Genetic variants of vascular endothelial growth factor gene polymorphism affect the risk and severity of ischemic stroke: a case–control study

Muhammad T. Abdel Ghafar[a], Osama Ragab[b], Ayman M. Al-Malt[b], Radwa A. Eissa[c], Sara Mina Samy[c], Sarah R. Abd El-Khalik[d], Hanem Rabah[d]

Departments of [a]Clinical Pathology [b]Neuropsychiatry [c]Microbiology & Immunology [d]Medical Biochemistry, Faculty of Medicine, Tanta University, Tanta, Egypt

Correspondence to Muhammad T. Abdel Ghafar MD, Department of Clinical Pathology, Faculty of Medicine, Medical Campus, Tanta University, Tanta, Egypt- 31511
Tel: +01 008 066 339; Fax: +20 403 282 344; e-mail: mohammedtarek5514@yahoo.com

Received 27 August 2020
Revised 12 October 2020
Accepted 01 December 2020
Published 21 July 2021

The Egyptian Journal of Laboratory Medicine 2020, 1:8–15

Background and objectives
Vascular endothelial growth factor (VEGF) is the key mediator of angiogenesis and atherosclerosis. Hence, it may play a major role in the pathogenic mechanisms underlying ischemic stroke (IS) progression. Thus, we conducted this case–control study to explore the genetic association of a single gene polymorphism of VEGF + 936C/T (rs3025039) with the risk and severity of IS and its relation with serum VEGF level.

Patients and methods
This study included a case series of 49 patients with IS with 41 controls. VEGF + 936C/T (rs3025039) variants were determined via Taqman allelic discrimination PCR. Serum VEGF level was estimated using enzyme-linked immunosorbent assay. The frequency of VEGF rs3025039 genotypes and alleles was calculated manually in both cases and controls. The risk ratio was estimated and represented by odds ratio (OR) and 95% confidence interval (CI) adjusted to the confounding variables via multinomial logistic regressions. The stroke severity was assessed by National Institute of Health Stroke Scale. Moreover, serum VEGF levels were compared between the studied groups and among different genotypes and alleles of IS cases.

Results
Our study detected significantly increased frequencies of TT genotype (P = 0.030) and T allele (P = 0.045) in IS cases when compared with the controls. TT genotype represents an increased adjusted risk for IS progression by 9.94 folds (OR: 9.938, 95% CI: 1.235–79.97) and T allele by 2.19 folds (OR: 2.191, 95% CI: 1.004–4.782) over the CC genotypes and C allele, respectively. Moreover, TT genotypes are associated with higher National Institute of Health Stroke Scale. The serum VEGF level was significantly lower in IS cases than controls, and a more decreased level was associated with the T allele carriers in IS cases groups.

Conclusions
VEGF rs3025039 gene polymorphism is associated with increased risk and severity of IS and decreased VEGF expression, which may play a major role in the pathogenesis of IS progression.

Keywords:
genotypes, ischemic stroke, polymorphism, risk, vascular endothelial growth factor

The Egyptian Journal of Laboratory Medicine 1:8–15

Introduction

Stroke is a highly prevalent health condition, presenting with the second highest mortality rate and the third highest disability rate after coronary heart disease worldwide [1]. Ischemic stroke (IS) occupies ~ 80% of this condition, which occurs as an end result of occlusion of the cerebral vessels [2]. It is a complex disorder with involvement of many etiological factors, including demographic, environmental, and morbidity conditions [3]. The demographic factors include age, sex, and race, whereas environmental factors involve smoking and alcohol consumption. The underlying morbidity conditions such as diabetes, hypertension, obesity, cardiovascular disorders, and dyslipidemia are also found to predispose to IS [4]. All of these risk factors cannot completely explain the high incidence of cerebral stroke in different populations. Thus, genetic background was found to influence the risk for IS progression [5]. Polymorphic variants affecting multiple genes such as apolipoprotein ε4 [6], nitric oxide synthase [7], and endothelin 1[8] are suggested to be associated with IS risk in different ethnic groups. Hence, investigating the genetic role in the pathogenesis of IS is required to improve the preventive and therapeutic measures for this disease.

Up to now, cerebral atherosclerosis is considered one of the major causes for IS with the proliferation and formation of new blood vessels in atheromatous plaque leading to microthrombi formation, as well as atheromatous plaque instability or rupture [9].

Vascular endothelial growth factor (VEGF) is the main mediator of angiogenesis [10]. However, its role in the pathogenesis of atherosclerosis is contradictory and needs to be further clarified [11]. It is encoded by a gene present on chromosome 6 (6p21.3) and is composed of 14-kb coding region with eight exons and seven introns [12]. The VEGF family is a group of seven proteins: six VEGF (A–F) and placental growth factor. Its action is mediated via interaction with high-affinity tyrosine kinases receptor VEGFR2 [13]. It is distributed mainly in the vascular endothelial cells, smooth muscle cells, and macrophages. However, it is also expressed in some malignant cells.

