Tumor Necrosis Factor-α–238G>A Promoter Polymorphism Is Associated With Increased Risk of New Hemorrhage in the Natural Course of Patients With Brain Arteriovenous Malformations

Achal S. Achrol, BS; Ludmila Pawlikowska, PhD; Charles E. McCulloch, PhD; K.Y. Trudy Poon, MS; Connie Ha, BS; Jonathan G. Zaroff, MD; S. Claiborne Johnston, MD, PhD; Chanhung Lee, MD, PhD; Michael T. Lawton, MD; Stephen Sidney, MD, MS; Douglas A. Marchuk, PhD; Pui-Yan Kwok, MD, PhD; William L. Young, MD; for the UCSF BAVM Study Project

Background and Purpose—Identification of single-nucleotide polymorphisms (SNPs) associated with increased risk of new intracranial hemorrhage (ICH) after brain arteriovenous malformation (BAVM) diagnosis would facilitate risk stratification and identify potential targets for therapeutic intervention.

Methods—Patients with BAVM were longitudinally followed. Primary outcome was new ICH after diagnosis; censoring events were last follow-up or any BAVM treatment. We genotyped 4 promoter SNPs in 2 inflammatory cytokine genes: interleukin-6 (IL-6–174G>C; IL-6–572G>C) and tumor necrosis factor-α (TNF-α–238G>A; TNF-α–308G>A). Association of genotype with risk of new ICH was screened using χ^2; SNPs associated with new ICH were further characterized using Cox proportional hazards.

Results—We genotyped 280 patients (50% female; 59% white, mean±SD age at diagnosis 37±17 years; 40% presenting with ICH). TNF-α–238G>A was associated with increased risk of new ICH after diagnosis (χ^2; $P=0.003$). After adjusting for age, race/ethnicity, and clinical presentation, the risk of new ICH was increased for patients with TNF-α–238 AG genotype (hazard ratio, 4.01; $P=0.015$). No other SNP was found to be associated with new ICH.

Conclusion—A TNF-α SNP was associated with increased risk of new ICH in the natural course of BAVMs. The role of inflammatory cytokines in the pathogenesis of BAVM hemorrhage merits further study. **(*Stroke*. 2006;37:231-234.)**

Key Words: cerebral hemorrhage ■ genetics ■ vascular malformations

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We previously reported on genes implicated in inflammatory and angiogenic pathways and found an interleukin-6 (IL-6) single-nucleotide polymorphism (SNP), IL-6–174G>C, was associated with presenting intracranial hemorrhage (ICH) at brain arteriovenous malformation (BAVM) diagnosis; a tumor necrosis factor-α (TNF-α) SNP, TNF-α–308G>A, showed a similar trend.[1]

In this study, we sought to examine whether promoter polymorphisms in these 2 genes (IL-6: -174G>C, -572G>C; TNF-α: -238G>A, -308G>A) were associated with risk of new ICH after diagnosis in the natural course of BAVMs.

Materials and Methods

With informed consent, we recruited BAVM patients at the University of California San Francisco (UCSF)[1] or at Kaiser Permanente Northern California (KPNC)[2] and classified them using standardized guidelines.[3] We genotyped 4 polymorphisms by template-directed dye-terminator incorporation assay with fluorescence polarization detection.[1] The primary outcome was occurrence of new ICH (symptomatic new hemorrhage with intracranial blood on computed tomography or MRI) after initial presentation, before any treatment.

Association of genotype with risk of new ICH after diagnosis was screened (χ^2 tests; *t* tests). SNPs found to have association with new ICH that survived Bonferroni correction ($P=0.0125$) were selected for survival analyses (Cox regression; Kaplan–Meier).

The period at risk was defined from date of BAVM diagnosis to date of an event (ie, onset of new [first subsequent] ICH) or censoring attributable either to initiation of first BAVM treatment (surgery, embolization, or radiosurgery) or loss to follow-up (using date of last available follow-up), whichever occurred first.

The predictors in our multivariate model were chosen based on their clinical/statistical importance to the outcome in an attempt to achieve maximum parsimony. The predictors included genotype, age at diagnosis (years), race/ethnicity (white versus nonwhite) and initial presentation (presenting ICH versus nonhemorrhagic presentation at diagnosis); their effects were reported as hazard ratios (HRs) and CIs.

