Association of cystathionine beta-synthase polymorphisms and aneurysmal subarachnoid hemorrhage

Philipp Hendrix, MD,1 Paul M. Foreman, MD,2 Mark R. Harrigan, MD,2 Winfield S. Fisher III, MD,2 Nilesh A. Vyas, MD,3 Robert H. Lipsky, PhD,3,4 Mingkuan Lin, PhD,4 Beverly C. Walters, MD, MSc, FRCSC,2-4 R. Shane Tubbs, PhD,5 Mohammadali M. Shoja, MD,6 Jean-Francois Pittet, MD,7 Mali Mathru, MD,7 and Christoph J. Griessenauer, MD1,9

1Department of Neurosurgery, Saarland University Medical Center and Saarland University Faculty of Medicine, Homburg/Saar, Germany; 2Department of Neurosurgery, University of Alabama at Birmingham, Alabama; 3Department of Neurosciences, Inova Health System, Falls Church; 4Department of Molecular Neuroscience, George Mason University, Fairfax, Virginia; 5Seattle Science Foundation, Seattle, Washington; 6Neurosurgery Research Center, Tabriz University of Medical Sciences, Tabriz, Iran; 7Department of Anesthesiology, University of Alabama at Birmingham, Alabama; 8Neurosurgical Service, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and 9Department of Neurosurgery, Geisinger Health System, Danville, Pennsylvania

OBJECTIVE Cystathionine beta-synthase (CBS) is involved in homocysteine and hydrogen sulfide (H2S) metabolism. Both products have been implicated in the pathophysiology of cerebrovascular diseases. The impact of CBS polymorphisms on aneurysmal subarachnoid hemorrhage (aSAH) and its clinical sequelae is poorly understood.

METHODS Blood samples from all patients enrolled in the CARAS (Cerebral Aneurysm Renin Angiotensin System) study were used for genetic evaluation. The CARAS study prospectively enrolled aSAH patients at 2 academic institutions in the United States from 2012 to 2015. Common CBS polymorphisms were detected using 5′exonuclease genotyping assays. Analysis of associations between CBS polymorphisms and aSAH was performed.

RESULTS Samples from 149 aSAH patients and 50 controls were available for analysis. In multivariate logistic regression analysis, the insertion allele of the 844ins68 CBS insertion polymorphism showed a dominant effect on aSAH. The GG genotype of the CBS G/A single nucleotide polymorphism (rs234706) was independently associated with unfavorable functional outcome (modified Rankin Scale Score 3–6) at discharge and last follow-up, but not clinical vasospasm or delayed cerebral ischemia (DCI).

CONCLUSIONS The insertion allele of the 844ins68 CBS insertion polymorphism was independently associated with aSAH while the GG genotype of rs234706 was associated with an unfavorable outcome both at discharge and last follow-up. Increased CBS activity may exert its neuroprotective effects through alteration of H2S levels, and independent of clinical vasospasm and DCI.

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KEY WORDS cystathionine beta-synthase; polymorphism; aneurysm; subarachnoid hemorrhage; outcome; vascular disorders

ABBREVIATIONS ADC = apparent diffusion coefficient; aSAH = aneurysmal subarachnoid hemorrhage; CARAS = Cerebral Aneurysm Renin Angiotensin System; CBS = cystathionine beta-synthase; DCI = delayed cerebral ischemia; mRS = modified Rankin Scale; SNP = single nucleotide polymorphism.


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thesis and plays a role in angiogenesis and functions as a neuromodulator. Following aSAH, H$_2$S appears protective against secondary brain injury. Additionally, there is evidence that H$_2$S is involved in collagen metabolism and regulation of the vascular wall. Hydrogen sulfide treatment induces angiogenesis after cerebral ischemia and may be of value in regenerative recovery after stroke.

Genetic polymorphisms of the genes encoding CBS may be associated with variable enzyme activity resulting in altered homocysteine and H$_2$S levels and may play a role in the pathogenesis of cerebrovascular disease. We report a prospective study of patients with aneurysmal subarachnoid hemorrhage (aSAH) to investigate the role of CBS polymorphisms in aSAH.

Methods

Blood samples from all aSAH patients and controls enrolled in the CARAS (Cerebral Aneurysm Renin Angiotensin System) study were used for genetic evaluation as previously described. The CARAS study prospectively enrolled aSAH patients at 2 academic institutions in the US from 2012 to 2015. Aneurysmal subarachnoid hemorrhage patients were treated in accordance with guidelines for the management of aSAH. The control group was composed of trauma patients, age ≥ 19 years, 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. Both aSAH patients and controls were enrolled within 72 hours of admission. Institutional review board approval was obtained at both institutions and written informed consent was obtained from individual participants or their proxy.

