

Received: 2019.08.01

Accepted: 2019.11.27

Available online: 2020.01.22

Published: 2020.03.13

Genetic Relationship Between Endothelin-1 Gene Polymorphisms and Intracerebral Hemorrhage Among Chinese Han People

Authors' Contribution:

Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

F **Wanzeng Zhang***
B **Wangmiao Zhao***
C **Chunyan Ge***
D **Xiaowei Li**
E **Xuehui Yang**
G **Yi Xiang**
A **Zhaosheng Sun**

Department of Neurosurgery, Harrison International Peace Hospital, Hengshui, Hebei, P.R. China

* Wanzeng Zhang, Wangmiao Zhao and Chunyan Ge are Co-first author

Corresponding Author: Zhaosheng Sun, e-mail: ytojfd@126.com

Source of support: Departmental sources

Background: The goal of the present study was to determine whether endothelin-1 (*EDN1*) variants are associated with intracerebral hemorrhage (ICH) risk among Chinese Han people.





Material/Methods: The genotyping of *EDN1* rs5370 and rs6458155 polymorphisms were conducted in 154 ICH patients and 168 healthy controls using polymerase chain reaction (PCR) and sequencing. Deviation for genotype frequencies in controls from Hardy-Weinberg equilibrium (HWE) was assessed. The genotype and allele distribution of *EDN1* polymorphisms was checked via χ^2 test between 2 groups. Strength of the association between *EDN1* polymorphisms and ICH risk is presented by odds ratio (OR) and 95% confidence interval (95% CI).

Results: Genotype distribution for rs5370 and rs6458155 polymorphisms in the control group both conformed to HWE ($P>0.05$). Only CC genotype and C allele frequencies of rs6458155 between ICH patients and healthy individuals were significantly different ($P=0.025$; $P=0.043$), indicating rs6458155 is associated with increased ICH onset (OR=2.214, 95% CI=1.009–4.461; OR=1.389, 95% CI=1.010–1.910). When adjusted by confounding factors, the significant correlations still existed between 2 groups ($P=0.028$, adjusted OR=2.217, 95% CI=1.092–4.500; $P=0.046$, adjusted OR=1.386, 95% CI=1.005–1.910).

Conclusions: *EDN1* rs6458155 polymorphism may be a risk factor of ICH among Chinese Han people.

MeSH Keywords: **Endothelin-1 • Intracranial Hemorrhage, Hypertensive • Polymorphism, Genetic**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/919110>

 1846  2  1  28



Background

Stroke is the most common cerebrovascular disease and is a leading cause of death and disability all over the world [1]. Intracerebral hemorrhage (ICH) is a primary subtype of stroke, and its incidence and fatality rates are increasing [2]. It is defined as hemorrhage caused by blood extravasation into the brain parenchyma. ICH pathogenesis is complicated and involves environmental and genetic components. The major modifiable risk factors are smoking, heavy alcohol intake, advanced age, hypertension, and diabetes, but they do not explain all cases of ICH [3–5], and genetic components in ICH are being increasingly studied [6,7].

Endothelin-1 (END1), encoded by the *EDN1* gene located on chromosome 6 (6p21–24), is a potent and long-lasting vasoconstrictor produced by endothelial cells of the vasculature, and it acts as a modulator of vasomotor tone and vascular remodeling [8,9]. Animal experiments demonstrated that the knockdown of *EDN1* in mice can cause hypertension, thus leading to cerebrovascular diseases [10]. *EDN1* is expressed in some tissues and cells, such as endothelial cells and cardiomyocytes [11]. Additionally, it has proatherogenic activity and regulates smooth-muscle cell proliferation through *EDN1* receptors. Furthermore, *EDN-1* causes the alteration of vascular structure and function in resistance vessels and acts as a proinflammatory factor in heart failure [12]. It has been demonstrated that *EDN1* has a number of polymorphisms involved in hypertension [13], body mass index [14], coronary heart disease [15], and ischemic stroke [16]. In aneurysmal subarachnoid hemorrhage patients, *EDN1* rs2070699 polymorphism was reported to be an independent risk indicator for aneurysm rebleeding [17]. Moreover, *EDN1* rs6912834 polymorphism is associated with angiographic vasospasm in aneurysmal subarachnoid hemorrhage patients [18]. In addition, the genetic association of *EDN1* polymorphism with the risk of neonatal intraventricular hemorrhage (IVH) was also reported in preterm infants in a white population [19]. Few published reports mention the correlation of *EDN1* polymorphisms with ICH susceptibility among Chinese Han people.

