhage and controls are shown in Table 1. The mean ages of the included patients with hypertensive intracerebral hemorrhage and controls were 64.60 ± 11.75 and 65.32 ± 10.92 years, respectively. Compared to the controls, patients with hypertensive intracerebral hemorrhage were more likely to have higher BMI, DBP, SBP, triglyceride levels, and LDL-C levels. They were also more likely to have a habit of tobacco use and alcohol consumption and develop diabetes mellitus ($P < 0.05$).

The genotype distributions of the *COL1A2* rs42524 polymorphism were found to adhere to the HWE in the control group (Table 2). Using the χ^2 test, we found that frequencies of the GG, GC and CC genotypes were significantly different between patients with hypertensive intracerebral hemorrhage and controls (χ^2 = 19.61, P < 0.001). Using logistic regression analysis, we found that the CC genotype was associated with increased risk of hypertensive intracerebral hemorrhage when compared to the GG genotype (OR = 12.67, 95%CI = 3.03-112.11). In a dominant model, the GC + CC genotype of the *COL1A2* rs42524 polymorphism was associated with a 2.57-fold increased risk of hypertensive intracerebral hemorrhage when compared with the GG genotype. In a recessive model, the CC genotype of the *COL1A2* rs42524 polymorphism correlated with a higher risk of hypertensive intracerebral hemorrhage when compared to the GG + GC genotype (OR = 12.07, 95%CI = 2.89-106.75).

Table 1. Baseline characteristics of patients with hypertensive intracerebral hemorrhage and controls.

Characteristics	Patients	%	Controls	%	*t* or χ^2 test	P value
Age (years)	64.60 ± 11.75		65.32 ± 10.92		0.68	0.25
Gender						
Female	142	62.56	142	62.56	0.00	1.00
Male	85	37.44	85	37.44		
BMI (kg/m^2)	23.83 ± 2.65		23.15 ± 2.56		2.78	0.006
Tobacco Use						
No	111	48.90	142	62.56	8.58	0.003
Yes	116	51.10	85	37.44		
Alcohol consumption						
No	147	64.76	165	72.69	3.32	0.007
Yes	80	35.24	62	27.31		
Diabetes mellitus						
No	154	67.84	199	87.67	25.79	<0.001
Yes	73	32.16	28	12.33		
DBP (mmHg)	165.52 ± 28.50		137.05 ± 20.65		12.19	<0.005
SBP (mmHg)	98.40 ± 19.42		83.52 ± 11.54		9.92	<0.001
Triglyceride (mM)	1.76 ± 1.21		1.42 ± 0.78		3.56	<0.001
Total cholesterol (mM)	4.55 ± 0.89		4.67 ± 0.92		1.41	0.08
HDL-C (mM)	1.36 ± 0.72		1.39 ± 0.85		0.41	0.34
LDL-C (mM)	3.42 ± 0.87		3.05 ± 0.81		4.67	<0.001

BMI = body mass index; DBP = diastolic blood pressure; SBP = systolic blood pressure; HDL-C = high-density lipoprotein cholesterol; LDL-C = low-density lipoprotein cholesterol.

Table 2. Association between the *COL1A2* rs42524 polymorphism and development of hypertensive intracerebral hemorrhage.

Polymorphism	Patients	%	Controls	%	HWE	χ^2 test	P value	Adjusted OR (95%CI)[1]	P value
COL1A2 rs42524									
Codominant									
GG	178	78.41	205	90.31				1.0 (Ref.)	-
GC	27	11.89	20	8.81				1.55 (0.81-3.03)	0.16
CC	22	9.69	2	0.88	0.07	19.61	<0.001	12.67 (3.03-112.11)	<0.001
Dominant									
GG	178	78.41	205	90.31				1.0 (Ref.)	-
GC + CC	49	21.59	22	9.69		12.17	<0.001	2.57 (1.45-4.63)	<0.005
Recessive									
GG + GC	205	90.31	225	99.12				1.0 (Ref.)	-
CC	22	9.69	2	0.88		17.59	<0.001	12.07 (2.89-106.75)	<0.001

[1]Adjusted for gender, age, body mass index, tobacco use, alcohol consumption, diabetes mellitus, diastolic blood pressure, systolic blood pressure, triglycerides, total cholesterol, high-density lipoprotein cholesterol and low-density lipoprotein cholesterol. OR = odds ratio; 95%CI = 95% confidence interval; HWE = Hardy-Weinberg equilibrium.

