Evaluation of VEGFA gene variants for possible roles in cerebral infarction diseases

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Abstract

Background: Cerebral infarction is one of the most common cerebral small vessel diseases. Many angiogenesis relevanted factors have special roles on the diseases pathogenesis. The vascular endothelial growth factor A (VEGFA) is the most prominent stimulating molecule factor for angiogenesis. So, we want to evaluate the correlation between VEGFA gene and this disease in this work.

Methods: The VEGFA gene was sequenced for 400 cerebral infarction patients and 600 normal controls. SPSS software (version 19.0), Plink (version 1.9), Haploview software and online software SNPSpD were used for the statistical analyses and Hardy-Weinberg equilibrium tests.

Results: We found variants rs10434, rs3025040, rs185218985, rs199971699, rs574579489, rs735286 and rs833061 within or near the VEGFA gene. The genetic heterozygosity of rs10434, rs3025040, rs735286 and rs833061 was very high. Statistical analysis showed that the variants rs10434 ($P=0.041$) and rs735286 ($P=0.034$) in the gene were associated with the risk of cerebral infarction diseases in the Chinese Han population.

Conclusions: VEGFA variants rs10434 and rs735286 were associated with the risk of cerebral infarction diseases in the Chinese Han population.

Introduction

The Cerebral small vessel diseases (CSVDs) refers to a group of prevalent brain vascular pathological processes, often involved in the cerebral small arteries, venules and capillaries, mainly causing cerebral white and deep grey matter damages^[1,2]. There are many types of the CSVDs, such as hypertension-related cerebral small vessel disease, age-related cerebral small vessel disease and cerebral amyloid angiopathy^[3,4]. Many brain illnesses including vascular dementia, cognitive impairment, stroke, are often also associated with CSVDs^[3-6].

Cerebral infarction is one of CSVDs, often characterized by mental severe hypoxic ischemic tissue necrosis and severe upper extremity impairments^[7]. Many diseases have been found lead to this disease, such as 20% of the cerebral infarction cases are cardiogenic cerebral infarction, and about 50% of the cardiogenic cerebral infarction cases are caused by nonvalvular atrial fibrillation^[8,9]. In other side, this disease also leading to other diseases, such as sensory disability, language barrier, repeated paralysis, dementia^[7,10,11]. Although a variety of therapies or methods have been applied to treat the disease in clinical^[12,13], but few are effective^[14,15].

Disclose the pathogenesis of cerebral infarction diseases (CID), would help exploit new treatment strategies. Patients mental blood vessels blockage is the direct cause of the cerebral infarction diseases, that is resulting from embolism or thrombosis and leading to cerebral ischemia^[16]. The cerebral ischemia may also induce immune responses and inflammation^[17-22]. The risk factors documented associated

with the disease include smoking, obesity, dyslipidemia, high blood pressure^[19, 23] and cellular apoptosis^[24, 25].

In the process of vascular cell proliferation, differentiation or apoptosis, many genes involved in, and that also involved in the etiology of the cerebral infarction^[19, 26, 27]. The expression of those genes is regulated by the Notch signaling pathway^[28–30]. This pathway also affects the proliferation of stem/progenitor cells and supports the central and peripheral nervous systems^[31]. In the previous study, we found that variants in the Notch3 and RBPJ genes are associated with the risk of cerebral infarction diseases^[19, 32]. The Notch3 and RBPJ mediates the process of Notch pathway functioning^[33, 34]. So cellular proliferation, differentiation or apoptosis, and Notch signaling pathway, many have special roles in the pathogenesis of the cerebral infarction diseases.

In the proliferation of many cells, including endothelial cells, neural stem cells and mature astrocytes, the vascular endothelial growth factor A (VEGFA) promotes this process^[35]. It is also the most prominent stimulating molecule factor for angiogenesis and neurogenesis^[36]. For example, VEGFA overexpression can improve cognitive performance and neovascularization, hippocampal neurogenesis; blocking the functions of VEGFA, impairs the angiogenesis and neurogenesis and cognitive performance^[37]. In other side, the VEGFA factor can be produced by many cells, and increasing the vascular permeability of endothelial cells^[38, 39]. However, the correlation between VEGFA and the pathogenesis of cerebral infarction diseases remains unclear.