Therefore, we explored the possible association of *EDN1* variants with susceptibility of individuals to ICH among Chinese Han people. We selected 2 common polymorphisms in *EDN1* – rs5370 and rs6458155.

Material and Methods

Study participants

Our research was approved by the Ethics Committee of Harrison International Peace Hospital, and written informed consent

was obtained from participants or their family members before sampling.

The case group consisted of a cohort of 154 consecutive ICH patients treated at the Neurology Department of Harrison International Peace Hospital. All included cases were confirmed based on their clinical features and computed tomography (CT) or/and magnetic resonance imaging (MRI) examination. Exclusion criteria for the case group were ICH resulting from trauma, neoplasms, anticoagulant therapy, coagulation disorders, aneurysms, and vascular malformations. The control group consisted of 168 healthy people seen at the health examination center of the same Hospital during the same period. Exclusion criteria for the control group included history of heart or vessel disorders, previous stroke incidents, and malignancy. Clinical characteristics for enrolled participants were investigated and documented by experienced doctors, including age, sex, body mass index (BMI), presence or absence of hypertension, ischemic heart disease and diabetes, alcohol intake, and current smoking. All enrolled subjects were unrelated Han Chinese people.

Genotyping

Firstly, 2 ml of peripheral blood was drawn from every participant in the morning after a 12-h fast into a blood-collection tube containing EDTA, and maintained at –80°C. Genomic DNA of peripheral blood samples was isolated by phenol-chloroform method. The integrity of DNA was verified by 1% agarose gel electrophoresis. Its purity and concentration were assessed using spectrophotometry in a Nanodrop 2000c device (Thermo Scientific, Suwanee, GA, USA).

Polymerase chain reaction-direct sequencing (PCR-DS) was used for the genotyping of *EDN1* rs5370 and rs6458155 polymorphisms. PCR primers of polymorphism were designed using Primer Premier 5.0 software and were generated by Shanghai Sangon Bio. Co. (Sangon, Shanghai). The specific primers of rs5370 and rs6458155 were:

rs5370 forward: 5'-TCTTTTGCCAAAGGGTGATT-3',

reverse: 5'-CAGGTTGGAGAGTGCAGAG-3';

rs6458155: forward: 5'-AAGGCTGTGTGGGAGATGAG-3',

reverse: 5'-CTTCTGGAACCTGCTCTG-3'.

The PCR system consisted of 25.0 µl mixed solution, including 20 ng DNA template, 12.5 µl PCR Master Mix, each 1.0 µl of forward and reverse primers and ddH₂O added to achieve a final volume. The PCR procedure was: 95°C for 3 min for pre-degeneration, 35 cycles of 94°C for 60 s for degeneration, 61°C or 57°C for 60 s for annealing, 72°C for 45 s for extension, and final extension at 72°C for 7 min, then stored at 4°C.

Acceptable PCR products were sent to Shanghai Sangon Bio. Co. for sequencing to determine genotypes for polymorphisms for all subjects.

Statistical analysis

Data synthesis was carried out with SPSS 18.0 software (SPSS, Inc., Chicago, IL, USA). Hardy-Weinberg equilibrium (HWE) status was checked in the control group by chi-square test. Categorical data are presented as numbers and proportions, while continuous data are presented as mean±SD (standard deviation). In subsequent analysis, the comparisons between cases and controls were analyzed using the *t* test. Genotype and allele frequencies for polymorphisms between ICH cases and healthy control subjects were compared using the chi-square test. The strength of associations between polymorphisms and ICH susceptibility was assessed by calculating odds ratio (OR) and 95% confidence interval (CI). Logistic regression analysis was applied to adjust the results by considering confounding factors. $P<0.05$ indicated a statistically significant difference.

Results

Basic features of study participants

Table 1 lists demographic and clinical information of study participants. The mean onset age of ICH patients was 50.92±5.91 years old. There were 137 ICH patients confirmed as having symptomatic intracerebral hemorrhage (SICH), while the other 17 patients were diagnosed with asymptomatic intracerebral hemorrhage (aSICH). Comparison analyses demonstrated that there was no significant dissimilarity between the 2 groups in age ($P=0.618$), sex ($P=0.376$), or BMI (body mass index) ($P=0.212$). However, in the case group there were significantly more ICH patients with hypertension ($P<0.001$), ischemic heart disease ($P<0.001$), and diabetes ($P=0.001$) than in the control group. Moreover, alcohol intake between cases and controls was also significantly different ($P<0.001$). For current smokers, no obvious difference was found between the 2 groups ($P=0.434$).

