Associations of renin-angiotensin system genetic polymorphisms and clinical course after aneurysmal subarachnoid hemorrhage

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OBJECTIVE Renin-angiotensin system (RAS) genetic polymorphisms are thought to play a role in cerebral aneurysm formation and rupture. The Cerebral Aneurysm Renin Angiotensin System (CARAS) study prospectively evaluated associations of common RAS polymorphisms and clinical course after aneurysmal subarachnoid hemorrhage (aSAH).

METHODS The CARAS study prospectively enrolled aSAH patients at 2 academic centers in the United States. A blood sample was obtained from all patients for genetic evaluation and measurement of plasma angiotensin converting enzyme (ACE) concentration. Common RAS polymorphisms were detected using 5' exonuclease genotyping assays and pyrosequencing. Analysis of associations of RAS polymorphisms and clinical course after aSAH were performed.

RESULTS A total of 166 patients were screened, and 149 aSAH patients were included for analysis. A recessive effect of allele I (insertion) of the ACE I/D (insertion/deletion) polymorphism was identified for Hunt and Hess grade in all patients (OR 2.76, 95% CI 1.17–6.50; $p = 0.0206$) with subsequent poor functional outcome. There was a similar effect on delayed cerebral ischemia (DCI) in patients 55 years or younger (OR 3.63, 95% CI 1.04–12.7; $p = 0.0439$). In patients older than 55 years, there was a recessive effect of allele A of the angiotensin II receptor Type 2 (AT2) A/C single nucleotide polymorphism (SNP) on DCI (OR 4.70, 95% CI 1.43–15.4; $p = 0.0111$).

CONCLUSIONS Both the ACE I/D polymorphism and the AT2 A/C single nucleotide polymorphism were associated with an age-dependent risk of delayed cerebral ischemia, whereas only the ACE I/D polymorphism was associated with poor clinical grade at presentation. Further studies are required to elucidate the relevant pathophysiology and its potential implication in the treatment of patients with aSAH.

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KEY WORDS renin; angiotensin; aneurysm; rupture; subarachnoid hemorrhage; vascular disorders

ABBREVIATIONS ACE = angiotensin-converting enzyme; AGT = angiotensinogen; aSAH = aneurysmal subarachnoid hemorrhage; AT1 = angiotensin II receptor Type 1; AT2 = angiotensin II receptor Type 2; CARAS = Cerebral Aneurysm Renin Angiotensin System; CTA = CT angiography; DCI = delayed cerebral ischemia; DSA = digital subtraction angiography; eNOS = endothelial nitric oxide synthase; FDR = false discovery rate; HWE = Hardy-Weinberg equilibrium; mRS = modified Rankin Scale; PCR = polymerase chain reaction; RAS = renin-angiotensin system; SNP = single nucleotide polymorphism; VSMC = vascular smooth muscle cell.

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THE renin-angiotensin system (RAS) plays a crucial role in physiological vasorelaxation/vasoconstriction of the cerebral vasculature, vascular remodeling, and maintenance of arterial wall integrity.^{2,9,14} Compared with the normal arterial wall, cerebral aneurysms, in particular ruptured aneurysms, express significantly less angiotensin-converting enzyme (ACE) and angiotensin II receptor Type 1 (AT1), resulting in lack of vascular remodeling and thinning of the arterial wall under hemodynamic stress.²³ Certain RAS genetic polymorphisms have been linked to aneurysmal subarachnoid hemorrhage (aSAH). In a meta-analysis, the II genotype of the ACE insertion/deletion (I/D) polymorphism was associated with aSAH (OR 1.64, 95% CI 1.24–2.17; $p = 0.009$) compared with controls.²⁴ In the Cerebral Aneurysm Renin Angiotensin System (CARAS) study, allele C of the X-linked angiotensin II receptor Type 2 (AT2) A/C single nucleotide polymorphism (SNP) demonstrated a dominant effect on aSAH (OR 3.48, 95% CI 1.23–9.84; $p = 0.0192$) (Griessenauer et al., unpublished data, 2016). The significance of RAS genetic polymorphisms on the clinical course of aSAH, however, has not been studied. We sought to evaluate the association between common RAS polymorphisms of angiotensinogen (AGT), ACE, AT1, AT2, and clinical course after aSAH.

Methods

Study Design

A prospective trial evaluating the role of common RAS genetic polymorphisms in aSAH was performed at 2 academic institutions in the United States from September 2012 to January 2015. The study group included all patients who presented with aSAH. The diagnosis of SAH was established by admission CT scan or xanthochromia of cerebrospinal fluid (CSF). A ruptured cerebral aneurysm was confirmed by CT angiography (CTA) or digital subtraction angiography (DSA). Exclusion criteria were age younger than 19 years and any associated genetic syndrome that could explain the presence of a cerebral aneurysm (such as polycystic kidney disease, Ehler-Danlos syndrome Type 4, and Marfan syndrome) or systemic diseases (e.g., congestive heart failure and cirrhosis) that could interfere with RAS activity. All patients were enrolled within 72 hours of admission.

General Management

Patients presenting with aSAH were treated in accordance with contemporary standards of care in the United States: ICU monitoring, treatment of hydrocephalus, early (< 48 hours) intervention for aneurysm treatment, oral nimodipine, maintenance of euvolemia, and surveillance for clinical vasospasm and delayed cerebral ischemia (DCI).⁶ Following discharge from the ICU, patients are transferred to a ward with personnel trained in managing aSAH patients.

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 that is not explained by an-

other clinical process including 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 CTA or DSA not due to atherosclerosis, catheter-induced vasospasm, or vessel hypoplasia. Additionally, vasospasm was diagnosed using transcranial Doppler ultrasound findings of a peak systolic middle cerebral artery of > 120 cm/sec with a Lindegaard ratio of > 3. CTA, DSA, and transcranial Doppler ultrasonography were performed 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.⁷ Hyperdynamic therapy included avoidance of hypovolemia with a goal systolic blood pressure of 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 scanning 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 an MR image 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 seen on postoperative Day 1 imaging were considered procedurally related and were not considered DCI.

