Associations between endothelin polymorphisms and aneurysmal subarachnoid hemorrhage, clinical vasospasm, delayed cerebral ischemia, and functional outcome

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OBJECTIVE Endothelin-1, a potent vasoconstrictor, and its receptors may be involved in the pathogenesis of aneurysmal subarachnoid hemorrhage (aSAH), clinical vasospasm, delayed cerebral ischemia (DCI), and functional outcome following aSAH. In the present study, common endothelin single nucleotide polymorphisms (SNPs) and their relation to aSAH were evaluated.

METHODS Blood samples from all patients enrolled in the Cerebral Aneurysm Renin Angiotensin System (CARAS) study were used for genetic evaluation. The CARAS study prospectively enrolled patients with aSAH at 2 academic institutions in the US from 2012 to 2015. Common endothelin SNPs were detected using 5' exonuclease (TaqMan) genotyping assays. Analysis of associations between endothelin SNPs and aSAH and its clinical sequelae was performed.

RESULTS Samples from 149 patients with aSAH and 50 controls were available for analysis. In multivariate logistic regression analysis, the TG (odds ratio [OR] 2.102, 95% confidence interval [CI] 1.048–4.218, $p = 0.036$) and TT genotypes (OR 7.884, 95% CI 1.003–61.995, $p = 0.05$) of the endothelin-1 T/G SNP (rs1800541) were significantly associated with aSAH. There was a dominant effect of the G allele (CG/GG genotypes; OR 4.617, 95% CI 1.311–16.262, $p = 0.017$) of the endothelin receptor A G/C SNP (rs5335) on clinical vasospasm. Endothelin SNPs were not associated with DCI or functional outcome.

CONCLUSIONS Common endothelin SNPs were found to be associated with presentation with aSAH and clinical vasospasm. Further studies are required to elucidate the relevant pathophysiology and its potential implications in the treatment of patients with aSAH.

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KEY WORDS endothelin; polymorphism; aneurysm; cerebral; intracranial; subarachnoid hemorrhage; vasospasm; delayed cerebral ischemia; rupture; vascular disorders

ANEURYSMAL subarachnoid hemorrhage (aSAH) is the common end point of environmental, biomechanical, cellular, molecular, and genetic processes that underlie the formation and rupture of cerebral

aneurysms. Genetic polymorphisms have been associated with aSAH and its sequelae, clinical vasospasm, and delayed cerebral ischemia (DCI).¹⁹ Endothelin-1 (END1), a potent vasoconstrictor, and its receptors may be com-

ABBREVIATIONS aSAH = aneurysmal subarachnoid hemorrhage; CARAS = Cerebral Aneurysm Renin Angiotensin System; CI = confidence interval; CONSCIOUS = Clazosentan to Overcome Neurological Ischemia and Infarct Occurring after Subarachnoid Hemorrhage; CTA = CT angiography; DCI = delayed cerebral ischemia; DSA = digital subtraction angiography; END1 = endothelin-1; ENDRA = endothelin receptor A; ENDRB = endothelin receptor B; ICU = intensive care unit; mRS = modified Rankin scale; NO = nitric oxide; OR = odds ratio; SNP = single nucleotide polymorphism; TCD = transcranial Doppler ultrasonography.

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ponents in this complex multifactorial process and have been investigated as therapeutic targets in aSAH. The purpose of the present study was to investigate associations of single nucleotide polymorphisms (SNPs) of *END1* and endothelin receptors A (*ENDRA*) and B (*ENDRB*) encoding genes with aSAH. The majority of these SNPs have been studied in the context of ischemic stroke²² but not in aSAH.