Definition of Clinical Vasospasm and Delayed Cerebral Ischemia

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. CT angiography, digital subtraction angiography, and transcranial Doppler ultrasound examinations 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 first line. Hyperdynamic therapy included avoidance of hypovolemia with a goal systolic blood pressure greater 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 scans or MRI were routinely performed when the patient was transferred from the intensive care unit to the ward with the purpose of imaging patients who receive permanent CSF diversion prior to transfer, to rule out occult hydrocephalus, and look for clinically silent cerebral infarction. Delayed cerebral ischemia (DCI) was defined as low-density areas on CT or an MRI study demonstrating a hyperintense area on a diffusion weighted imaging sequence with a corresponding hypointense apparent diffusion coefficient (ADC) sequence that corresponded with a vascular territory. Infarctions or contusion seen on post-operative Day 1 imaging were considered procedurally related and were not considered DCI.

Functional Outcome Assessment

Functional outcome was recorded 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.

Laboratory and Genetic Evaluation

Common CBS polymorphisms (Table 1) were detected using 5’ exonuclease (Taqman) genotyping assays [844ins68 CBS insertion polymorphism W>I, CBS 699 G>A (c.699C>T) single nucleotide polymorphism (SNP) (rs234706), and CBS 1080 C>T SNP (rs1801181)]. 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 mean ± standard deviation, and categorical variables are presented as frequency and percentage. Analyses were carried out using the unpaired Wilcoxon rank-sum test, Student t-test, chi-square test, and Fisher exact test, as appropriate. Patient characteristics and CBS polymorphisms were tested in univariable analysis to determine predictors of aSAH. Patient and aneurysm characteristics and CBS polymorphisms were tested in univariable analysis to determine predictors of the following dependent variables: clinical vasospasm, DCI, unfavorable functional outcome at discharge (mRS Score 3–6) and upon follow-up. Factors predictive in univariable analysis (p < 0.15) were entered into a multivariable logistic regression analysis. A p value ≤ 0.05 was considered statistically significant.

Results

Patient and Control Characteristics

Blood samples from 149 aSAH patients and 50 controls were analyzed. The mean ages of aSAH patients and controls were 54.9 ± 12.5 and 50.6 ± 18.6 years, respectively.

<table>
<thead>
<tr>
<th>TABLE 1. Common CBS polymorphisms</th>
</tr>
</thead>
<tbody>
<tr>
<td>CBS Polymorphism</td>
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<tr>
<td>------------------</td>
</tr>
<tr>
<td>844ins68</td>
</tr>
<tr>
<td>rs234706 (699G&gt;A) (c.699C&gt;T)</td>
</tr>
<tr>
<td>rs1801181 (1080C&gt;T)</td>
</tr>
</tbody>
</table>

*= Heterozygote insertion frequency from Tsai et al.† From http://useast.ensembl.org/Homo_sapiens/Info/Index.
Cystathionine β-synthase polymorphisms and aSAH

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Sex was evenly distributed between the two groups (p = 0.086). 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%). The Hunt and Hess grade was I–III in 77.2% of cases and the modified Fisher CT grade was 1–2 in 49.2%. Clinical vasospasm and DCI occurred in 22.8% and 21.2%, respectively. Favorable functional outcome (mRS Score 0–2) at hospital discharge and last follow-up was achieved in 47.7% and 61.7% of patients, respectively (Table 2).

Association of CBS Polymorphisms and aSAH

All CBS polymorphisms were in Hardy-Weinberg equilibrium in patients and controls with the exception of rs1801181 in aSAH patients (p = 0.009) (Table 3). In multivariate logistic regression analysis, the insertion allele of the 844ins68 CBS insertion polymorphism showed a dominant effect on aSAH (OR 3.046, 95% CI 1.049–8.846, p = 0.041) in a model that included age, race, and antithrombotic use (Table 4).

Association of CBS Polymorphisms and Clinical Course After aSAH

In multivariate logistic regression analysis, the GG genotype of rs234706 was associated with an unfavorable outcome at discharge (OR 5.295, 95% CI 1.571–17.840, p = 0.007). Other predictors of an unfavorable functional outcome (p = 0.07). Sex was evenly distributed between the two groups (p = 0.086). 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%). The Hunt and Hess grade was I–III in 77.2% of cases and the modified Fisher CT grade was 1–2 in 49.2%. Clinical vasospasm and DCI occurred in 22.8% and 21.2%, respectively. Favorable functional outcome (mRS Score 0–2) at hospital discharge and last follow-up was achieved in 47.7% and 61.7% of patients, respectively (Table 2).