Outcome Measures in aSAH Patients

Outcome measures included clinical vasospasm, DCI, mortality, and functional outcome at the time of discharge from the acute hospital setting, at 12 months, and at last follow-up using the modified Rankin Scale (mRS). Poor outcome was defined as death or severe disability (mRS Score 4–6). All outcome data were obtained blinded to the results of the genetic analysis. Functional outcome was assessed either in the clinic or via telephone interview with the patient or with a surrogate if the patient was unable to participate.³

Laboratory and Genetic Evaluation

A blood sample was obtained from all patients within 72 hours of admission for genetic evaluation and measurement of plasma ACE concentration. Common RAS genetic polymorphisms were detected using 5' exonuclease (TaqMan) genotyping assays (SNPs AGT C/T [rs699], AT1 A/C [rs5186], AT2 A/C [rs11091046], and G/A [rs1403543]) and restriction fragment length polymorphism analysis (ACE I/D [rs4340]). 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. For the ACE I/D polymorphism, a polymerase chain reaction (PCR)-based gel assay was used for the first round of genotyping with a second-round replication performed using an

FIG. 1. Flowchart of participant enrollment.

amplification reaction containing 5 ng/μl of genomic DNA, 2 μM of forward and reverse primers in a FailSafe Premix B 1× reaction (Epicentre). PCRs were performed in a C100 Touch Thermocycler (Bio-Rad), using cycling conditions as follows: 30 cycles with denaturation at 94°C for 1 minute, annealing at 58°C for 1 minute, and extension at 72°C for 2 minutes. PCR primers were as follows: forward primer, sense oligo 5'CTGGAGACCACTCCC ATCCTTCT3'; reverse primer, 5'GATGTGGCCATCA CATTCGTCAGAT3'. PCR products were resolved using the QIAxcel system (QIAGEN) to discriminate 490 bp (insertion) from 202 bp (deletion) alleles.

Statistical Analysis

Genotype frequencies for the individual SNPs and the ACE I/D were analyzed for all patients and then by age (patients ≤ 55 years or > 55 years; 55 years was the median age in the cohort). Sample size calculation was performed. Assuming a 50% rate of clinical vasospasm, with an alpha of 0.05 and power of 90%, a total sample size of 146 is needed to detect genotype associated with a 25% rate of vasospasm. Hardy-Weinberg equilibrium (HWE) was assessed using the chi-square test in aSAH patients

and controls separately. To identify potential confounding variables, we tested for associations between patient characteristics, outcome measures, and genotype. Categorical variables and numerical variables were compared using Fisher's exact test and 1-way ANOVA, respectively. The association of genotype and outcome measures was tested using binary logistic regression with a standard method for dominant and recessive models. Multivariate logistic regression was subsequently performed to control for race and hypertension for all comparisons with $p < 0.1$. Adjustment of the false discovery rate (FDR) associated with multiple comparisons was performed using the Benjamini-Hochberg procedure.¹ The FDR was set to 10%. Unless specifically indicated, associations were not statistically significant after FDR correction.

Results

Between September 2012 and February 2015, 166 aSAH patients were screened for inclusion. Nine patients who were screened were excluded. A total of 157 aSAH patients were enrolled. One aSAH patient withdrew from the study, and blood samples of 7 aSAH patients could not be processed (Fig. 1).

TABLE 1. Patient characteristics and outcome measures in 149 patients with aSAH

Variable	Value*
Patient characteristics	
Mean age in yrs	54.9 ± 12.5
Race	
White	85 (57.0)
African American	60 (40.3)
Other	4 (2.7)
Sex	
Male	35 (23.5)
Female	114 (76.5)
Ischemic vascular disease	13 (8.7)
Hypertension	90 (60.4)
ACE inhibitor or ARB	42 (28.2)
Smoker	
Never	58 (38.9)
Former	18 (12.1)
Current	73 (49.0)
Unruptured aneurysm(s)	32 (21.5)
Family history of intracranial aneurysms	14 (9.4)
Aneurysm size in 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)
Treatment†	
Microsurgical clipping	76 (52.4)
Endovascular treatment	69 (47.6)
Rerupture	7 (4.7)
Outcome measures	
Clinical vasospasm	34 (22.8)
DCI‡	31 (21.2)
Mean ICU stay in days	11.9 ± 7.9
Mean hospital stay in days	15.9 ± 11.3
Discharge mRS score	
0–3	92 (61.7)
4–6	57 (38.3)
mRS score at last follow-up	
0–3	120 (80.5)
4–6	29 (19.5)
Mean length of follow-up in days	249.7 ± 187.8

ARB = angiotensin receptor blocker.

* Values are presented as the number of patients (%) unless noted otherwise. Mean values are presented as the mean ± SD.

† Data were not available for 4 aneurysms.

‡ Data were not available for 3 aneurysms.

Patient Characteristics and Outcome Measures

The mean age of aSAH patients was 54.9 ± 12.5 years (male/female ratio 1:3.26). The majority (62.4%) of ruptured aneurysms were smaller than 7 mm in size and were located in the anterior circulation (80.5%). Favorable Hunt and Hess Grades I–III and Fisher CT Grades 1 and 2 were present in 77.2% and 15.4% of patients, respectively. Microsurgical clipping and endovascular treatment were performed in 52.4% and 47.6%, respectively, and 4.7% of patients experienced rerupture of the aneurysm prior to treatment. Clinical vasospasm, DCI, and poor functional outcome at last follow-up occurred in 22.8%, 21.2%, and 19.5% of patients, respectively (Table 1).

Association of RAS Polymorphisms and Clinical Course

Genotype frequencies of the individual SNPs and ACE I/D polymorphism were found to be in HWE. The X-linked AT2 SNPs were in HWE in males and females.

Angiotensinogen C/T Polymorphism (rs699)

Univariable analysis showed statistically significant associations of genotype with race ($p < 0.0001$) that persisted after FDR correction. The association with aneurysm size ($p = 0.0226$) was not significant after FDR correction (Table 2). In patients older than 55 years of age, genotype was associated with clinical vasospasm ($p = 0.0369$) and DCI ($p = 0.0173$); however, this was not significant after FDR correction (Table 3).

In logistic regression, there was a statistically significant recessive effect of the C allele (CC vs CT + TT) on aneurysm size (< 7 mm vs ≥ 7 mm) (OR 2.63, 95% CI 1.06–6.51; $p = 0.0367$) after controlling for race and performing FDR correction (Table 4). There was also a non-significant dominant effect of the T allele (CC vs CT + TT) on clinical vasospasm (OR 4.62, 95% CI 0.719–29.7; $p = 0.107$) and DCI (OR 9.54, 95% CI 0.878–105; $p = 0.0651$) in patients older than 55 years after controlling for race and hypertension (Table 4).

Angiotensin Converting Enzyme (ACE) I/D Polymorphism (rs4340)

Univariable analysis showed a trend toward a statistically significant association of genotype with Hunt and Hess grade ($p = 0.0682$) (Table 2) and mRS score at last follow-up ($p = 0.129$) (Table 3). The ACE concentration was the lowest in aSAH patients with II genotype (17.6 ± 8.0 U/L) ($p = 0.0195$) (Table 2).

In logistic regression, there was a recessive effect of the I allele (II vs ID + DD) on Hunt and Hess grade (OR 2.76, 95% CI 1.17–6.50; $p = 0.0206$) that remained significant after FDR correction. After controlling for Hunt and Hess grade, the effect on mRS was no longer present (OR 1.54, 95% CI 0.498–4.74, $p = 0.455$). In patients 55 years or younger, there was a similar effect of the I allele on clinical vasospasm and DCI (OR 3.63, 95% CI 1.04–12.7; $p = 0.0439$); this remained significant after FDR correction (Table 4).

