Published in final edited form as:
Stroke. 2008 April ; 39(4): 1103–1108. doi:10.1161/STROKEAHA.107.496596.

Endothelial nitric oxide synthase polymorphism (-786T>C) and increased risk of angiographic vasospasm after aneurysmal subarachnoid hemorrhage

Nerissa U. Ko, MD[1], **Pam Rajendran, MD**[1], **Helen Kim, PhD**[5], **Martin Rutkowski, BA**[1], **Ludmila Pawlikowska, PhD**[6], **Pui-Yan Kwok, MD, PhD**[6], **Randall T. Higashida, MD**[2], **Michael T. Lawton, MD**[3], **Wade S. Smith, MD PhD**[1], **Jonathan G. Zaroff, MD**[4], and **William L. Young, MD**[1,3,5]

1*Department of Neurology, University of California, San Francisco*

2*Department of Radiology, University of California, San Francisco*

3*Department of Neurological Surgery, University of California, San Francisco*

4*Department of Medicine/Cardiology Division, University of California, San Francisco*

5*Department of Anesthesia and Perioperative Care/Center for Cerebrovascular Research, University of California, San Francisco*

6*Cardiovascular Research Institute, University of California, San Francisco*

Abstract

Background and Purpose—Vasospasm following aneurysmal subarachnoid hemorrhage (SAH) remains a leading cause of death and disability after aneurysm rupture. Decreased availability of nitric oxide (NO) may be crucial in the pathogenesis. We hypothesize that endothelial NO synthase (*eNOS*) polymorphisms may determine susceptibility to vasospasm in SAH patients.

Methods—We conducted a prospective cohort study of SAH patients, and determined vasospasm by cerebral angiography. We genotyped three *eNOS* polymorphisms: intron 4 variable-number-tandem-repeat (VNTR), promoter single-nucleotide-polymorphism (-786T>C SNP), and coding SNP in exon 7 (894G>T encoding E298D). Using multivariable logistic regression, we quantified the association of *eNOS* polymorphisms in patients with vasospasm confirmed by cerebral angiogram.

Results—For the *eNOS* promoter -786T>C SNP, the presence of the CC genotype compared to any T genotype (CT or TT) was associated with increased odds of vasospasm (OR 2.97, 95%CI 1.32-6.67, p=0.008). No association with vasospasm was observed for the *eNOS* 894G>T or VNTR polymorphisms.

Conclusions—These findings suggest that genetic variation influencing NO regulation contributes to risk of angiographic vasospasm in patients with SAH. The specific role of the promoter SNP (-786T>C) may determine the effect of NO regulated by this pathway distinct from other known *eNOS* polymorphisms.

Send correspondence to: Nerissa U. Ko, MD, 505 Parnassus Avenue, M830, San Francisco, CA 94143-0114, Tel: 415-353-8897, Fax: 415-353-8705, E mail: kon@ucsfmedctr.org.

Role of the funding sources: There was no role for any of the study sponsors in the study design, in the collection, analysis, and interpretation of the data, in the writing of the report, or in the decision to submit the paper for publication.

There are no conflicts of interest among the authors.

Keywords

Endothelial nitric oxide; Genetics; Subarachnoid hemorrhage; Vasospasm

Introduction

Cerebral vasospasm is a common complication after SAH, with a prevalence measured by cerebral angiogram approaching 70% during the first two weeks.[1] Unfortunately, despite maximal medical therapy, 20-30% of patients with angiographic vasospasm will experience delayed ischemic neurologic deficits and cerebral infarcts.[2] In more recent studies and clinical trials, angiographic vasospasm occurred in 88%, symptomatic vasospasm in 33-66%, and delayed cerebral infarct in 16-44%.[3-7] Among patients who survive with good outcomes, vasospasm contributes to increased hospital length of stay, long-term disability and diminished quality of life.[8-10] Although early detection and initiation of treatment to prevent vasospasm may reduce both acute and long-term disability, current methods do not accurately predict which patients will have clinically significant vasospasm warranting potentially invasive treatments.