More than 30 VEGF gene single nucleotide polymorphisms (SNPs) have been identified and found to be associated with multiple disorders such as osteosarcoma, renal cell carcinoma, diabetic retinopathy, and autoimmune disorders [14–17]. Among these polymorphisms, three common polymorphisms were extensively studied and found to affect the VEGF expression level or signaling activity, such as +936C/T (rs3025039), −2578C/A (rs699947), and − 1154G/A (rs1570360) [18]. Some of these *VEGF* gene SNPs had been suggested to be related with IS risk in several studies [19]. Thus, we conduct this case–control study to explore the possible association of a single nucleotide polymorphism +936C/T (rs3025039) with genetic susceptibility and severity of IS and to assess its relation to VEGF expression via estimation of its serum level.

Patients and methods

Study population

This case–control study was performed on 49 cases (group I) with IS who were admitted to the ICU of Neuropsychiatry Department at Tanta University Hospitals and were diagnosed clinically by a neurologist based on the presence of clinical signs of acute neurological deficit that were continued for more than 1 day and confirmed by either brain MRI and/or cranial computed tomography (CT) scan according to the updated definition of stroke of American Stroke Association [20]. The different pathological types of IS were determined according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria[21] and cases with cardio-embolic, lacunar subtypes were excluded. We excluded also all other cases suspected to have cranial hemorrhage, coronary artery disease, arteritis, tumors, and head trauma. All patients presented beyond the thrombolytic therapy time window, so they had not received recombinant tissue plasminogen activator or mechanical thrombectomy. Patients with IS were treated according to American Stroke Association guideline for management of IS [22].

Furthermore, we recruited 41 participants from the Outpatient Clinic of Neuropsychiatry Department at Tanta University Hospitals experiencing neurological disorders other than stroke with no previous history of any types of cerebrovascular diseases, tumors, and coronary artery diseases. They served as a study control (group II). The recruitment started in June 2018 till May 2019. All participants were asked to sign an informed consent. The study protocol was approved by Ethics Committee of Faculty of Medicine, Tanta University, and the study was performed in accordance with Helsinki Declaration.

Data collection and assessment

Full medical data were obtained from all participants. A questionnaire concerning the family history of IS and history of smoking, diabetes, or hypertensive disorder was applied. A complete clinical examination with measurement of blood pressure and calculation of BMI was performed. All participants were examined by ultrasonography to evaluate the carotid system and measure the intima-media thickness (IMT) of common carotid artery, using Ultrasound Philips HD (22100 Bothell Everett Hwy, Bothell, WA 98021, USA) 11 linear array transducer of multifrequency (3–12 MHz), in real time and in sagittal, coronal, and axial views. IMT considered abnormal if more 0.9 mm and indicate the presence of atherosclerosis [23]. Echocardiography to exclude cardio-embolic causes of stroke, lipid profile, fasting blood glucose (FBS), and glycated hemoglobin (HbA1C) were done. Stroke severity was assessed by the validated Arabic version of the National Institute of Health Stroke Scale (NIHSS) on admission and 3 months after stroke onset [24].

Sampling

For the laboratory analysis, all participants were asked for a venous blood sample. Overall, 7–10 ml was withdrawn by a sterile syringe and evacuated into one plain vacutainer tube, and then into two K3 EDTA vaccutainer tubes. The plain tubes were centrifuged for 15 min at 3000 rpm, and the serum was separated into two aliquots, where one was used for immediate biochemical analysis for FBS and lipids, and the other aliquot was stored at − 20°C for estimation of VEGF serum level. One of the EDTA tubes was used for HBA1C determination, and the other was stored at − 20°C for VEGF rs3025039 (+936C/T) genotyping.

Routine biochemical analysis

FBS, total cholesterol, triglyceride, high-density

lipoprotein cholesterol, and low-density lipoprotein cholesterol were measured on automated chemistry analyzer, Konelab-60I (Thermo Scientific, Vantaa, Finland), using commercial kits provided by Thermo-Fisher Scientific (Waltham, Massachusetts, USA). The HbA1C was measured by immune-turbidimetric method using commercial HbA1C kits on Twin A1c semi-automated analyzer, provided by Spectrum Diagnostics (Obour City, Egypt).

