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Genetic variation in soluble epoxide hydrolase is associated with outcome after aneurysmal subarachnoid hemorrhage

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Abstract

Object—Patients with aneurysmal subarachnoid hemorrhage (SAH) are at high risk for delayed cerebral ischemia (DCI) and stroke. Epoxyeicosatrienoic acids (EETs) play an important role in cerebral blood flow regulation and neuroprotection after brain injury. Polymorphisms in the gene for the enzyme soluble epoxide hydrolase (sEH), which inactivates EETs, are associated with ischemic stroke risk and neuronal survival after ischemia. In this prospective observational study of patients with SAH we compare vital and neurologic outcomes based on functional polymorphisms of sEH.

Methods—Allelic discrimination based on quantitative real-time PCR was used to differentiate wild type (WT) sEH from K55R heterozygotes (predictive of increased sEH activity and reduced EETs) and R287Q heterozygotes (predictive of decreased sEH activity and increased EETs). The primary outcome was new stroke after SAH. Secondary outcomes were mortality, Glasgow outcome scale (GOS) score and neurologic deterioration attributable to delayed cerebral ischemia (DCI).

Results—Multivariable logistic regression models adjusted for admission age and Glasgow coma scale revealed an increase in the odds of new stroke (OR 5.48 (1.51–19.91) and mortality (OR 7.62 (1.19–48.7) in the K55R group, but no change in the odds of new stroke 0.56 (0.16–1.96) or death 3.09 (0.51–18.52) in patients with R287Q genotype, compared to wild-type sEH. R287Q genotype was associated with reduced odds of having a GOS ≤ 3 (0.23 (0.06–0.82)). There were no significant differences in the odds of neurologic deterioration due to DCI.

Conclusions—Genetic polymorphisms of sEH are associated with neurologic and vital outcomes after aneurysmal subarachnoid hemorrhage.

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Disclosures/Conflict of Interest

None

Keywords

soluble epoxide hydrolase; epoxyeicosatrienoic acids; subarachnoid hemorrhage

Introduction

Pathologic alterations in cerebral blood flow are common after subarachnoid hemorrhage (SAH), and often manifest as delayed cerebral ischemia (DCI).[29] The incidence of DCI is highest in the first two weeks after the initial hemorrhage, and may lead to cerebral infarction or poor outcome.[21, 23] DCI after SAH has a broad pathogenesis and few therapies have demonstrated benefit in its prevention or treatment.[24, 29]

Recent work has focused on the role of cytochrome p450 metabolites of arachadonic acid, epoxyeicosatrienoic acids (EETs), in neuroprotection and regulation of cerebral blood flow (CBF) after brain injury. EETs may have properties that mitigate the risk of DCI after SAH such as promotion of cerebral vasodilation and inhibition of inflammation.[7, 11, 15, 17] EETs are synthesized by P450 epoxygenase and metabolized primarily by the enzyme soluble epoxide hydrolase (sEH).[26]

Pharmacologic inhibitors of sEH have demonstrated success in reducing infarct size after stroke in animal models.[33, 34] Several polymorphisms that alter the structure and function of the enzyme have also been identified in the gene responsible for sEH transcription, designated EPHX2.[25, 27] Overall, 26 different EPHX2 variants have been identified, which population studies have reported frequencies up to 20% for the most common polymorphisms.[9] One variant conferring increased sEH activity and inactivation of EETs, known as K55R, has been linked to hypertension, ischemic stroke and coronary artery disease.[6, 10, 19] Another form, R287Q, results in reduced sEH activity and potentially higher EETs levels. This variant has been associated with equivocal ischemic stroke risk but enhanced neuronal survival after ischemic injury in vitro.[10, 16, 32]

It is unknown how genetic variation in sEH is associated with the risk of DCI, stroke or death in patients with SAH. In the present study, we compare two common genetic variants to wild-type sEH and determine the association between sEH variants and outcome in patients with subarachnoid hemorrhage.