The mechanism of injury from vasospasm remains poorly understood, but endothelial nitric oxide (NO) appears important in vasospasm pathogenesis.[11-14] NO is a potent vasodilator and inhibitor of inflammation, smooth muscle proliferation and platelet aggregation.[15] Polymorphisms in endothelial nitric oxide synthase (*eNOS*) have been associated with increased susceptibility to cerebral aneurysm rupture and risk of vasospasm after SAH in small clinical series.[16-18] In a prospective cohort of SAH patients, we examined the association between angiographic vasospasm and three previously investigated *eNOS* polymorphisms: a variable-number-tandem-repeat (VNTR) in intron 4,[19-21] a promoter single-nucleotide-polymorphism (−786T>C SNP),[22-26] and a coding SNP in exon 7 (894G>T encoding E298D).[24, 27-31]

Materials and Methods

Subjects

This was a prospective cohort study of adult patients with aneurysmal SAH followed for acute cardiac events and long-term neurological outcomes. Patients were admitted to a tertiary care referral center with SAH confirmed by non-contrast computed tomography (CT). Lumbar puncture was used in cases with negative head CT but high clinical suspicion of SAH.[32] All patients had cerebral aneurysm identified as the source of hemorrhage primarily by cerebral angiogram. Patients were excluded if they had a history of antecedent head trauma or non-aneurysmal source of hemorrhage, and if they could not undergo at least one angiogram during the vasospasm period, typically 3-14 days after SAH. After approval from the institutional review board, consent was obtained from each patient or an appropriate surrogate.

Management of vasospasm

Ruptured aneurysms were treated with either surgical clipping or endovascular coiling within the first 48 hours of admission. All patients were managed in the Neurointensive Care unit. Follow-up head CT and cerebral angiograms were obtained if vasospasm was suspected by elevated TCD velocities and ratios[33-35] or clinical neurologic deterioration not explained by other causes (rebleeding, hydrocephalus, seizures, or metabolic disturbances). All patients received standard medical management for vasospasm with volume resuscitation and induced hypertension.[36] Nimodipine was administered to all patients.[37, 38] Patients who had evidence

of angiographic spasm and were failing standard medical therapy were considered for interventional treatment with intraarterial verapamil or transluminal angioplasty.

Measurement of vasospasm and clinical risk factors

For our primary outcome, cerebral vasospasm was defined by the gold standard: cerebral angiography.[39, 40] Patients were considered to have vasospasm if there was evidence of arterial narrowing compared to the normal vessel caliber (mild <30%, moderate 30-60%, severe 61-99%) as determined by one of three attending neurointerventional radiologists. Follow-up CT scans in a subset of 235 subjects were reviewed by a trained stroke neurologist (NUK, PR). Cerebral infarction on head CT was defined as any new hypodensity in a vascular distribution. Hypodensities that may have been related to post-operative edema or procedural complications were excluded. All outcome measures were performed by investigators blinded to genotype.

We measured known risk factors associated with vasospasm after SAH including age, sex, hypertension, tobacco, and stimulant drug use.[41, 42] We also included SAH characteristics including clinical severity measured by the presenting Hunt-Hess grade,[43] volume of hemorrhage by Fisher group score,[44] and aneurysm treatment and location.

Polymorphism selection and genotyping

Three *eNOS* polymorphisms previously reported to be associated with vascular disease were chosen for analysis (Table 1). Genomic DNA was extracted from peripheral blood lymphocytes (Gentra Systems). Polymorphisms were genotyped by template-directed dye-terminator incorporation assay with fluorescence polarization detection (FP-TDI), using the AcycloPrime-FP kit (Perkin-Elmer).[45] The *eNOS* VNTR was genotyped by the UCSF Genomics Core on a 3700 DNA Analyzer with GeneScan and GeneMapper v3.0 software (Applied Biosystems). Oligonucleotide primer sequences are shown in Table A (online only). Average genotype success rate was 94% per 96-well plate, and individual plates pass quality control screening above 85%. In 90 subjects, genotype data was available only for the -786T>C SNP, accounting for the variation in number of subjects for the three eNOS genotypes. Genotyping was performed by investigators blinded to vasospasm outcomes.

Statistical analysis

The primary outcome variable was presence of vasospasm on cerebral angiogram. Our secondary outcome was presence of infarct on delayed CT. Chi-square and Wilcoxon rank-sum tests were used to compare baseline characteristics of subjects with and without angiographic vasospasm and with and without infarct on head CT.

Each polymorphism was tested for adherence to Hardy-Weinberg equilibrium using χ^2 goodness-of-fit test ($P<0.05$) for the entire cohort and within each racial subgroup. We examined the relationship between angiographic vasospasm and cerebral infarct with the putative high-risk alleles: “C” allele for −786T>C, the short, 4-repeat (“a”) allele for the VNTR, and the “T” allele for 894G>T (Glu298Asp).