In this study, we evaluated VEGFA gene variants rs10434, rs3025040, rs735286 and rs833061 for their possible roles in the cerebral infarction disease risk in the Chinese Han population and also demonstrated the relationship between VEGFA factors, vascular cell and cerebral infarction diseases.

Methods

Study population

At the Department of Neurology and Medical Examination Center of the Daqing Oilfield General Hospital, Daqing, China, we collected a total of 400 cerebral infarction disease patients and 600 normal controls for this study. We also recorded the clinical features and medical histories of the all the enrolled participants in detail. In the clinical examination, those enrolled participants also carried out the MRI and physical examinations. The detailed clinical features of those enrolled participants were presented in the previous study^[19]. The experiments were performed in accordance with relevant guidelines and regulations, and we also obtained a written informed consent from each participant. The Ethics Committee of HMU approved this work, which is consistent with the 1975 Declaration of Helsinki.

DNA analysis

From the peripheral blood of those participants, we extracted the genomic DNA [40, 41]. Then we were amplified the splicing sites and transcribed regions of VEGFA gene using the primers shown in Table 1, and as previously shown for mutational analysis we were sequenced the PCR products [42].

Table 1
PCR primers used for VEGFA gene sequence analysis

Exon	Forward primer	Reverse primer	Size	Tm
1a	GCGAGCAGCGAAAGCGACAG	GCAGCAAGGCAAGGCTCCAAT	694	64.1
1b	GGCTCATGGACGGGTGAGG	ACTCCGGTGGGTGGGCAG	493	63.0
2	AGGGAAGTGAGGAGGGAG	TCACGAAACTGAGGGTGAA	312	55.4
3	TTACAGAGCTGGGTGGAGA	GAGCCAGACAAGGCAAAT	383	54.9
4	GTATGGCTGGCTGGGTCA	GCCAAAGGTCACATAGCG	248	53.1
5	CACCATCTTAACCCTTCC	ACTAGCCCTAGAGCCATC	269	52.5
6	GCTTTGCTTTGGTCGTTC	GGTGCCAATTAGTGGAGTG	340	54.8
7	GGGTGTTGCATGGTGATT	CAGGCAGGCAGCTAGAAA	354	53.8
8a	TGGGGCTGTTCTCATACT	CTCTTCCCTGTCAGGATC	742	52.0
8b	TATTGGTGTCTTCACTGGA	CAGGGGATGGAGGAAGGT	679	54.1
8c	CTCATCCTCTTCCTGCTC	GTATTTCTTTGTCGTTGTTT	870	47.7

SNP genotyping analysis

We determined the variations of the VEGFA gene in the enrolled cerebral infarction patients and controls, and the measurements were conducted by two researchers independently. Finally overall the genetics correlation analysis was conducted.

Statistical analysis

We were conducted the statistical analyses and Hardy-Weinberg equilibrium tests of the study participants using SPSS software (version 19.0) [43] and Plink (version 1.9) [44, 45]. And as previously study shown, using the online software SNPSpD and Haploview software, we were calculated the experiment-wide significance threshold and the matrix of mpirwise linkage-disequilibrium (LD) correlation [46].

Results

SNP analyses

In this work, we sequenced the VEGFA gene, in order to evaluate variations in VEGFA gene for their possible associations with the cerebral infarction diseases susceptibility. We were characterized the variants rs10434, rs3025040, rs185218985, rs199971699, rs574579489, rs735286 and rs833061 within or near the VEGFA gene (Fig. 1A). Farther analysis showed that the genetic heterozygosity of rs10434, rs3025040, rs735286 and rs833061 was very high, whereas that of rs185218985, rs199971699 and rs574579489 was very low and were excluded from further analysis (Table 2).

Table 2

The genotype and allele frequency of rs10434, rs3025040, rs735286 and rs833061 variants in 400 Chinese Han cerebral infarction and 600 normal agedness controls.