Methods

Patient population

Adult patients with aneurysmal SAH on cerebral angiography between December 1, 2008 and November 1 2013 were recruited from the Neurosciences Intensive Care Unit at Oregon Health & Science University (OHSU), a 572-bed stroke referral center in Portland, OR. Patients were excluded if they were pregnant, had non-aneurysmal or spontaneous subarachnoid hemorrhage, or had severe preexisting neurologic or cardiac disease. Patients who died in the first 72 hours of their ICU stay were not enrolled. The study was approved by the Institutional Review Board and informed consent was obtained. Patients were co-

managed by neurosurgeons and neuro-intensivists based on established guidelines for the management of SAH.[4]

Genotyping

We selected two of the most common EPHX2 polymorphisms, K55R and R287Q for analysis based on prevalence reported in previous studies, a documented effect on sEH enzyme activity, and previous associations with disease states.[6, 19, 32] Blood samples were collected at the time of informed consent. DNA was extracted and purified from peripheral leukocytes using the QIAamp DNA Micro Kit protocol (Qiagen). Allelic discrimination of EPHX2 was performed by TaqMan amplification (Applied Biosystems) using a PRISM 7000 sequence detection system (Applied Biosystems). Following quantitative PCR (qPCR) allelic discrimination, 10% of the DNA samples were sequenced for verification of process integrity and quality control. All sequenced samples corresponded to specific alleles found with the qPCR allelic discrimination method.

Data collection

Baseline demographic and physiologic data were retrieved from electronic medical records. The simple acute physiology score (SAPS II) was calculated from the worst laboratory values in the first 24 hours following admission.[18] Stress cardiomyopathy was defined as wall motion abnormalities in a nonvascular distribution diagnosed on 2D echocardiography with a documented resolution during the hospital course.[14] Neurogenic fever was defined as a body temperature greater than 38.3°C documented on at least one measurement during the first 72 hours of the hospital stay without documented infection.[22] Development of acute respiratory distress syndrome (ARDS), cerebral salt wasting (CSW) or syndrome of inappropriate secretion of anti-diuretic hormone (SIADH) were determined from diagnoses in progress notes and were not based on direct evaluation of objective data.

Transcranial Doppler (TCD) and angiographic vasospasm data are reported as positive if maximum middle cerebral artery (MCA) velocity on TCD was greater than or equal to 200mL/sec and/or Lindegaard ratio >3.0 and as any degree of blood vessel narrowing identified on the angiogram report, respectively.[20] Interventional angiography was defined as intra-arterial verapamil of any dose, or rarely, as angioplasty.

Outcomes

Based on recent multicenter consensus guidelines for outcome studies after SAH, the primary outcome of interest was ‘new stroke attributable to DCI’.[31] A ‘new stroke’ was defined as a cerebral infarction identified on hospital discharge or follow-up that was not present on imaging between 24–48 hours after aneurysm occlusion, and not attributable to other causes such as surgical clipping or endovascular treatment. Hypodensities resulting from extraventricular drains or residual intraparencyhmal hematomas were excluded.

Secondary outcomes included mortality, functional status at discharge, and the development of an acute neurologic deterioration attributable to DCI. Hospital mortality was inclusive of patients for whom care was withdrawn. Functional outcome was determined based on the Glasgow Outcome Scale (GOS) at hospital discharge or six-week follow-up, whichever was

later. 'Neurologic deterioration' was defined as the development of focal neurologic signs or decreased level of consciousness that resulted in a decrease in the GCS of two points, lasting at least one hour, and not attributable to infection, changes in sedation, or non-ischemic cerebral pathology such as acute hydrocephalus.

To detect the possibility that patients with high grade SAH had poor outcomes not modifiable by genotype, we did a sensitivity analysis excluding Hunt & Hess grade 4 and 5 patients.

Statistical Analysis

SAH patients were separated into groups based on EPHX2 genotypes. One-way analysis of variance (ANOVA) was used to detect differences between groups for continuous variables. Kruskal-Wallis ANOVA by ranks was used to detect differences between groups for non-parametric distributions of data. To allow for unequal sample size between groups, the Tukey-Kramer *post hoc* pairwise comparison test was used to assess for significance at a level of α=.025. Associations between categorical variables were investigated using χ^2 statistic. Categorical variables are presented as frequency distributions and continuous variables as either mean ± standard deviation or medians (interquartile range), depending on distribution.