Angiotensin 2 Type 1 Receptor (AT1) A/C Polymorphism (rs5186)

No genotype associations with patient characteris-

TABLE 2. Patient demographics and characteristics by AGT C/T (rs699), ACE I/D (rs4340), and AT1 A/C (rs5186) genotype*

Variable	AGT C/T (rs699)				ACE I/D (rs4340)				AT1 A/C (rs5186)			
	CC (n = 58)	CT (n = 60)	TT (n = 31)	p Value	DD (n = 52)	I/D (n = 66)	II (n = 31)	p Value	AA (n = 93)	AC (n = 49)	CC (n = 7)	p Value
HWE	p = 0.0412				p = 0.241				p = 0.867			
Mean age in yrs	53.7 ± 13.7	55.4 ± 12.2	56.1 ± 10.8	0.648	53.2 ± 12.7	56.2 ± 13.0	54.7 ± 11.0	0.435	54.6 ± 12.2	54.5 ± 12.6	61.4 ± 15.9	0.366
Race	<0.0001†				0.376				<0.0001†			
White	11 (19.0)	45 (75.0)	29 (93.5)		26 (50.0)	40 (60.6)	19 (61.3)		36 (38.7)	42 (85.7)	7 (100.0)	
African American	44 (75.9)	14 (23.3)	2 (6.5)		25 (48.1)	25 (37.9)	10 (32.3)		55 (59.1)	5 (10.2)	0 (0.0)	
Other	3 (5.2)	1 (1.7)	0 (0.0)		1 (1.9)	1 (1.5)	2 (6.5)		2 (2.2)	2 (4.1)	0 (0.0)	
Sex	0.189				0.376				0.373			
Male	13 (22.4)	11 (18.3)	11 (35.5)		11 (21.2)	19 (28.8)	5 (16.1)		24 (25.8)	11 (22.4)	0 (0.0)	
Female	45 (77.6)	49 (81.7)	20 (64.5)		41 (78.8)	47 (71.2)	26 (83.9)		69 (74.2)	38 (77.6)	7 (100.0)	
Ischemic vascular disease	5 (8.6)	6 (10.0)	2 (6.5)	0.933	4 (7.7)	7 (10.6)	2 (6.5)	0.806	11 (11.8)	1 (2.0)	1 (14.3)	0.0730
Hypertension	40 (69.0)	34 (56.7)	16 (51.6)	0.209	34 (65.4)	38 (57.6)	18 (58.1)	0.675	59 (63.4)	27 (55.1)	4 (57.1)	0.617
Mean ACE level‡	24.0 ± 13.0	23.3 ± 12.4	20.8 ± 12.4	0.566	26.6 ± 14.2	22.5 ± 12.1	17.6 ± 8.0	0.0195				
Smoker	0.269				0.976				0.759			
Never	24 (41.4)	21 (35.0)	13 (41.9)		21 (40.4)	24 (36.4)	13 (41.9)		36 (38.7)	18 (36.7)	4 (27.1)	
Former	3 (5.2)	11 (18.3)	4 (12.9)		6 (11.5)	8 (12.1)	4 (12.9)		12 (12.9)	5 (10.2)	1 (14.3)	
Current	31 (53.4)	28 (46.7)	14 (45.2)		25 (48.1)	34 (51.5)	14 (45.2)		45 (48.4)	26 (53.1)	2 (28.6)	
Family history	5 (8.6)	8 (13.3)	1 (3.2)	0.319	5 (9.6)	6 (9.1)	3 (9.7)	1	9 (9.7)	5 (10.2)	0 (0.0)	1
Aneurysm size	0.0226				0.997				0.373			
<7 mm	44 (75.9)	32 (53.4)	17 (54.8)		33 (63.5)	41 (62.1)	19 (61.3)		61 (65.6)	27 (55.1)	5 (71.4)	
7–12 mm	10 (17.2)	26 (43.3)	13 (41.9)		17 (32.7)	21 (31.8)	11 (35.5)		28 (30.1)	20 (40.8)	1 (14.3)	
13–24 mm	3 (5.2)	2 (3.3)	1 (3.2)		2 (3.8)	3 (4.5)	1 (3.2)		3 (3.2)	2 (4.1)	1 (14.3)	
≥25 mm	1 (1.7)	0 (0.0)	0 (0.0)		0 (0.0)	1 (1.5)	0 (0.0)		1 (1.1)	0 (0.0)	0 (0.0)	
Location	1				0.769				0.552			
Anterior	47 (81.0)	48 (80.0)	25 (80.6)		40 (76.9)	54 (81.8)	26 (83.9)		77 (82.8)	38 (77.6)	5 (71.4)	
Posterior	11 (19.0)	12 (20.0)	6 (19.4)		12 (23.1)	12 (18.2)	5 (16.1)		16 (17.2)	11 (22.4)	2 (28.6)	
Hunt & Hess grade	0.144				0.0682				0.743			
I–III	45 (77.6)	50 (83.3)	20 (64.5)		43 (82.7)	53 (80.3)	19 (61.3)		73 (78.5)	37 (75.5)	5 (71.4)	
IV & V	13 (22.4)	10 (16.7)	11 (35.5)		9 (17.3)	13 (19.7)	12 (38.7)		20 (21.5)	12 (24.5)	2 (28.6)	
Fisher grade	0.838				0.186				0.373			
1 & 2	10 (17.2)	8 (13.3)	5 (16.1)		12 (23.1)	8 (12.1)	3 (9.7)		15 (16.1)	6 (12.2)	2 (28.6)	
3 & 4	48 (82.8)	52 (86.7)	26 (83.9)		40 (76.9)	58 (87.9)	28 (90.3)		78 (83.9)	43 (87.8)	5 (71.4)	
Treatment§	0.694				0.0743				0.0334			
Clipping	28 (49.1)	30 (52.6)	18 (58.1)		32 (64.0)	32 (50.0)	12 (38.7)		52 (57.8)	19 (38.8)	5 (83.3)	
Endovascular	29 (50.9)	27 (47.4)	13 (41.9)		18 (36.0)	32 (50.0)	19 (61.3)		38 (42.2)	30 (61.2)	1 (16.7)	
Rerupture	0 (0.0)	4 (6.7)	3 (9.7)	0.0465	1 (1.9)	4 (6.1)	2 (6.5)	0.539	5 (5.4)	1 (2.0)	1 (14.3)	0.273

* Values indicate number of patients (%) unless specified otherwise. Mean values are presented as the mean ± SD.

† Significant after FDR correction.

‡ Data were not available for 28 aneurysms.

§ Data were not available for 4 aneurysms.

