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# Common Variants in Interleukin-1-Beta Gene Are Associated with Intracranial Hemorrhage and Susceptibility to Brain Arteriovenous Malformation

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**Key Words**
Arteriovenous malformations · Cerebrovascular disease · Inflammation · Single nucleotide polymorphisms

**Abstract**
***Background:*** Polymorphisms in the proinflammatory cytokine interleukin (IL)-1β gene have been associated with systemic atherogenesis, thrombosis and rupture. The aim of this study was to investigate associations between single nucleotide polymorphisms (SNPs) in IL-1β and intracranial hemorrhage (ICH) in the natural course of brain arteriovenous malformation (BAVM) patients. ***Method:*** Two IL-1β promoter SNPs (–511C→T, –31T→C) and 1 synonymous coding SNP in exon 5 at +3953C→T (Phe) were genotyped in 410 BAVM patients. We performed a survival analysis of time to subsequent ICH, censoring cases at first treatment, death or last follow-up. A Cox regression analysis was performed to obtain hazard ratios (HRs) for genotypes adjusted for age, sex, Caucasian race/ethnicity and hemorrhagic presentation. ***Results:*** Subjects with the –31 CC genotype (HR = 2.7; 95% CI 1.1–6.6; p = 0.029) or the –511 TT genotype (HR = 2.6; 95% CI 1.1–6.5; p = 0.039) had a greater risk of subsequent ICH compared with reference genotypes, adjusting for covariates. The +3953C→T SNP was not significantly associated with an increased ICH risk (p = 0.22). The IL-1β promoter polymorphisms were also associated with BAVM susceptibility among a subset of 235 BAVM cases and 255 healthy controls of Caucasian race/ethnicity ($p < 0.001$). ***Conclusion:*** IL-1β promoter polymorphisms were associated with an increased risk of ICH in BAVM clinical course and with BAVM susceptibility. These results suggest that inflammatory pathways, including the IL-1β cytokine, may play an important role in ICH.



## Introduction

Brain arteriovenous malformation (BAVM) is a rare but important cause of hemorrhagic stroke in young adults. Most cases are sporadic, but BAVM also frequently occurs in patients with hereditary hemorrhagic telangiectasia [1], an autosomal dominant disorder (OMIM

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**Table 1.** Properties of IL-1β polymorphisms selected for genotyping

| Position relative to first base of exon 1 | dbSNP ID | Change | Function |
|---|---|---|---|
| –511 | rs16944 | C→T | promoter polymorphism |
| –31 | rs1143627 | T→C | promoter polymorphism |
| +3953 | rs1143634 | C→T | synonymous polymorphism at an amino acid coding site |

dbSNP ID = SNP database identification.

No. 600376) involving loss-of-function mutations in transforming growth factor-β pathway genes. Although the etiology of BAVM is obscure, genetic susceptibility may play a role in both susceptibility and disease progression [2].

Proinflammatory cytokines, including interleukins (ILs), have been reported to be involved in brain ischemic injury; increased availability of ILs in the brain tends to exacerbate damage from ischemic insult [3–5]. The effect of IL-1β during the ischemic insult appears to increase, owing to the synergistic effect of other cytokines [6]. Moreover, BAVM tissue specimens express 100- to 1,000-fold higher protein levels of inflammatory markers (myeloperoxidase, matrix metalloproteinase 9 and IL-6) compared with control brain tissue [7, 8].

Recently, common functional polymorphisms in the IL-1β gene have been associated with various cerebrovascular phenotypes, including ischemic stroke [9] and aneurysmal subarachnoid hemorrhage [10]. Therefore, we hypothesized that IL-1β polymorphisms may influence the natural course of BAVM and the risk of subsequent intracranial hemorrhage (ICH) and/or disease susceptibility.

