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Endothelin-1 and Endothelin Receptor Gene Variants and Their Association With Negative Outcomes Following Aneurysmal Subarachnoid Hemorrhage

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Abstract

Aneurysmal subarachnoid hemorrhage (aSAH) is a devastating disease that affects approximately 30,000 people a year in the United States. Delayed cerebral ischemia (DCI) and cerebral vasospasm (CV) are common complications after aSAH. In addition, aSAH patients have a high risk of poor long-term outcomes. Endothelin-1 (ET-1), a potent vasoconstrictor, or its two types of receptors, ET receptor A (ET_A) and ET receptor B (ET_B), may play a role in the pathogenesis of DCI and CV. Genetic variations within the *ET-1, ET_A*, or *ET_B* genes may also account for variance observed in the outcomes of aSAH patients. The purpose of this study was to describe the distribution of the Lys198Asn polymorphism, a known functional SNP in the *ET-1* gene, and tagging SNPs of the *ET-1, ET_A*, and *ET_B* genes in individuals recovering from aSAH. This study also investigated the relationships among the *ET* polymorphisms, DCI, and global functional outcomes measured at 3 and 6 months after aSAH. Participants included individuals aged 18–75 years with a diagnosis of aSAH. There was a trend found between the variant allele of an *ET-1* SNP (rs6912834) and angiographic vasospasm. There were also associations found between two *ET_B* SNPs (rs9574124 and rs3027111) and poor outcomes as measured by the Glasgow Outcome scale at 3 months. These findings support the role of ET-1 and ET_B in recovery following aSAH.

Keywords

aneurysmal subarachnoid hemorrhage; cerebral vasospasm; delayed cerebral ischemia; endothelin-1; endothelin receptor A; endothelin receptor B; functional outcomes

There are approximately 25,000 cases of subarachnoid hemorrhage (SAH) in the United States each year (Roger et al., 2011), most of which occur in women at an average age of 50 years (Anderson, Anderson, & Bonita, 2000). Ruptured cerebral aneurysms are the cause for 85% of spontaneous SAH cases (van Gijn, Kerr, & Rinkel, 2007). The morbidity and mortality rates of aneurysmal subarachnoid hemorrhage (aSAH) have decreased little over the past 40 years (Stegmayr, Eriksson, & Asplund, 2004) despite the development of new early intervention methods designed to improve survival rates following the initial injury.

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While early intervention has decreased the risk of aneurysmal rebleed, the frequency of secondary injuries has become evident, and the survival rate from aSAH has not improved.

Cerebral vasospasm (CV), a decrease in diameter of the lumen of cerebral blood vessels, is one mechanism that can lead to delayed cerebral ischemia (DCI) following aSAH, furthering neurological damage and possibly causing ischemic stroke. Worse clinical outcomes have been associated with the presence of CV compared to aSAH patients without CV (Goto & Yamagata, 2006; Ratsep & Asser, 2001). CV has been correlated with a 1.5- to 3-fold increase in mortality in the first 2 weeks after aSAH (Treggiari-Venzi et al., 2001). Angiographic vasospasm or arterial vasospasm indicating vascular constriction is present in approximately 70% of aSAH patients (Dorsch, 2002). Approximately 20–30% of these patients display signs of neurological deficit termed symptomatic or clinical vasospasm.

While DCI may be caused by factors such as CV or arterial narrowing, it can also occur in the absence of radiologically confirmed arterial narrowing (Vergouwen et al., 2010). Researchers have investigated numerous possible causal factors for DCI (Cohen & Vanhoutte, 1995; Hatake, Wakabayashi, Kakishita, & Hishida, 1992; Kasuya et al., 1995; Kim, Schini, Sundt, & Vanhoutte, 1992; Onoue et al., 1995; Sobey, Heistad, & Faraci, 1996; Yamamoto, Nishizawa, Yokoyama, Ryu, & Uemura, 1997). Endothelin-1 (ET-1) is a long-lasting vasoconstrictor produced by the endothelial cells of the vasculature (Petzold, Einhaupl, Dirnagl, & Dreier, 2003; Suzuki, Sato, Suzuki, Oka, et al., 1990). Suzuki and associates reported increased levels of ET-1 in the cerebrospinal fluid (CSF) of aSAH patients (Suzuki, Sato, Suzuki, Takekoshi, et al., 1990). Other researchers have found that CSF ET-1 levels are associated with the development of CV after aSAH (Kessler, Pacheco, Lozzi, de Araujo, & Onishi, 2005; Thampatty et al., 2011), thus supporting the role of ET-1 in the pathogenesis of CV and DCI.