Serum vascular endothelial growth factor estimation

The quantitative estimation of VEGF was performed via enzyme immunoassay sandwich technique (enzyme-linked immunosorbent assay) for all of the study participants in the serum sample using DRG Human VEGF ELISA Kit, USA, catalog no EIA-4819 (304 Route 22 West, Springfield, NJ 07081, USA). The instructions for reagent preparation and assay procedure were obtained from the package insert, and accordingly, the standard provided was reconstituted in 1-ml sample diluent to obtain a 4200 pg/ml concentrate, and then diluted serially to obtain seven concentrations starting from 0 to 1200 pg/ml. In each well of 96 precoated microtiter plate with monoclonal antibody specific for human VEGF, 50 µl of each standard and samples was added. After washing, biotin-conjugated VEGF antibody and HRP-streptavidin were added sequentially. After repeated washing, the enzyme detection was performed via adding the substrate (TMB), and then the reaction was stopped by acidification. The color developed was measured as absorbance on Tecan Spectra II micro-plate reader (Switzerland) at 450 nm. The standard curve was displayed in logit-log manner with standard concentrations versus their absorbance values, and the sample concentration was calculated from the curve. The interassay and intraassay CVs were less than 10.0 and less than 6.0%, respectively.

Determination of vascular endothelial growth factor rs3025039 (+936C/T) polymorphism

The genomic DNA was isolated from the peripheral blood leukocytes using human DNA extraction kit, GeneJET genomic DNA purification kit (Thermo-Fisher Scientific), according to the manufacturer's protocol. The DNA yield was quantified on the ultraviolet spectrophotometer (Jenway, Staffordshire, UK), and the purity was determined as 260/280 ratio. The DNA yield was used as a template for amplification and determination of VEGF rs3025039 (+936C/T) via Taqman SNP real-time PCR allele discrimination method. The primers used for the amplification was provided in the following sequences (For. 5′-AAGGAAGA GGAGACTCTGCGCAGAGC-3′ and Rev.5′- CCTGTAGACACACCCAC CCACATACATACATTTA-3′). The reaction was prepared in 25-µl mixture comprising the following: 2 × Taqman genotyping master mix, Taqman SNP genotyping assay (Thermo-Fisher Scientific); 20 ng of the extracted genomic DNA; and nuclease-free water. The amplification protocol was an initial degeneration (95°C/10 min) and then 40 cycles (95°C/15 s, 60°C/1 min). In all samples, cycle threshold was detected for each genotype, and the results were displayed on a multicomponent plot using Applied Biosystem (1241 E Hillsdale Blvd #270, Foster City, CA 94404, USA), step I version software analysis modules.

Statistical analysis

The genotype and allele frequency of VEGF gene SNP (rs3025039) were calculated by manual counting. The genotype distribution was analyzed according to the Hardy–Weinberg equilibrium in the control group. The relative risk of VEGF gene SNP (rs3025039) for IS was expressed as odds ratio (OR) with the corresponding 95% confidence interval (95% CI). Multiple logistic regression analysis was used for adjustment of the risk ratio for the confounding variables such as age, sex, BMI, family history, smoking, history of diabetes mellitus and hypertension, and IMT. Other results of this study were statistically analyzed using Student *t* test for normally distributed variables, Mann–Whitney *U* test for non-normally distributed variables, and χ^2 for nominal variables. Analysis of variance test was used for comparing the basic characteristics of IS cases among the different genotypes. Two-sided *P* value was employed, with value less than 0.05 as significant. The statistical analysis was performed using SSPS software, version 22, and the graph was designed using graphpad software (IBM Corp., version 22.0, Armonk, NY, USA).

Results

Basic features of the study population

In this case–control study, 49 cases with IS were included in addition to 41 participants as controls. The IS cases were aged 57.1 ± 5.52 years, with 28 males and 21 females. They were matched with the control group regarding the age and sex distribution. Overall, 57.1% of the IS cases were diabetic and 49.0% were hypertensive. NIHSS of IS cases was 15.29 ± 4.6. The

case group had a significantly increased BMI, systolic blood pressure, diastolic blood pressure, IMT, FBS, HbA1C, and serum lipids when compared with the control group (Table 1).

Distribution of vascular endothelial growth factor rs3025039 (+936C/T) genotypes between the studied groups

We detected that the dominant TT genotype is significantly higher than the CC genotype in the IS cases when compared with the controls (OR: 4.800, 95% CI: 1.137–20.27). Moreover, the T allele presents 1.83-fold increased risk for IS over the C allele (OR: 1.828, 95% CI: 1.009–3.313). After adjustment for the confounding variables such as age, sex, BMI, family history, smoking, history of diabetes mellitus and hypertension, and IMT, this association increases to 9.94 folds for TT genotypes and to 2.19 folds for T allele when compared with CC genotype and C allele, respectively (Table 2).