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TABLE 3. Genotype frequencies of CBS polymorphisms

<table>
<thead>
<tr>
<th>Polymorphism &amp; Genotype</th>
<th>aSAH Pts</th>
<th>Controls</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>844ins68*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WW</td>
<td>95</td>
<td>44</td>
<td>0.004</td>
</tr>
<tr>
<td>WI</td>
<td>43</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>7</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>p = 0.462</td>
<td>p = 0.707</td>
<td></td>
</tr>
<tr>
<td>rs234706†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GG</td>
<td>74</td>
<td>24</td>
<td>0.926</td>
</tr>
<tr>
<td>AG</td>
<td>59</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>AA</td>
<td>14</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>p = 0.655</td>
<td>p = 0.842</td>
<td></td>
</tr>
<tr>
<td>rs1801181†</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CC</td>
<td>73</td>
<td>20</td>
<td>0.562</td>
</tr>
<tr>
<td>CT</td>
<td>51</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>TT</td>
<td>23</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>HWE</td>
<td>p = 0.009</td>
<td>p = 0.326</td>
<td></td>
</tr>
</tbody>
</table>

HWE = Hardy-Weinberg equilibrium; pts = patients.
* Alleles were not available for 4 aSAH patients and 1 control.
† Alleles were not available for 2 aSAH patients and 1 control.

outcome at discharge were days for symptom onset to admission (OR 1.377, 95% CI 1.025–1.851, p = 0.034), higher Hunt and Hess grade (OR 2.798, 95% CI 1.397–5.604, p = 0.004), higher modified Fisher CT grade (OR 2.304, 95% CI 1.293–4.107, p = 0.005), and antiepileptic drug administration (OR 4.419, 95% CI 1.329–14.697, p = 0.015). Hyponatremia was protective in the model (OR 0.202, 95% CI 0.062–0.658, p = 0.008) (Table 5).

The GG genotype of rs234706 was also associated with an unfavorable outcome at last follow-up (OR 3.008, 95% CI 1.062–0.658, p = 0.008) (Table 5).

None of the CBS polymorphisms were associated with clinical vasospasm, DCI, or aneurysm rebleeding.

Discussion

Cystathionine β-synthase polymorphisms appear to play a role in the pathophysiology of aSAH and its clinical sequelae. The insertion allele of the 844ins68 CBS insertion polymorphism was associated with aSAH, while the GG genotype of rs234706 was associated with an unfavorable outcome both at discharge and last follow-up.

Cystathionine β-Synthase and aSAH

The insertion allele of the 844ins68 CBS insertion polymorphism was found to be significantly associated with aSAH. In North America, 11.7% of the population is heterozygous for 844ins68. The frequency of the insertion allele of 844ins68 ranges between 5% and 40% among different ethnicities worldwide. While the insertion allele of 844ins68 has been linked to a gain-of-function of the CBS enzyme, others have reported that this polymorphism had no effect on plasma homocysteine levels. Cystathionine β-synthase also catalyzes the formation of H2S. Endogenous H2S, as well as carbon monoxide (CO) and nitric oxide (NO), play a role in pulmonary artery collagen remodeling in rats with high pulmonary blood flow. Hydrogen sulfide also has a regulatory effect on vascular collagen content in hypertensive rats. Polymorphisms of CBS may be associated with altered H2S levels that subsequently impact collagen metabolism in cerebral vessels. This alteration in the composition of the blood vessel may contribute to aneurysm formation and aSAH.

CBS and Clinical Course After Subarachnoid Hemorrhage

Grobelny et al. found the insertion allele of the 844ins68 CBS polymorphism to be protective against DCI following aSAH, while the TT genotype of rs1801181 was associated with an increased risk of DCI. Collectively, they hypothesized that gain-of-function CBS polymorphisms increased H2S, which subsequently protects against DCI. However, they also observed a trend for an increased risk for angiographic cerebral vasospasm associated with CBS gain-of-function polymorphisms. Hence, they concluded that the protective effect against DCI is independent of the protective effect against large-vessel cerebral vasospasm.