Researchers have investigated a number of predictors of DCI, including amount and distribution of blood on computed tomography (CT) scan (categorized by Fisher grade), age, hypertension, cigarette smoking, and cocaine use (Conway & Tamargo, 2001; Qureshi et al., 2001); however, none of these risk factors accurately predict the occurrence of DCI. Determination of genetic susceptibility may allow for more accurate identification of aSAH patients at high risk of poor outcomes, thus allowing for closer monitoring and more aggressive interventions. The purpose of the present study was to describe the distribution of the Lys198Asn polymorphism, a known functional SNP in the *ET-1* gene, and tagging SNPs of the *ET-1,* ET_A, and ET_B genes in individuals recovering from aSAH. We also investigated the relationships among the ET polymorphisms, DCI, and global functional outcomes measured at 3 and 6 months after aSAH.

Materials and Methods

Subjects

Following institutional review board approval and after obtaining informed consent from the patient or next of kin, we recruited 250 patients as part of an ongoing National Institutes of Health (NIH)-funded study (RO1NR004339). Inclusion criteria were adult patients (ages 18–75 years) with the diagnosis of aSAH verified by digital subtraction angiography or CT angiography (CTA) with a Fisher grade > 1 and/or a Hunt and Hess score > 1. We did not exclude any people based on ethnic background in the recruiting process. However, because of known allele frequency distribution difference based on ancestry, we limited our analyses for this project to non-Hispanic Whites in an attempt to address population stratification. We excluded patients from the study if they had a preexisting neurological disorder. We collected a blood sample from each patient to obtain a deoxyribonucleic acid (DNA) sample for analysis.

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Participants were admitted to the neurovascular intensive care unit (NV-ICU) at the University of Pittsburgh Medical Center (UPMC) from September 2000 to April 2006. All received standard nursing and medical care in the NV-ICU, including triple-H therapy (hypertension, hypervolemia, and hemodilution), nimodipine, and early surgical or endovascular intervention. We used this sample of 250 patients to describe the distribution of *ET-1, ET_A*, and *ET_B* polymorphisms in the aSAH population.

Measures

Demographic information—We collected age in years, race, sex, Fisher grade, and Hunt and Hess grade from medical records. The admitting neurosurgeon assigned the Fisher and Hunt and Hess grades.

DCI—For the purpose of this study, we used the definition of DCI presented in Crago et al. (2011), such that, for DCI to occur there must be a neurological deterioration in the presence of impaired cerebral blood flow. We defined neurologic deterioration as a change in level of consciousness, pupil changes, presence of a new focal neurologic deficit, or worsening Glasgow Coma scale or National Institutes of Health Stroke scale (NIHSS) scores. We did not classify neurological deterioration as DCI if the deterioration could be associated with medication administration (e.g., narcotics), hydrocephalus, or any other non-blood-flow causes of deterioration. We assessed cerebral blood flow by cerebral angiography, transcranial Doppler (TCD) and CT or magnetic resonance imaging perfusion scans.

Angiographic vasospasm—Neurosurgeons blinded to participant identity read participants' cerebral angiograms and coded them as either "negative" (0–24% narrowing of cerebral blood vessels) or "positive" (>25% narrowing of cerebral blood vessels).

To determine CV, we examined the following variables: (1) angiographic results, when present and (2) daily TCD ultrasound results (mean systolic velocities >200 and/or Lindegaard ratios >3) accompanied by clinical deterioration as recorded by the nurse (a decrease in the NIHSS score of 2 or more from the previous assessment; Sloan et al., 1989; Spilker et al., 1997). We classified patients as either (1) clinical vasospasm positive—elevated vessel velocities on TCD ultrasound and/or angiographic vasospasm with corresponding clinical deterioration or (2) clinical vasospasm negative—the absence of neurologic deterioration or neurologic deterioration with no changes in cerebral vessel diameter.