Logistic regression analysis was performed to determine the effect of genotype on angiographic vasospasm or cerebral infarct using odds ratios (OR) and 95% confidence intervals (CI). Clinical covariates with a univariate P value <0.10 were included in multivariable logistic regression models to adjust for potential confounders, in addition to age, gender, and race/ethnicity. Significant interactions among predictor variables and *eNOS* polymorphisms were evaluated using Mantel-Haenszel χ^2 test for homogeneity. Additional models were developed to measure the effect of the polymorphisms in whites only, our largest racial subgroup.

To minimize the possibility of type 1 error from multiple comparisons, we used a Bonferroni correction testing three polymorphisms (Bonferroni-adjusted P<0.017). All statistical analyses were performed using commercially-available software (Intercooled STATA® version 9, College Station, TX).

Results

In our cohort of 347 subjects, 71% were female, mean age was 54.7 +/- 13 years, 73% had anterior aneurysm location and 62% were treated with surgical clipping. Mean Hunt-Hess grade was 2.18 and mean Fisher group score was 2.53. Angiographic vasospasm was present in 48%, and cerebral infarct was documented in 35% of 235 subjects with head CT scans obtained after initial presentation. Use of pressors was noted in 53% of subjects. Among those with angiographic vasospasm, subjects were generally younger with anterior aneurysms, and history of stimulant use. Patients were more likely to present with Hunt-Hess grades III or IV and Fisher group 3 (Table 2).

Genotype frequencies, listed in Table 3, were in Hardy-Weinberg equilibrium. For the *eNOS* promoter, -786T>C SNP, 14% of subjects with vasospasm had the CC genotype, compared to 8% without vasospasm (p=0.15). Unadjusted OR for the CC vs. TT genotype was 2.10 (95% CI 0.97-4.53, p=0.06), while the CT vs. TT genotype was 1.02 (95%CI 0.64-1.65, p=0.90). Therefore, we used CC vs. any T genotype (CT or TT) for our multivariable models. There were no differences in genotype frequencies for the 894G>T SNP (p=0.60) or VNTR (p=0.87). The unadjusted OR for 894G>T and VNTR were 0.91 (95%CI 0.43-1.95, p=0.81) and 0.82 (95%CI 0.19-3.49, p=0.78) respectively. Similarly, there were no significant differences in any of the *eNOS* genotype frequencies between subjects with and without infarct (Table 3). No significant interactions between *eNOS* polymorphisms and clinical characteristics were observed (P<0.05).

For -786T>C, the CC genotype compared to any T genotype was associated with a 2.97 increased odds of vasospasm (95%CI 1.32-6.67, p=0.008) after adjustment for age, gender, race/ethnicity, Hunt-Hess grade and Fisher group. We performed a subset analysis of Whites only, our largest ethnic subgroup, to minimize the confounding effect of population stratification. The association of CC genotype with vasospasm in 206 White subjects had a similar effect size (OR 2.09, 95%CI 0.88-4.98, p=0.09; Table 4).

Discussion

We report the first quantitative estimation of the association of *eNOS* polymorphisms with risk of angiographic vasospasm after aneurysmal SAH. The CC genotype of the promoter -786T>C SNP was associated with nearly three-fold increased odds of angiographic vasospasm. The association of vasospasm with the CC genotype was consistent in all our logistic regression models and remained significant even after Bonferroni correction for multiple comparisons. This association was similar in our analysis of Whites only.

To date, the -786T>C SNP is the only *eNOS* polymorphism associated with cerebral vasospasm. Our results replicate the findings of the first study to report an association with this promoter SNP on 28 cases selected by CT criteria for the highest risk of vasospasm.[16] The strengths of our study are a large prospective cohort and detailed radiographic outcome assessment.

We were able to show an association for the promoter polymorphism (-786T>C) with a relatively small sample size. For the -786T>C and 894G>T polymorphisms, our sample size could detect an effect size greater than 1.5-2.0. However, for the VNTR, we could only detect an effect size greater than 2-3.[46] We defined our outcome using the gold standard cerebral

angiography for vasospasm, and included only cases that were eligible for at least one cerebral angiogram during the vasospasm risk period. Using this outcome measure would likely miss milder cases of vasospasm that responded to medical therapy alone and did not require angiography or interventional treatments. Excluding milder cases as having no vasospasm likely biases our results toward the null hypothesis. We did not find any association of *eNOS* genotype with cerebral infarct. Although this may also be a result of too small a sample size to detect an effect, progression to ischemia and infarct likely involves mechanisms distinct from NO pathways that may be involved in angiographic vasospasm.