Vascular endothelial growth factor rs3025039 (+936C/T) genotypes and the characteristics of the ischemic stroke case group

In this study, a higher percentage of VEGF rs3025039 TT genotype was observed in patients with IS who were hypertensive, smoker, or had a positive family history for IS. Furthermore, TT genotype was associated with a significantly higher NIHSS (P < 0.001), IMT (P = 0.007), FBS (P = 0.005), HbA1C (P = 0.007), total cholesterol (P = 0.009), and low-density lipoprotein cholesterol (P = 0.004) levels and lower high-density lipoprotein cholesterol (P = 0.008) level than the CC genotype in the IS cases (Table 3).

Vascular endothelial growth factor rs3025039 (+936C/T) polymorphism and stroke severity

NIHSS has been used for assessment of the stroke severity among the case group at admission (baseline) and after 3 months following treatment (follow-up),

Table 1 Basic characteristics of the studied groups

Characteristic	Group I (SI cases) (*n*=49)	Group II (controls) (*n*=41)	*P*
Age (years)	57.1±5.52	58.1±7.65	0.500
Sex			
Male	28 (57.1)	25 (61.0)	0.713
Female	21 (42.9)	16 (39.0)	
Positive smoking	21 (42.9)	16 (39.0)	0.713
Positive diabetes history	28 (57.1)	12 (29.3)	0.008*
Positive hypertension history	24 (49.0)	12 (29.3)	0.057
Positive family history	15 (30.6)	9 (22.0)	0.355
SBP (mmHg)	138.3±14.0	124.3±8.58	<0.001*
DBP (mmHg)	96.3±13.3	83.8±6.38	<0.001*
BMI	26.02±3.81	23.17±2.45	<0.001*
FBS (mg/dl)	132.6±40.72	98.5±19.39	<0.001*
HbA1C (%)	6.56±1.56	5.45±0.76	<0.001*
TC (mg/dl)	237.2±43.36	172.3±31.06	<0.001*
TAG	194.5±66.53	136.3±47.2	<0.001*
HDL-C	30.9±5.08	41.34±8.21	<0.001*
LDL-C	167.4±43.93	103.7±33.66	<0.001*
NIHSS	15.29±4.6	NA	
IMT	1.16±0.23	0.89±0.16	<0.001*

Data are presented as mean±SD and *n* (%). DBP, diastolic blood pressure; FBS, fasting blood sugar; HbA1C, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; IMT, intima-media thickness; LDL-C, low-density lipoprotein cholesterol; NIHSS, National Institute of Health Stroke Scale; SBP, systolic blood pressure; TAG, triglyceride; TC, total cholesterol. *P<0.05 versus control group is significant.

Table 2 Distribution of vascular endothelial growth factor rs3025039 (+936C/T) genotypes and alleles between the studied groups

	Group I (SI cases) (*n*=49)	Group II (healthy control) (*n*=41)	Crude OR (95% CI)	*P*	Adjusted OR (95% CI)	*P*
VEGF rs3025039 (+936C/T) genotypes						
CC	5 (10.2)	9 (22.0)	Reference			
CT	28 (57.1)	26 (63.4)	1.938 (0.574-6.544)	0.286	2.791 (0.541-14.38)	0.220
TT	16 (32.7)	6 (14.6)	4.800 (1.137-20.27)	0.033*	9.938 (1.235-79.97)	0.030*
HWE p	0.078					
VEGF rs3025039 (+936C/T) alleles						
C	38 (38.8)	44 (53.7)	Reference			
T	60 (61.2)	38 (46.3)	1.828 (1.009-3.313)	0.047*	2.191 (1.004-4.782)	0.045*

Data are presented as *n* (%). CI, confidence interval; HC, healthy control; HWE; Hardy-Weinberg equilibrium; OR, odds ratio; VEGF, vascular endothelial growth factor. *P<0.05 is significant.

and the difference was expressed as a percent of change. Among the different VEGF rs3025039 (+936C/T) genotypes, TT genotype carriers were associated with a higher NIHSS at admission and after 3 months following treatment when compared with CC genotype carriers. The percent of improvement in NIHSS was significantly lower in TT genotypes than CC genotypes (Table 4).