In the present study, the GG genotype of rs234706 was associated with an unfavorable outcome both at discharge and at last follow-up. This accounts not only for an increased risk for DCI but also for an increased risk of an unfavorable outcome at discharge and follow-up (Fig. 1). Grobelny et al. hypothesized that the protective mechanism of gain-of-function polymorphisms lies in the production of H2S. Recent findings by Cui et al. support this hypothesis. In a mouse model, aSAH caused decreased H2S and CBS levels that were restored following administration of exogenous NaHS, which serves as an H2S donor. NaHS administration increased blood-brain barrier integrity, restored plasma inflammatory cytokine levels, and attenuated apoptosis and cerebral vasospasm. Li et al. also reported neuroprotective effects of H2S administration in a rat aSAH model. Administration of NaHS after SAH decreased mortality, attenuated brain edema, decreased neuronal cell death, reduced neurological deficits, and improved neurobehavioral outcome. Despite promising findings in aSAH, H2S was found to actually have deleterious effects in acute ischemic stroke. In a study by Wong et al. involving a rat stroke model, endogenous H2S was elevated following stroke and cysteine administration increased infarct volume, potentially by increasing H2S. This effect was abolished by application of a CBS inhibitor. Exogenous administration of NaHS significantly increased cerebral ischemic damage. Chemically, H2S is a.
gaseous transmitter that is difficult to measure and acts on vascular smooth-muscle cells, resulting in vasodilatation similar to NO and CO. Hydrogen sulfide has been shown to have both vasodilating and vasoconstricting effects depending on type of vessel, endothelial function, and H$_2$S concentration. The interplay of gaseous transmitters and their functions remains poorly understood. Thus, we cannot draw final conclusions on how H$_2$S alters the clinical course of patients who suffered from aSAH. However, common CBS polymorphisms may result in alterations of homocysteine and particularly hydrogen sulfide (H$_2$S) levels that affect the clinical course.

**Limitations**

This study’s small sample size is a limitation as larger samples are preferred for association studies. As such, findings should be interpreted with caution until larger data sets are available for analysis. Race distribution was

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

<table>
<thead>
<tr>
<th>Outcome &amp; Variable</th>
<th>OR (95% CI)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Unfavorable outcome at discharge (mRS Score 3–6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days for symptom onset to admission</td>
<td>1.377 (1.025–1.851)</td>
<td>0.034</td>
</tr>
<tr>
<td>Higher Hunt &amp; Hess grade</td>
<td>2.798 (1.397–5.604)</td>
<td>0.004</td>
</tr>
<tr>
<td>Higher modified Fisher CT grade</td>
<td>2.304 (1.293–4.107)</td>
<td>0.005</td>
</tr>
<tr>
<td>Higher Hijdra grade</td>
<td>1.405 (1.067–1.850)</td>
<td>0.016</td>
</tr>
<tr>
<td>Hyponatremia</td>
<td>0.202 (0.062–0.658)</td>
<td>0.008</td>
</tr>
<tr>
<td>AED administration</td>
<td>4.419 (1.329–14.697)</td>
<td>0.015</td>
</tr>
<tr>
<td>GG vs GA &amp; AA genotype (rs234706)</td>
<td>5.295 (1.571–17.840)</td>
<td>0.007</td>
</tr>
<tr>
<td><strong>Unfavorable outcome at follow-up (mRS Score 3–6)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DCI</td>
<td>4.436 (1.608–12.238)</td>
<td>0.004</td>
</tr>
<tr>
<td>Higher Hunt &amp; Hess grade</td>
<td>2.772 (1.732–4.436)</td>
<td>0.000</td>
</tr>
<tr>
<td>Posterior location</td>
<td>4.619 (1.631–13.078)</td>
<td>0.004</td>
</tr>
<tr>
<td>GG vs GA &amp; AA genotype (rs234706)</td>
<td>3.008 (1.243–7.276)</td>
<td>0.015</td>
</tr>
</tbody>
</table>

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**FIG. 1.** Proposed mechanism of CBS activity in aSAH.
not well matched between aSAH patients and controls. The association of the insertion allele of the 844ins68 CBS insertion polymorphism with aSAH, however, remained significant in a multivariable regression model that included race. The study did not assess whether common CBS polymorphisms altered transcription or the biological character of the proteins encoded. Neither substrates nor products of the herein assessed CBS polymorphisms such as homocysteine or H$_2$S were measured. One SNP was 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 survival disadvantage following aSAH or result in a decreased risk for cerebral aneurysm formation or rupture.

**Conclusions**

The insertion allele of the 844ins68 CBS insertion polymorphism was independently associated with aSAH. This gain-of-function polymorphism is hypothesized to influence collagen metabolism, predisposing to aneurysm formation and rupture. The GG genotype of rs234706 was associated with an unfavorable outcome both at discharge and last follow-up, potentially reflecting a role of H$_2$S in neuroprotection following aSAH.

**Acknowledgments**

We would like to thank the participants in this study and the efforts of the neurosurgical research coordinators at Inova Health System for their work and contribution to the CARAS Study. We would also like to thank the Department of Anesthesiology at the University of Alabama at Birmingham, the Brain Aneurysm Foundation, and the family of Timothy P. Susco for their generous support of the present study.

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**Disclosures**

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

**Author Contributions**


**Correspondence**

Christoph J. Griessenauer, Beth Israel Deaconess Medical Center, Harvard Medical School, 110 Francis St., Ste. 3B, Boston, MA 02215. email: christoph.griessenauer@gmail.com.