The small sample size of ethnic minorities limited our ability to evaluate associations with *eNOS* polymorphisms within ethnic subgroups. There are clear differences in genotype frequency among ethnic groups.[47-49] Our minor allele frequencies were consistent with the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/) and previously published studies.[16, 47-49] Our strongest association was with the CC genotype (-786C>T) while previous studies reported the highest risk for CT heterozygotes.[16, 22] In these previous studies among Japanese and Korean populations, CC genotypes occurred in less than 1% of subjects.[47, 49] We need to confirm our findings in larger cohorts of non-White ethnic subgroups.

We hypothesize that *eNOS* polymorphisms contribute to individual variability in angiographic vasospasm, and our study supports the hypothesis that NO is important in the pathogenesis of angiographic vasospasm. Biological evidence from animals and humans suggests that differential expression of eNOS leads to decreased NO levels after SAH.[50] Specifically, the promoter -786T>C polymorphism has been associated with decreased eNOS promoter activity and lower nitrite/nitrate levels in subjects with coronary spasm.[23, 25, 51]

Identifying patients at high risk for angiographic vasospasm and improving our ability to select patients for potentially harmful treatment has important clinical relevance. Vasospasm remains one of the most challenging management issues for clinicians since clinical and radiographic factors have been unreliable in predicting which patients will have significant clinical consequences from cerebral ischemia and infarct.[41] Individual genetic variability in the response to SAH likely contributes to the clinical variability seen among subjects with similar presenting symptoms. Early initiation of treatment for vasospasm may prevent morbidity and mortality after aneurysm rupture and improve outcomes. Conversely, better selection of patients may prevent complications of aggressive therapy such as induced hypertension, hypervolemia, and interventional therapies. Genetic screening for high risk individuals may become clinically available in the near future. Future studies of the specific role of the promoter polymorphism (−786T>C) may also determine the effect of NO regulated by this pathway, and identify potential targets for treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

The following members of the UCSF Stroke Sciences Group and BAVM Study Project (<http://avm.ucsf.edu>) contributed to portions of this work: Suzannah Stason, Annie Poon, Nancy Quinnine, Frankye Pang; Brad Dispensa, Philippe Jolivalt, and Achal Achrol.

This study was supported in part by grants from the National Institutes of Health (NINDS): K23 NS044014 (NUK), and R01 NS34949, NS41877, P01 NS44155 (WLY).

