Ryanodine Receptor 1 Polymorphism Is Not Associated with Aneurysmal Subarachnoid Hemorrhage or its Clinical Sequelae

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■ OBJECTIVE: **The pathophysiologic mechanisms underlying cerebral vasospasm after aneurysmal subarachnoid hemorrhage (aSAH) remain poorly understand. Ryanodine receptors (RYR) are intracellular calcium channels involved in the regulation of vascular smooth muscle cells and cerebrovascular tone and diameter. Previous work reported an association between an RYR polymorphism and cerebral vasospasm. Here, we sought to assess the impact of that RYR polymorphism on aSAH and its clinical sequelae.**

■ METHODS: **Blood samples from all patients enrolled in the CARAS (Cerebral Aneurysm Renin Angiotensin System) study were used for genetic evaluation. The RYR1 single nucleotide polymorphism (SNP) rs35364374 was detected using 5′exonuclease (Taqman) genotyping assays. Associations between the RYR1 polymorphism and aSAH and its clinical sequelae were analyzed.**

■ RESULTS: **Samples from 149 patients with aSAH and 50 controls were available for analysis. Multivariable regression analysis did not show an association of RYR1 SNP rs35364374 with aSAH. Moreover, there was no association of RYR1 SNP rs35364374 with clinical vasospasm, delayed cerebral ischemia, functional outcome at discharge, or functional outcome at last follow-up.**

■ CONCLUSIONS: **Contrary to a previous report, the RYR1 SNP rs35364374 was not associated with aSAH or its clinical sequelae.**

INTRODUCTION

Ryanodine receptors (RYR) are intracellular Ca^{2+} channels that function in muscle contraction. In the skeletal muscles, RYR1 is the predominant channel triggering Ca^{2+} release from the sarcoplasmic reticulum facilitating muscle contraction. Despite physiologic differences between the contraction of smooth and skeletal muscle, the RYR is also involved in Ca^{2+} signaling in vascular smooth muscle cells. This signaling is believed to play a role in regulating vascular tone and diameter in the cerebral parenchymal microcirculation. Whereas RYR elicit skeletal muscle excitation via Ca^{2+} release from the sarcoplasmic reticulum, RYR stimulation in the cerebral vasculature results in vasodilation by activation of Ca^{2+}-induced potassium (BK or big potassium) channels counteracting pressure-induced depolarization and vasoconstriction.[1-4]

Cerebral vasospasm after aneurysmal subarachnoid hemorrhage (aSAH) may cause neurologic deterioration and delayed cerebral ischemia (DCI). Although clinical risk factors for cerebral vasospasm and DCI have been identified,[5] the molecular mechanisms

Key words
- Aneurysm
- Clinical vasospasm
- Delayed cerebral ischemia
- Functional outcome
- Polymorphism
- Ryanodine receptor
- Subarachnoid hemorrhage

Abbreviations and Acronyms
aSAH: Aneurysmal subarachnoid hemorrhage
CARAS: Cerebral Aneurysm Renin Angiotensin System
CT: Computed tomography
DCI: Delayed cerebral ischemia
mRS: modified Rankin Scale
RYR: Ryanodine receptor
SNP: Single nucleotide polymorphism

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Citation: World Neurosurg. (2017) 100:190-194.
http://dx.doi.org/10.1016/j.wneu.2016.12.132

*Journal homepage: **www.WORLDNEUROSURGERY.org***

*Available online: **www.sciencedirect.com***

underlying these conditions remain poorly understood. The RYR1 single nucleotide polymorphism (SNP) rs35364374 may alter the regulation of vascular smooth muscle cells, cerebrovascular tone, and diameter. Here, we assessed associations of RYR1 SNP rs35364374 with aSAH and its clinical sequelae using specimens obtained in the CARAS (Cerebral Aneurysm Renin Angiotensin System) study.[5-8]

METHODS

The CARAS study prospectively enrolled patients with aSAH at 2 academic institutions in the United States from 2012 to 2015. Blood samples from all patients with aSAH and controls enrolled in the CARAS study were assessed for genetic evaluation as previously described.[5-8] Patients with aSAH were treated in accordance with guidelines for the management of aSAH.[9] The control group was comprised of patients with trauma, age ≥19 years, with unremarkable computed tomography (CT) angiography of the head and neck and without known genetic disorders associated with cerebral aneurysm formation. Enrollment of both patients with aSAH and controls was performed within 72 hours of admission. Institutional review board approval was obtained at both participating institutions. Participants or their proxy were required to provide informed consent.