## Methods

*Study Population*

BAVM cases were recruited at the University of California, San Francisco (UCSF) or the Kaiser Permanente Medical Care Plan (KPMCP) of Northern California. Clinical presentation and morphological characteristics were recorded as described previously [11, 12], using standardized definitions [13]. All subjects provided informed consent, and the studies were approved by the institutional review boards of the UCSF and the KPMCP.

*Genotyping*

Three polymorphic variants in IL-1β (–511C→T, –31T→C and +3953C→T) were selected on the basis of the putative functional effect and previous associations with related phenotypes (table 1) [9, 10]. Genotyping was performed using a template-directed dye-terminator incorporation assay with fluorescence polarization detection [14]. Positive and negative controls were included on all plates to minimize genotyping errors, and any discrepancies were repeated.

*Statistical Analysis*

Linkage disequilibrium, i.e. pairwise correlation, between single nucleotide polymorphisms (SNPs) was determined using both D′ and $r^2$ measures [15]. The $\chi^2$ goodness-of-fit test was used to assess whether genotypes were in Hardy-Weinberg equilibrium (HWE) within each and pooling across all race/ethnic groups.

*Cohort Analysis*

We performed the Kaplan-Meier survival analysis and log-rank test of time to subsequent ICH (primary outcome), defined as a symptomatic event with signs of new intracranial blood on computed tomographic or magnetic resonance imaging, after initial presentation but prior to any intervening treatment. Patients were censored at either date of first treatment, last follow-up or death. Clinical presentation leading to diagnosis was coded dichotomously as hemorrhagic or nonhemorrhagic.

Univariate and multivariate Cox regression analysis was performed to obtain hazard ratios (HRs) and 95% confidence intervals (CIs), adjusting for age, sex, Caucasian race/ethnicity and initial hemorrhagic presentation. The HR describes the relative risk of ICH based on the comparison of ICH rates in the high-risk versus low-risk (reference) genotype groups.

*Case-Control Analysis*

We also conducted a secondary case-control analysis of 235 BAVM patients and 255 healthy controls of self-reported Caucasian ancestry. Controls were healthy volunteers from the same clinical catchment area without a significant past medical history recruited for a pharmacogenetics study conducted at the UCSF [16]. Logistic regression analysis was performed to obtain odds ratios (ORs) and 95% CIs for each polymorphism, adjusting for age and sex.

*Meta-Analysis*

As a sensitivity analysis, we conducted a random effects meta-analysis to validate our case-control findings using the pooled IL-1β –31 CC genotype frequency of Caucasian controls from 14 published studies [17–30]. A restricted maximum likelihood

method was used to estimate the between-study variance as implemented in the Stata metareg package [31]. All statistical analyses were conducted using Intercooled Stata version 9 (StataCorp LP, College Station, Tex., USA).

## Results

Demographic and clinical characteristics of the BAVM cohort are shown in table 2. A total of 27 ICH events occurred during a total of 1,296 person-years at risk, with a mean (standard deviation) follow-up of 3.1 (7.5) years. The mean age of BAVM patients was 35.6 (17.4) years, 55% were of self-reported Caucasian race/ethnicity, 52% were females, and 42% presented with hemorrhage.

Genotype frequencies of IL-1β polymorphisms were in HWE among BAVM patients within and across all race/ethnic categories ($p > 0.05$). The 2 promoter SNPs were strongly correlated and in almost perfect linkage disequilibrium ($D' = 0.98$, $r^2 = 0.96$), whereas the exon 5 SNP was more weakly correlated with the promoter SNPs ($D' = 0.74$, $r^2 = 0.08$).

*Cohort Analysis*

Kaplan-Meier hemorrhage-free survival curves and corresponding log-rank tests by IL-1β genotypes are shown in figure 1, with numbers at risk shown at each 2-year time interval. Patients with the IL-1β –31 CC genotype ($p = 0.008$) or the –511 TT genotype ($p = 0.013$) had a greater rate of subsequent ICH compared with reference genotypes (fig. 1a, b). No significant difference in the rate of subsequent ICH was observed for the IL-1β +3953C→T polymorphism ($p = 0.145$; fig. 1c).