TABLE 3. Outcome measures by AGT C/T (rs699), ACE I/D (rs4340), and AT1 A/C (rs5186) genotype for all patients, patients older than 55 years, and patients 55 years or younger*

Age Group & Variable	AGT C/T (rs699)				ACE I/D (rs4340)				AT1 A/C (rs5186)			
	CC	CT	TT	p Value	DD	I/D	II	p Value	AA	AC	CC	p Value
All patients												
No. of patients	58	60	31		52	66	31		93	49	7	
Clinical vasospasm	10 (17.2)	14 (23.3)	10 (32.3)	0.273	9 (17.3)	15 (22.7)	10 (32.3)	0.291	21 (22.6)	13 (26.5)	0 (0.0)	0.367
DCIT	7 (12.3)	14 (24.1)	10 (32.3)	0.122	10 (19.6)	11 (17.2)	10 (32.3)	0.257	19 (20.9)	11 (22.4)	1 (16.7)	0.301
Mean ICU stay in days	11.8 ± 6.5	11.9 ± 9.5	11.9 ± 6.8	1	10.5 ± 5.7	13.4 ± 9.3	11.0 ± 7.4	0.115	12.0 ± 8.0	12.4 ± 8.0	6.7 ± 2.8	0.200
Mean hospital stay in days	16.1 ± 9.6	15.8 ± 13.7	15.7 ± 9.1	0.984	14.0 ± 7.5	18.3 ± 13.8	13.8 ± 9.7	0.0654	16.2 ± 12.2	16.3 ± 9.8	8.1 ± 3.0	0.180
Discharge mRS score				0.910				0.388				0.917
0-3	37 (63.8)	37 (61.7)	18 (58.1)		35 (67.3)	41 (62.1)	16 (51.6)		57 (61.3)	30 (61.2)	5 (71.4)	
4-6	21 (36.2)	23 (38.3)	13 (41.9)		17 (32.7)	25 (37.9)	15 (48.4)		36 (38.7)	19 (38.8)	2 (28.6)	
mRS score at last follow-up				0.620				0.129				0.669
0-3	48 (82.8)	49 (81.7)	23 (74.2)		45 (86.5)	54 (81.8)	21 (67.7)		74 (79.6)	41 (83.7)	5 (71.4)	
4-6	10 (17.2)	11 (18.3)	8 (25.8)		7 (13.5)	12 (18.2)	10 (32.3)		19 (20.4)	8 (16.3)	2 (28.6)	
Mean length of follow-up in days	248 ± 201	258 ± 181	237 ± 181	0.880	276 ± 180	243 ± 194	219 ± 188	0.373	238 ± 185	268 ± 193	269 ± 200	0.642
≤55 yrs												
No. of patients	33	27	13		27	31	15		48	23	2	
Clinical vasospasm	8 (24.2)	4 (14.8)	3 (23.1)	0.687	4 (14.8)	5 (16.1)	6 (40.0)	0.156	11 (22.9)	4 (8.3)	0 (0.0)	0.849
DCI	6 (18.2)	6 (22.2)	6 (46.2)	0.864	6 (22.2)	3 (9.7)	6 (40.0)	0.0573	12 (25.0)	3 (13.0)	0 (0.0)	0.595
Mean ICU stay in days	10.6 ± 4.7	10.3 ± 7.8	12.0 ± 5.2	0.699	9.8 ± 4.2	11.9 ± 9.5	11.9 ± 6.8	1	11.3 ± 6.8	10.0 ± 4.5	7.5 ± 2.1	0.533
Mean hospital stay	14.6 ± 7.0	12.5 ± 7.6	16.5 ± 9.2	0.284	13.4 ± 6.3	15.8 ± 13.7	15.7 ± 9.1	0.984	15.0 ± 8.6	12.8 ± 5.3	8.5 ± 0.7	0.305
Discharge mRS score				0.699				0.632				0.455
0-3	24 (72.7)	20 (74.1)	8 (66.7)		18 (66.7)	24 (77.4)	10 (66.7)		33 (68.8)	18 (78.3)	1 (50.0)	
4-6	9 (27.3)	7 (25.9)	5 (41.7)		9 (33.3)	7 (22.6)	5 (33.3)		15 (31.3)	5 (21.7)	1 (50.0)	
mRS score at last follow-up				0.335				1				0.0374
0-3	30 (90.9)	25 (92.6)	10 (76.9)		24 (88.9)	28 (90.3)	13 (86.7)		41 (85.4)	23 (100.0)	1 (50.0)	
4-6	3 (9.1)	2 (7.4)	3 (23.1)		3 (11.1)	3 (9.7)	2 (13.3)		7 (14.6)	0 (0.0)	1 (50.0)	
Mean length of follow-up in days	267 ± 226	246 ± 172	198 ± 176	0.570	294 ± 209	258 ± 181	237 ± 181	0.880	224 ± 190	303 ± 209	133 ± 188	0.207
>55 yrs												
No. of patients	25	33	18		25	35	16		45	26	5	
Clinical vasospasm	2 (8.0)	10 (30.3)	7 (38.9)	0.0369	5 (20.0)	10 (28.6)	4 (25.0)	0.742	10 (22.2)	9 (34.6)	0 (0.0)	0.236
DCIT	1 (4.2)	8 (25.8)	7 (38.9)	0.0173	4 (16.7)	8 (24.2)	4 (25.0)	0.122	7 (16.3)	8 (30.8)	1 (25.0)	0.314
Mean ICU stay in days	13.4 ± 8.1	13.2 ± 10.7	11.8 ± 7.9	0.828	11.2 ± 7.0	11.9 ± 9.5	11.9 ± 6.8	1	12.7 ± 9.0	14.5 ± 9.8	6.4 ± 3.2	0.188
Mean hospital stay in days	18.1 ± 12.0	18.4 ± 16.9	15.1 ± 9.2	0.697	14.6 ± 8.8	15.8 ± 13.7	15.7 ± 9.1	0.984	17.5 ± 15.2	19.4 ± 11.7	8.0 ± 3.7	0.238
Discharge mRS score				1				0.157				0.407
0-3	13 (52.0)	17 (51.5)	10 (55.6)		17 (68.0)	17 (48.6)	6 (37.5)		24 (53.3)	12 (46.2)	4 (80.0)	
4-6	12 (48.0)	16 (48.5)	8 (44.4)		8 (32.0)	18 (51.4)	10 (62.5)		21 (46.7)	14 (53.8)	1 (20.0)	

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TABLE 3. Outcome measures by AGT C/T (rs699), ACE I/D (rs4340), and AT1 A/C (rs5186) genotype for all patients, patients older than 55 years, and patients 55 years or younger*

Age Group & Variable	AGT C/T (rs699)			ACE I/D (rs4340)			AT1 A/C (rs5186)				
	CC	CT	TT	p Value	DD	I/D	II	AA	AC	CC	p Value
>55 yrs (<i>continued</i>)											
mRS score at last follow-up				1							0.917
0–3	18 (72.0)	24 (72.7)	13 (72.2)		21 (84.0)	26 (74.3)	8 (50.0)	33 (73.3)	18 (69.2)	4 (80.0)	
4–6	7 (28.0)	9 (27.3)	5 (27.8)		4 (16.0)	9 (25.7)	8 (50.0)	12 (26.7)	8 (30.8)	1 (20.0)	
Mean length of follow-up in days	224 ± 163	268 ± 190	265 ± 184	0.625	257 ± 143	258 ± 181	237 ± 181	254 ± 182	237 ± 175	324 ± 195	0.620

* Values represent number of patients (%) unless noted otherwise. Mean values are presented as the mean ± SD.