References

1. Kassell NF, Sasaki T, Colohan AR, Nazar G. Cerebral vasospasm following aneurysmal subarachnoid hemorrhage. Stroke 1985;16:562–572. [PubMed: 3895589]
2. Haley EC Jr, Kassell NF, Torner JC. The international cooperative study on the timing of aneurysm surgery. The north american experience. Stroke 1992;23:205–214. [PubMed: 1561649]
3. Lanzino G, Kassell NF, Dorsch NW, Pasqualin A, Brandt L, Schmiedek P, Truskowski LL, Alves WM. Double-blind, randomized, vehicle-controlled study of high-dose tirilazad mesylate in women with aneurysmal subarachnoid hemorrhage. Part i. A cooperative study in europe, australia, new zealand, and south africa. J Neurosurg 1999;90:1011–1017. [PubMed: 10350245]
4. McGirt MJ, Blessing R, Alexander MJ, Nimjee SM, Woodworth GF, Friedman AH, Graffagnino C, Laskowitz DT, Lynch JR. Risk of cerebral vasopasm after subarachnoid hemorrhage reduced by statin therapy: A multivariate analysis of an institutional experience. J Neurosurg 2006;105:671–674. [PubMed: 17121126]
5. Singhal AB, Topcuoglu MA, Dorer DJ, Ogilvy CS, Carter BS, Koroshetz WJ. Ssri and statin use increases the risk for vasospasm after subarachnoid hemorrhage. Neurology 2005;64:1008–1013. [PubMed: 15781818]
6. Vajkoczy P, Meyer B, Weidauer S, Raabe A, Thome C, Ringel F, Breu V, Schmiedek P. Clazosentan (axv-034343), a selective endothelin a receptor antagonist, in the prevention of cerebral vasospasm following severe aneurysmal subarachnoid hemorrhage: Results of a randomized, double-blind, placebo-controlled, multicenter phase iia study. J Neurosurg 2005;103:9–17. [PubMed: 16121967]
7. Wurm G, Tomancok B, Nussbaumer K, Adelwohrer C, Holl K. Reduction of ischemic sequelae following spontaneous subarachnoid hemorrhage: A double-blind, randomized comparison of enoxaparin versus placebo. Clin Neurol Neurosurg 2004;106:97–103. [PubMed: 15003298]
8. Claassen J, Bernardini GL, Kreiter K, Bates J, Du YE, Copeland D, Connolly ES, Mayer SA. Effect of cisternal and ventricular blood on risk of delayed cerebral ischemia after subarachnoid hemorrhage: The fisher scale revisited. Stroke 2001;32:2012–2020. [PubMed: 11546890]
9. Richardson JT. Cognitive performance following rupture and repair of intracranial aneurysm. Acta Neurol Scand 1991;83:110–122. [PubMed: 2017896]
10. Stenhouse LM, Knight RG, Longmore BE, Bishara SN. Long-term cognitive deficits in patients after surgery on aneurysms of the anterior communicating artery. J Neurol Neurosurg Psychiatry 1991;54:909–914. [PubMed: 1744646]
11. Schwartz AY, Sehba FA, Bederson JB. Decreased nitric oxide availability contributes to acute cerebral ischemia after subarachnoid hemorrhage. Neurosurgery 2000;47:208–214. [PubMed: 10917364]discussion 214-205
12. Sehba FA, Chereshnev I, Maayani S, Friedrich V Jr, Bederson JB. Nitric oxide synthase in acute alteration of nitric oxide levels after subarachnoid hemorrhage. Neurosurgery 2004;55:671–677. [PubMed: 15335435]discussion 677-678
13. Sehba FA, Schwartz AY, Chereshnev I, Bederson JB. Acute decrease in cerebral nitric oxide levels after subarachnoid hemorrhage. J Cereb Blood Flow Metab 2000;20:604–611. [PubMed: 10724124]
14. Woszczyk A, Deinsberger W, Boker DK. Nitric oxide metabolites in cisternal csf correlate with cerebral vasospasm in patients with a subarachnoid haemorrhage. Acta Neurochir (Wien) 2003;145:257–263. [PubMed: 12748885]discussion 263-254
15. Moncada S, Palmer RM, Higgs EA. Nitric oxide: Physiology, pathophysiology, and pharmacology. Pharmacol Rev 1991;43:109–142. [PubMed: 1852778]
16. Khurana VG, Sohni YR, Mangrum WI, McClelland RL, O'Kane DJ, Meyer FB, Meissner I. Endothelial nitric oxide synthase gene polymorphisms predict susceptibility to aneurysmal subarachnoid hemorrhage and cerebral vasospasm. J Cereb Blood Flow Metab 2004;24:291–297. [PubMed: 15091109]
17. Khurana VG, Sohni YR, Mangrum WI, McClelland RL, O'Kane DJ, Meyer FB, Meissner I. Endothelial nitric oxide synthase t-786c single nucleotide polymorphism: A putative genetic marker differentiating small versus large ruptured intracranial aneurysms. Stroke 2003;34:2555–2559. [PubMed: 14576373]