Unadjusted regression models that included only the main predictor of interest were fitted to estimate the association between EPHX2 genotype and outcomes. The models were then tested for fit with *a priori* selected confounding factors. Admission GCS, SAPS II, Fisher and Hunt/Hess scores were highly collinear. SAPS II, troponin, and mechanical ventilation did not contribute to the model whereas GCS and age improved the fit of the model. The adjusted models included the genotype, age and admission GCS.

To assess the significance of differences in outcomes across groups, and as a surrogate for a post-hoc power analysis, we performed an analysis of the minimum effect size detectable between groups for the primary outcome.

All p-values are two sided with a 0.05 level of significance. A commercially available statistical program was used for all analyses. (STATA version 12.0; StataCorp, College Station TX). PASS version 12 (PASS 12. NCSS, LLC. Kaysville, Utah, USA) was used to calculate minimum effect size.

Results

Study population

During the study period, 95 patients with a diagnosis of aneurysmal subarachnoid hemorrhage met the inclusion criteria and were consented. Of those, 57 (60.0%) had WT sEH, 14 (14.7%) were K55R heterozygous, 23 (24.2%) were R287Q heterozygous, and 1 (1.1%) was R287Q homozygous. The R287Q genotypes were combined for analysis (25.3%).

Demographic and physiologic data

Demographic and baseline physiologic characteristics of the study population are shown in Table 1. No significant differences were noted with respect to age, sex, weight, distribution and severity of SAH, or neurologic presentation at admission. Among the K55R group, 100% were Caucasian compared to 82.4% in the wild-type group and 58.3% in the R287Q group (p=0.006). Groups were similar in mean SAPS II scores at admission, incidence of EVD placement, and prior anticoagulation. Similar proportions of patients had aneurysms surgically clipped and coiled endovascularly. There was a trend toward increased white blood cell count in the K55R group, but this did not reach statistical significance.

A summary of ICU complications and interventions stratified by EPHX2 genotype are shown in Table 2. No significant differences in the frequency of ICU interventions or drug therapy were noted between the groups. No differences were noted in the incidence of stress-induced cardiomyopathy, neurogenic fever, or CSW/SIADH. Significantly more patients with K55R genotype developed acute respiratory distress syndrome (ARDS) when compared to the other genotypes (p=0.012). There were no differences in the cumulative 7-day fluid balance between the groups.

Study Endpoints

Stroke and Mortality—There was a significant increase in the odds of new stroke in patients with K55R sEH compared to wild type (OR 5.04 (1.45– 17.52)), but no change in patients with R287Q genotype compared to wild type (OR 0.56 (0.16 – 1.91)). These differences persisted after adjustment for age and admission GCS (K55R OR_{adj} 5.48 (1.51–19.91); R287Q OR_{adj} 0.56 (0.16–1.96)

Mortality in patients with K55R genotype sEH was 28.6%, compared to 5.3% in the WT group and 12.5% in the R287Q group (p=0.037). The odds of death were significantly increased in patients with the K55R genotype (OR 7.2 (1.39–37.19)), but unchanged with R287Q compared to WT sEH (OR 2.57 (0.48–13.77)). These differences persisted after adjustment for age and admission GCS (K55R OR_{adj} 7.52 (1.27–44.46); R287Q OR_{adj} 3.09 (0.51–18.52).

Neurologic Outcome—No significant differences were noted in the incidence of TCD or angiographic vasospasm, or the use of angiographic interventions for vasospasm between the groups (Table 3). Patients in the K55R group had significantly worse ICU discharge GCS when compared with WT patients (p=0.025). In the K55R genotype group, 71.4% had a GOS ≤ 3 at hospital discharge or follow-up, compared to 64.9% in the WT group and 37.5% in the R287Q group (p=0.022; post-hoc wild type vs R287Q p=0.023). There was no difference in the incidence of clinical neurologic deterioration attributable to DCI. Patients with R287Q genotype sEH had significantly decreased hospital length of stay. (p=0.009) compared to WT. There were no significant differences in disposition location based on genotypes.