Global functional outcomes—We collected the global functional outcome scores at 3 months and 6 months after aneurysm rupture. The Glasgow Outcome scale (GOS), a clinical observation scale, categorizes functional outcomes into five levels, with 1 indicating *death* and 5 indicating *good recovery* (Jennett, 1976). The GOS has wide acceptance and established validity (Jennett & Bond, 1975; Maas, Braakman, Schouten, Minderhoud, & van Zomeren, 1983). The GOS correlates well with severity of illness. Interrater reliability has been reported from 68% to 95% with κ values from .62 to .79 (Gennarelli et al., 1982) and is within the acceptable range when there is adherence to assessment guidelines, administration of a structured interview, and training of examiners (Wilson, Pettigrew, & Teasdale, 1998). We dichomotized scores on the GOS into good outcome (score of 4 to 5) and poor outcome (score of 1 to 3).

We used the Modified Rankin Scale (MRS) as a measure of functional recovery. This scale incorporates mental and physical adaptations to the neurological deficits following a neurological injury. The MRS is easy to use and has been widely adopted for use in stroke trials. Scores range from 0 (*no symptoms at all*) to 6 (*death*). Researchers have reported the

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interobserver κ to be .56 and weighted κ to be .91 (Sulter, Steen, & De Keyser, 1999). We also dichotomized the MRS scores into good outcome (score of 0 to 3) and poor outcome (score of 4 to 6). A trained neuropsychological technician who was blinded to genotype collected both global functional outcome scores.

Mortality—We obtained mortality status from medical records or caregiver reports at 3 and 6 months.

Procedures

DNA specimens—We drew 3 cc of whole blood from subjects within 48 hr of their admission. The tube of blood we delivered to the genetics laboratory had no personal identifiers and was labeled only with the study participant's identification number, date, and time of sample collection. We logged in and centrifuged the sample, removed the white cells, and extracted DNA from the white cells using a simple salting out procedure (Miller, Dykes, & Polesky, 1988). We stored all DNA in 1X TE buffer at 4 °C until genotyping was performed.

Genotyping—We used an ABI Prism® 7000 Sequence Detection System (Applied Bioscience, Carlsbad, CA, USA) to conduct allele discrimination using TaqMan® allele discrimination assays. We classified tagging SNPs of ET-1, ET_A, ET_B, and Lys198Asn into variant positive or variant negative. Variant positive genotypes were either homozygous variant or heterozygous. Variant negative genotypes were homozygous wild type.

Haplotype calculation—We performed haplotype calculation using THESIAS 3.1 software (Tregouet & Garelle, 2007). For the ET_A and ET_B genes, we used all SNPs in the calculation for each gene independently. Because of the number of SNPs for the *ET-1* gene, we used a “rolling haplotype.” For each haplo-type calculation, we used three SNPs, starting at the 3’ end and moving to the 5’ end of the gene. For example, the first calculation was SNP 1, SNP 2, and SNP 3. The second calculation was SNP 2, SNP 3, and SNP 4.

Statistical Analysis

Statistical Package for the Social Sciences (SPSS) version 16.0 was used for all analyses. Values are presented in the tables and text as mean ± *SD*. Differences in demographics based on CV status, DCI, outcome scores, and genotypes were investigated using chi-square or Fisher analysis for categorical variables, and independent *t*-test for continuous variables.

Binary logistic regression was used to evaluate the relationship between genotype and CV or DCI controlling for age and Fisher grade. Outcomes at 3 months and at 6 months were evaluated using multinomial logistic regression. In this analysis, age, Hunt and Hess grade, and CV status were controlled for in the model. A significant difference was defined as a $p \leq .05$.

Results

There were 235 subjects with genotype, demographic, DCI, and functional outcome data available. These subjects were predominantly female ($n = 165$; 70.2%) and all were non-Hispanic Whites. The mean subject age was 53.37 ± 11.23 years. Table 1 presents demographic information in relationship to DCI. There were no significant differences in gender or age between the two groups. However, we did find significant differences between groups when we compared Hunt and Hess grade and Fisher grade. Table 2 presents the genotype distributions. Of the 19 SNPs we investigated, 18 were in Hardy Weinberg equilibrium, with SNP rs1626492 being the one exception. There were no significant

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differences in genotype distribution of the *ET-1, ET_A*, or *ET_B* SNPs for this sample when compared to the general population.