† Data were not available for 3 aneurysms.

tics and outcome measures were identified in univariable analysis for the entire patient group and those older than 55 years. In patients 55 years or younger, genotype was associated with mRS score at last follow-up ($p = 0.0374$) (Table 3). This association was not statistically significant following FDR correction or logistic regression.

AT2 A/C Polymorphism (rs11091046)

No genotype associations with aSAH were identified in univariable analysis for the entire patient group and patients 55 years or younger (Table 5). In patients older than 55 years, genotype was associated with clinical vasospasm ($p = 0.0277$) and DCI ($p = 0.0332$), but this was not significant after FDR correction (Table 6).

In logistic regression, there was a recessive effect of allele A ($X^A X^A + X^A Y$ vs $X^A X^C + X^C X^C + X^C Y$) on clinical vasospasm (OR 3.04, 95% CI 0.990–9.30; $p = 0.0523$) and DCI (OR 4.70, 95% CI 1.43–15.4; $p = 0.0111$); these findings remained significant after FDR correction (Table 4).

AT2 G/A Polymorphism (rs1403543)

No genotype associations with aSAH were identified in univariable analysis for the entire patient group and subgroups of patients ≤ 55 years or younger and those older than 55 years (Tables 5 and 6).

Discussion

The CARAS^{10,11} study prospectively enrolled aSAH patients and controls to evaluate associations among common RAS genetic polymorphisms and identified a dominant effect of allele C of AT2 A/C SNP on aSAH in patients older than 55 years (Griessenauer et al., unpublished data, 2016). Whereas systemic RAS is a potent vasoregulator, local RAS plays a significant role in vascular remodeling. Selective association of this receptor polymorphism with aSAH suggests that antitrophic effects mediated through AT2 receptors on vascular smooth muscle cells (VSMCs) may be relevant to the formation and rupture of cerebral aneurysms. In the present study, there were significant associations of RAS polymorphisms with the clinical course of aSAH. The I allele of the ACE I/D polymorphism was associated with poor Hunt and Hess grade and subsequent poor functional outcome. Both the I allele of the ACE I/D polymorphism and the A allele of the AT2 A/C SNP demonstrated age-dependent associations with clinical vasospasm and DCI.

Renin-Angiotensin Polymorphisms and Risk Factors for aSAH

This study identified important associations for the AGT C/T and ACE I/D polymorphism and risk factors for aSAH. Patients homozygous for the C allele of the AGT C/T polymorphism experienced a higher rate of rupture from aneurysms smaller than 7 mm. Identification of risk factors for rupture of small aneurysms is critically important, as size remains a controversial issue in aneurysm management. While the average size of ruptured aneurysms measures 6 to 7 mm,^{15,21} natural history studies of unruptured aneurysms indicate a benign course for small aneurysms. The ISUIA (International Study of Unrup-

TABLE 4. Dominant and recessive effects of RAS polymorphisms on neurological status at presentation and outcome measures in aSAH

Polymorphism	Outcome	Age	Effect	OR (95% CI)	p Value*
AGT G/A (rs699)	Aneurysm size <7 mm	All patients	Recessive effect of the C allele (CC vs CT + TT)	2.69 (1.86–3.91)†	0.0077
AGT G/A (rs699)	Clinical vasospasm	>55 yrs	Dominant effect of the T allele (TT + CT vs CC)	5.75 (1.21–27.4)‡	0.0278
AGT G/A (rs699)	DCI	>55 yrs	Dominant effect of the T allele (TT + CT vs CC)	10.1 (1.25–82.0)§	0.0300
ACE I/D (rs4340)	Hunt & Hess grade	All patients	Recessive effect of the I allele (II vs ID + DD)	2.76 (1.17–6.50)	0.0206
ACE I/D (rs4340)	mRS score at last follow-up	All patients	Recessive effect of the I allele (II vs ID + DD)	2.48 (1.01–6.10)¶	0.0475
ACE I/D (rs4340)	Hunt & Hess grade	>55 yrs	Recessive effect of the I allele (II vs ID + DD)	4.00 (1.25–12.8)	0.0198
ACE I/D (rs4340)	mRS score at last follow-up	>55 yrs	Recessive effect of the I allele (II vs ID + DD)	3.61 (1.14–11.5)**	0.0294
ACE I/D (rs4340)	Clinical vasospasm	≤55 yrs	Recessive effect of the I allele (II vs ID + DD)	3.63 (1.04–12.7)	0.0439
ACE I/D (rs4340)	DCI	≤55 yrs	Recessive effect of the I allele (II vs ID + DD)	3.63 (1.04–12.7)	0.0439
AT2 A/C (rs11091046)	Clinical vasospasm	>55 yrs	Recessive effect of the A allele ($X^A X^A + X^A Y$ vs $X^A X^C + X^C X^C + X^C Y$)	3.04 (0.990–9.30)	0.0523
AT2 A/C (rs11091046)	DCI	>55 yrs	Recessive effect of the A allele ($X^A X^A + X^A Y$ vs $X^A X^C + X^C X^C + X^C Y$)	4.70 (1.43–15.4)	0.0111

* p values significant after FDR correction.

† After adjusting for race, OR 2.63, 95% CI 1.06–6.51; $p = 0.0367$, statistically significant.‡ After adjusting for race and hypertension, OR 4.62, 95% CI 0.719–29.7; $p = 0.107$.§ After adjusting for race and hypertension, OR 9.54, 95% CI 0.878–105; $p = 0.0651$.¶ After adjusting for Hunt and Hess grade, OR 1.54, 95% CI 0.498–4.74; $p = 0.455$.** After adjusting for Hunt and Hess grade, OR 2.12, 95% CI 0.519–8.71; $p = 0.295$.