The odds of developing a neurologic deterioration attributable to DCI were unchanged with either genotype when compared to WT sEH, even after adjustment for potential confounders

at admission (Table 4). The odds of a discharge GOS ≤ 3 were significantly reduced in patients with R287Q genotype (OR .32 (.12-.87), an association that was maintained after adjustment for potential confounders (OR_{adj} 0.23 (0.06–0.82).

When patients with Hunt and Hess scores of 4 and 5 were excluded, the results were similar.

Effect size

The minimum detectible effect size of genotypes on the primary outcome (new stroke) was a 32.3% increase in the risk of stroke attributable to DCI.

Discussion

We report important differences in outcome of patients with aneurysmal SAH based on genetic polymorphisms of sEH. A polymorphism predictive of increased sEH activity compared to WT, K55R, was associated with increased odds of new ischemic stroke, and increased odds of mortality, without a change in the odds of poor functional status at discharge or neurologic deterioration during ICU stay. A polymorphism predictive of decreased activity, R287Q, was associated with decreased odds of a poor functional outcome, but there was no difference in the odds of new stroke or death compared to WT.

Increased metabolism of EETs and outcomes after SAH

K55R genotype sEH was associated with increased odds of stroke and death. Outcome after SAH in patients with K55R sEH may be worse due to enhanced metabolism and inactivation of EETs; K55R genotype sEH has been previously shown to metabolize EETs more efficiently than WT sEH.[19] EETs play an important role in CBF regulation and protection from ischemic injury, and reduced levels have been associated with vascular dysfunction and stroke.[12] Interestingly, we detected differences in new stroke and mortality, but these are not explained by differences in the incidence neurologic deteriorations or vasospasm between K55R and WT sEH. One possible explanation for this finding is that it is often challenging to detect DCI in critically ill, brain injured patients until a stroke has already occurred. Another explanation is that in addition to having potent vasoactive properties, EETs are anti-inflammatory, promote angiogenesis and axonal regeneration and inhibit platelet aggregation.[1, 7, 8, 12, 15, 17] It is possible that abnormalities in CBF or metabolism as a result of microthrombosis, inflammation, and impaired neurovascular coupling are more prevalent in patients with the K55R genotype, and influence risk of stroke without detectible, acute neurologic changes or vasospasm. To support this hypothesis we also found a significantly larger subgroup of patients with K55R genotype who presented with admission WBC >20,000. (Leukocytosis has also previously been independently associated with DCI after SAH.)[13] We also report a significant increase in the incidence of ARDS in patients with K55R genotype sEH, which provides additional evidence that inflammatory changes modulated by EETs may be important in the development of DCI and poor outcome.

A protective role for EETs?

Patients with R287Q genotype sEH had decreased odds of a poor functional neurologic outcome without changes in the odds of stroke or death. There were no differences in the incidence of acute neurologic deterioration or vasospasm. Perhaps more durable EETs signaling and beneficial microvascular regulation of blood flow influence longer-term neurologic function in these patients. One study has shown that R287Q sEH is associated with improved neuronal survival after ischemic conditions *in vitro.*[16] Population studies have also shown equivocal risk of developing an ischemic stroke with R287Q genotype sEH.[10, 32] We demonstrate an improved neurologic outcome, but no other indicators of benefit. An alternative explanation is that while decreased levels of EETs are somehow harmful, increased EETs levels are not necessarily beneficial. It is possible that less sEH activity merely shifts EETs metabolism to other, non-enzymatic pathways.