18. Khurana VG, Meissner I, Sohni YR, Bamlet WR, McClelland RL, Cunningham JM, Meyer FB. The presence of tandem endothelial nitric oxide synthase gene polymorphisms identifying brain aneurysms more prone to rupture. J Neurosurg 2005;102:526–531. [PubMed: 15796389]
19. Wang XL, Sim AS, Badenhop RF, McCredie RM, Wilcken DE. A smoking-dependent risk of coronary artery disease associated with a polymorphism of the endothelial nitric oxide synthase gene. Nat Med 1996;2:41–45. [PubMed: 8564837]
20. Wang XL, Wang J. Endothelial nitric oxide synthase gene sequence variations and vascular disease. Mol Genet Metab 2000;70:241–251. [PubMed: 10993711]
21. Hou L, Osei-Hyiaman D, Yu H, Ren Z, Zhang Z, Wang B, Harada S. Association of a 27-bp repeat polymorphism in ecnos gene with ischemic stroke in chinese patients. Neurology 2001;56:490–496. [PubMed: 11222793]
22. Nakayama M, Yoshimura M, Sakamoto T, Shimasaki Y, Nakamura S, Ito T, Abe K, Yamamuro M, Miyamoto Y, Saito Y, Nakao K, Yasue H, Ogawa H. Synergistic interaction of t-786-->c polymorphism in the endothelial nitric oxide synthase gene and smoking for an enhanced risk for coronary spasm. Pharmacogenetics 2003;13:683–688. [PubMed: 14583681]
23. Yoshimura M, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Nakamura S, Ito T, Mizuno Y, Harada E, Yasue H, Miyamoto Y, Saito Y, Nakao K. A t-786-->c mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene and coronary arterial vasomotility. Am J Cardiol 2000;85:710–714. [PubMed: 12000044]
24. Yoshimura M, Yasue H, Nakayama M, Shimasaki Y, Ogawa H, Kugiyama K, Saito Y, Miyamoto Y, Ogawa Y, Kaneshige T, Hiramatsu H, Yoshioka T, Kamitani S, Teraoka H, Nakao K. Genetic risk factors for coronary artery spasm: Significance of endothelial nitric oxide synthase gene t-786-->c and missense glu298asp variants. J Investig Med 2000;48:367–374.
25. Nakayama M, Yasue H, Yoshimura M, Shimasaki Y, Kugiyama K, Ogawa H, Motoyama T, Saito Y, Ogawa Y, Miyamoto Y, Nakao K. T-786-->c mutation in the 5′-flanking region of the endothelial nitric oxide synthase gene is associated with coronary spasm. Circulation 1999;99:2864–2870. [PubMed: 10359729]
26. Howard TD, Giles WH, Xu J, Wozniak MA, Malarcher AM, Lange LA, Macko RF, Basehore MJ, Meyers DA, Cole JW, Kittner SJ. Promoter polymorphisms in the nitric oxide synthase 3 gene are associated with ischemic stroke susceptibility in young black women. Stroke 2005;36:1848–1851. [PubMed: 16100023]
27. Hingorani AD, Liang CF, Fatibene J, Lyon A, Monteith S, Parsons A, Haydock S, Hopper RV, Stephens NG, O'Shaughnessy KM, Brown MJ. A common variant of the endothelial nitric oxide synthase (glu298-->asp) is a major risk factor for coronary artery disease in the uk. Circulation 1999;100:1515–1520. [PubMed: 10510054]
28. Ogimoto A, Shigematsu Y, Nakura J, Hara Y, Ohtsuka T, Kohara K, Hamada M, Miki T, Higaki J. Endothelial nitric oxide synthase gene polymorphism (glu298asp) in patients with coexistent hypertrophic cardiomyopathy and coronary spastic angina. J Mol Med 2005;83:619–625. [PubMed: 15778808]
29. Chang K, Baek SH, Seung KB, Kim PJ, Ihm SH, Chae JS, Kim JH, Hong SJ, Choi KB. The glu298asp polymorphism in the endothelial nitric oxide synthase gene is strongly associated with coronary spasm. Coron Artery Dis 2003;14:293–299. [PubMed: 12826928]
30. Elbaz A, Poirier O, Moulin T, Chedru F, Cambien F, Amarenco P. Association between the glu298asp polymorphism in the endothelial constitutive nitric oxide synthase gene and brain infarction. The genic investigators. Stroke 2000;31:1634–1639. [PubMed: 10884465]
31. Lembo G, De Luca N, Battagli C, Iovino G, Aretini A, Musicco M, Frati G, Pompeo F, Vecchione C, Trimarco B. A common variant of endothelial nitric oxide synthase (glu298asp) is an independent risk factor for carotid atherosclerosis. Stroke 2001;32:735–740. [PubMed: 11239195]
32. Edlow JA, Caplan LR. Avoiding pitfalls in the diagnosis of subarachnoid hemorrhage. N Engl J Med 2000;342:29–36. [PubMed: 10620647]
33. Lindegaard KF, Nornes H, Bakke SJ, Sorteberg W, Nakstad P. Cerebral vasospasm diagnosis by means of angiography and blood velocity measurements. Acta Neurochir (Wien) 1989;100:12–24. [PubMed: 2683600]