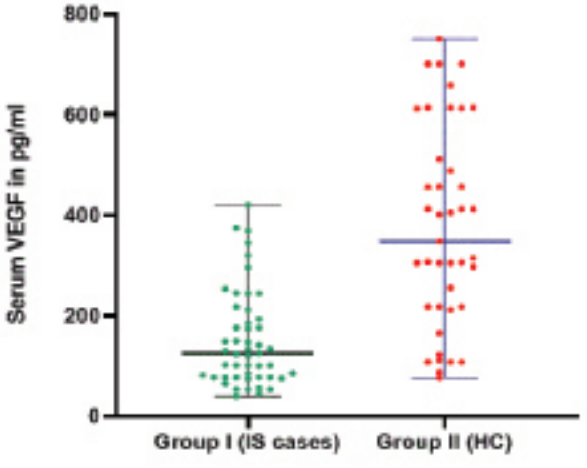
Vascular endothelial growth factor rs3025039 (+936C/T) polymorphism and serum vascular endothelial growth factor level

The median serum VEGF level in IS cases was 126.0 pg/ml, with a range of 40.0–421.0, and in the control group was 348.0 pg/ml, with a range of 77.0–751.0. The median serum VEGF level in the IS group was significantly lower than that in the control group ($P < 0.001$) (Fig. 1). No significant differences were detected in serum VEGF levels among the three VEGF rs3025039 (+936C/T) genotypes in IS cases; however, patients with T allele had a significantly lower serum VEGF level than C allele ($P = 0.021$) (Table 5).

Discussion

The pathogenesis of acute IS is heterogeneous with involvement of multiple factors. The genetic epistasis may play a role in the predisposition for IS, which is different in various ethnic groups. Recognition of these genetic factors is important for early detection of IS in the risky persons, and hence, effective preventative and curative measures could be applied. As the vascular dysfunction is the main mechanism involved in development of the cerebrovascular disorders, VEGF with its genetic variants had been suggested to contribute for the enhanced IS risk. In the hypoxic state, VEGF enhances the angiogenesis and neovascularization with increased vascular permeability. Thus, it is suggested to have a role in the pathogenesis of many ischemic disorders, specifically the cerebrovascular diseases. Several polymorphisms had been described for VEGF gene, with + 936C/T (rs3025039) being commonly detected to be associated with the susceptibility

Figure 1



Serum VEGF level between the studied groups. VEGF, vascular endothelial growth factor.

Table 3 Distribution of the basic characteristics of the ischemic stroke cases among the different vascular endothelial growth factor rs3025039 (+936C/T) genotypes

Characteristic	TT genotype (*n*=16)	CT genotype (*n*=28)	CC genotype (*n*=5)	*P*
Age (years)	56.6±4.9	57.3±5.8	57.8±6.6	0.881
Sex				
Male	8 (53.3)	17 (58.6)	3 (60.0)	
Female	7 (46.7)	12 (41.4)	2 (40.0)	0.936
Positive smoking	13 (86.7)	7 (24.1)	1 (20.0)	<0.001*
Positive diabetes history	13 (86.7)	14 (48.3)	1 (20.0)	0.011*
Positive hypertension history	10 (66.7)	11 (37.9)	3 (60.0)	0.171
Positive family history	11 (73.3)	4 (13.8)	0	<0.001*
SBP (mmHg)	141.6±15.1	136.3±12.9	139.2±17.8	0.488
DBP (mmHg)	102.2±13.8	94.1±12.5	89.8±2.1	0.078
BMI	26.8±3.3	25.9±4.1	23.8±3.6	0.308
FBS (mg/dl)	158.3±43.3	120.3±34.4	115.2±28.6	0.005*
HbA1C (%)	7.53±1.72	6.11±1.26	5.94±1.35	0.007*
TC (mg/dl)	262.8±45.0	227.1±39.7	211.6±13.6	0.009*
TAG	201.3±70.5	180.5±61.8	251.0±56.6	0.079
HDL-C	28.3±4.6	32.8±4.9	28.6±2.9	0.008*
LDL-C	194.2±44.3	158.2±39.9	132.8±8.68	0.004*
NIHSS	19.81±3.08	13.92±2.98	8.4±2.07	<0.001*
IMT	1.28±0.28	1.14±0.18	0.92±0.025	0.007*

Data are presented as mean±SD and *n* (%). DBP, diastolic blood pressure; FBS, fasting blood sugar; HbA1C, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; IMT, intima-media thickness; NIHSS, National Institute of Health Stroke Scale; SBP, systolic blood pressure; TAG, triglyceride; TC, total cholesterol. *P<0.05 is significant.

Table 4 Distribution of NHISS in ischemic stroke cases among the different vascular endothelial growth factor rs3025039 (+936C/T) genotypes

NIHSS	TT genotype (*n*=16)	CT genotype (*n*=28)	CC genotype (*n*=5)	*P*
Baseline	19.81±3.08	13.92±2.98	8.4±2.07	<0.001*
After 3 months	13.38±2.58	7.96±2.33	4.2±0.84	<0.001*
Percent of change	−32.5±8.27	-43.4±7.92	−49.5±4.19	<0.001*

NIHSS, National Institute of Health Stroke Scale. *P<0.05 is significant.