tured Intracranial Aneurysms) reported a 5-year cumulative rupture rate of 1.5% and 0% for aneurysms smaller than 7 mm in the anterior circulation in patients with and without a history of SAH, respectively.³¹ The UCAS (Natural Course of Unruptured Cerebral Aneurysm Study of Japan) found annual rupture rates of 0.36% and 0.5% for aneurysms measuring 3–4 mm and 5–6 mm, respectively.²² Both studies, however, were likely influenced by treatment selection bias. In UCAS, 69.7% of all aneurysms selected for elective treatment were smaller than 7 mm in maximum diameter. Factors currently considered in the decision to electively treat small aneurysms include female sex, young age, smoking, hypertension, history of aSAH, and aneurysm morphology.^{5,19} Whereas a family history of aSAH is a recognized risk factor, specific genetic information is not currently part of routine aneurysm management. Associations between genetic risk factors and aneurysm size at the time of rupture have been investigated. Of the 7 SNPs found to be associated with aneurysms in genome-wide association studies, none exerted influence on the aneurysm size at rupture.¹⁸ These genetic risk loci, however, only explain approximately 5% of the genetic risk for aneurysms¹⁸ and did not include the AGT C/T polymorphism. The AGT TT genotype is associated with hypertension in women and increased plasma AGT levels.^{25,27} The CC genotype may result in decreased AGT levels and subsequently decreased local RAS activity. Re-

duced vascular remodeling due to locally downregulated RAS may lead to thinning and weakening of the arterial wall and aneurysm rupture at a smaller size. Another SNP linked to aneurysm size at rupture is the endothelial nitric oxide synthase (eNOS) T786C SNP. Patients homozygous for either T or C alleles experienced rupture of smaller aneurysms compared with heterozygotes.¹⁷ Interestingly, neither AGT C/T (Griessenauer et al., unpublished data, 2016) nor eNOS was associated with aSAH as compared with controls.¹⁷

The present study also found recessive effects of the I allele of the ACE I/D polymorphism on Hunt and Hess grade and subsequent functional outcome. Whereas the II genotype was found to be associated with aSAH,^{16,24,27} no such effect was found in CARAS.²⁴ The II genotype is associated with the lowest ACE activity; thus, bradykinin levels are predicted to be increased, resulting in more vascular dilation and less VSMC proliferation locally.³² While aneurysm formation and rupture may be associated with decreased local RAS activity, decreased systemic RAS activity linked to the I allele may be responsible for the association with poor Hunt and Hess grade. In the acute setting of the aneurysm rupture there is a sudden surge in intracranial pressure and deterioration of cerebral autoregulation.⁴ Thus, systemic vasoregulation is critical for maintaining cerebral perfusion. Decreased systemic RAS activity associated with the I allele may complicate main-

TABLE 5. Patient demographics and characteristics by AT2 A/C (rs11091046) and AT2 G/A (rs1403543) genotype*

Variable	AT2 A/C (rs11091046)†					AT2 G/A (rs1403543)					p Value	p	p Value
	X ^A X ^A (n = 23)	X ^A X ^C (n = 59)	X ^C X ^C (n = 32)	X ^A Y (n = 19)	X ^C Y (n = 16)	X ^A X ^A (n = 21)	X ^A X ^G (n = 52)	X ^G X ^G (n = 41)	X ^A Y (n = 20)	X ^G Y (n = 15)			
HWE	p = 0.280 (males), 0.261 (females)					p = 0.364 (males), 0.473 (females)							
Mean age in yrs	53.9 ± 13.7	55.8 ± 13.1	56.0 ± 11.5	50.1 ± 12.4	56.1 ± 10.3	57.6 ± 14.2	55.0 ± 12.2	55.0 ± 12.7	53.6 ± 13.3	51.9 ± 9.5	0.456	0.259	0.722
Race													0.172
White	11 (47.8)	33 (55.9)	18 (56.3)	15 (78.9)	8 (50.0)	14 (66.6)	30 (57.7)	18 (43.9)	13 (65.0)	10 (66.7)			
African American	12 (52.2)	25 (42.4)	13 (40.6)	3 (15.8)	7 (43.8)	6 (28.6)	21 (40.4)	23 (56.1)	6 (30.0)	4 (26.7)			
Other	0 (0.0)	1 (1.7)	1 (3.1)	1 (5.3)	1 (6.3)	1 (4.8)	1 (1.9)	0 (0.0)	1 (5.0)	1 (6.7)			
Sex													<0.0001‡
Male	0 (0.0)	0 (0.0)	0 (0.0)	19 (100)	16 (100)	0 (0.0)	0 (0.0)	0 (0.0)	20 (100.0)	15 (100.0)			
Female	23 (100)	59 (100)	32 (100)	0 (0)	0 (0)	21 (100.0)	52 (100.0)	41 (100.0)	0 (0.0)	0 (0.0)			
Ischemic vascular disease	1 (4.3)	3 (5.1)	3 (9.4)	3 (15.8)	3 (18.8)	1 (4.8)	2 (3.8)	4 (9.8)	3 (15.0)	3 (20.0)	0.273		0.188
Hypertension	13 (56.5)	35 (59.3)	22 (68.8)	9 (47.4)	11 (68.8)	15 (71.4)	29 (55.8)	26 (63.4)	14 (70.0)	6 (40.0)	0.582		0.298
Smoker													0.162
Never	8 (34.8)	22 (37.3)	17 (53.1)	8 (42.1)	3 (18.8)	9 (42.9)	21 (40.4)	17 (41.4)	7 (35.0)	4 (26.7)	0.374		
Former	3 (13.0)	5 (8.5)	3 (9.4)	4 (21.1)	3 (18.8)	4 (19.0)	3 (5.8)	4 (9.8)	6 (30.0)	1 (6.7)			
Current	12 (52.2)	32 (54.2)	12 (37.5)	7 (36.8)	10 (62.5)	8 (38.1)	28 (53.8)	20 (48.8)	7 (35.0)	10 (66.7)			
Family history	0 (0.0)	6 (10.2)	5 (15.6)	1 (5.3)	2 (12.5)	4 (19.0)	3 (5.8)	4 (9.8)	2 (10.0)	1 (6.7)	0.305		0.512
Aneurysm size													0.202
<7 mm	15 (65.2)	35 (59.3)	18 (56.3)	12 (63.2)	13 (81.3)	10 (47.6)	33 (63.5)	25 (61.0)	16 (80.0)	9 (60.0)	0.523		
7–12 mm	7 (30.4)	23 (39.0)	11 (34.4)	6 (31.6)	2 (12.5)	11 (52.4)	17 (32.7)	13 (31.7)	2 (10.0)	6 (40.0)			
13–24 mm	1 (4.3)	1 (1.7)	2 (6.3)	1 (5.3)	1 (6.3)	0 (0.0)	2 (3.8)	2 (4.9)	2 (10.0)	0 (0.0)			
≥25 mm	0 (0.0)	0 (0.0)	1 (3.1)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (2.4)	0 (0.0)	0 (0.0)			
Location													0.295
Anterior	19 (82.6)	43 (72.9)	28 (87.5)	17 (89.5)	13 (81.3)	18 (85.7)	37 (71.2)	35 (85.4)	16 (80.0)	14 (93.3)	0.432		
Posterior	4 (17.4)	16 (27.1)	4 (12.5)	2 (10.5)	3 (18.8)	3 (14.3)	15 (28.8)	6 (14.6)	4 (20.0)	1 (6.7)			
Hunt & Hess grade													0.775
I–III	18 (78.3)	48 (81.4)	23 (71.9)	14 (73.7)	12 (75.0)	15 (71.4)	43 (82.7)	31 (75.6)	15 (75.0)	11 (73.3)	0.832		
IV & V	5 (21.7)	11 (18.6)	9 (28.1)	5 (26.3)	4 (25.0)	6 (28.6)	9 (17.3)	10 (24.4)	5 (25.0)	4 (26.7)			
Fisher grade													0.410
1 & 2	4 (17.4)	10 (16.9)	4 (12.5)	3 (15.8)	2 (12.5)	6 (28.6)	6 (11.5)	6 (14.6)	2 (10.0)	3 (20.0)	0.984		
3 & 4	19 (82.6)	49 (83.1)	28 (87.5)	16 (84.2)	14 (87.5)	15 (71.4)	46 (88.5)	35 (85.4)	18 (90.0)	12 (80.0)			
Treatment§													0.730
Clipping	11 (52.4)	30 (50.8)	16 (53.3)	10 (52.6)	9 (56.3)	8 (38.1)	29 (55.8)	20 (54.1)	11 (55.0)	8 (53.3)	0.999		
Endovascular	10 (47.6)	29 (49.2)	14 (46.7)	9 (47.4)	7 (43.8)	13 (61.9)	23 (44.2)	17 (45.9)	9 (45.0)	7 (46.7)			
Rerupture	2 (8.7)	1 (1.7)	2 (6.3)	1 (5.3)	1 (6.3)	2 (9.5)	1 (1.9)	2 (4.9)	2 (10.0)	0 (0.0)	0.423		0.321