The univariate and multivariate HRs for IL-1β polymorphisms are summarized in table 3. Patients with the –31 CC genotype (HR = 2.7; 95% CI 1.1–6.6; $p = 0.029$) or the –511 TT genotype (HR = 2.6; 95% CI 1.1–6.5; $p = 0.039$) had a greater risk of subsequent ICH compared with reference genotypes, adjusting for covariates. A nonsignificant increased risk was observed in those with the +3953 CC genotype (HR = 2.2; 95% CI 0.6–7.7; $p = 0.218$).

Similar effect sizes for the IL-1β –31 CC genotype were observed when restricting analyses to Caucasians (HR = 2.4; 95% CI 0.7–8.3; $p = 0.175$) or after further adjustments for deep venous drainage and BAVM size (HR = 2.5; 95% CI 0.9–6.9; $p = 0.078$). However, these estimates were nonsignificant due to the reduced sample size from examining 1 race/ethnic group or missing angiographic data.

**Table 2.** Characteristics of 410 BAVM patients by new ICH status during the natural history course

| Characteristic | No new ICH | | New ICH | | Total | |
|---|---|---|---|---|---|---|
| | n = 383 | % | n = 27 | % | n = 410 | % |
| Race/ethnicity | | | | | | |
| African American | 16 | 4.2 | 3 | 11.1 | 19 | 4.6 |
| White | 210 | 54.8 | 15 | 55.6 | 225 | 54.9 |
| Asian/Pacific Islander | 49 | 12.8 | 2 | 7.4 | 51 | 12.4 |
| Hispanic | 97 | 25.3 | 7 | 25.9 | 104 | 25.4 |
| Native American | 5 | 1.3 | 0 | 0.0 | 5 | 1.2 |
| Other/unknown | 6 | 1.6 | 0 | 0.0 | 6 | 1.5 |
| Gender | | | | | | |
| Male | 182 | 47.5 | 13 | 48.2 | 195 | 47.6 |
| Female | 201 | 52.5 | 14 | 51.9 | 215 | 52.4 |
| Initial presentation | | | | | | |
| Nonhemorrhagic | 220 | 57.4 | 17 | 63.0 | 237 | 57.8 |
| Hemorrhagic | 163 | 42.6 | 10 | 37.0 | 173 | 42.2 |
| BAVM size | | | | | | |
| <3 cm | 179 | 46.7 | 10 | 37.0 | 189 | 46.1 |
| ≥3 cm | 143 | 37.3 | 14 | 51.9 | 157 | 38.3 |
| Missing | 61 | 15.9 | 3 | 11.1 | 64 | 15.6 |
| Venous drainage | | | | | | |
| Superficial | 169 | 44.1 | 13 | 48.2 | 182 | 44.4 |
| Superficial and deep | 107 | 27.9 | 7 | 25.9 | 114 | 27.8 |
| Deep | 59 | 15.4 | 3 | 11.1 | 62 | 15.1 |
| Missing | 48 | 12.5 | 4 | 14.8 | 52 | 12.7 |
| IL-1β –31T→C | | | | | | |
| TT | 116 | 30.3 | 10 | 37.0 | 126 | 30.7 |
| CT | 178 | 46.5 | 6 | 22.3 | 184 | 44.9 |
| CC | 80 | 20.9 | 10 | 37.0 | 90 | 22.0 |
| Missing | 9 | 2.3 | 1 | 3.7 | 10 | 2.4 |
| IL-1β –511C→T | | | | | | |
| CC | 116 | 30.3 | 11 | 40.8 | 127 | 31.0 |
| CT | 177 | 46.2 | 5 | 18.5 | 182 | 44.4 |
| TT | 76 | 19.8 | 9 | 33.3 | 85 | 20.7 |
| Missing | 14 | 3.7 | 2 | 7.4 | 16 | 3.9 |
| IL-1β +3953C→T | | | | | | |
| CC | 268 | 70.0 | 21 | 77.8 | 289 | 70.5 |
| CT | 100 | 26.1 | 3 | 11.1 | 103 | 25.1 |
| TT | 8 | 2.1 | 0 | 0 | 8 | 2.0 |
| Missing | 7 | 1.8 | 3 | 11.1 | 10 | 2.4 |