Table 5 Serum vascular endothelial growth factor level distribution among different vascular endothelial growth factor rs3025039 genotypes and alleles in ischemic stroke cases

	Serum VEGF level (*n*=49)	*P*
VEGF rs3025039 (+936C/T) genotypes		
CC	185.0 (78.0-320.0)	-
CT	134.0 (54.0-421.0)	0.679
TT	78.0 (40.0-218.0)	0.075
VEGF rs3025039 (+936C/T) alleles		
C	145.5 (54.0-421.0)	0.021*
T	102.0 (40.0-421.0)	

VEGF, vascular endothelial growth factor. *P<0.05 is significant.

of various disorders, such as malignancies [25,26], endometriosis [27], and other vascular disorders [28,29].

In this study, we aimed to explore the association of VEGF single gene polymorphism + 936C/T (rs3025039) with the risk and severity of IS and to assess its relation to VEGF expression via estimation of its serum level.

We detected that the frequency of VEGF rs3025039 TT genotype was significantly higher in IS cases when compared with CC genotypes. Moreover, the carriers of T allele had higher risk for IS by 2.19 folds more than the C allele carriers. These results were consistent with the results of other studies previously conducted and found that the VEGF rs3025039 TT genotype is associated with IS [19,30]. The same findings were detected collectively in a meta-analysis performed by Xu *et al.*[31] on 3747 IS cases and 2868 controls, particularly in the Asian population after stratification to the ethnic variables. Furthermore, the frequency of T allele was elevated and associated with hypertensive cerebellar hemorrhage in the findings of He *et al.*[32] study. Contradictory to our study findings, no association was detected between VEGF rs3025039 polymorphism and either the risk of IS in Chinese population[33] or aneurysmal subarachnoid hemorrhage in Italian population [34].

Our study detected that the VEGF rs3025039 TT genotype carriers were significantly associated with a higher IS severity and a lower percent of improvement as assessed by NHISS than CC genotype carriers. These findings agree with Zhao *et al.* [33], who detected that TT and CT genotypes were associated with increased IS severity and poor outcome after adjustment for the confounding risk factors.

Several studies had assessed the role of VEGF in IS and have found that it may be under debate whether it is predisposing or protective [11]. VEGF can enhance the progression of atheroma via stimulation of endothelial cells migration, smooth muscle cell proliferation, plaque neovascularization, and increasing the vascular permeability that predispose to brain edema and IS [35,36]. On the contrary, it has an anti-atherosclerotic effect through enhancing the re-endothelialization, reducing intimal thickening, and inhibiting thrombus formation [37,38]. Moreover, it may promote the resolution of the cerebral infarction region via stimulation of neovascularization with restoration of adequate blood supply in the ischemic areas [39].

In our study, the VEGF rs3025039 TT genotype was significantly associated with a higher IMT when compared with CC genotype. Moreover, the serum VEGF was significantly lower in IS cases than controls. Thus, we suggested that decreased VEGF level may be a risk factor for the progression of IS. Moreover, we detected that the T allele is associated with lower VEGF serum level when compared with C allele in IS cases. The same association was detected in two previous studies [18,26], which suggest the possible influence of this polymorphism on VEGF either at transcriptional or post-transcriptional level. This can be explained by the presence of this polymorphism in the 3'UTR which subsequently interferes with the binding of AP-4 [26,40] or hypoxia-induced protein, resulting in a marked reduction of the VEGF mRNA half-life [41].

These findings may support the contradictory role of VEGF in the pathogenesis of IS with more acceptance for its anti-atherosclerotic and angiogenesis role over its effect in the propagation of the atheromatous plaque. Our findings were also confirmed by a previous study, which found an improved neurological outcomes of patients with IS following recombinant human VEGF administration [42].

Conclusion

In conclusion, VEGF rs3025039 gene polymorphism

is associated with increased susceptibility and severity of IS and decreased VEGF expression, which may play a major role in the pathogenesis of IS. There are some imitations in this study. First, the included participants were from a single center, arising a selection bias; however, the distribution of genotypes were in accordance with Hardy–Weinberg equilibrium that reflects a sufficient presentation. Second, the number of included participants was small; therefore, we recommend further studies to be carried out on larger or different populations to support our results.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

References

1 Katan M, Luft A. Global Burden of Stroke. Semin Neurol. 2018;38:208-11.

2 Candelario-Jalil E. Injury and repair mechanisms in ischemic stroke: considerations for the development of novel neurotherapeutics. Curr Opin Investig Drugs. 2009;10:644-54.

3 Bevan S, Traylor M, Adib-Samii P, Malik R, Paul NL, Jackson C, *et al*. Genetic heritability of ischemic stroke and the contribution of previously reported candidate gene and genomewide associations. Stroke. 2012;43:3161-7.