Variations	Group		Genotype frequency (%)			Allele frequency (%)	
rs10434	Genotype		G/G	G/A	A/A	G	A
	CID	400	256(64.0%)	138(34.5%)	6(1.5%)	650(81.3%)	150(18.8%)
	Control	600	394(65.7%)	182(30.3%)	24(4.0%)	970(80.8%)	230(19.2%)
rs3025040	Genotype		C/C	C/T	T/T	C	T
	CID	400	246(61.5%)	143(35.8%)	11(2.8%)	635(79.4%)	165(20.6%)
	Control	600	394(65.7%)	188(31.3%)	18(3.0%)	976(81.3%)	224(18.7%)
rs735286	Genotype		G/G	G/A	A/A	G	A
	CID	400	127(31.8%)	212(53.0%)	61(15.3%)	466(58.3%)	334(41.8%)
	Control	600	239(39.8%)	281(46.8%)	80(13.3%)	759(63.3%)	441(36.8%)
rs833061	Genotype		T/T	T/C	C/C	T	C
	CID	400	187(46.8%)	175(43.8%)	38(9.5%)	549(68.6%)	251(31.4%)
	Control	600	250(41.7%)	283(47.2%)	67(11.2%)	783(65.3%)	417(34.8%)
CID: cerebral infarction diseases.							

Polymorphism-disease association analyses

In order to, evaluate their possible associations with the cerebral infarction disease, we conducted analyses on those SNPs and found rs10434 ($P = 0.041$) and rs735286 ($P = 0.034$) within VEGFA gene were associated with the risk of this disease in Chinese Han population (Tables 2, 3). At the same time, we also conducted the Hardy-Weinberg equilibrium test for the cerebral infarction disease and control populations, and found they were in line with equilibrium (Tables 4).

Table 3

Varients rs10434 and rs735286 within the VEGFA gene were associated with risk of cerebral infarction in Chinese Han populations

Title		Pearson Chi-square				Risk		
Genotyped SNP	Statistical Types	Value	Min count ^a	df	Asymp. Sig. (2-sided)	Value	95%CI-low	95%CI-up
rs10434	Genotype	6.405	12.0	2	0.041*	–	–	–
	Allele	0.054	152.0	1	0.816	1.027	0.818	1.291
rs3025040	Genotype	2.117	11.6	2	0.347	–	–	–
	Allele	1.175	155.6	1	0.278	0.883	0.706	1.106
rs735286	Genotype	6.761	56.4	2	0.034*	–	–	–
	Allele	5.056	310.0	1	0.025*	0.811	0.675	0.974
rs833061	Genotype	2.666	42.0	2	0.264	–	–	–
	Allele	2.458	267.2	1	0.117	1.165	0.962	1.410
a: The minimum expected count; *: statistically significant, P < 0.05								

Table 4

Hardy-Weinberg equilibrium test for the study population groups

SNPs	Genotype	H-W equilibrium Testing		
	Homo/Hetero/Homozygote	O(HET)	E (HET)	P
rs10434	30/320/650	0.320	0.3078	0.2577
rs3025040	29/331/640	0.331	0.3133	0.0858
rs735286	141/493/366	0.493	0.4747	0.2312
rs833061	105/458/437	0.458	0.4449	0.3936

The experiment-wide significance threshold required to keep Type I error rate at 5% of rs10434, rs3025040, rs735286 and rs833061 within the VEGFA gene was 0.018. The variants rs10434, rs3025040, rs735286 and rs833061 LD analysis was conducted using the Haploview software, and it was consistent with the HapMap CHB population data (Fig. 2). The genotype frequencies of cerebral infarction disease and control groups were also analyzed by trend, dominant and recessive models, and all those results indicated rs10434 and rs735286 were associated with the risk of cerebral infarction diseases (Table 5).

Table 5
SNP rs10434 and rs735286 within VEGFA gene associated with the risk of
cerebral infarction diseases

SNPs	Value	Trend model	Dominant model	Recessive model
rs10434	ChisQ	0.0564	0.2930	5.1550
	P	0.8123	0.5883	0.0232*
rs735286	ChisQ	5.2590	6.7580	0.7279
	P	0.0218*	0.0093*	0.3936
*: statistically significant				

Discussion

Cerebral blood vessels systems characteristics changes is the main cause of cerebral infarction diseases, and many physiologic or pathologic conditions have been found contribute to vascular characteristics changes or cerebral infarction^[16, 47]. The VEGFA factor can strengthen neoangiogenesis under those conditions and maintain the blood vessels homeostasis^[48], VEGFA may also mediate the response of increases in permeability and angiogenesis, especially in brain^[49]. In the endothelial cells, VEGFA can facilitating cellular migration and sprouting^[50], and also have some roles in cancer pathology, inflammation and wound healing^[51, 52].