Definition of Clinical Vasospasm and DCI

Clinical vasospasm was diagnosed in case of a new focal or global neurologic deficit, or deterioration of at least 2 points on the Glasgow Coma Scale and additional exclusion of other clinical conditions that potentially cause neurologic deterioration such as hydrocephalus, aneurysm rerupture, electrolyte disturbance, seizure, infection, fever, metabolic disturbance, cerebral edema, or surgical complication. Corroborating evidence of angiographic vasospasm was defined as arterial narrowing on CT angiography or digital subtraction angiography not caused by atherosclerosis, catheter-induced vasospasm, or vessel hypoplasia. In addition, vasospasm was diagnosed with transcranial Doppler ultrasonographic findings of a peak systolic middle cerebral artery of >120 with a Lindegaard ratio of >3. CT angiography, digital subtraction angiography, and transcranial Doppler ultrasonography were performed at the discretion of the treating neurosurgeon. Clinical vasospasm diagnosis was adjudicated by consensus of the study team and treated with hyperdynamic therapy as first line[10]: avoidance of hypovolemia with a goal systolic blood pressure >160 mm Hg, accomplished with either permissive hypertension or vasopressor therapy. Endovascular treatment was performed in patients refractory to medical treatment at the discretion of the treating neurosurgeon. CT scans or magnetic resonance imaging were routinely performed when the patient was transferred from the intensive care unit to the ward.

DCI was defined as low-density areas on CT that corresponded to a vascular distribution or a magnetic resonance imaging showing a hyperintense area on a diffusion-weighted imaging sequence with a corresponding hypointense apparent diffusion coefficient sequence correlate that corresponds with a vascular territory. Infarctions or contusions seen on postoperative day 1 imaging were considered procedurally related.

Table 1. Ryanodine Receptor 1 Polymorphism

Ryanodine Receptor 1 Polymorphism	Alleles	Minor Allele Frequency*
rs35364374 (c.6178G>T)	G/T	MAF (T) 0.05

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

Functional Outcome Assessment

Using the modified Rankin Scale (mRS), functional outcome was recorded at the time of discharge from the acute hospital setting and at last follow-up. Scores of 0–2 were defined as a favorable outcome whereas scores of 3–6 were considered as an unfavorable outcome.

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

The RYR1 polymorphism (Table 1) was detected using 5′exonuclease (Taqman) genotyping assays (c.6178G>T RYR 1 SNP rs35364374). Commercial Taqman assays were designed and performed according to the vendor (Thermo Fisher Scientific Inc., Cambridge, Massachusetts, USA). Approximately 10% of the DNA samples were randomly selected to test reproducibility of Taqman assays. All the replication samples produced concordant genotypes.

Statistical Analysis

Continuous variables are presented as mean ± standard deviation and categorical variables are presented as frequency and percent. Analyses were carried out using unpaired Wilcoxon rank sum, Student t test, χ^2, and Fisher exact tests, as appropriate. Patient characteristics and RYR1 polymorphism were tested in univariable analysis to determine predictors of aSAH, clinical vasospasm, DCI, unfavorable functional outcome (mRS score, 3–6) at discharge and at last follow-up. Factors predictive in univariable analysis ($P < 0.15$) were entered into a multivariable logistic regression analysis. P values of ≤ 0.05 were considered statistically significant.

RESULTS

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

Patient and Control Characteristics

Age, gender, history of ischemic vascular disease, and smoking did not differ between patients with aSAH and controls. African American race and hypertension were more common in the aSAH group (Table 2).

Association of RYR1 Polymorphism and ASAH

The RYR1 SNP rs35364374 was in Hardy-Weinberg equilibrium in patients with aSAH and controls. In the entire study sample, the GT genotype was identified in 16 patients (8.2%). Eight patients with aSAH (5.4%) and 8 controls (16.3%) carried the T allele, respectively. All patients with the GT polymorphism were white (Table 3).