4 Hankey GJ. Potential new risk factors for ischemic stroke: what is their potential? Stroke. 2006;37:2181-8.

5 Hachiya T, Kamatani Y, Takahashi A, Hata J, Furukawa R, Shiwa Y, *et al*. Genetic Predisposition to Ischemic Stroke: A Polygenic Risk Score. Stroke. 2017;48:253-8.

6 Kumar A, Kumar P, Prasad M, Misra S, Kishor Pandit A, Chakravarty K. Association between Apolipoprotein epsilon4 Gene Polymorphism and Risk of Ischemic Stroke: A Meta-Analysis. Ann Neurosci. 2016;23:113-21.

7 Dai Y, He Z, Sui R, Jiang Z, Ma S. Association of nNOS gene polymorphism with ischemic stroke in Han Chinese of North China. ScientificWorldJournal. 2013;2013:891581.

8 Dubovyk YI, Oleshko TB, Harbuzova VY, Ataman AV. Positive Association between EDN1 rs5370 (Lys198Asn) Polymorphism and Large Artery Stroke in a Ukrainian Population. Dis Markers. 2018;2018:1695782.

9 Libby P. Inflammation in atherosclerosis. Arterioscler Thromb Vasc Biol. 2012;32:2045-51.

10 Wang S, Li X, Parra M, Verdin E, Bassel-Duby R, Olson EN. Control of endothelial cell proliferation and migration by VEGF signaling to histone deacetylase 7. Proc Natl Acad Sci U S A. 2008;105:7738-43.

11 Ma WQ, Wang Y, Han XQ, Zhu Y, Liu NF. Association of genetic polymorphisms in vascular endothelial growth factor with susceptibility to coronary artery disease: a meta-analysis. BMC Med Genet. 2018;19:108.

12 Brogan IJ, Khan N, Isaac K, Hutchinson JA, Pravica V, Hutchinson IV. Novel polymorphisms in the promoter and 5' UTR regions of the human vascular endothelial growth factor gene. Hum Immunol. 1999;60:1245-9.

13 Tamura K, Amano T, Satoh T, Saito D, Yonei-Tamura S, Yajima H. Expression of rigf, a member of avian VEGF family, correlates with vascular patterning in the developing chick limb bud. Mech Dev. 2003;120:199-209.

14 Hu GL, Ma G, Ming JH. Impact of common SNPs in VEGF gene on the susceptibility of osteosarcoma. Genet Mol Res. 2015;14:14561-6.

15 Kim HW, Ko GJ, Kang YS, Lee MH, Song HK, Kim HK, *et al*. Role of the VEGF 936 C/T polymorphism in diabetic microvascular complications in type 2 diabetic patients. Nephrology (Carlton). 2009;14:681-8.

16 Wei H, Liu L, Chen Q. Selective removal of mitochondria via mitophagy: distinct pathways for different mitochondrial stresses. Biochim Biophys Acta. 2015;1853:2784-90.

17 Xian W, Zheng H, Wu WJ. Predictive value of vascular endothelial growth factor polymorphisms on the risk of renal cell carcinomas. Genet Mol Res. 2015;14:7634-42.

18 Al-Habboubi HH, Sater MS, Almawi AW, Al-Khateeb GM, Almawi WY. Contribution of VEGF polymorphisms to variation in VEGF serum levels in a healthy population. Eur Cytokine Netw. 2011;22:154-8.

19 Kim OJ, Hong SH, Oh SH, Kim TG, Min KT, Oh D, Kim NK. Association between VEGF polymorphisms and homocysteine levels in patients with ischemic stroke and silent brain infarction. Stroke. 2011;42:2393-402.

20 Sacco RL, Kasner SE, Broderick JP, Caplan LR, Connors JJ, Culebras A, et al. An updated definition of stroke for the 21st century: a statement for healthcare professionals from the American Heart Association/American Stroke Association. Stroke. 2013;44:2064-89.

21 Adams HP, Jr., Bendixen BH, Kappelle LJ, Biller J, Love BB, Gordon DL, March EE. Classification of subtype of acute ischemic stroke. Definitions for use in a multicenter clinical trial. TOAST. Trial of Org 10172 in Acute Stroke Treatment. Stroke. 1993;24:35-41.

22 Powers WJ, Rabinstein AA, Ackerson T, Adeoye OM, Bambakidis NC, Becker K, *et al*. 2018 Guidelines for the Early Management of Patients With Acute Ischemic Stroke: A Guideline for Healthcare Professionals From the American Heart Association/American Stroke Association. Stroke. 2018;49:e46-e110.

23 Homma S, Hirose N, Ishida H, Ishii T, Araki G. Carotid plaque and intima-media thickness assessed by b-mode ultrasonography in subjects ranging from young adults to centenarians. Stroke. 2001;32:830-5.