DCI

All 235 subjects had DCI data available for analyses. There were no significant associations between DCI and genotype while controlling for age and Fisher grade. We conducted haplotype analyses on each of the three genes investigated (*ET-1, ET_A*, and *ET_B*). There were no haplotypes that were significantly associated with the occurrence of DCI.

Angiographic CV

There were 146 subjects with angiographic data. The subjects were mostly female ($n = 105$, 71.9%). The mean age of the subjects was 53.62 ± 11.4 years. Of these 146 subjects, 63 (43.2%) had angiographic vasospasm. There was a trend associated with subjects who were variant allele positive for the rs6912834 SNP (*ET-1* gene) to develop angiographic vasospasm ($p = .051$). However, there were no other SNPs associated with angiographic vasospasm. Age and Fisher grade were controlled for in this analysis. There were no haplotypes of the three genes investigated that were significantly associated with the occurrence of DCI.

GOS/MRS

At the 3-month outcome measure, there were 191 subjects with outcome data available. The majority of these subjects were female (134, 70.2%), and their mean age was 53.70 ± 10.9 years. We present the GOS/MRS scores in relation to DCI in Table 3. A total of 33 (17.3%) subjects had a poor outcome as measured by MRS and 37 (19.4%) as measured by GOS. Using binary logistic regression while controlling for age, Hunt and Hess score, and presence of DCI, we found two SNPs with variant alleles associated with a poor score on the GOS: rs9574124 ($p = .014$) and rs3027111 ($p = .015$), both of which are on the ET receptor B gene. When using the MRS to score the subjects, we found that only one of the SNP's (rs3027111; $p = .038$) variant allele was associated with poor outcomes. There were no haplotypes associated with poor outcomes measured by GOS or MRS.

At 6 months post aneurysm rupture, 161 subjects had GOS and MRS data available. These subjects were predominantly female (114, 70.8%) with a mean age of 54.66 ± 10.9 years. Of these, 31 (19.3%) had poor outcomes measured by both the MRS and the GOS instruments (Table 3). No variant alleles of the SNPs were associated with poor outcomes measured at 6 months. There were also no haplotypes that were associated with poor outcomes at this time period.

Mortality

We collected mortality rates at both 3 and 6 months post aneurysm rupture (Table 3). There were 24 deaths (12.6%) at the 3-month outcome data collection and 26 deaths (16.1%) at the 6-month outcome data collection. The variant allele of SNP rs3027111 (*ET_B* gene) was associated with increased risk of death ($p = .018$) at 3 months post rupture when controlling for age, Hunt and Hess Score, and DCI. There was a trend toward association for the variant allele of the rs3027111 SNP and death at 6 months ($p = .058$) when controlling for age, Hunt and Hess Score, and DCI.

Discussion

The major finding of the present study is that there are numerous gene polymorphisms associated with outcomes following aSAH. There was a trend toward association between the minor allele of the rs6912834 SNP (*ET-1* gene) and angiographic vasospasm. Poor

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global functional outcomes measured at 3 months were associated with the minor alleles of the SNPs rs9574124 and rs3027111, both on the *ET_B* gene. Finally, the minor allele of SNP rs3027111 (*ET_B* gene) was associated with a higher risk of mortality at the 3-month outcome measure. All of these findings have clinical relevance. This study is the first to identify minor alleles of the *ET-1* and *ET_B* genes that are associated with poor outcomes following aSAH. In the future, these minor alleles may be used as a screening tool to determine which patients may be at higher risk of poor outcomes. Such a tool will allow nurses and other health care professionals to focus resources on these at-risk patients to improve their outcomes following aSAH.

ET-1 is a well-known, potent vasoconstrictor produced by the endothelial cells of the vasculature (Suzuki, Sato, Suzuki, Oka, et al., 1990; Yanagisawa et al., 1988). Researchers have also implicated ET-1 as a mediator of CV and therefore DCI (Mascia et al., 2001; Seifert, Loffler, Zimmermann, Roux, & Stolke, 1995; Yamaji et al., 1990; Yanagisawa et al., 1988). Suzuki and associates reported increased levels of ET-1 in the CSF of aSAH patients (Suzuki, Sato, Suzuki, Takekoshi, et al., 1990). Recent studies showed that increased CSF ET-1 levels are associated with the development of CV after aSAH (Kessler, Pacheco, Lozzi, de Araujo, Onishi, de Mello, et al., 2005), thus supporting the role of ET-1 in the pathogenesis of CV.