* Values indicate number of patients (%) unless specified otherwise. Mean values are presented as the mean ± SD.

† Genotype was not available for 2 patients.

‡ Significant after FDR correction.

§ Data were not available for 4 aneurysms.

TABLE 6. Outcome measures by AT2 A/C (rs11091046) and AT2 G/A (rs1403543) genotype for all patients, patients older than 55 years, and patients 55 years or younger*

Age Group & Variable	AT2 A/C (rs11091046)†						AT2 G/A (rs1403543)					
	X ^A X ^A	X ^A X ^C	X ^C X ^C	X ^A Y	X ^C Y	p Value	X ^A X ^A	X ^A X ^C	X ^C X ^C	X ^A Y	X ^C Y	p Value
All patients												
No. of patients	23	59	32	19	16		21	52	41	20	15	
Clinical vasospasm	8 (34.8)	10 (16.9)	10 (31.3)	5 (26.3)	1 (6.3)	0.130	5 (23.8)	10 (19.2)	13 (31.7)	3 (15.0)	3 (20.0)	0.603
DCI‡	7 (31.8)	9 (15.3)	9 (30.0)	5 (26.3)	1 (6.3)	0.150	5 (23.8)	10 (19.2)	10 (26.3)	3 (15.0)	3 (20.0)	0.881
Mean ICU stay in days	13.1 ± 9.5	11.8 ± 7.7	15.0 ± 7.0	11.8 ± 7.2	12.3 ± 10.9	0.913	12.0 ± 6.9	11.9 ± 7.5	11.2 ± 8.0	11.9 ± 8.5	12.2 ± 9.8	0.954
Mean hospital stay in days	19.3 ± 19.2	15.0 ± 9.1	11.0 ± 5.4	16.1 ± 10.1	15.4 ± 12.2	0.628	16.7 ± 7.5	15.0 ± 8.9	16.6 ± 15.5	16.1 ± 10.7	15.4 ± 11.5	0.957
Discharge mRS score						0.792						0.729
0–3	16 (69.6)	36 (61.0)	21 (65.6)	10 (52.6)	9 (56.3)		15 (71.4)	33 (63.5)	25 (61.0)	10 (50.0)	9 (60.0)	
4–6	7 (30.4)	23 (39.0)	11 (34.4)	9 (47.4)	7 (43.8)		6 (28.6)	19 (36.5)	16 (39.0)	10 (50.0)	6 (40.0)	
mRS score at last follow-up						0.753						0.619
0–3	17 (73.9)	50 (84.7)	26 (81.3)	15 (78.9)	12 (75.0)		17 (81.0)	45 (86.5)	31 (75.6)	16 (80.0)	11 (73.3)	
4–6	6 (26.1)	9 (15.3)	6 (18.8)	4 (21.1)	4 (25.0)		4 (19.0)	7 (13.5)	10 (24.4)	4 (20.0)	4 (26.7)	
Mean length of follow-up in days	224 ± 171	273 ± 184	286 ± 188	155 ± 171	239 ± 218	0.109	252 ± 151	282 ± 196	256 ± 183	184 ± 164	206 ± 236	0.307
≤ 55 yrs												
No. of patients	11	29	13	12	8		9	27	17	9	11	
Clinical vasospasm	2 (18.2)	6 (20.7)	3 (23.1)	3 (25.0)	1 (12.5)	0.991	0 (0.0)	5 (18.5)	6 (35.3)	1 (11.1)	3 (27.3)	0.265
DCI	1 (9.1)	5 (17.2)	5 (38.5)	3 (25.0)	1 (12.5)	0.150	1 (11.1)	6 (22.2)	4 (23.5)	1 (11.1)	3 (27.3)	0.905
Mean ICU stay in days	10.5 ± 4.2	10.5 ± 5.8	9.2 ± 3.1	11.5 ± 4.5	13.4 ± 12.7	0.633	9.4 ± 3.0	10.4 ± 5.8	10.2 ± 4.2	10.2 ± 4.8	13.9 ± 10.6	0.461
Mean hospital stay in days	13.6 ± 4.3	13.3 ± 6.9	13.3 ± 6.4	16.4 ± 9.7	15.8 ± 12.4	0.750	12.6 ± 3.0	13.2 ± 6.9	14.0 ± 6.7	15.0 ± 7.7	17.1 ± 12.7	0.653
Discharge mRS score						0.562						0.313
0–3	9 (81.8)	19 (65.5)	11 (84.6)	7 (58.3)	6 (75.0)		9 (100.0)	18 (66.7)	12 (70.6)	6 (66.7)	7 (63.6)	
4–6	2 (18.2)	10 (34.5)	2 (15.4)	5 (41.7)	2 (25.0)		0 (0.0)	9 (33.3)	5 (29.4)	3 (33.3)	4 (36.4)	
mRS score at last follow-up						0.753						0.214
0–3	9 (81.8)	27 (93.1)	12 (92.3)	10 (83.3)	7 (87.5)		9 (100.0)	25 (92.6)	14 (82.4)	9 (100.0)	8 (72.7)	
4–6	2 (18.2)	2 (6.9)	1 (7.7)	2 (16.7)	1 (12.5)		0 (0.0)	2 (7.4)	3 (17.6)	0 (0.0)	3 (27.3)	
Mean length of follow-up in days	240 ± 201	258 ± 175	314 ± 219	99 ± 131	327 ± 244	0.0414	256 ± 153	280 ± 211	256 ± 181	152 ± 161	221 ± 250	0.562
> 55 yrs												
No. of patients	12	30	19	7	8		12	25	24	11	4	
Clinical vasospasm	6 (50.0)	4 (13.3)	7 (36.8)	2 (28.6)	0 (0.0)	0.0277	5 (41.7)	5 (20.0)	7 (29.2)	2 (18.2)	0 (0.0)	0.492
DCI‡	6 (54.5)	4 (13.3)	4 (23.5)	2 (28.6)	0 (0.0)	0.0332	4 (33.3)	4 (16.0)	6 (28.6)	2 (18.2)	0 (0.0)	0.608
Mean ICU stay in days	15.5 ± 12.3	12.9 ± 9.2	12.3 ± 6.3	12.4 ± 10.8	11.1 ± 9.6	0.856	15.5 ± 8.0	16.8 ± 10.5	11.9 ± 9.9	13.3 ± 10.7	7.5 ± 6.0	0.617
Mean hospital stay in days	24.4 ± 25.7	16.7 ± 10.7	16.2 ± 7.3	15.6 ± 11.5	15.1 ± 12.8	0.458	19.8 ± 8.4	13.4 ± 8.8	18.5 ± 19.4	17.0 ± 13.0	10.8 ± 6.9	0.835
Discharge mRS score						0.900						0.790
0–3	7 (58.3)	17 (56.7)	10 (52.6)	3 (42.9)	3 (37.5)		6 (50.0)	15 (60.0)	13 (54.2)	4 (36.4)	2 (50.0)	
4–6	5 (41.7)	13 (43.3)	9 (47.4)	4 (57.1)	5 (62.5)		6 (50.0)	10 (40.0)	11 (45.8)	7 (63.6)	2 (50.0)	