34. Sloan MA, Haley EC Jr, Kassell NF, Henry ML, Stewart SR, Beskin RR, Sevilla EA, Torner JC. Sensitivity and specificity of transcranial doppler ultrasonography in the diagnosis of vasospasm following subarachnoid hemorrhage. Neurology 1989;39:1514–1518. [PubMed: 2682350]
35. Vora YY, Suarez-Almazor M, Steinke DE, Martin ML, Findlay JM. Role of transcranial doppler monitoring in the diagnosis of cerebral vasospasm after subarachnoid hemorrhage. Neurosurgery 1999;44:1237–1247. [PubMed: 10371622]discussion 1247-1238
36. Romner B, Reinstrup P. Triple h therapy after aneurysmal subarachnoid hemorrhage. A review. Acta Neurochir Suppl 2001;77:237–241. [PubMed: 11563296]
37. Allen GS, Ahn HS, Preziosi TJ, Battye R, Boone SC, Boone SC, Chou SN, Kelly DL, Weir BK, Crabbe RA, Lavik PJ, Rosenbloom SB, Dorsey FC, Ingram CR, Mellits DE, Bertsch LA, Boisvert DP, Hundley MB, Johnson RK, Strom JA, Transou CR. Cerebral arterial spasm--a controlled trial of nimodipine in patients with subarachnoid hemorrhage. N Engl J Med 1983;308:619–624. [PubMed: 6338383]
38. Pickard JD, Murray GD, Illingworth R, Shaw MD, Teasdale GM, Foy PM, Humphrey PR, Lang DA, Nelson R, Richards P, et al. Effect of oral nimodipine on cerebral infarction and outcome after subarachnoid haemorrhage: British aneurysm nimodipine trial. Bmj 1989;298:636–642. [PubMed: 2496789]
39. Okada Y, Shima T, Nishida M, Yamane K, Hatayama T, Yamanaka C, Yoshida A. Comparison of transcranial doppler investigation of aneurysmal vasospasm with digital subtraction angiographic and clinical findings. Neurosurgery 1999;45:443–449. [PubMed: 10493365]discussion 449-450
40. Lysakowski C, Walder B, Costanza MC, Tramer MR. Transcranial doppler versus angiography in patients with vasospasm due to a ruptured cerebral aneurysm: A systematic review. Stroke 2001;32:2292–2298. [PubMed: 11588316]
41. Harrod CG, Bendok BR, Batjer HH. Prediction of cerebral vasospasm in patients presenting with aneurysmal subarachnoid hemorrhage: A review. Neurosurgery 2005;56:633–654. [PubMed: 15792502]discussion 633-654
42. Janjua N, Mayer SA. Cerebral vasospasm after subarachnoid hemorrhage. Curr Opin Crit Care 2003;9:113–119. [PubMed: 12657973]
43. Hunt WE, Hess RM. Surgical risk as related to time of intervention in the repair of intracranial aneurysms. J Neurosurg 1968;28:14–20. [PubMed: 5635959]
44. Fisher CM, Kistler JP, Davis JM. Relation of cerebral vasospasm to subarachnoid hemorrhage visualized by computerized tomographic scanning. Neurosurgery 1980;6:1–9. [PubMed: 7354892]
45. Hsu TM, Chen X, Duan S, Miller RD, Kwok PY. Universal snp genotyping assay with fluorescence polarization detection. Biotechniques 2001;31:560–562. 564–568. [PubMed: 11570500]passim
46. Dichgans M, Markus HS. Genetic association studies in stroke: Methodological issues and proposed standard criteria. Stroke 2005;36:2027–2031. [PubMed: 16051898]
47. Akagawa H, Kasuya H, Onda H, Yoneyama T, Sasahara A, Kim CJ, Lee JC, Yang TK, Hori T, Inoue I. Influence of endothelial nitric oxide synthase t-786c single nucleotide polymorphism on aneurysm size. J Neurosurg 2005;102:68–71. [PubMed: 15658098]
48. Krex D, Fortun S, Kuhlisch E, Schackert HK, Schackert G. The role of endothelial nitric oxide synthase (enos) genetic variants in european patients with intracranial aneurysms. J Cereb Blood Flow Metab 2006;26:1250–1255. [PubMed: 16467782]
49. Song MK, Kim MK, Kim TS, Joo SP, Park MS, Kim BC, Cho KH. Endothelial nitric oxide gene t-786c polymorphism and subarachnoid hemorrhage in korean population. J Korean Med Sci 2006;21:922–926. [PubMed: 17043430]
50. Hanggi D, Steiger HJ. Nitric oxide in subarachnoid haemorrhage and its therapeutics implications. Acta Neurochir (Wien) 2006;148:605–613. [PubMed: 16541208]discussion 613
51. Nakayama M, Yoshimura M, Sakamoto T, Abe K, Yamamuro M, Shono M, Suzuki S, Nishijima T, Miyamoto Y, Saito Y, Nakao K, Yasue H, Ogawa H. A -786t>c polymorphism in the endothelial nitric oxide synthase gene reduces serum nitrite/nitrate levels from the heart due to an intracoronary injection of acetylcholine. Pharmacogenet Genomics 2006;16:339–345. [PubMed: 16609365]