Polymorphisms in the *ET-1* gene have been investigated in numerous vascular conditions, including essential hypertension (Stevens & Brown, 1995), preeclamptic pregnancy (Barden et al., 2001), cerebral small vessel disease (Gormley, Bevan, Hassan, & Markus, 2005), and arterial stiffness (Iemitsu et al., 2006), to name a few. A functional polymorphism (Lys198Asn) of the gene encoding ET-1 has shown differences in vasculature reactivity (Iglarz et al., 2002). The increase in vasculature reactivity caused by this polymorphism may have an effect on the development or severity of DCI, thereby influencing long-term outcomes in aSAH. Along with this known functional polymorphism, other polymorphisms in the gene for ET-1 may influence DCI and long-term outcomes. It is also important to investigate the genetic variability associated with the receptor, *ET_A* and *ET_B*, polymorphisms as they moderate the effects of ET-1 in the vasculature. The gene that encodes for ET_A is located on Chromosome 4. In recent studies, polymorphisms of this receptor have been associated with increased diastolic blood pressure in subjects with essential hypertension (Benjafield, Katyk, & Morris, 2003; Stevens & Brown, 1995). *ET_A* polymorphisms have also been associated with pulse pressure (Nicaud et al., 1999) and prediction of survival in patients with idiopathic dilated cardiomyopathy (Herrmann et al., 2001). The gene for ET_B is located on Chromosome 13.

By exploring all of the tagging SNPs of *ET-1, ET_A*, and *ET_B*, it is possible to account for genetic variance in each of the genes, including potentially functional polymorphisms. To date, there are no published studies investigating *ET-1, ET_A*, or *ET_B* tagging SNPs or functional SNPs and their associations with DCI or outcomes following aSAH.

The distribution of the genotypes for the sample of 253 non-Hispanic White subjects in the present study was not significantly different when compared to the HAP-MAP database of the general non-Hispanic White population. From this finding, we conclude that genetic variation in *ET-1, ET_A*, or *ET_B* plays no role in aneurysm formation, development, or rupture. If these genes were involved in aneurysm formation or rupture, we would have expected the genotype distribution to be different in this sample when compared to the general population. There was one SNP that was not in Hardy Weinberg equilibrium. This SNP, rs1626492 (*ET-1* gene), may not be in equilibrium for numerous reasons. It may appear to be in genetic drift because of the small number in this sample. Carriers of this SNP

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may also be at a disadvantage for survival of the original bleed following aneurysm rupture or may be at decreased risk of aneurysm development/rupture.

The genotype distribution of the subset of 191 subjects with 3-month outcome data had a similar distribution to that of the general population. The minor allele of the SNP rs3027111 (*ET_B* gene) was associated with poor outcomes when measured by both the GOS and the MRS. However, the variant allele for SNP rs9574124 (*ET_B* gene) was only associated with a poor outcome when measured by the GOS. This discrepancy is likely due to the difference in sensitivity between the two scales. Subjects with the variant allele for rs3027111 were 3.637 times (95% confidence interval [CI] [1.077, 12.284]) more likely to have a poor outcome when measured by the MRS at 3 months post aneurysm rupture than those without the allele. When outcome was measured by GOS, the subjects were 4.485 times (95% CI [1.346, 14.948]) more likely to have a poor outcome. Subjects with the rs9574124 variant allele were 3.074 times (95% CI = 1.261 to 7.496) more likely to have a poor outcome measured by the GOS at 3 months following aneurysm rupture than those without the allele.

Contrary to our initial hypothesis, our findings show that there were no significant associations between genotype and DCI. DCI is a complicated phenomenon. *ET-1* and the ET receptor genes are mainly involved in vasoconstriction. Therefore, it is more likely that these genes would be involved in angiographic CV. Many of our subjects with angiographic CV did not show clinical signs of CV. Therefore, there are other factors (genes/proteins) influencing DCI. Some subjects with angiographic CV are able to compensate through other mechanisms, allowing them to show no signs of DCI, while others do not have the ability to compensate and therefore show signs of DCI.