Connection for *EDN1* variants to ICH risk

As shown in Figure 1, the genotypes of *EDN1* polymorphisms were identified by direct sequencing. Table 2 shows the genotype and allele distribution for *EDN1* variants among the 2 groups. Genotype distributions for rs5370 and rs6458155 polymorphisms in the control group both conformed to Hardy-Weinberg equilibrium, indicating that the study group was from the same Mendelian genetic population ($P>0.05$).

Table 1. Basic characteristics of ICH patients and controls.

Characteristics	Cases (n=154, %)	Controls (n=168, %)	P value
Mean age (years±SD)	50.92±5.91	50.54±6.11	0.618
Gender			
Male	86	102	0.376
Female	68	66	
BMI (kg/m ² , mean±SD)	24.60±1.82	23.61±1.55	0.212
Hypertension			
No	90	133	<0.001
Yes	64	35	
Ischemic heart disease			
Absent	137	168	<0.001
Present	17	0	
Diabetes			
Absent	144	168	0.001
Present	10	0	
Alcohol intake			
Absent	107	147	<0.001
Present	47	21	
Current smoking			
Absent	134	141	0.434
Present	20	27	
Pathological subtype			
SICH	137		
aSICH	17		

BMI – body mass index; SD – standard deviation; SICH – symptomatic intracerebral hemorrhage; aSICH – asymptomatic intracerebral hemorrhage.

GT and TT genotypes of rs5370 were more frequent among cases than among controls, but the difference was not statistically significant. Similar results were observed in the allele distribution of rs5370 ($P>0.05$). Following adjusted by age, sex and BMI, the results were not obviously changed (adjusted $P>0.05$). However, CC genotype of rs6458155 in cases was more frequent than in controls ($P=0.025$). Similarly, its C allele was also significantly higher in the case group than in controls ($P=0.043$). Therefore, CC genotype and C allele of rs6458155 increased ICH risk (CC vs. TT: OR=2.214, 95% CI=1.099–4.461; C vs. T: OR=1.389, 95% CI=1.010–1.910). Moreover, after adjustment for age, sex, and BMI, the significant differences still existed