Table 1

Endothelial nitric oxide synthase (eNOS) polymorphisms

Polymorphism	Location	Alleles[^]	Clinical rationale	References
−786 T>C	promoter	C, T	C allele associated with: post-SAH vasospasm, coronary artery vasospasm	18-20, 24-28
894 G>T	exon 7	T, G	T allele associated with: coronary artery disease, carotid atherosclerosis, myocardial infarction	26, 29-33
VNTR[*]	intron 4	a, b	a allele associated with: coronary artery disease, myocardial infarction, cerebral aneurysm rupture, aortic aneurysm rupture	21-23

[*] 27-base variable-number-tandem-repeat

[^] C, cytosine; T, thymine; G, guanine; a=4 repeats; b=5 or 6 repeats

Table 2
Characteristics of SAH patients with and without angiographic vasospasm

	No Vasospasm n=181	Vasospasm n=166	p value
Mean age in years +/- SD	57±14	52±12	<0.001
Female	125 (69)	121 (73)	0.43
Surgical treatment	102(56)	112(67)	0.19
Anterior location	120(66)	131(79)	0.006
History of hypertension	70(39)	73(44)	0.32
History of smoking	60(33)	55(33)	1.00
History of stimulant use	9(5)	18(11)	0.04
Race/Ethnicity			0.47
Caucasian	119(66)	100(60)	
Latino	22(12)	24(14)	
Asian	16(9)	23(14)	
African-American	19(10)	13(8)	
Other	5(3)	6(4)	
Admission Hunt-Hess Grade			0.014
I	86(48)	58(35)	
II	33(18)	27(16)	
III	37(20)	51(31)	
IV	17(9)	23(14)	
V	7(4)	6(4)	
Fisher Group			0.016
1	14(8)	2(1)	
2	86(48)	70(42)	
3	66(36)	82(49)	
4	14(8)	12(7)	

Table 3

Genotype frequency of *eNOS* polymorphisms by primary outcome vasospasm and secondary outcome cerebral infarct

	All	No Vasospasm	Vasospasm	p-value	No Infarct	Infarct	p-value
	n(%)	n(%)	n(%)		n(%)	n(%)	
eNOS -786T>C				0.15			0.74
TT	167(53)	91(54)	76(50)		77(53)	41(56)	
CT	119(37)	64(38)	55(36)		53(37)	23(32)	
CC	33(10)	12(8)	21(14)		15(10)	9(12)	
TOTALS	319	167	152		145	73	
eNOS 894G>T (E298D)				0.60			0.25
GG	141(60)	77(57)	64(63)		84(59)	44(60)	
GT	63(27)	39(29)	24(24)		43(30)	16(22)	
TT	32(13)	19(14)	13(13)		16(11)	13(18)	
TOTAL	236	135	101		143	73	
eNOS VNTR*				0.87			0.65
bb	184(75)	107(76)	77(74)		114(77)	55(71)	
ab	53(22)	29(20)	24(23)		29(20)	19(25)	
aa	8(3)	5(4)	3(3)		5(3)	3(4)	
TOTAL	245	141	104		148	77	

*a=4 allele, b=5 or 6 allele, as 6 is very rare occurring in only 6 cases

Table 4

Association of *eNOS* -786T>C polymorphism with angiographic vasospasm

	Unadjusted			Adjusted			Whites Only		
	OR	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**	**OR**	**95% CI**	**p-value**
eNOS -786T>C(CC vs. any T)	2.09	0.99-4.40	0.05	2.97	1.32-6.67	0.008	2.09	0.88-4.98	0.09
Fisher Group 3	--	--	--	2.06	1.25-3.42	0.005	1.86	1.00-3.46	0.05
Female gender	--	--	--	1.30	0.77-2.21	0.32	1.36	0.70-2.64	0.36
Age, year	--	--	--	0.96	0.94-0.98	<0.001	0.95	0.93-0.98	<0.001
White vs. non-White	--	--	--	0.71	0.43-1.17	0.18	--	--	--
Hunt-Hess Grade <3	--	--	--	0.49	0.30-0.81	0.006	0.56	0.30-1.05	0.07