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TABLE 6. Outcome measures by AT2 A/C (rs11091046) and AT2 G/A (rs1403543) genotype for all patients, patients older than 55 years, and patients 55 years or younger*

Age Group & Variable	AT2 A/C (rs11091046)†				AT2 G/A (rs1403543)				p Value	X ^G Y	p Value
	X ^A X ^A	X ^A X ^C	X ^C X ^C	X ^A Y	X ^C Y	X ^A X ^A	X ^A X ^G	X ^G X ^G	X ^A Y	X ^G Y	
>55 yrs (continued)											
mRS score at last follow-up											0.833
0–3	8 (66.7)	23 (76.7)	14 (73.7)	5 (71.4)	5 (62.5)	8 (66.7)	20 (80.0)	17 (70.8)	7 (63.6)	3 (75.0)	
4–6	4 (33.3)	7 (23.3)	5 (26.3)	2 (28.6)	3 (37.5)	4 (33.3)	5 (20.0)	7 (29.2)	4 (36.4)	1 (25.0)	
Mean length of follow-up in days	209 ± 146	288 ± 195	268 ± 168	251 ± 198	150 ± 155	249 ± 156	284 ± 183	257 ± 188	209 ± 170	165 ± 223	0.677

* Values represent number of patients (%) unless noted otherwise. Mean values are presented as the mean ± SD.

† Genotype was not available for 2 patients.

‡ Data were not available for 3 aneurysms.

tenance of cerebral perfusion and render the patient more susceptible to transient ischemia. This process may be exacerbated if the patient also suffers from hydrocephalus. The ACE I/D genotype was not independently associated with functional outcome, and poor functional outcome associated with the II genotype may be an effect of poor Hunt and Hess grade.

Clinical Vasospasm and DCI

Once the aneurysm is secured, DCI represents the most significant threat to a patient's clinical course. The current study identified important associations among RAS polymorphisms, clinical vasospasm, and DCI. While there were recessive effects of the I allele of the ACE I/D polymorphism in patients 55 years and younger, DCI in older patients was associated with the A allele of the AT2 A/C SNP. Both systemic and local RAS modulation may be involved in the development of DCI. Younger patients homozygous for the I allele may have difficulties mounting a systemic response to cerebral vasospasm, making them susceptible to DCI. The C allele of the AT2 SNP has been associated with trophic effects in the local RAS.⁸ While antitrophic effects on the VSMCs appear to predispose to aneurysm formation and rupture, overactivation of the VSMCs via the local AT2 receptor may exacerbate vasospasm and DCI. Activation of RAS in aSAH occurs around 4 to 6 days after aneurysm rupture as a response to sodium and water loss triggered by brain and atrial natriuretic peptides.²⁰ This delayed RAS activation coincides with the onset of cerebral vasospasm, and it is plausible that in an interrelated system of enzymes and substrates such as RAS, a change in activity or level of individual components may alter the susceptibility for clinical vasospasm and DCI. Other genetic polymorphisms associated with vasospasm and DCI include endothelin-1,¹² cystathionine b-synthase,¹³ eNOS,^{26,29} haptoglobin,²⁶ and plasminogen activator inhibitor-1.³⁰ How these polymorphisms relate to the RAS-related effects identified in the present study remains to be determined.

Limitations and Future Directions

There are limitations to the present study. Patients who died prior to or soon after admission could not be consented to participate in the study. Thus, findings only apply to aSAH patients who survived the acute phase. Methodologically, multiple testing may find false-positive associations of genotypes with outcome measures by chance. In an effort to reduce the rate of false-positive findings, we performed adjustment of the FDR associated with multiple comparisons.¹ The population and sample size was relatively small, a recognized limitation of studying rare disease processes. The patient population represented individuals presenting to a tertiary referral center in the eastern and southeastern United States, thus limiting broad generalizability. Lastly, the associations identified in the present study occurred on the genomic level. Future work will focus on gene expression profiles and protein activity associated with the polymorphism investigated to delineate the precise mechanism by which RAS polymorphisms may affect the clinical course after aSAH.

Conclusions

The RAS appears to play a role in the clinical course of aSAH patients. Both the ACE I/D polymorphism and the AT2 A/C SNP were associated an age-dependent risk of DCI, whereas only the ACE I/D polymorphism was associated with poor clinical grade at presentation. Further studies are required to elucidate the relevant pathophysiology and its potential implication in treatment of patients with aSAH.

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Disclosures

The authors report no conflict of interest concerning the materi-

als or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Griessenauer, Tubbs, Shoja. Acquisition of data: Griessenauer, Foreman, Vyas. Analysis and interpretation of data: Griessenauer, Foreman, Chua, Vyas, Lipsky, Lin, Iyer, Haridas, Chaudry, Malieva, Wilkins, Shoja. Drafting the article: Griessenauer, Foreman. Critically revising the article: Griessenauer, Tubbs, Foreman, Chua, Lipsky, Lin, Shoja. Reviewed submitted version of manuscript: Griessenauer, Tubbs, Foreman, Shoja. Approved the final version of the manuscript on behalf of all authors: Griessenauer. Statistical analysis: Griessenauer, Chua. Administrative/technical/material support: Griessenauer, Tubbs, Vyas, Lipsky, Lin, Iyer, Haridas, Walters, Harrigan, Fisher, Shoja. Study supervision: Griessenauer, Walters, Harrigan, Fisher, Shoja.

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