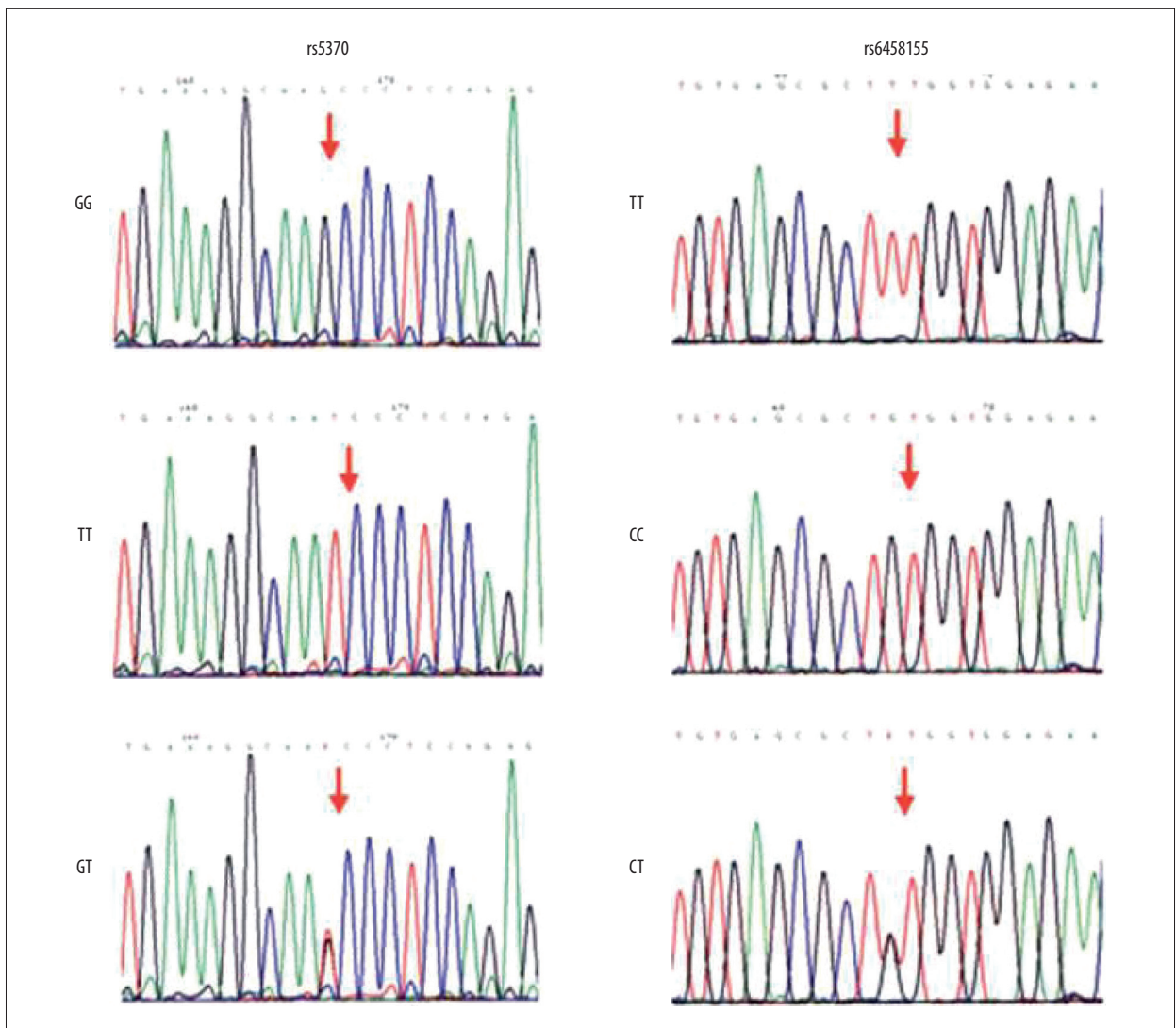


Figure 1. The sequencing results of *EDN1* rs5370 and rs6458155 polymorphisms.

(CC vs. TT: adjusted OR=2.217, 95% CI=1.092–4.500, $P=0.028$;
C vs. T: adjusted OR=1.386, 95% CI=1.005–1.910, $P=0.046$).

Discussion

ICH is a common type of stroke and is also considered to be a multifactorial disorder involving environmental and genetic elements. A number of factors have been reported to be associated with ICH occurrence, such as hypertension, cardiac disease, diabetes, and alcohol intake. However, few people exposed to these factors develop ICH. Therefore, genetic factors are the most important influences on ICH etiology. Therefore, to predict individual and population susceptibility and explore the pathological mechanism of ICH occurrence, it is of great importance to assess the polymorphisms that affect gene function or expression and which are associated with

ICH risk. Most patients with ICH have elevated blood pressure, and studies have indicated that the genetic predisposition to high blood pressure can explain much of the genetic susceptibility to ICH [20].

EDN1 is the most potent vasoconstrictor and mediates the 2-way effect of contraction after relaxation in endothelium-intact vessels through binding endothelin receptor. It is also reported to be related to vascular tone and blood pressure modulation [21]. Various polymorphisms in *EDN1* have been confirmed and they have been investigated in a number of vascular conditions, such as essential hypertension [22], pre-eclamptic pregnancy [23], and cerebral small-vessel disease [24]. Among them, rs5370 is a widely studied polymorphism located on the exon5 region of *EDN1*, causing the substitution of guanine/thymine. It can alter the function of *EDN1* in humans [25]. Schiffrin et al. revealed that *EDN1* acts as a central

Table 2. Association of *EDN1* polymorphisms with ICH patients susceptibility.

Genotype/allele	Cases n=154 (%)	Controls n=168 (%)	P value	OR (95% CI)	P value*	OR (95% CI)*
rs5370						
GG	98 (63.64)	116 (69.05)	–	–	–	–
GT	51 (33.12)	49 (29.17)	0.389	1.232 (0.766–1.982)	0.384	1.238 (0.766–2.001)
TT	5 (3.25)	3 (1.79)	0.352	1.973 (0.460–8.464)	0.420	1.832 (0.421–7.971)
G	247 (80.19)	281 (83.63)	–	–	–	–
T	61 (19.81)	55 (16.37)	0.257	1.262 (0.844–1.887)	0.274	1.253 (0.836–1.879)
P_{HWE}	–	0.40				
rs6458155						
TT	16 (10.39)	32 (19.05)	–	–	–	–
CT	76 (49.35)	80 (47.62)	0.061	1.900 (0.965–3.740)	0.065	1.904 (0.960–3.777)
CC	62 (40.26)	56 (33.33)	0.025	2.214(1.099–4.461)	0.028	2.217(1.092–4.500)
T	108 (35.06)	144 (42.86)	–	–	–	–
C	200 (64.94)	192 (57.14)	0.043	1.389(1.010–1.910)	0.046	1.386(1.005–1.910)
P_{HWE}	–	0.72				

