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Original Article

Predictive significance of *VEGFA* variations in intracranial aneurysm

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Abstract: Aim: This study was aimed to predict the significance of vascular endothelinal growth factor A (*VEGFA*) gene variations (rs3025039 and rs201096) in intracranial aneurysm (IA). Methods: 114 IA patients and 128 healthy controls were enrolled in this case-control study. Cases and controls were age- and gender- matched. All of the participants were unrelated Han Chinese. Strength of the association between the *VEGFA* variations and IA risk was presented by odds ratios (ORs) and 95% confidence intervals (CIs). Results: Frequency of rs3025039 TT genotype obviously higher in cases than in controls, indicating a statistical significance in the development of IA (P=0.025, OR=3.090, 95% CI=1.113-8.580). Meanwhile, the T allele of rs3025039 variation might act as a risk factor of IA (P=0.006, OR=1.812, 95% CI=1.182-2.776). Stratified analysis showed significant association of rs3025039 with the aneurysms number and size (P<0.05). *VEGFA* rs2010963 had no significant association with the IA both in the susceptibility and the clinical features of IA. Conclusion: *VEGFA* rs3025039 might predict the development of IA, including the susceptibility, aneurysms number and size. Further studies were needed to certify this results.

Keywords: *VEGFA*, variations, intracranial aneurysm

Introduction

Intracranial aneurysm (IA), also called cerebral or brain aneurysm, is defined as the tumor-simulating protuberance in the cerebral vascular. The alteration is caused by the local abnormal changes of intracranial blood vessels which lead to the dilation or bulging of cystic artery walls. IA mainly occurs in the Circle of Willis [1]. Rupture of IA will lead to the subarachnoid hemorrhage (SAH). So IA is one of the most destructive cerebrovascular diseases at present [2]. Prognosis of IA is very poor, it has a high mortality after the rupture [3]. Specific molecular mechanism of IA is extremely complex [4]. The formation and rupture of IA is a pathological process affected by many factors [5, 6]. Risk factors of IA could divide into 3 phases: ① factors promote the aneurysm development; ② factors alter the growth or morphology of IA; and ③ factors lead to the rupture [7].

Vascular endothelinal growth factor A (VEGFA), a member of platelet-derived growth factor (PDGF)/VEGF family, contribute to the development of many diseases [8-11]. As a glycosylated mitogen, VEGFA influences the endothelial cells and mediates the vascular permeability, vasculogenesis, angiogenesis and endothelial cell growth, promoting cell migration, and inhibiting apoptosis [12-16]. VEGF signaling could modify the endothelial function and the microvessel permeability in a mice model [17]. In addition, a vitro study showed that VEGF and VEGFR regulated the proliferation of vascular endothelial cells and vascular angiogenesis [18]. According to the function, VEGFA might affect the aneurysm development and the growth of IA. Variations of *VEGFA* gene will alter the expression and the function of encoded protein, then lead to the changes of serum levels of VEGFA, and finally influence the development of diseases [14, 19, 20]. *VEGFA* variations contribute to the development of abdominal aortic aneurysms [21]. So we speculated that polymorphisms of *VEGFA* gene might involve in the development of IA too. However the exact role of VEGFA in the progress of IA is still unknown.

Table 1. Features of the participants

Features	Case n=114 (%)	Control n=128 (%)	*P*
Age			0.856
<50	53 (46.49)	61 (47.66)	
≥50	61 (53.51)	67 (52.34)	
Gender			0.916
Male	50 (43.56)	57 (44.53)	
Female	64 (56.14)	71 (55.47)	
History of hypertension			0.689
No	72 (63.16)	84 (65.62)	
Yes	42 (36.84)	44 (34.38)	
History of diatetes			0.075
No	108 (94.74)	113 (88.28)	
Yes	6 (5.26)	15 (11.72)	
Family history of IA			0.132
No	112 (98.25)	128 (100.00)	
Yes	2 (1.75)	0 (0.00)	
Aneurysm number			
1	103 (90.35)	-	
>1	11 (9.65)	-	
Rupture			
No	13 (11.40)	-	
Yes	101 (88.60)	-	
Site of IAs			
ACA	54 (47.37)	-	
MCA	26 (22.81)	-	
ICA	21 (18.42)	-	
Others	13 (11.40)	-	
Shape of aneurysm			
Saccular	91 (79.82)	-	
Fusiform	17 (14.91)	-	
Others	6 (5.26)	-	
Size of aneurysm			
<15 mm	80 (70.17)	-	
15-25 mm	29 (25.44)	-	
>25 mm	5 (4.39)	-	

Notes: ACA, anterior cerebral artery; MCA, middle cerebral artery; ICA, internal carotid artery.

Therefore, in this case-control study we selected two single nucleotide polymorphisms (SNPs) rs3025039 and rs201096 of *VEGFA* gene to explore the mechanism of IA in Chinese Han population.

Materials and methods

Study objects

Local ethic committee approved this study. Participants understood this study and provided the informed consent. Study process and the sample collection conformed with the declaration of Helsinki. Controls and cases matched with each other in age and gender. All of the subjects were Chinese Han population and had no blood relation among them.

