The genetics of sporadic ruptured and unruptured intracranial aneurysms: a genetic meta-analysis of 8 genes and 13 polymorphisms in approximately 20,000 individuals

Clinical article

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Object. Intracranial aneurysms (IAs) are thought to have a multifactorial origin. The authors undertook a comprehensive meta-analysis on all genes investigated using a case-control model in ruptured (subarachnoid hemorrhage) and unruptured aneurysms.

Methods. Electronic databases were searched until and including July 2008 for any candidate gene studied in IA or subarachnoid hemorrhage using a case-control model. The ORs and 95% CIs were determined for each gene-disease association using fixed and random effect models.

Results. Thirty studies of 8 genes and 13 polymorphisms were analyzed among 19,961 individuals (6622 cases and 13,339 controls). Two genes and 3 polymorphisms were associated with IA. The eNOS gene T786C polymorphism (OR 1.24, 95% CI 1.0–1.54; p = 0.05) and IL-6 gene G572C polymorphism (OR 7.08, 95% CI 2.85–17.57; p < 0.0001) both showed a significant association with ruptured/unruptured IA. The IL-6/G174C polymorphism exerted a significant protective effect against IA (OR 0.49, 95% CI 0.25–0.95; p = 0.04). The other candidate genes investigated (ACE, endoglin, APOE, elastin, MMP-3, and SERPINA3) showed no significant associations.

Conclusions. There is a likely genetic basis to sporadic IAs. However, the evidence base is small when compared against other complex disorders. (DOI: 10.3171/2009.8.JNS092)

Key Words • intracranial aneurysm • ruptured aneurysm • unruptured aneurysm • polymorphism • single nucleotide • meta-analysis

It is estimated that ~2% of the general population have an IA\(^9\) whose rupture results in an SAH.\(^31\) These IAs may occur alone (70–75%) or as multiple aneurysms (25–30%).\(^31\) Risk factors for the formation, growth, and rupture of IAs include age, sex, cigarette smoking, hypertension, and atherosclerosis,\(^23\) although aneurysm size, cigarette smoking, and age are the most important risk factors for rupture.\(^41\) Familial aneurysms tend to be larger at time of rupture and are more likely to be multiple, suggesting a genetic liability.\(^51\) The prognosis of SAH is poor with a mortality rate of ~50% within 1 month of rupture; 20% of patients ultimately remain dependent for activities of daily living.\(^18,20,36,51\)

Case-control studies using an allelic-association model in IA have identified a number of potential genetic predisposing SNPs. However, due to lack of reproducibility, uncertainty remains about the nature and number of genes contributing to risk of the aneurysm.\(^6\) By using all available published data, meta-analyses can increase statistical power, thus allowing risks to be quantified with greater precision.

We therefore performed a comprehensive meta-analysis of all genetic case-control studies of ruptured and unruptured IAs.

Methods

Data Sources

Electronic searches using PubMed, Google Scholar, Yahoo, and EMBASE were conducted to identify all published case-control studies evaluating any candidate gene
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in IA and SAH in humans published until and including June 2008. Letters and abstracts were included in the searches. The Medical Subject Headings and text words used for the search were “intracranial aneurysm,” “berry aneurysm,” “saccular aneurysm,” or “subarachnoid hemorrhage” in combination with “genetics,” “gene,” “single nucleotide polymorphism,” “SNP,” “polymorphisms,” or “genetic linkage.” Search results were limited to humans. All languages were searched. The references of all identified publications were hand-searched for additional studies, and the PubMed option “related articles” was used to examine all relevant articles.

Study Selection

Selection criteria included case-control studies in which the aneurysm was characterized as a dichotomous trait. Studies were only selected if an unruptured aneurysm was diagnosed using conventional angiography, 3D CT scanning, MR imaging, or MR angiography. The SAH studies were included if the hemorrhage was verified by lumbar puncture, CT scanning, or during surgical repair. Studies were excluded if patients had any history of connective tissue disorder or polycystic kidney disease, the genotype frequency was not reported and could not be obtained by contacting authors, and quantitative traits or intermediate phenotypes were investigated. For studies with > 1 publication describing results among the same or overlapping groups of patients or controls, only the largest of the available published data sets was included.

Statistical Analysis

Data were analyzed using software for preparing and maintaining Cochrane reviews (Review Manager version 5.0, Cochrane Collaboration, http://www.cc-ims.net/RevMan) and Comprehensive Meta Analysis version 2.2.023 (Biodata, http://www.biodata.org). To determine the strength of genetic association, a pooled OR was calculated for each gene variant by using fixed- and random-effects models, in addition to the calculation of 95% CIs. Fixed-effects summary ORs were calculated using the Mantel-Haenszel method and the DerSimonian and Laird method was used to calculate random-effects summary ORs. The frequencies of at-risk genotypes were compared between cases and controls for each SNP analyzed. Tests for heterogeneity were performed for each polymorphic meta-analysis with significance set at p ≤ 0.05. Publication bias was assessed using funnel plots while the Egger regression asymmetry test was conducted for each SNP with ≥ 3 publications. Iterative sensitivity analysis was carried out in cases in which there was heterogeneity in the initial meta-analysis.