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From the Departments of Anesthesia and Perioperative Care and Center for Cerebrovascular Research (A.S.A., K.Y.P., C.L., W.L.Y.), Cardiovascular Research Institute (L.P., C.H., P.Y.K.), Departments of Epidemiology and Biostatistics (C.E.M., S.C.J.), Neurology (S.C.J., W.L.Y.), Medicine (J.G.Z.), Neurological Surgery (M.T.L., W.L.Y.), University of California, San Francisco, California; Division of Research, Kaiser-Permanente Medical Care Program, Oakland, Calif (S.S.); and the Department of Genetics, Duke University Medical Center, Durham, NC (D.A.M.).

Correspondence to William L. Young, MD, UCSF, 1001 Potrero Ave, Room 3C-38, San Francisco, CA 94110. E-mail ccr@anesthesia.ucsf.edu

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TABLE 1. Demographics, BAVM Characteristics, and IL-6 and TNF-α Polymorphism Genotypes

	New ICH Events	No New ICH Events	Total	% New ICH	*P* Value
Race					
White	11	153	164	6.7	
Black	2	10	12	16.7	
Asian/Pacific Islander	1	33	34	2.9	
Hispanic	4	55	59	6.8	
Other/unknown	0	11	11	0.0	
Total	18	262	280	6.4	0.468
Gender					
Female	9	132	141	6.4	
Male	9	130	139	6.5	
Total	18	262	280	6.4	0.975
Age, y					
Mean±SD	36.8±16.8	37.0±17.4	37.0±17.3		
n	18	262	280		0.803
Follow-up time, y					
Median (interquartile range)	3.66 (19.12)	0.27 (0.81)	0.31 (1.40)		
n	18	262	280		<0.001*
Size of arteriovenous malformation					
<3 cm	5	103	108	4.6	
3 cm+	10	96	106	9.4	
Total	15	199	214	7.0	0.169
Venous drainage					
Any superficial	12	179	191	6.3	
Deep only	2	36	38	5.3	
Total	14	215	229	6.1	0.811
Initial presentation					
ICH presentation	6	105	111	5.4	
Non-ICH presentation	12	157	169	7.1	
Total	18	262	280	6.4	0.572
IL-6–174G>C					
Any C	7	103	110	6.4	
GG	10	148	158	6.3	
Total	17	251	268	6.3	0.991
IL-6–572G>C					
Any C	6	72	78	7.7	
GG	11	187	198	5.6	
Total	17	259	276	6.2	0.506
TNF-α–238G>A					
AG	6	26	32	18.8	
GG	12	236	248	4.8	
Total	18	262	280	6.4	0.003
TNF-α–308G>A					
AG	4	55	59	6.8	
GG	14	199	213	6.6	
Total	18	254	272	6.6	0.955

Some subgroups do not sum to 280 because of missing data (*P*; χ^2 test for categorical variables; *t* test for continuous variables; *Mann–Whitney *U* test).

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TABLE 2. Impact of TNF-α–238 AG Genotype on Risk of New ICH (n=280)

Cox Proportional Hazard Estimates	Variable(s) in Model	*P* Value	HR	95% CI
Univariate	TNF-α–238 AG genotype	0.071	2.53	0.92–6.90
Multivariate	TNF-α–238 AG genotype	0.015	4.01	1.31–12.29
	White race	0.788	0.86	0.28–2.61
	Age, y	0.055	0.97	0.83–1.00
	Presentation with ICH	0.074	3.23	0.89–11.63