The genotype distribution of the subset of 146 subjects with angiographic data was also similar to that of the general population. The functional SNP (Lys198Asn) had no association with angiographic CV after aSAH. Therefore, the difference in vascular response to ET-1, as reported by Iglarz et al. (2002), does not appear to play a part in the etiology of CV. After controlling for age and Fisher score, we found a trend for an association between the presence of the variant allele of the tagging SNP rs6912834 (*ET-1* gene) and angiographic CV. Subjects with the variant allele were 2.714 times (C.I. = 0.994 to 7.410) more likely to have angiographic vasospasm when compared to subjects without a variant allele. This genotype association supports the role of *ET-1* in the development of angiographic CV following aSAH and the proposition that the gene product associated with the variant allele of the rs6912834 SNP may lead to more potent vasoconstriction. While the SNP rs6912834 is within an intron of the *ET-1* gene and has no known direct effect on the mature protein, the region of the *ET-1* gene that it tags extends to regions of the gene that may affect the mature ET-1 protein. The lack of association in the present study between *ET_A* and *ET_B* with angiographic CV does not rule out the possibility that these genes play a role in CV.

The genotype distribution of the subset of 161 subjects with 6-month outcome data was similar to that of the general population. We found no significant associations between the *ET-1, ET_A,* or *ET_B* genes and functional outcomes measured at 6 months.

Long-term recovery from aSAH is complex and multifactorial. Numerous genes are involved in recovery and cell repair. The *ET_B* receptor gene is one of the genes that are involved in recovery within the first 3 months of aneurysm rupture. However, after that time period, it appears that *ET_B* is not as important in functional recovery. Our findings lead to the conclusion that the tagging SNPs of the *ET-1* and *ET_A* genes are most likely not major direct contributors to long-term recovery after aSAH. However, as Thampatty et al. (2011)

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showed in a previous study, ET-1 protein levels in the CSF are associated with outcomes following aSAH.

Finally, the rs3027111 (ET_B gene) variant allele was associated with a higher risk of mortality at the 3-month outcome measure. These subjects were 13.601 times (95% CI [1.550, 119.338]) more likely to die within the first 3 months following aneurysm rupture than those without the allele. There was a trend for this SNP to be associated with increased risk of mortality at the 6-month outcome measurement as well ($p = .058$). These subjects were 4.137 times (95% CI [0.955, 17.918]) more likely to die within the first 6 months following aneurysm rupture. This finding supports the hypothesis that the ET_B gene plays a part in mortality following aSAH.

One of the limitations to this study was small sample size. A larger sample size is preferred in association studies. We continue to recruit at the study site to increase the number of subjects with aSAH. A second limitation is the lack of minorities in our sample. We did not present minority subjects in this study because the size of the minority sample available to us would make it a very underpowered analysis. The minority population is small in the Pittsburgh region and therefore our minority sample is less than 10% of the overall aSAH population. In the future, as we are able to recruit a larger number of minorities, we will be able to report the genetic characteristics of the *ET-1* SNPs for this aSAH population.

Another limitation is that patients with a cerebral angiography may have had this procedure due to a worsening in their clinical assessment (e.g., change in level of consciousness). By including patients that have a need for angiography, we skew the sample toward subjects that are in a more critical state. Patients that were in a less critical state may have differences in their genotype distributions when compared to the more critical patients. Such differences in genotype distribution may account for differences in the overall state of the patients. In addition, we were only able to include patients that had follow-up visits with the neuropsychological technician. In the overall study population, 21% of patients were not available for 3-month follow-up and 24% were not available for 6-month follow-up; thus we could not include them in this sample.

In summary, we found that the variant allele of tagging SNP rs6912834 trended toward an association with an increased incidence of CV following aSAH. This finding suggests that the ET-1 protein does play a role in the development of CV after aSAH. Further investigation is needed to find the functional SNP that is within the region of the *ET-1* gene represented by that tagging SNP. In addition, two SNPs of the ET_B gene were associated with poor outcomes at 3 months after aneurysm rupture, suggesting that ET_B plays a role in both functional outcomes and mortality after aSAH. These findings are of high clinical significance. Using these alleles as a screening tool to identify patients who are at higher risk of death and other poor outcomes may allow nurses and other health professionals to focus resources on these at-risk patients, thus improving these outcomes.