Our findings support an association between sEH activity and outcome after SAH, and contribute to a growing body of literature that suggest a role for manipulation of sEH activity in the secondary prevention of complications after brain injury.[11] Inhibitors of sEH are biologically active with respect to the regulation of vascular tone, hyperglycemia, and reduction of inflammatory response to insults.[2] In experimental stroke in animal models, sEH inhibitors decrease infarct size without increasing measured CBF when compared to untreated animals.[33] These findings could partially explain the differences in outcome we observe, without differences in vasospasm or acute neurologic deterioration.

Selection of outcomes

The goal of this study was to identify clinical outcomes related to delayed ischemia that may be influenced by EETs and sEH genetic polymorphisms. Translating observational physiologic variables into a summary definition of DCI, however, can be challenging and imprecise, especially given that EETs have many potential roles. We chose to identify new stroke as the primary outcome and mortality as a secondary outcome because they are the most objectively quantifiable, and based on the recommendations of a multicenter consensus statement for the study of DCI after SAH. [31] These outcomes do not provide a precise explanation of causality. DCI has a broad pathogenesis, including neurovascular uncoupling, cortical spreading depression, microthrombosis, microvascular spasm and large vessel vasospasm. [5, 28, 29, 30] These pathologic disturbances are sometimes undetectable with standard monitoring until an infarction has occurred.

To more sensitively detect episodes of ischemia that may not have resulted in infarction, we selected acute neurologic deterioration in the ICU and functional neurologic status at discharge as secondary outcomes. However, many non-ischemic processes (such as hydrocephalus, fever, electrolyte abnormalities) can result in neurologic changes, and a change in exam can be imperceptible in patients who are intubated, sedated, or have poor exams at baseline. We also included objective measures of vasospasm considering it is an important, albeit incomplete, contributor to DCI. [29].

Other Limitations

There are several limitations of the data presented. As a measure of neurologic function, the GOS is imprecise. The data we present were collected at hospital discharge, due to different follow-up intervals and a proportion of patients lost to follow-up, which could have influenced the results. Many patients' discharge neurologic status may not be reflective of their eventual neurologic recovery, and other patients may have later died that we were unable to quantify.

We chose to report only two genetic polymorphisms of EPHX2 because they are the most common, and previously cited as biologically important. [11] There are additional polymorphisms of EPHX2 that have been associated with sEH activity,[19] and it is possible that these polymorphisms exist in our WT group and are not detected. When compared with previous population studies, the frequencies of genotypes we report are similar: 15.3% for K55R and 19.4% for R287Q.

Perhaps most importantly, caution should be taken with interpreting the magnitude of associations we present. There is insufficient sample size to establish precise estimates of outcome odds ratios based on genotype, as evidenced by the wide confidence intervals. Still, we do present significantly different odds of important outcomes based on sEH genotype. That these differences are significantly different despite wide confidence intervals suggests either an unrecognized type I error, or that sEH polymorphisms strongly influence outcomes after SAH. We performed a statistical estimation of effect size for the primary outcome of new stroke, which revealed that we are able to detect at minimum, a 33% difference in the odds of new stroke between the K55R and WT groups.

Conclusions

We demonstrate an association between genetic sEH enzyme hyperactivity (K55R) and low EETs levels with new stroke and mortality; enzyme dysfunction (R287Q), representative of higher EETs, was associated with improved neurologic outcome after SAH. While this study represents a small sample of observational data with several limitations, it supports a future examination of the role of EETs in neuroprotection after SAH. It remains to be determined how different genotypes of sEH influence *in vivo* EETs levels after brain injury such as SAH, and whether EETs levels after injury are directly associated with clinical outcomes. If maintained levels of EETs after injury are associated with improved outcome, already-existing inhibitors of sEH may represent an important therapeutic intervention for the prevention and management of neurologic complications after SAH.