It is important to explore the pathogenesis of VEGFA in those diseases, such as cerebral infarction diseases. It has been found that VEGFA factors can disrupt vascular pericytes coverage and stop the newborn vessels maturation^[53]. However, after cerebral ischemia, VEGFA factors can also induce the proliferation of endothelial cells in angiogenesis, and after subarachnoid hemorrhage, the factors can promote the formation of brain edema^[53, 54]. The expression level of VEGFA factors may also increase the increase the risk of cerebral infarction diseases^[54] and plaque rupture^[55]. Because in the brain infarct area, the new small blood vessels can improve blood supply, but also cause bleeding due to the vascular rupture^[56]. So the relationship between the VEGFA factors, vascular development and cerebral infarction diseases was very complex, it is may depend on the VEGFA factors released place, time and level. The results of this work, further confirmed the special roles of VEGFA in pathogenesis of cerebral infarction diseases.

In the development of the vessel, vascular cell proliferation, differentiation and apoptosis take it, and those cellular life process are regulated by Notch signaling pathway^[28]. Many variations in the genes that interact with the pathway could result in severe defects in the angiogenic vascular remodeling and lead to embryonic lethality^[57, 58]. Our previous works have showed the importance of Notch signaling pathway factors Notch3 and RBPJ in the pathogenesis of the cerebral infarction diseases^[19, 32]. It is interesting that

the VEGFA factor also promotes the proliferation of endothelial cells, neural stem cells and mature astrocytes^[35], and also stimulates molecule factor for angiogenesis and neurogenesis^[36]. Those results further proved the important roles of normal vascular cell life process in the pathogenesis of the cerebral infarction diseases.

In this study, we identified genetic variations rs10434 and rs735286 in the VEGFA gene were related with the risk of cerebral infarction diseases. Variation rs735286 was located within the third intron. It has been found that some untranslated regions of the gene includes key regulatory elements that is responsive to hypoxia^[59]. VEGFA gene variation + 936C/T resides in the 3-untranslated region, is associated with the risk of stroke^[60] and VEGFA serum levels^[61]. Variation – 2578C/A located at the VEGFA gene promoter region is correlated with a decreased VEGF expression^[62] and the risk of stroke^[60]. We found the site of variation rs10434 was within the 3'UTR of the gene, and it is associated with the risk of cerebral infarction diseases. However the specific functions of rs10434 in the 3'UTR region or the gene, is not completely clear. Combined the fact that VEGFA factor mediates angiogenesis was very complex, more works on the functions of special region of VEGFA may be needed.

Conclusion

We evaluated the risk between rs10434 and rs735286 in VEGFR gene and cerebral infarction diseases in Chinese Han population and demonstrated the relationship between VEGFA factors, vascular cell and cerebral infarction diseases.

Declarations

Ethics approval and consent to participate: Ethics Committee of Harbin Medical University. The experiments were performed in accordance with relevant guidelines and regulations, and all participants in this study signed a written informed consent.

Consent to publish: Not applicable.

Availability of data and materials: Not applicable.

Competing interests: The authors have declared that no competing interests exist.

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Figures

Figure 1

Schematic diagrams and DNA sequence chromatograms. A: Schematic diagrams of rs10434, rs3025040, rs185218985, rs199971699, rs574579489, rs735286 and rs833061 within or near the VEGFA gene; B: Three genotypes of DNA sequence chromatograms of rs735286 and rs10434.

Figure 2

LD analysis for rs10434, rs3025040, rs735286 and rs833061 within VEGFA gene. A: Data analysis between the cerebral infarction diseases patients and controls in present study; B: Data from HapMap CHB. The data of this work and from HapMap CHB were consistent.