Methods

Blood samples from all patients and controls enrolled in the Cerebral Aneurysm Renin Angiotensin System (CARAS) study were used for genetic evaluation; much of the method has previously been reported.^{5–7,10} In the CARAS study, the diagnosis of aSAH was established on the basis of the admission CT scan or xanthochromia of CSF. A ruptured aneurysm as the source of hemorrhage was confirmed by CT angiography (CTA) or digital subtraction angiography (DSA). Exclusion criteria were age under 19 years and any associated genetic predisposition that could contribute to cerebral aneurysm formation (polycystic kidney disease, Turner syndrome, Noonan syndrome, Ehler-Danlos syndrome Type 4, Marfan syndrome, neurofibromatosis Type 1, etc.). The control group was composed of trauma patients, at least 19 years old, with unremarkable CT angiograms of the head and neck (no cerebral aneurysm or other vascular malformation), and without known genetic risk factors for cerebral aneurysm formation. Controls underwent CTA due to suspicion for traumatic cerebrovascular injury. Patients with any abnormalities of the cerebral vasculature were excluded. Efforts were made to match aSAH patients and controls for age and sex. Both aSAH patients and controls were enrolled within 72 hours of admission.

General Management

Patients were treated in accordance with guidelines for the management of aSAH, including intensive care unit (ICU) monitoring, treatment of hydrocephalus, early (< 48-hour) intervention for aneurysm treatment, oral nimodipine, maintenance of euvolemia, and surveillance for clinical vasospasm and DCI.²

Definition of Clinical Vasospasm and DCI

Clinical vasospasm was defined as a new focal or global neurological deficit, or deterioration of at least 2 points on the Glasgow Coma Scale, not explained by another clinical process including hydrocephalus, aneurysm rebleeding, electrolyte disturbance, seizure, infection, fever, metabolic disturbance, cerebral edema, or surgical complication. Corroborating evidence of angiographic vasospasm was defined as arterial narrowing on CTA or DSA not due to atherosclerosis, catheter-induced vasospasm, or vessel hypoplasia. Additionally, vasospasm was diagnosed with transcranial Doppler ultrasonography (TCD) findings of a mean systolic middle cerebral artery (MCA) pressure of more than 120 cm/sec with a Lindegaard ratio more than 3. CTA, DSA, and TCD were obtained at the discretion of the treating neurosurgeon. The diagnosis of clinical vasospasm was adjudicated by consensus of the study team

and treated with hyperdynamic therapy as the first line of treatment.³ Hyperdynamic therapy included avoidance of hypovolemia with a goal systolic blood pressure of more than 160 mm Hg, accomplished with either permissive hypertension or vasopressor therapy. Patients with clinical vasospasm refractory to medical treatment were treated in the endovascular suite at the discretion of the treating neurosurgeon.

CT or MRI was routinely performed when the patient was transferred from the ICU to the ward. DCI was defined as low-density areas on CT that corresponded to a vascular distribution, or MRI demonstrating a hyperintense area on a diffusion-weighted imaging sequence with a corresponding hypointense apparent diffusion coefficient sequence correlate that corresponded with a vascular territory. Infarctions or contusions noted on postoperative Day 1 imaging were considered procedurally related and were not considered DCI. Images were evaluated locally and blinded to the genetic analysis.

Outcome Measures in Patients With aSAH

Outcome measures included clinical vasospasm, DCI, and functional outcome at the time of discharge from the acute hospital setting, and at last follow-up using the modified Rankin scale (mRS). Unfavorable outcome was defined as mRS score 3–6. All outcome data were obtained blinded to the results of the genetic analysis. Functional outcome was assessed either in clinic or via telephone interview with the patient or with a surrogate if the patient was unable to participate.¹

Laboratory and Genetic Evaluation

Common endothelin SNPs (Table 1) were detected using 5' exonuclease (TaqMan) genotyping assays (SNPs *END1* [rs1800541, rs2070699, rs5370; Lys198Asn, K198N], *ENDRA* [rs1801708, rs5333, rs5335], and *ENDRB* [rs3818416, rs5351]; http://useast.ensembl.org/Homo_sapiens/Info/Index). Commercial TaqMan assays were designed and performed according to the vendor (Thermo Fisher Scientific, Inc.). Approximately 10% of the DNA samples were randomly selected to test reproducibility of TaqMan assays. All of the replication samples produced concordant genotypes.