*Case-Control Analysis*

To determine if IL-1β SNPs were associated with disease susceptibility, we performed a case-control analysis restricted to Caucasians. IL-1β –31 CC or –511 TT genotypes were present at a much higher frequency in BAVM cases than in controls (table 4). The risk of BAVM was higher in subjects with the putative high-risk IL-1β genotypes after adjusting for age and sex: OR of 3.2 (95% CI 1.7–6.1; $p < 0.001$) for the –31 CC genotype and for the –511 TT genotype, respectively, and 1.8 (95% CI 1.2–2.7; $p = 0.004$) for the +3953 CC genotype (table 4).

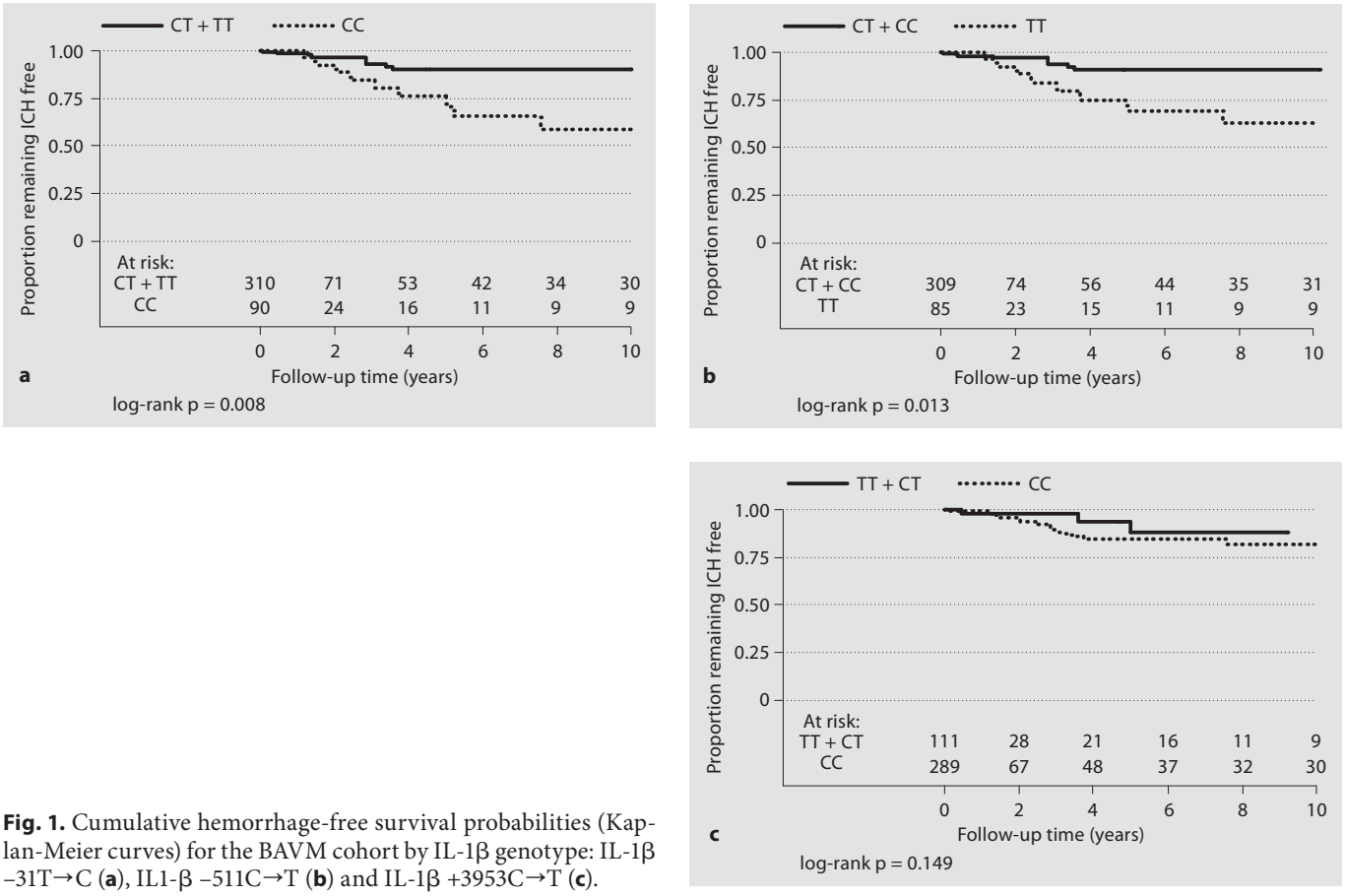


**Fig. 1.** Cumulative hemorrhage-free survival probabilities (Kaplan-Meier curves) for the BAVM cohort by IL-1β genotype: IL-1β –31T→C (**a**), IL1-β –511C→T (**b**) and IL-1β +3953C→T (**c**).

**Table 3.** Cox regression analysis of time to new ICH and IL-1β polymorphism genotype in BAVM patients

| SNP | Cases | Univariate | | | Multivariate[1] | | |
|---|---|---|---|---|---|---|---|
| | | HR | 95% CI | p | HR | 95% CI | p |
| IL-1β –31 CC | 400 | 3.00 | 1.27–7.09 | 0.012 | 2.71 | 1.11–6.60 | 0.029 |
| IL-1β –511 TT | 394 | 2.90 | 1.20–7.01 | 0.018 | 2.62 | 1.05–6.53 | 0.039 |
| IL-1β +3953 CC | 400 | 2.38 | 0.71–8.33 | 0.158 | 2.17 | 0.63–7.69 | 0.218 |

[1] Adjusted for age at diagnosis, gender, white race and hemorrhagic presentation.