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Table 1

Demographic Characteristics of Subjects With Outcomes Measurement Categorized by Delayed Cerebral Ischemia (DCI; $N = 235$)

Characteristic	DCI negative ($n = 122$)	DCI positive ($n = 113$)	p value
Sex, female (n [%])	86 (70.5%)	79 (69.9%)	.924
Age (mean [SD])	53.2 (10.9)	53.55 (11.6)	.811
Hunt and Hess grade (n [%])			.007 *
1	23 (18.9%)	13 (11.5%)	
2	46 (37.7%)	24 (21.2%)	
3	37 (30.4%)	48 (42.5%)	
4	12 (9.8%)	20 (17.7%)	
5	4 (3.2%)	8 (7.1%)	
Fisher grade (n [%])			.002 *
1	0 (0%)	0 (0%)	
2	49 (40.2%)	25 (22.1%)	
3	61 (50.0%)	63 (55.8%)	
4	12 (9.8%)	25 (22.1%)	

* p values considered significant at $p \leq .05$.

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Table 2

Genotype Distribution

	Genotype			
Gene and SNPs	**Homozygous wild-type**	**Homozygous variant**	**Heterozygous**	**HW *p* value**
Endothelin-1				
rs5370	145 (G/G)	14 (T/T)	74 (G/T)	.257
rs2071943	144 (G/G)	16 (A/A)	73 (A/G)	.111
rs1626492	114 (G/G)	12 (A/A)	107 (A/G)	<.001 *
rs5369	174 (G/G)	5 (A/A)	54 (A/G)	.769
rs1476046	138 (G/G)	15 (A/A)	79 (A/G)	.265
rs2070699	72 (G/G)	45 (T/T)	117 (G/T)	.944
rs6912834	182 (A/A)	2 (G/G)	49 (G/T)	.410
rs3087459	153 (A/A)	5 (C/C)	75 (A/C)	.294
rs1800541	154 (T/T)	5 (G/G)	73 (G/T)	.357
Endothelin receptor Type A				
rs6841799	115 (C/C)	14 (G/G)	100 (C/G)	.239
rs5342	90 (A/A)	30 (G/G)	111 (A/G)	.695
rs10305860	112 (G/G)	14 (A/A)	102 (A/G)	.106
rs6537484	85 (C/C)	25 (G/G)	116 (C/G)	.081
rs6812093	115 (A/A)	14 (T/T)	102 (A/T)	.195
rs10008744	126 (A/A)	11 (C/C)	91 (A/C)	.345
Endothelin receptor Type B				
rs9574124	114 (C/C)	24 (C/G)	93 (G/G)	.678
rs4885493	129 (C/C)	14 (G/G)	73 (C/G)	.457
rs4885491	171 (G/G)	3 (A/A)	48 (A/G)	.767
rs3027111	154 (C/C)	5 (T/T)	61 (C/T)	.599

Note. HW = Hardy Weinberg equilibrium.

* Significant (i.e., not in Hardy Weinberg equilibrium).

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Table 3

Glasgow Outcome Scale (GOS) and Modified Rankin Scale (MRS) scores and Mortality by Delayed Cerebral Ischemia (DCI; $N = 235$)

Outcome variable	DCI negative *n* (%)	DCI positive *n* (%)	*p* value
GOS at 3 months			.011*
“Good” outcome	90 (87.4%)	64 (72.7%)	
“Bad” outcome	13 (12.6%)	24 (27.3%)	
MRS at 3 months			.026*
“Good” outcome	91 (88.3%)	67 (76.1%)	
“Bad” outcome	12 (11.7%)	21 (23.9%)	
Mortality at 3 months			.084
Alive	94 (91.3%)	73 (83.0%)	
Dead	9 (8.7%)	15 (17.0%)	
GOS at 6 months			.014*
“Good” outcome	78 (87.6%)	52 (72.2%)	
“Bad” Outcome	11 (12.4%)	20 (27.8%)	
MRS at 6 months			.014*
“Good” outcome	78 (87.6%)	52 (72.2%)	
“Bad” outcome	11 (12.4%)	20 (27.8%)	
Mortality at 6 months			.060*
Alive	79 (88.8%)	56 (77.8%)	
Dead	10 (11.2%)	16 (22.2%)	

*Significant at $p < .05$.