Statistical Analysis

Continuous variables are presented as means $\pm$ standard deviations and categorical variables are presented as frequencies and percentages. Analyses were performed using unpaired Wilcoxon rank-sum, chi-square, and Fisher's exact tests, as well as the Student t-test, as appropriate. Patient characteristics and endothelin SNPs were tested in univariate analysis to determine predictors of aSAH. Patient and aneurysm characteristics and endothelin SNPs were tested in univariate analysis to determine predictors of the following dependent variables: clinical vasospasm, DCI, and unfavorable functional outcome at discharge (mRS score 3–6) and at follow-up. Factors predictive in univariate analysis ($p < 0.15$) were entered into a multivariate logistic regression analysis; p values ≤ 0.05 were considered statistically significant.

TABLE 1. Common endothelin SNPs

Gene name	rsID (variant)	Alleles	Minor Allele Frequency*
Endothelin 1 (END1)	rs1800541	T/G	0.28 (G)
Endothelin 1 (END1)	rs2070699	G/T	0.36 (T)
Endothelin 1 (END1)	rs5370 (Lys198Asn, K198N)	G/T	0.25 (T)
Endothelin receptor A (EDNRA)	rs1801708	G/A	0.48 (G)
Endothelin receptor A (EDNRA)	rs5333	T/C	0.38 (C)
Endothelin receptor A (EDNRA)	rs5335	G/C	0.42 (G)
Endothelin receptor B (ENDRB)	rs3818416	A/C	0.22 (A)
Endothelin receptor B (ENDRB)	rs5351	T/C/G	0.46 (T)

* http://useast.ensembl.org/Homo_sapiens/Info/Index?redirect=no.

Results

Blood samples were analyzed from 149 patients with aSAH and 50 controls enrolled between September 2012 and February 2015.

Characteristics of Patients and Controls

The mean ages of aSAH patients and controls were 54.9 ± 12.5 and 50.6 ± 18.6 years, respectively ($p = 0.07$). There was a higher proportion of African Americans in the aSAH group as compared with the control group ($p = 0.003$). Sex was evenly distributed between the two groups ($p = 0.086$). A history of hypertension ($p = 0.013$) and a family history of cerebral aneurysms ($p = 0.025$) were more frequent in the aSAH group. There was no difference in the rate of current or former smokers ($p = 0.426$). The majority of ruptured aneurysms were less than 7 mm in maximum diameter (62.4%) and located in the anterior circulation (80.5%). Hunt & Hess Grades I–III and modified Fisher CT grades 1–2 made up 77.2% and 49.2%, respectively, of the study population. Clinical vasospasm and DCI occurred in 22.8% and 21.2%, respectively. Favorable functional outcome (mRS 0–2) at hospital discharge and last follow-up was achieved in 47.7% and 61.7% of patients, respectively (Table 2).

Association of Endothelin SNPs and aSAH

All SNPs were in Hardy-Weinberg equilibrium with the exception of rs2070699 in patients ($p = 0.001$) and controls ($p = 0.007$), rs1801708 in patients ($p < 0.001$) and controls ($p = 0.002$), and rs3818416 in controls ($p = 0.035$; Table 3). In multivariate logistic regression analysis, the TG (odds ratio [OR] 2.102, 95% confidence interval [CI] 1.048–4.218, $p = 0.036$) and TT genotypes (OR 7.884, 95% CI 1.003–61.995, $p = 0.05$) of *END1* T/G SNP (rs1800541) were significantly associated with aSAH along with older age, African American race, and antithrombotic use (Table 4).