24 Hussein HM, Abdel Moneim A, Emara T, Abd-Elhamid YA, Salem HH, Abd-Allah F, *et al*. Arabic cross cultural adaptation and validation of the National Institutes of Health Stroke Scale. J Neurol Sci. 2015;357:152-6.

25 Kim JG, Chae YS, Sohn SK, Cho YY, Moon JH, Park JY, *et al*. Vascular endothelial growth factor gene polymorphisms associated with prognosis for patients with colorectal cancer. Clin Cancer Res. 2008;14:62-6.

26 Krippl P, Langsenlehner U, Renner W, Yazdani-Biuki B, Wolf G, Wascher TC, *et al*. A common 936 C/T gene polymorphism of vascular endothelial growth factor is associated with decreased breast cancer risk. Int J Cancer. 2003;106:468-71.

27 Ikuhashi Y, Yoshida S, Kennedy S, Zondervan K, Takemura N, Deguchi M, *et al*. Vascular endothelial growth factor +936 C/T polymorphism is associated with an increased risk of endometriosis in a Japanese population. Acta Obstet Gynecol Scand. 2007;86:1352-8.

28 Atzeni F, Boiardi L, Vaglio A, Nicoli D, Farnetti E, Palmisano A, *et al*. TLR-4 and VEGF polymorphisms in chronic periaortitis. PLoS One. 2013;8:e62330.

29 Song GG, Kim JH, Lee YH. Vascular endothelial growth factor gene polymorphisms and vasculitis susceptibility: A meta-analysis. Hum Immunol. 2014;75:541-8.

30 Yadav BK, Yadav R, Shin B-S. Single-nucleotide Polymorphisms in Vascular Endothelial Growth Factor Gene Associated with Stroke Subtype in LAA and SVO. International Journal of Gerontology. 2017;11:16-21.

31 Xu B, Zhan R, Mai H, Wu Z, Zhu P, Liang Y, Zhang Y. The association between vascular endothelial growth factor gene polymorphisms and stroke: A PRISMA-compliant meta-analysis. Medicine (Baltimore). 2019;98:e14696.

32 He QS, Yang LF, Wang WB, Yuan B, Zhang LY, Guo XJ. Vascular endothelial growth factor gene is associated with hypertensive cerebellar hemorrhage and rehabilitative treatment. Genet Mol Res. 2015;14:9849-57.

33 Zhao J, Bai Y, Jin L, Weng Y, Wang Y, Wu H, et al. A functional variant in the 3'-UTR of VEGF predicts the 90-day outcome of ischemic stroke in Chinese patients. PLoS One. 2017;12:e0172709.

34 Fontanella M, Gallone S, Panciani PP, Garbossa D, Stefini R, Latronico N, *et al*. Vascular endothelial growth factor gene polymorphisms and intracranial aneurysms. Acta Neurochir (Wien). 2013;155:1511-5.

35 Kolodgie FD, Gold HK, Burke AP, Fowler DR, Kruth HS, Weber DK, *et al*. Intraplaque hemorrhage and progression of coronary atheroma. N Engl J Med. 2003;349:2316-25.

36 Grunewald M, Avraham I, Dor Y, Bachar-Lustig E, Itin A, Jung S, *et al*. VEGF-induced adult neovascularization: recruitment, retention, and role of accessory cells. Cell. 2006;124:175-89.

37 Grosskreutz CL, Anand-Apte B, Duplaa C, Quinn TP, Terman BI, Zetter B, *et al*. Vascular endothelial growth factor-induced migration of vascular smooth muscle cells in vitro. Microvasc Res. 1999;58:128-36.

38 Howell WM, Ali S, Rose-Zerilli MJ, Ye S. VEGF polymorphisms and severity of atherosclerosis. J Med Genet. 2005;42:485-90.

39 Gora-Kupilas K, Josko J. The neuroprotective function of vascular endothelial growth factor (VEGF). Folia Neuropathol. 2005;43:31-9.

40 Renner W, Kotschan S, Hoffmann C, Obermayer-Pietsch B, Pilger E. A common 936 C/T mutation in the gene for vascular endothelial growth factor is associated with vascular endothelial growth factor plasma levels. J Vasc Res. 2000;37:443-8.

41 Maeda M, Yamamoto I, Fujio Y, Azuma J. Homocysteine induces vascular endothelial growth factor expression in differentiated THP-1 macrophages. Biochim Biophys Acta. 2003;1623:41-6.

42 Sun Y, Jin K, Xie L, Childs J, Mao XO, Logvinova A, Greenberg DA. VEGF-induced neuroprotection, neurogenesis, and angiogenesis after focal cerebral ischemia. J Clin Invest. 2003;111:1843-51.