Between January 2013 and June 2015, 114 new diagnosed IA patients and 128 controls were recruited from Affiliated Hospital of Hebei University of Engineering, Patients were diagnosed by computed tomography angiography (CTA), magnetic resonance sngiography (MRA) or digital subtraction angiography (DSA). Controls were take in a healthy check-up in the hospital, and had no histories of IA, stroke and other cerebrovascular diseases.

Genotype analysis of VEGFA polymorphisms

5mL peripheral venous blood was collected from every fasting participator using an EDTA anticoagulation vacuum tube, and then stored at -70°C until to use. Genomic DNA was extracted by a Blood Genomic DNA Kit (Sigma, USA). *VEGFA* rs3025039 and rs2010963 polymorphisms were detected by MassARRAY Assay Design Version (Sequenom, USA).

Statistical analysis

Genotype and allele distributions were detected by Hardy-Weinberg equilibrium (HWE) test. Frequencies of genotype and alleles were calculated by direct counting. Differences of the characteristics between the case and control groups were assessed by the t test or χ^2. Relative risk of IA was presented by odds ratios (ORs) and corresponding 95% confidence intervals (CIs).

Results

Features of the participants

Basic features had no significant differences between the case and control groups (**Table 1**). The clinical features were also listed in the **Table 1**. Most IA patients had one aneurysm. Aneurysms mainly located at anterior cerebral artery (ACA), middle cerebral artery (MCA), and internal carotid artery (ICA). Most of the aneu-

Table 2. Association between *VEGFA* SNPs (rs3025039 and rs2010963) and IA risk

SNP	Case n=114 (%)	Control n=128 (%)	*P*	OR (95% CI)
rs3025039				
CC	61 (53.51)	87 (67.97)		
CT	40 (35.09)	35 (27.34)	0.086	1.630 (0.932-2.852)
TT	13 (11.40)	6 (4.69)	0.025	3.090 (1.113-8.580)
C	162 (71.05)	209 (81.64)		
T	66 (28.95)	47 (18.36)	0.006	1.812 (1.182-2.776)
rs2010963				
GG	37 (32.46)	43 (33.59)		
GC	59 (51.75)	71 (55.47)	0.903	0.966 (0.552-1.689)
CC	18 (15.79)	14 (10.94)	0.339	1.494 (0.655-3.410)
G	133 (58.33)	157 (61.33)		
C	95 (41.67)	99 (38.67)	0.502	1.133 (0.787-1.630)

rysms were saccular, small (<15 mm) and ruptured.

Association between VEGFA variations (rs3025039 and rs2010963) and IA risk

Genotype distributions of rs3025039 and rs2010963 SNPs were not deviated from the HWE test both in cases and controls, indicating the subjects could represent the general population.

For rs3025039, the frequencies of CT and TT genotypes were respectively 35.09%, 11.40% in cases and 27.34%, 4.69% in controls (**Table 2**). Genotype frequencies of CT and TT were higher in cases than in controls, but only the difference of TT genotype was statistically significant (*P*=0.025). This obvious difference showed a close association between rs3025039 TT genotype and the risk of IA (OR=3.090, 95% CI=1.113-8.580). At the same time, T allele of rs3025039 polymorphism was significantly higher in case group than in control group (*P*=0.006), demonstrated that T allele positively associated with the susceptibility of IA (OR=1.812, 95% CI=1.182-2.776). Conversely, all of the genotypes and alleles of rs2010963 variation had no significant correlation with the risk of IA (*P*>0.05).

Correlation between VEGFA variations (rs3025039 and rs201096) and the features of IA patients

In order to explore the exact role of *VEGFA* SNPs in the development of IA, we detected the correlation between *VEGFA* variations and the clinical features of IA (**Table 3**). Afterwards, we found that genotypes of rs3025039 significantly related to the number and size of aneurysms (*P*<0.05), but not the rupture, site and shape of aneurysms (*P*>0.05). Meanwhile, no significant association was observed between rs2010963 polymorphism and the clinical characteristics of IA (*P*>0.05).

Discussion

IA supply blood to the brain with the dilated artery. Saccular or berry form is the most common shape of IA. It also divided into unruptured and ruptured IA [22]. Unruptured IA usually is asymptomatic, before a larger aneurysm rupture, patients may undergo the symptoms such as nausea, vomiting, severe headache, vision impairment, and loss of consciousness. Unruptured IA patients are often found through screening high risk patients or as purely incidental findings of other neurological symptoms [22]. If an aneurysm ruptures, blood spilled into the space around the brain, this symptom is called SAH. Prognosis of ruptured IA patients depends on many factors. However, most IA patients with ruptures had poor prognosis [23, 24]. Most aneurysms are unobserved until they have been ruptured. There is no effective predictive and therapy method for IA. So it is urgent to explore the mechanism of IA, so as to looking for a predictive marker of IA. Epidemiology researches showed that IA rarely occurs in pediatric populations, and the development of it is influenced by multiple factors [5, 6]. Among these factors, genetic factors are the crucial factor for the onset and development of IA. Genetic factors result in the dilated artery may be the basic factor for IA progression.