* Adjusted by age, gender and BMI; OR – odds ratios; 95% CI – 95% confidence interval.

pivot in the process of elevated blood pressure and vascular proliferation [26]. Zhang et al. showed that *EDN1* rs5370 and rs2070699 polymorphisms are risk factors for ischemic stroke in the male Han population in northern China [27]. Carlos et al. found that the paternal rs5370 polymorphism in *EDN1* is associated with reduced risk of preeclampsia [28]. Rs6458155, a mutation in 5'UTR of *EDN1* inducing the replacement of C/T, has been reported to influence transcriptional activity of *EDN1* and change the concentration of circulating ET-1 [15]. Therefore, *EDN1* rs6458155 and rs5370 polymorphisms were selected to study the association with ICH risk.

In the present study, we analyzed the potential function of *EDN1* polymorphisms in the occurrence and development of ICH in a Chinese Han population. Significant associations were detected between ICH occurrence and hypertension, ischemic heart disease, diabetes, and alcohol intake. We also found that carrying the CC genotype and C allele in the *EDN1* rs6458155 polymorphism significantly increased the risk of ICH compared with TT genotype and T allele carriers in the Chinese Han population. After adjustment for confounding factors, CC genotype and C allele of rs6458155 were associated with 2.217-fold and 1.386-fold, respectively, higher ICH risk compared with the corresponding controls. However, no significant correlation was observed between rs5370 and ICH susceptibility. Our findings are consistent with previous studies. For instance, Liang et al. demonstrated that rs6458155 polymorphism in the *EDN1* gene can affect susceptibility to coronary artery disease among

Chinese Han people [15]. A study by Fang et al. found no independent association between *EDN1* rs5370 or rs10478694 polymorphisms and risk of developing hypertension in the Chinese population [22].

Although our study produced important results, several limitations should be considered. First, the sample size was relatively small, which could lead to low statistical power for identifying differences between the 2 groups. Second, the study population was homogenous and only included Chinese Han people, but because the distribution of polymorphisms differs with ethnicity, the accuracy of our data is limited. Third, ICH is influenced by a variety of factors, but we did not perform detailed analysis of gene-gene and gene-environment interactions. Consequently, future research should have larger sample sizes, include various ethnic groups, and considering more environmental factors.

Conclusions

Our findings show that *EDN1* rs6458155 polymorphism may play a role in the onset of ICH in the Chinese Han population, and rs5370 was not found to be associated with risk of ICH. These findings will be valuable for future studies to research the molecular mechanism of *EDN1* polymorphisms in ICH development.

References:

1. Thrift AG, Thayabaranathan T, Howard G et al: Global stroke statistics. *Int J Stroke*, 2017; 12: 13–32
2. Egashira Y, Hua Y, Keep RF, Xi G: Intercellular cross-talk in intracerebral hemorrhage. *Brain Res*, 2015; 1623: 97–109
3. Woo D, Sauerbeck LR, Kissela BM et al: Genetic and environmental risk factors for intracerebral hemorrhage: preliminary results of a population-based study. *Stroke*, 2002; 33: 1190–95
4. Garg RK, Ouyang B, Pandya V et al: The influence of weather on the incidence of primary spontaneous intracerebral hemorrhage. *J Stroke Cerebrovasc Dis*, 2019; 28: 405–11
5. Radmanesh F, Falcone GJ, Anderson CD et al: Rare coding variation and risk of intracerebral hemorrhage. *Stroke*, 2015; 46: 2299–301
6. Xia C, Lin S, Yang J et al: Genetic variations of COL4A1 gene and intracerebral hemorrhage outcome: A cohort study in a Chinese Han population. *World Neurosurg*, 2018; 113: e521–28
7. Szepecht D, Szymankiewicz M, Seremak-Mrozikiewicz A, Gadzinowski J: The role of genetic factors in the pathogenesis of neonatal intraventricular hemorrhage. *Folia Neuropathol*, 2015; 53: 1–7
8. Yanagisawa M, Kurihara H, Kimura S et al: A novel potent vasoconstrictor peptide produced by vascular endothelial cells. *Nature*, 1988; 332: 411–15
9. Rankinen T, Church T, Rice T et al: Effect of endothelin 1 genotype on blood pressure is dependent on physical activity or fitness levels. *Hypertension*, 2007; 50: 1120–25
10. Ge Y, Bagnall A, Stricklett PK et al: Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol*, 2008; 295(6): F1635–40
11. Brunner F, Bras-Silva C, Cerdeira AS, Leite-Moreira AF: Cardiovascular endothelins: Essential regulators of cardiovascular homeostasis. *Pharmacol Ther*, 2006; 111: 508–31
12. von Websky K, Heiden S, Pfab T, Hofer B: Pathophysiology of the endothelin system – lessons from genetically manipulated animal models. *Eur J Med Res*, 2009; 14: 1–6
13. Fan XH, Wang H, Gao LG et al: The association of an adenine insertion variant in the 5'UTR of the endothelin-1 gene with hypertension and orthostatic hypotension. *Arch Med Sci*, 2012; 8: 219–26
14. Barath A, Endreffy E, Bereczki C et al: Endothelin-1 gene and endothelial nitric oxide synthase gene polymorphisms in adolescents with juvenile and obesity-associated hypertension. *Acta Physiol Hung*, 2007; 94: 49–66
15. Liang LL, Chen L, Zhou MY et al: Genetic susceptibility of five tagSNPs in the endothelin-1 (EDN1) gene to coronary artery disease in a Chinese Han population. *Biosci Rep*, 2018; 38: pii: BSR20171320
16. 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
17. Foreman PM, Starke RM, Hendrix P et al: Endothelin polymorphisms as a risk factor for cerebral aneurysm rebleeding following aneurysmal subarachnoid hemorrhage. *Clin Neurol Neurosurg*, 2017; 157: 65–69
18. Gallek M, Alexander S, Crago E et al: Endothelin-1 and endothelin receptor gene variants and their association with negative outcomes following aneurysmal subarachnoid hemorrhage. *Biol Res Nurs*, 2013; 15: 390–97
19. Szepecht D, Gadzinowski J, Seremak-Mrozikiewicz A et al: Role of endothelial nitric oxide synthase and endothelin-1 polymorphism genes with the pathogenesis of intraventricular hemorrhage in preterm infants. *Sci Rep*, 2017; 7: 42541
20. Chen YC, Chang KH, Chen CM: Genetic polymorphisms associated with spontaneous intracerebral hemorrhage. *Int J Mol Sci*, 2018; 19: pii: E3879
21. Rautureau Y, Schiffrin EL: Endothelin in hypertension: An update. *Curr Opin Nephrol Hypertens*, 2012; 21: 128–36
22. Fang Z, Li M, Ma Z, Tu G: Association of endothelin-1 gene polymorphisms with essential hypertension in a Chinese population. *Genet Mol Res*, 2017; 16(3)
23. Barden AE, Herbison CE, Beilin LJ et al: Association between the endothelin-1 gene Lys198Asn polymorphism blood pressure and plasma endothelin-1 levels in normal and pre-eclamptic pregnancy. *J Hypertens*, 2001; 19: 1775–82
24. Gormley K, Bevan S, Hassan A, Markus HS: Polymorphisms in genes of the endothelin system and cerebral small-vessel disease. *Stroke*, 2005; 36: 1656–60
25. Zang X, Zhou Y, Huang Z, Zhang C: Endothelin-1 single-nucleotide polymorphisms and risk of pulmonary metastatic osteosarcoma. *PLoS One*, 2013; 8: e73349
26. Schiffrin EL, Deng LY, Sventek P, Day R: Enhanced expression of endothelin-1 gene in resistance arteries in severe human essential hypertension. *J Hypertens*, 1997; 15: 57–63
27. Zhang L, Sui R: Effect of SNP polymorphisms of EDN1, EDNRA, and EDNRB gene on ischemic stroke. *Cell Biochem Biophys*, 2014; 70: 233–39
28. Galaviz-Hernandez C, Arambula-Meraz E, Medina-Bastidas D et al: The paternal polymorphism rs5370 in the EDN1 gene decreases the risk of pre-eclampsia. *Pregnancy Hypertens*, 2016; 6: 327–32