However, the observed high-risk genotype frequencies for the 2 promoter SNPs in our Caucasian control population were lower than those previously reported in the published literature and were also out of HWE (p = 0.009). Therefore, we conducted a meta-analysis to validate our IL-1β –31T→C findings using the pooled genotype frequency of controls reported in 14 published studies conducted in Caucasians [17–30]. The IL-1β –31 CC genotype association remained when comparing the BAVM genotype frequency with the published controls. On average, the –31 CC genotype frequency was 5.1% higher in BAVM cases (17%) compared with pooled controls (12%), accounting for variation across studies (p = 0.042).

**Table 4.** IL-1β genotype frequency and risk of BAVM among Caucasian cases and controls

| SNP | Cases | | Controls | | OR[1] | 95% CI | p value |
|---|---|---|---|---|---|---|---|
| | n | % | n | % | | | |
| IL-1β –31T→C | | | | | | | |
| TT | 91 | 38.7 | 107 | 42.3 | reference | | |
| CT | 103 | 43.8 | 129 | 51.0 | 1.07 | 0.71–1.60 | 0.753 |
| CC | 41 | 17.5 | 17 | 6.7 | 3.35 | 1.71–6.57 | <0.001 |
| Total | 235 | | 253 | | | | |
| CC versus any T | | | | | 3.23 | 1.72–6.10 | <0.001 |
| IL-1β –511C→T | | | | | | | |
| CC | 89 | 38.5 | 110 | 43.1 | reference | | |
| CT | 102 | 44.2 | 128 | 50.2 | 1.10 | 0.73–1.65 | 0.660 |
| TT | 40 | 17.3 | 17 | 6.7 | 3.40 | 1.72–6.71 | <0.001 |
| Total | 231 | | 255 | | | | |
| TT versus any C | | | | | 3.23 | 1.70–6.14 | <0.001 |
| IL-1β +3953C→T | | | | | | | |
| CC | 160 | 68.4 | 143 | 55.8 | reference | | |
| CT | 67 | 28.6 | 99 | 38.7 | 0.58 | 0.39–0.87 | 0.008 |
| TT | 7 | 3.0 | 14 | 5.5 | 0.41 | 0.15–1.11 | 0.080 |
| Total | 234 | | 256 | | | | |
| CC versus any T | | | | | 1.79 | 1.21–2.66 | 0.004 |

[1] Adjusted for age and sex.