Associations of Endothelin SNPs and Clinical Course After aSAH

In multivariate logistic regression analysis, there was a dominant effect of the G allele (CG/GG genotypes; OR 4.617, 95% CI 1.311–16.262, $p = 0.017$) of *EDNRA* G/C SNP (rs5335) on clinical vasospasm. Other predictors of clinical vasospasm were African American race (OR 0.318, 95% CI 0.128–0.795, $p = 0.014$) and higher Hunt and Hess grade (OR 1.861, 95% CI 1.274–2.716, $p = 0.001$). DCI was associated with higher Hunt & Hess grade (OR 1.761, 95% CI 1.077–2.881, $p = 0.024$) and higher modified Fisher CT grade (OR 1.867, 95% CI 1.099–3.172, $p = 0.021$), but none of the endothelin SNPs. Functional outcome at follow-up was associated with ischemic vascular disease (OR 188.109, 95% CI 9.879–3581.984, $p < 0.001$), higher Hunt and Hess grade (OR 4.384, 95% CI 1.734–11.085, $p = 0.002$), higher modified Fisher grade (OR 3.392, 95% CI 1.062–10.838, $p = 0.039$), larger aneurysm size (OR 1.192, 95% CI 1.005–1.413, $p = 0.043$), and rebleeding (OR 62.005, 95% CI 3.037–1265.997, $p = 0.007$). Endothelin SNPs did not predict unfavorable outcome at hospital discharge or last follow-up (Table 5).

Discussion

The present study analyzed common endothelin SNPs and their association with aSAH and its sequelae. The T allele of the *END1* T/G SNP (rs1800541) was associated with aSAH when compared with controls. The G allele of the *EDNRA* G/C SNP (rs5335) was associated with clinical vasospasm. This represents the first investigation of a majority of common endothelin SNPs in the context of aSAH.

Endothelin, Cerebral Aneurysm Development, and Cerebral Vasospasm

END1 and its receptors have been known to play a significant role in cerebral aneurysm development.¹⁵ END1 is produced by vascular endothelial cells and is a potent vasoconstrictor that mediates its effects via 2 receptor subtypes, EDNRA and ENDRB. EDNRA is predominantly found on vascular smooth muscle cells of the cerebral vasculature and regulates vasoconstriction and vascular smooth muscle cell proliferation.²¹ The interplay of END1, EDNRA, and ENDRB is critically important for the regulation of vasoconstriction and vasodilatation in response to hemodynamic stress. Endothelin also helps regulate nitric oxide (NO) and alterations in NO SNPs have been associated with aneurysm formation and vasospasm following aSAH.^{13,14,20} The G allele of the *EDNRA* G/C SNP has been associated with hypertension.¹⁸ In the setting of aSAH, the cerebrovascular circulation of homo- and heterozygotes for the G allele may be more susceptible to the circulating END1 resulting in vasoconstriction and clinical vasospasm mediated at the receptor level. Elevated levels of END1 in CSF and plasma were found in aSAH patients with cerebral vasospasm and DCI.^{4,12} Hemolysis of SAH induces END1 production in activated mononuclear leukocytes.⁴ These discoveries and a number of precursor trials led to the design of the Clazosentan to Overcome Neurological Ischemia and Infarct Occurring after Subarachnoid Hemorrhage (CONSCIOUS)-2 trial,

TABLE 2. Patient and control characteristics

Characteristic	aSAH (%)	Controls (%)	p Value
No. of patients	149	50	
Mean age $\pm$ SD (yrs)	54.9 $\pm$ 12.5	50.6 $\pm$ 18.6	0.07
Race			
Caucasian	85 (57.0)	41 (82)	0.003
African American	60 (40.3)	7 (14)	
Other	4 (2.7)	2 (4)	
Sex			
Male	35 (23.5)	18 (36)	0.086
Female	114 (76.5)	32 (64)	
Ischemic vascular disease	13 (8.7)	8 (16)	0.153
Hypertension	90 (60.4)	20 (40)	0.013
Antithrombotics			
None	132 (88.6)	39 (78.0)	0.044
Antiplatelets	14 (9.4)	11 (22.0)	
Anticoagulation	3 (2.0)	0	
Smoking			
Never	58 (38.9)	21 (42)	0.426
Former	18 (12.1)	9 (18)	
Current	73 (49)	20 (40)	
Family history of cerebral aneurysms	14 (9.4)	0 (0)	0.025
Mean days for symptom onset to admission $\pm$ SD	0.9 $\pm$ 2.7		
Aneurysm size (mm)			
<7	93 (62.4)		
7–12	49 (32.9)		
13–24	6 (4.0)		
≥ 25	1 (0.7)		
Location			
Anterior	120 (80.5)		
Posterior	29 (19.5)		
Hunt & Hess grade			
I–III	115 (77.2)		
IV–V	34 (22.8)		
Fisher grade			
1–2	23 (15.4)		
3–4	126 (84.6)		
Modified Fisher grade*			
1–2	59 (49.2)		
3–4	61 (50.8)		
Hijdra scale*			
0	29 (24.2)		
1	10 (8.3)		
2	24 (20.0)		
3	20 (16.7)		
4	7 (5.8)		
5	4 (3.3)		
6	11 (9.2)		
7	5 (4.2)		
8	4 (3.3)		
9	0		
10	5 (4.2)		
11	1 (0.8)		