VEGFA, a highly conserved homodimer glycoprotein, is linked by disulfide bond. Proliferation and migration of vascular endothelial cells may be induced by VEGFA factor [10]. Whilst, VEGFA is essential for both physiological and pathological of angiogenesis [11]. VEGFA might play a potential role in the development of IA. A vitro study suggested that recombinant human VEGF (rhVEGF) is effective for the therapy of

Table 3. Correlation between *VEGFA* SNPs (rs3025039 and rs2010963) and the features of IA patients

	NO.	rs3025039				rs2010963			
		CC n=61 (%)	CT n=40 (%)	TT n=13 (%)	*P*	GG n=37 (%)	GC n=59 (%)	CC n=18 (%)	*P*
IA number					<0.001				0.81
1	103	60 (98.36)	38 (95.00)	5 (38.46)		33 (89.19)	53 (89.83)	17 (94.44)	
>1	11	1 (1.64)	2 (5.00)	8 (61.54)		4 (10.81)	6 (10.17)	1 (5.56)	
Rupture					0.868				0.987
No	13	7 (11.48)	4 (10.00)	2 (15.38)		4 (10.81)	7 (11.86)	2 (11.11)	
Yes	101	54 (88.52)	36 (90.00)	11 (84.62)		33 (89.19)	52 (88.14)	16 (88.890)	
Site of IAs					0.982				0.758
ACA	54	28 (45.90)	20 (50.00)	6 (46.15)		17 (45.95)	28 (47.46)	9 (50.00)	
MCA	26	14 (22.95)	9 (22.50)	3 (23.08)		8 (21.62)	13 (22.03)	5 (27.78)	
ICA	21	11 (18.03)	8 (20.00)	2 (15.38)		9 (24.32)	11 (18.64)	1 (5.55)	
Others	13	8 (13.11)	3 (7.50)	2 (15.38)		3 (9.11)	7 (11.86)	3 (16.67)	
Aneurysm shape					0.912				0.759
Saccular	91	48 (78.69)	33 (82.50)	10 (76.92)		30 (81.08)	47 (79.66)	14 (77.78)	
Fusiform	17	9 (14.75)	6 (15.00)	2 (15.38)		6 (16.22)	9 (15.25)	2 (11.11)	
Others	6	4 (6.56)	1 (2.50)	1 (7.69)		1 (2.70)	3 (5.08)	2 (11.11)	
Aneurysm size					0.014				0.098
<15 mm	80	44 (72.13)	28 (70.00)	8 (61.54)	-	26 (70.27)	41 (69.49)	13 (72.22)	-
15-25 mm	29	16 (26.23)	11 (27.50)	2 (15.38)		9 (24.32)	15 (25.42)	5 (27.78)	
>25 mm	5	1 (1.64)	1 (2.50)	3 (23.08)		2 (5.41)	3 (5.08)	0 (0.00)	

aneurysms [25]. A previous study showed that expression of VEGF was altered in the injured brains [26]. However, Sandalcioglu et al. indicated that plasma VEGF level was not significantly associated with aneurysm include the number and location in unruptured IA patients [27]. Recent years, polymorphism becomes the hotspot in the exploration of the mechanism of many diseases. Besides, many researches revealed that variations of *VEGFA* gene correlated with the occurrence of cerebrovascular diseases [21]. *VEGFA* rs3025039 and rs201096 variations, respectively located in the 3' and 5' untranslated regions (UTRs) of *VEGFA* gene, associated with many diseases [28-30]. Because the role of *VEGFA* gene in IA onset is not clear, we selected these two SNPs of *VEGFA* gene to certify the pathogenesis of IA.

In this study we found that rs3025039 TT genotype increased the IA risk about 3.090 times compared with the CC genotype. T allele of rs3025039 variation enhanced 1.812 times risk of IA. These results were different from a study performed in Italy which suggested that *VEGF* +936C>T and 18 bp microdeletion polymorphisms were not significantly associated with the susceptibility and clinical features of aneurysmal SAH [31]. But He et al. [29] indicated that T allele increased the risk of hypertensive cerebellar hemorrhage. This variation also positively associated with the risk of glioma [28]. These results provide an evidence that rs3025039 associated with the susceptibility of IA from other aspect. However, no significant association was observed between rs2010963 variation and the IA susceptibility. This result accorded with the previous study which suggested that *VEGFA* -634GG genotype significantly enhanced the risk of abdominal aortic aneurysm [21]. But it was different from the role in glioma [28] and hypertensive cerebellar hemorrhage [29]. Further stratified analysis revealed that rs3025039 variation correlated with the aneurysms number and size, but not other features. Meanwhile, rs2010963 polymorphism was not related to the clinical characteristics of IA.

In summary, *VEGFA* rs3025039 variation may be predictive to the development of IA, including susceptibility, number and size of IA. Present results suggested that *VEGFA* rs3025039 variation could predict the onset and development of IA. However, the small sample size, unadjusted result, as well as other con-

founding factors may lead to the limitations of present study. Therefore, further studies are necessary to understand the mechanism of IA.

Disclosure of conflict of interest

None.

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