## Discussion

We provide the first report of an association between polymorphic variants in the IL-1β gene with both ICH risk in the natural course of BAVM patients and BAVM susceptibility. Several lines of evidence support our findings. First, all 3 SNPs have previously been implicated in various cerebrovascular phenotypes, including ischemic injury [3], stroke [9, 32] and aneurysmal rupture [10]. For example, the TT genotype of the IL-1β –511C→T polymorphism was significantly associated with an increased risk of subarachnoid hemorrhage [10] and small vessel disease stroke [32] in a Polish population, but with a decreased risk of ischemic stroke in an Italian population [9]. Second, animal studies have shown that IL-1β expression levels are significantly upregulated after intracerebral hemorrhage [33]. Third, all 3 polymorphisms are located in known functional regions of the IL-1β gene (the promoter and an exon), and several studies have demonstrated a genotype-phenotype association. The T allele of the –511 promoter SNP is correlated with enhanced IL-1β production in vivo [34], and the –31 promoter SNP is located in a TATA box transcription initiation site [28]. The +3953C→T (Phe) variant has been shown to influence in vitro IL-1β production in some [35, 36] but not in all studies [37]. Haplotypes containing both –511T and –31C alleles have been shown to have increased transcriptional activity in vitro [38]. Thus, the increased ICH risk in IL-1β high-risk genotype carriers may be due to increased local or peripheral inflammation influencing AVM disease progression.

The IL-1β gene located on chromosome 2q14 is approximately 7 kb long with 7 exons. However, it sits in an IL-1 cytokine cluster spanning approximately 430 kb, containing genes for IL-1α, IL-1β and IL-1 receptor antagonist. Therefore, the association we observed with IL-1β variants could possibly be due to linkage disequilibrium (LD) with variants in these related IL-1 genes or additional variants in the IL-1β gene. As expected, analyses of both IL-1β promoter SNPs yielded similar findings due to the strong LD. Thus, we cannot distinguish whether 1 of the 2 promoter SNPs, or another SNP in the same LD block, is responsible for the observed associations with BAVM susceptibility and ICH.

An increasing number of reports suggest a role of inflammatory signaling in BAVM pathogenesis. Activin-like kinase 1, a transforming growth factor-β superfamily member, is mutated in hereditary hemorrhagic telan-

giectasia; a common activin-like kinase 1 polymorphism is also strongly associated with sporadic BAVM [39, 40]. The GG genotype of the IL-6 174G→C promoter polymorphism was associated with clinical presentation of ICH in BAVM patients [41] and with highest IL-6 expression levels in BAVM tissue [8]; IL-6 levels correlated strongly with IL-1β mRNA levels [8]. Compared with control brain (structurally normal cortex removed during temporal lobectomy), BAVM tissue had an order of magnitude higher expression of the leukocyte marker myeloperoxidase, which highly correlated and colocalized with matrix metalloprotease 9 and IL-6 expression [7]. Finally, a promoter polymorphism in the tumor necrosis factor-α (–238G→A) gene was associated with new hemorrhage in the natural course of a sample of 280 BAVM cases; the presence of the A allele increases the risk of ICH [12].

Together, these results suggest that inflammatory mechanisms are involved in BAVM susceptibility and progression and warrant replication and further investigation into the precise mechanism of how proinflammatory pathways are involved. A better understanding of BAVM pathogenesis is needed to prevent ICH and potentially help develop novel therapeutics for patient management.

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