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Table 1

Baseline characteristics at ICU admission of patients with subarachnoid hemorrhage stratified by EPHX2 genotype

	EPHX2 Genotype			
Characteristics*	**Wild Type (n=57)**	**K55R (n=14)**	**R287Q (n=24)**	**p-value**
Age, years	54 ± 12	60 ± 15	53 ± 9	0.22
Male, n (%)	17 (29.8)	4 (28.6)	8 (33.3)	0.94
Caucasian, n (%)	47 (82.4)	14 (100)	14 (58.3)	0.006
Weight, kg	77 ± 20	78 ± 25	81 ± 15	0.66
Aneurysm Location, n (%)				0.73
ACA	2 (3.5)	0 (0)	0 (0)	
ACOM	18 (31.6)	8 (57.1)	6 (25.0)	
ICA/MCA	20 (35.1)	3 (21.4)	8 (33.3)	
PCOM	7 (12.3)	2 (14.3)	4 (16.7)	
PCA	1 (1.8)	0 (0)	1 (4.2)	
Vertebro-basilar	9 (15.8)	1 (7.1)	5 (20.8)	
Fisher grade ≥ 3	51 (89.5)	14 (100)	22 (91.7)	0.45
Hunt/Hess score, n (%)				0.70
1	6 (10.5)	1 (7.1)	3 (12.5)	
2	15 (26.3)	2 (14.3)	7 (29.2)	
3	12 (21.1)	7 (50.0)	6 (25.0)	
4	14 (24.6)	2 (14.3)	5 (20.8)	
5	10 (17.5)	2 (14.3)	2 (12.5)	
Glasgow Coma Scale, median (IQR)	12 (6–15)	12 (8–14)	13.5 (6–15)	0.93
SAPS II	30.0 ± 13.5	32.6 ± 9.0	27.3 ± 14.1	0.46
Hematocrit, %	39.7 ± 4.2	40.1 ± 3.6	38.5 ± 4.6	0.46
WBC count, x $10^3/\mu L$	15.6 ± 5.2	18.4 ± 14.1	13.6 ± 5.5	0.16
WBC count > 20,000/μL, n (%)	24 (42.1)	8 (57.1)	4 (16.7)	0.027
Troponin > 0.40 ng/ml, n (%)	8 (14.0)	4 (28.6)	4 (16.7)	0.43
Serum glucose, mg/dl	155 ± 43	179 ± 79	152 ± 35	0.21
Serum sodium, mEq/dl	137 ± 4.4	139 ± 3.0	138 ± 3.5	0.80
Serum creatinine, mg/dl	0.74 ± .18	0.81 ± 0.37	.76 ± 0.26	0.62
MAP, mmHg	87 ± 17	84 ± 12	86 ± 15	0.76
Prior anti-coagulation, n (%)	5 (8.8)	2 (14.3)	3 (12.5)	0.78
Intervention, n (%)				0.96
Craniotomy	39 (68.4)	10 (71.4)	17 (70.8)	
Coil embolization	18 (31.6)	4 (26.7)	7 (29.2)	

ACA anterior cerebral artery, ACOM anterior communication, ICA internal carotid artery, IQR interquartile range, MCA middle cerebral artery, PCOM posterior communicating, PCA posterior cerebral artery, SAPS II simplified acute physiology score, WBC peripheral white blood cell, MAP mean arterial pressure

* Data are expressed as mean ± standard deviation unless otherwise specified

Table 2

Summary of ICU complications and interventions stratified by EPHX2 genotype

	EPHX2 Genotype			
Characteristics*	**Wild Type (n=57)**	**K55R (n=14)**	**R287Q (n=24)**	**p-value**
Mechanically ventilated	29 (50.8)	9 (64.3)	11 (45.8)	0.54
Duration, hours, med (IQR)	46 (32–186)	192 (132–216)	144 (30–264)	0.23
Extraventricular drains	43 (75.4)	14 (92.9)	17 (70.8)	0.27
Stress-induced cardiomyopathy	4 (7.0)	2 (14.3)	2 (8.3)	0.68
ARDS	1 (1.8)	3 (21.4)	1 (4.2)	0.012
Neurogenic fever	8 (14.0)	4 (28.6)	5 (20.8)	0.41
Vasopressor use	26 (45.6)	7 (50.0)	8 (33.3)	0.51
Hypertonic saline	44 (77.2)	9 (64.3)	16 (66.7)	0.47
Sodium handling				0.42
CSW	15 (26.3)	4 (28.6)	10 (41.7)	
SIADH	7 (12.3)	0 (0)	2 (8.3)	
Blood product use	21 (36.8)	6 (42.9)	8 (33.3)	0.84
7-day fluid balance, l, mean ± SD	3.5 ± 3.5	4.4 ± 2.1	2.7 ± 3.1	0.32