Results

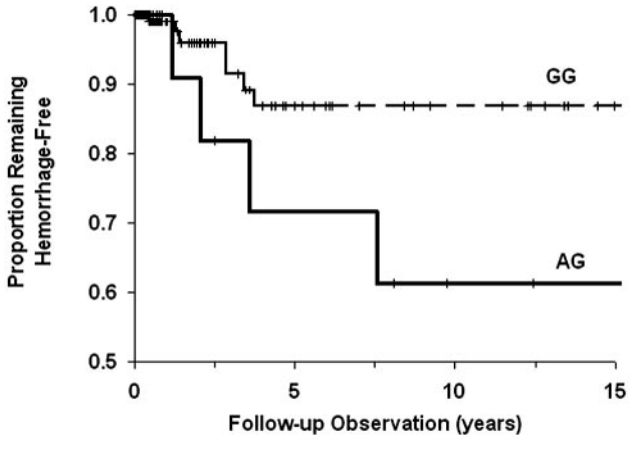
We genotyped 280 patients (Table 1); 189 were evaluated at UCSF and 91 at KPNC. As reported previously,[1] patients homozygous for IL-6–174G had a greater risk of presenting with ICH than IL-6–174C carriers (univariate logistic regression analysis; HR, 2.00 [CI, 1.20 to 3.35]; $P=0.008$). Neither the more distal IL-6–572G>C SNP nor the 2 TNF-α SNPs were associated with ICH presentation.

In contrast, only the TNF-α promoter SNP TNF-α–238G>A was associated with increased risk of new ICH after diagnosis (mean±SD follow-up 2.7±6.7 years). Overall, 224 (80.0%) cases were followed until censoring treatment (74 surgery, 107 embolization, and 43 radiosurgery), and 38 (13.6%) were untreated at last follow-up. New ICH in the natural course after diagnosis occurred in 18 (6.4%) patients and was associated with the AG genotype of TNF-α–238G>A (χ^2; $P=0.003$); this association survived correction for multiple testing.

TNF-α–238G>A was selected for further analysis to estimate prediction of BAVM hemorrhage risk (Table 2). In Cox regression analysis, risk of new ICH was increased for patients with TNF-α–238 AG genotype, adjusting for race/ethnicity, age, and presenting ICH (HR, 4.01 [CI, 1.31 to 12.29]; $P=0.015$; Table 2).

Kaplan–Meier analysis exploring association of TNF-α–238 AG genotype with new ICH supported this trend (Figure; $P=0.06$).

Results were unaffected by including center of ascertainment (UCSF versus KPNC) as a factor in the models.



Kaplan–Meier statistics.

TNF-α–238 AG genotype conferred similar magnitude of risk in subset analysis within the major ethnic group (whites) and in stratified analysis irrespective of presenting ICH versus non-ICH presentation (data not shown). Haplotype analysis was performed for TNF-α and IL-6 genes; results were consistent with single-SNP results but did not contribute additional information (data not shown).

Discussion

This study demonstrates the first evidence of an association between genotype and risk of new ICH in the natural course of BAVMs. The AG genotype of the TNF-α–238G>A promoter polymorphism was associated with a 4-fold increase in risk of new ICH. This finding further implicates inflammatory processes in the pathogenesis of vessel rupture. TNF-α is a proinflammatory and immunomodulatory cytokine implicated in inflammatory conditions that involve proteolytic processes.[4] TNF-α–238 AG genotype has been associated with a high cytokine expressor phenotype.[5]

As an upstream modulator for many inflammatory cytokines and proteolytic enzymes, TNF-α induces IL-6 and matrix metalloproteinases, a family of proteolytic enzymes that degrade extracellular matrix around blood vessels and damage endothelial cells, which could result in destabilization and potential weakening of the vessel wall, passive dilation, and rupture.[6] Surgical BAVM specimens display elevated matrix metalloproteinase expression.[6] IL-6 expression appears to be increased as well and is associated with IL-6–174 GG genotype.[7]

Although these observations suggest a common pathway whereby genetic variation in IL-6 and TNF-α influences hemorrhagic risk, it remains unclear why only the IL-6–174 GG genotype was associated with hemorrhagic presentation at diagnosis, whereas the current study only implicates TNF-α–238 AG genotype in increased risk of new ICH in the natural course of disease after presentation.

A limitation of the current study is that the results depend on events in a small subset of our patients, which makes it difficult to assess more complicated models and fully explore possible confounders. Another consequence is a wide CI for the HR for TNF-α in Table 2 (1.31–12.29), although this does bound the HR to be ≥1.31. Nevertheless, we believe these findings indicate that the role of inflammatory cytokines in the pathogenesis of BAVM hemorrhage merits further study.

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