We further analyzed the association between the *COL1A2* rs42524 polymorphism, tobacco use and alcohol consumption, and the risk of hypertensive intracerebral hemorrhage (Table 3). We found that the GC + CC genotype of the *COL1A2* rs42524 polymorphism was associated with an increased risk of hypertensive intracerebral hemorrhage in smokers (OR = 3.71, 95%CI = 1.48-10.56). Moreover, we found a significant association between the *COL1A2* rs42524 polymorphism and alcohol consumption in the risk of hypertensive intracerebral hemorrhage (OR = 4.89, 95%CI = 1.65-17.39).

Table 3. Association between the *COL1A2* rs42524 polymorphism and tobacco use and alcohol consumption in the risk of hypertensive intracerebral hemorrhage.

Characteristics	COL1A2 rs42524				OR (95%CI)[1]	P value
	GG		GC + CC			
	Patients	Controls	Patients	Controls		
Tobacco use						
Non-smokers	91	127	20	15	1.86 (0.85-4.12)	0.09
Smokers	87	78	29	7	3.71 (1.48-10.56)	0.002
Alcohol consumption						
Non-drinkers	122	148	25	17	1.78 (0.88-3.69)	0.08
Drinkers	56	57	24	5	4.89 (1.65-17.39)	0.001

[1]Adjusted for gender and age.

DISCUSSION

Polymorphisms can have an effect on gene expression and contribute to differences between individuals in susceptibility to and severity of disease. Hypertensive intracerebral hemorrhage is generally believed to be a disease influenced by interactions between genes and the environment, resulting in high mortality and disability. However, the etiology of hypertensive intracerebral hemorrhage is unclear; many recent studies have reported that molecular factors may play an important role in the development of the disease. In our study, we investigated whether the *COL1A2* rs42524 polymorphism alters susceptibility to hypertensive intracerebral hemorrhage. We found that the *COL1A2* rs42524 polymorphism was associated with the development of hypertensive intracerebral hemorrhage in codominant, dominant, and recessive models.

It is well known that collagen can reduce the strength of the vascular wall and cause aneurysms. *COL1A2* contributes to the expression of collagen type I *in vivo* and this gene plays an important role in human tissue repair and development (Niederreither et al., 1992; Ponticos et al., 2004). Previous studies have reported that the *COL1A2* gene plays an essential role in the development, stabilization, maturation, and remodeling of blood vessels (Allt and Lawrenson, 2001; Vontell et al., 2006). The *COL1A2* gene is also associated with many cardiovascular diseases, such as stroke, myocardial infarction, and intracranial aneurysm (Roos et al., 2004; Yoneyama et al., 2004; Lindahl et al., 2009; Zuo et al., 2012). Yoneyama et al. (2004) conducted a case-control study in a Japanese population and reported that *COL1A2* polymorphisms could be a genetic risk factor for patients with a family history of intracranial aneurysms. Lindahl et al. (2009) conducted a study in a Swedish population and found that the heterozygous genotype of the *COL1A2* rs42524 polymorphism resulted in an increased risk of stroke and myocardial infarction. Zuo et al. (2012) conducted a case-control study in a Chinese population and found that the *COL1A2* rs42524 polymorphism is a risk factor for neovascular age-related macular degeneration.

In our study, we found that the *COL1A2* rs42524 polymorphism correlated with an increased risk of hypertensive intracerebral hemorrhage when compared with the wide-type genotype. Only

one previous study has reported the association between the *COL1A2* rs42524 polymorphism and the development of intracerebral hemorrhage. Liu et al. (2012) conducted a case-control study with 393 patients with primary intracerebral hemorrhage and 486 controls, and they reported that the *COL1A2* rs42524 polymorphism could contribute to the development of intracerebral hemorrhage. Our study is in line with the aforementioned study; however, further studies with larger sample sizes are greatly needed to confirm our results.

There were two limitations to our study. First, the patients with hypertensive intracerebral hemorrhage and controls were both selected from the same hospital, and thus selection bias could not be avoided in this study. Second, the sample size of this study was relatively small, which may not represent the general population and could limit the statistical power to find differences between groups.

In conclusion, our study suggests that the *COL1A2* rs42524 polymorphism is associated with the development of hypertensive intracerebral hemorrhage, particularly in those who use tobacco and consume alcohol. Further studies with larger sample sizes are highly warranted to further elucidate our findings.

Conflicts of interest

The authors declare no conflict of interest.

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