CONTINUED IN NEXT COLUMN »

» CONTINUED FROM PREVIOUS COLUMN

TABLE 2. Patient and control characteristics

Characteristic	aSAH (%)	Controls (%)	p Value
Treatment†			
Microsurgical clipping	76 (52.4)		
Endovascular treatment	69 (47.6)		
Rebleeding	7 (4.7)		
Clinical vasospasm	34 (22.8)		
DCI‡	31 (21.2)		
Hyponatremia	56 (37.6)		
Infection (blood stream, pneumonia, urinary tract)	61 (40.9)		
Ventriculitis	4 (2.7)		
Antiepileptic drug administration	62 (41.6)		
Mean ICU stay $\pm$ SD (days)	11.9 $\pm$ 7.9		
Mean hospital stay $\pm$ SD (days)	15.9 $\pm$ 11.3		
Discharge mRS score			
0–2	71 (47.7)		
3–6	78 (52.3)		
mRS score at last follow-up			
0–2	92 (61.7)		
3–6	57 (38.3)		
Mean length of follow-up $\pm$ SD (days)	249.7 $\pm$ 187.8		

* Data not available for 29 patients.

† Data not available for 4 aneurysms.

‡ Data not available for 3 patients.

a randomized, double-blind, placebo-controlled, Phase 3 study assessing the efficacy of clazosentan in patients in whom the ruptured aneurysm was secured by microsurgical clipping.¹⁶ The CONSCIOUS-3 trial, including patients treated with endovascular coil embolization, was halted prematurely after CONSCIOUS-2 failed to show a benefit in terms of all-cause mortality and vasospasm-related morbidity at 6 weeks.¹⁷ Failure of the trial was attributed to beneficial effects of rescue therapy for angiographic vasospasm that was used more frequently in the placebo group and may have mitigated benefits of clazosentan. Successful rescue therapy in the present study may have also decreased the incidence of DCI and explain the lack of associations of the *EDNRA* G/C SNP with DCI. Also, mechanisms other than large vessel vasospasm may result in delayed ischemic neurological deficit, DCI, and poor functional outcome. Whereas angiographic vasospasm was significantly decreased by clazosentan in CONSCIOUS-1, microvascular processes such as microthromboembolism, microcirculatory dysfunction, cortical spreading ischemia, and delayed neuronal injury contributing to aforementioned outcome measures may have not been affected.¹⁶ Lastly, polymorphisms in genes encoding *END1* and endothelin receptors or adjacent regulatory regions may change their biological characteristics and alter their response to a drug such as clazosentan.