ARDS acute respiratory distress syndrome, CSW cerebral salt wasting, SIADH syndrome of inappropriate anti-diuretic hormone secretion

*Data are expressed as n (%) frequency distributions unless otherwise specified

Table 3

Summary of stroke, vasospasm, delayed cerebral ischemia, and outcomes stratified by EPHX2 genotype

	EPHX2 Genotype			
Characteristics*	**Wild Type (n=57)**	**K55R (n=14)**	**R287Q (n=24)**	**p-value**
Vasospasm				
Severe TCD	17 (29.8)	4 (28.6)	9 (37.5)	0.77
Any Angiographic	30 (52.6)	5 (35.7)	11 (45.8)	0.50
Angiographic intervention	24 (42.1)	2 (14.3)	6 (25.0)	0.083
Neurologic deterioration d/t DCI	21 (37.0)	3 (21.4)	5 (20.8)	0.26
Strokes				
Stroke at admission	5 (8.8)	1 (7.1)	2 (8.3)	0.98
Stroke after intervention	28 (49.1)	3 (21.4)	10 (41.7)	0.17
New stroke at discharge/follow up	15 (26.3)	9 (64.3)	4 (16.7)	0.006
Any stroke at follow up	41 (71.9)	12 (85.7)	13 (54.2)	0.10
ICU discharge GCS, mean ± SD	13.4 ± 2.6	10.6 ± 5.2	13.2 ± 3.6	0.022†
Discharge GOS ≤ 3, n (%)	37 (64.9)	10 (71.4)	9 (37.5)	0.043‡
Hospital length of stay, mean ± SD	22 ± 11	21 ± 15	17 ± 7	0.009
Mortality at follow-up	3 (5.3)	4 (28.6)	3 (12.5)	0.037§
Disposition				0.058
Home / rehab	34 (59.7)	5 (35.7)	18 (75.0)	
SNF/LTAC/dead	23 (40.4)	9 (64.3)	6 (25.0)	

DCI delayed cerebral ischemia, GCS Glasgow coma scale, GOS Glasgow outcome scale, LTAC long term acute care hospital, SNF skilled nursing facility, TCD transcranial Doppler

*Data are expressed as frequency distributions unless otherwise specified

†*post hoc* p=0.025 for wild type vs. K55R genotype

‡*post hoc* p=0.023 for wild type vs. R287Q genotype

§*post hoc* p=0.009 for wild type vs. K55R genotype

Table 4

Unadjusted and adjusted odds ratios for primary and secondary outcome measures.

	New Stroke	Mortality	Neurologic deterioration	GOS ≤ 3
Unadjusted Model*				
K55R	5.04 (1.45– 17.52)	7.2 (1.39–37.19)	0.47 (0.12–1.86)	1.35 (.38–4.86)
R287Q	0.56 (0.16 – 1.91)	2.57 (0.48–13.77)	0.57 (0.20 – 1.66)	.32 (.12-.87)
Adjusted Model*				
K55R	5.48 (1.51–19.91)	7.52 (1.27–44.46)	0.49 (0.12–2.09)	1.25 (0.30–5.20)
R287Q	0.56 (0.16–1.96)	3.09 (0.51–18.52)	0.56 (0.18 – 1.71)	0.23 (0.06–0.82)
Age	1.00 (0.96–1.04)	1.06 (1.00–1.14)	0.98 (0.94–1.02)	1.02 (0.98–1.06)
Admission GCS	0.91 (0.81–1.01)	0.63 (0.68–0.99)	0.86 (0.77–0.96)	0.72 (0.62–0.84)

GCS Glasgow coma scale, GOS Glasgow outcome scale

*Data presented as Odds ratios with 95% confidence intervals OR (95% CI).