TABLE 3. Genotype frequencies of endothelin SNPs

Polymorphism	aSAH (%)	Controls (%)	p Value
rs1800541			0.016
Genotype			
GG	69 (46.3)	34 (68.0)	
TG	64 (43.0)	15 (30.0)	
TT	16 (10.7)	1 (2)	
HWE (p value)	0.840	0.656	
rs2070699			0.111
Genotype			
GG	117 (78.5)	33 (66.0)	
GT	25 (16.8)	11 (22.0)	
TT	7 (4.7)	6 (12.0)	
HWE (p value)	0.001	0.007	
rs5370			0.33
Genotype			
GG	88 (59.0)	35 (70.0)	
GT	56 (37.6)	13 (26.0)	
TT	5 (3.4)	2 (4)	
HWE (p value)	0.273	0.578	
rs1801708			0.571
Genotype			
AA	1 (0.7)	0	
AG	99 (66.4)	30 (60)	
GG	49 (32.9)	20 (40)	
HWE (p value)	< 0.001	0.002	
rs5333*			0.615
Genotype			
CC	19 (12.9)	9 (18.4)	
CT	61 (41.5)	18 (36.7)	
TT	67 (45.6)	22 (44.9)	
HWE (p value)	0.389	0.142	
rs5335*			0.058
Genotype			
CC	30 (20.4)	17 (34.7)	
CG	70 (47.6)	20 (40.8)	
GG	47 (32.0)	12 (24.5)	
HWE (p value)	0.674	0.220	
rs3818416*			0.34
Genotype			
CC	15 (10.2)	6 (12.2)	
CA	56 (38.1)	13 (26.5)	
AA	76 (51.7)	30 (61.3)	
HWE (p value)	0.335	0.035	
rs5351*			0.27
Genotype			
TT	51 (34.7)	12 (24.5)	
TG	65 (44.2)	28 (57.1)	
GG	31 (21.1)	9 (18.4)	
HWE (p value)	0.230	0.303	

HWE = Hardy-Weinberg equilibrium

* Alleles were not available for 2 patients with aSAH and 1 control.

TABLE 4. Predictors of aSAH in multivariate logistic regression analysis

Variable	OR (95% CI)	p Value
Older age	1.031 (1.006–1.058)	0.016
African American	3.293 (1.329–8.158)	0.01
Antithrombotics	0.279 (0.106–0.735)	0.01
TG genotype (rs1800541)	2.102 (1.048–4.218)	0.036
TT genotype (rs1800541)	7.884 (1.003–61.995)	0.05

Endothelin SNPs and aSAH

Endothelin SNPs have not been extensively studied in the setting of aSAH. Through a genome-wide association study and imputation analysis, 4 SNPs, 1 in an intron (rs17612742) and 3 upstream (rs6841581, 1878406, and rs6842241) of the *EDNRA* gene on chromosome 4, were found to be associated with cerebral aneurysm development.¹⁵ Using cell line material, potential effects of these SNPs on the transcriptional activity of *EDNRA* were studied. A dual-luciferase reporter assay was used to detect differences in the transcriptional activity and found significantly lower activity of the G allele of rs6841581 when compared with the A allele. This suggests that the 5' flanking region of the *EDNRA* gene containing rs6841581 might function as a transcriptional repressor and that this SNP is a functional variant that may be involved in cerebral aneurysm development.¹⁵ Whether the G allele of the *EDNRA* G/C SNP (rs5335) confers similar functional alterations relevant to clinical vasospasm remains to be determined.

Gallek et al. examined a number of other *END1* and endothelin receptor SNPs and their association with negative outcome after aSAH.⁸ Similar to the present study, their analysis included rs5370, or Lys198Asn, a known functional SNP in the *END1* gene associated with differences in vascular reactivity,¹¹ and a number of other tagging SNPs of *END1*, *ENDRA*, and *ENDRB*. Neither their study nor the present study found any association of rs5370 with aSAH or its sequelae. They did, however, identify a trend between the *END1* A/G SNP (rs6912834) and angiographic vasospasm. There were also associations found between two *ENDRB* SNPs (rs9574124 and rs3027111) and poor functional outcomes at 3 months, with the *ENDRB* C/T SNP (rs3027111) being associated with a higher risk of mortality at 3 months. Gallek et al. did not collect samples from a control group, but compared their patients with aSAH to a representative control population from the HapMap database (<https://www.ncbi.nlm.nih.gov/probe/docs/projhapmap/>). The distribution of genotypes between study and control patients did not differ and the authors concluded that common endothelin SNPs did not play a role in aneurysm development and aSAH.⁸ In the present study, the T allele of the *END1* T/G SNP (rs1800541) conferred a risk for aSAH even after controlling for racial differences and other potential confounders between patients with aSAH and controls. This and other studies may result in the development of a screening tool to identify patients with cerebral aneu-

TABLE 5. Predictors of outcome measures in aSAH in multivariate logistic regression analysis

Predictor	OR (95% CI)	p Value
Clinical vasospasm		
African American	0.318 (0.128–0.795)	0.014
Higher Hunt & Hess grade	1.861 (1.274–2.716)	0.001
Dominant effect G allele (CG & GG vs CC; rs5335)	4.617 (1.311–16.262)	0.017
DCI		
Higher Hunt & Hess grade	1.761 (1.077–2.881)	0.024
Higher modified Fisher grade	1.867 (1.099–3.172)	0.021
Rebleeding	10.773 (1.027–113.050)	0.047
Hyponatremia	0.312 (0.103–0.941)	0.039
Unfavorable outcome at discharge (mRS score 3–6)		
Days for symptom onset to admission	1.631 (1.118–2.379)	0.011
Higher Hunt & Hess grade	18.077 (3.456–94.546)	0.001
Higher modified Fisher grade	2.280 (1.162–4.474)	0.017
Higher Hijdra grade	1.437 (1.102–1.874)	0.007
Hyponatremia	0.190 (0.049–0.740)	0.017
Ventriculitis	27.213 (1.180–627.701)	0.039
Antiepileptic drug administration	8.360 (2.350–29.730)	0.001
Unfavorable outcome at follow-up (mRS score 3–6)		
Ischemic vascular disease	188.109 (9.879–3581.984)	<0.001
Higher Hunt & Hess grade	4.384 (1.734–11.085)	0.002
Higher modified Fisher grade	3.392 (1.062–10.838)	0.039
Larger aneurysm size	1.192 (1.005–1.413)	0.043
Rebleeding	62.005 (3.037–1265.997)	0.007

rysms at risk for aSAH, and patients with aSAH who are at risk for developing clinical vasospasm, DCI, and unfavorable functional outcome.

Limitations of the Study

The small sample size is a limitation as larger samples are preferred for association studies. As such, the findings should be interpreted with caution until larger data sets are available for analysis. The study did not assess whether endothelin SNPs altered transcription or the biological character of the proteins encoded. Some SNPs were not in Hardy-Weinberg equilibrium. This may be a result of genetic drift or variation in the relative frequency of different genotypes in a small population due to the chance that particular genes disappear as individuals die or do not reproduce. Certain genotypes may also represent a disadvantage to survive aSAH or result in a decreased risk for cerebral aneurysm formation or rupture. Another limitation is the determination of clinical vasospasm, i.e., its diagnosis is based on the clinical process as a whole, including the

neurological examination and corroborating imaging evidence, and as such is vulnerable to subjective opinion.

Conclusions

Common endothelin SNPs were found to be associated with aSAH and its sequelae. The T allele of the *END1* T/G SNP (rs1800541) was associated with aSAH. The G allele of the *EDNRA* G/C SNP (rs5335) was associated with clinical vasospasm. Further studies are required to elucidate the relevant pathophysiology and its potential implication in the treatment of patients with aSAH.

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Disclosures

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Author Contributions

Conception and design: Griessenauer. Acquisition of data: Griessenauer, Foreman, Vyas. Analysis and interpretation of data: Griessenauer, Starke, Foreman, Lipsky, Lin. Drafting the article: Griessenauer, Starke. Critically revising the article: Griessenauer, Starke, Foreman, Hendrix, Pittet, Mathru. Reviewed submitted version of manuscript: Griessenauer, Foreman, Hendrix. Approved the final version of the manuscript on behalf of all authors: Griessenauer. Statistical analysis: Griessenauer, Starke. Administrative/technical/material support: Griessenauer, Pittet, Mathru. Study supervision: Griessenauer, Harrigan, Fisher, Walters, Pittet, Mathru.

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