The proportion of aneurysm cases in the population that could be attributed to a particular gene variant (PAR) was estimated as \( PAR = 100 \times \frac{\text{prevalence} \times (OR - 1)}{\text{prevalence} \times (OR - 1) + 1} \). For this calculation the fixed-effects model was used, and the prevalence of exposure was estimated as the genotype frequency among controls.

Results

From 671 papers identified in our primary search, 86 met the initial inclusion criteria. Following examination of the complete texts, 24 did not meet our end inclusion criteria, leaving 62 candidate-gene case-control studies in which a ruptured or unruptured intracerebral aneurysm was analyzed in a dichotomous manner. In total, 91 polymorphisms in 23 genes were identified. Only genes with at least 2 publications on any single SNP were included in our analyses, leaving a total of 30 publications addressing 13 polymorphisms in 8 genes (Fig. 1).

Four of the 13 polymorphisms in the 8 genes analyzed in detail (6622 cases and 13,339 controls), the mean number of studies per polymorphism was 3.5. Seven of the 13 meta-analyses had > 500 cases, and 10 had a total participant size of > 1000. Table 1 summarizes the genotypic ORs for the 13 polymorphisms and modes of inheritance evaluated.

Five studies evaluated the association between the T786C polymorphism on the eNOS gene and ruptured/unruptured IA. Significant association was seen in the dominant 786C model (Fig. 2) (OR 1.24, 95% CI 1.0–1.54; p = 0.05) but not in the recessive 786CC model (OR 0.83, 95% CI 0.38–1.83; p = 0.64). There was no significant interstudy heterogeneity in the dominant model. Following iterative sensitivity analysis, the statistical significance of our finding was maintained in the recessive model (p = 0.15) model.

Three studies were evaluated that identified the G572C polymorphism of the IL-6 gene and its association with ruptured/unruptured IA. In assessing the dominant 572C model, there was no significant association with IA when compared against homozygous G carriers (OR 2.12, 95% CI 0.65–6.78; p = 0.21). However, the recessive 572CC model showed significant association when compared against the homozygote (G572G) and heterozygote (G572C) state (OR 7.08, 95% CI 2.85–17.57; p < 0.0001). Significant interstudy heterogeneity was observed in the dominant (pHet < 0.00001) but not the recessive (pHet = 0.15) model. Following iterative sensitivity analysis, the statistical significance of our finding was maintained in the recessive model. Funnel plots were symmetrical and the Egger test for both the dominant (p = 0.17) and recessive (p = 0.648) models showed no significance, suggesting little evidence of publication bias.

In the case of the G174C polymorphism on the IL-6 gene, 2 studies were identified that evaluated its association with ruptured/unruptured IA. No significant association with IA was observed in the dominant model (OR 0.71, 95% CI 0.41–1.25; p = 0.04). However there was a significant association in the recessive model (OR 0.49, 95% CI 0.25–0.95; p = 0.04) with the polymorphism exerting a protective effect. Interstudy heterogeneity was not observed in either the dominant (pHet = 0.07) or recessive (pHet = 0.22) model. Following iterative sensitivity analysis, the statistical significance of our finding was not maintained. Publication bias could not be assessed as there were only 2 studies.
Six studies were identified that investigated the Endo-
glin intron 7, 23 bp insertion polymorphism and its asso-
ciation with ruptured/unruptured IAs.22,32,46,47,60 No sig-
ificant association was observed in either the dominant
(OR 1.00, 95% CI 0.82–1.22; p = 0.99) or recessive (OR
1.41, 95% CI 0.79–2.52; p = 0.25) model. Funnel plots
were symmetrical, and the Egger test for the dominant
(p = 0.954) and recessive (p = 0.286) models showed no
significance, suggesting a low probability of publication
bias.

Four studies evaluating the ACE/I polymorphism
and its association with ruptured/unruptured IA were
identified.26,34,44,55 No significant association was ob-
served when ACE/I was analyzed in the dominant model
(OR 1.23, 95% CI 0.82–1.85; p = 0.31) or recessive model
ACE/II (OR 1.58, 95% CI 0.98–2.57; p = 0.06). Signifi-
cant interstudy heterogeneity was observed (p_Het = 0.02).
Funnel plots were symmetrical and the Egger test for the
dominant (p = 0.894) and recessive (p = 0.756) models
showed no significance, suggesting a low probability of
publication bias.

A total of 4 studies were identified evaluating the
G894T polymorphism on the eNOS gene and its associa-
tion with ruptured/unruptured IA.27,30,34,43 In assessing the
dominant 894T model, there was not a significant asso-
ciation with IA when compared against the homozygous
GG genotype (OR 0.89, 95% CI 0.57–1.40; p = 0.61).
Similarly, the recessive 894TT model showed no signifi-
cant association when compared against the homozygote
(G894G) and heterozygote (G894C) states (OR 0.85, 95%
CI 0.36–1.96; p = 0.70). Funnel plots were symmetrical,
and the Egger test for both the dominant (p = 0.629) and
recessive (p = 0.295) models showed no significance, sug-
gestive little evidence of publication bias.

Three studies evaluated the Intron 4 variable number
tandem repeat polymorphism on the eNOS gene and its
association with ruptured/unruptured IA.27,30,34 No signif-
icant association was observed in the dominant 4A model
(OR 1.43, 95% CI 0.70–2.89; p = 0.32) or recessive model
4A4A (OR 0.77, 95% CI 0.33–1.83; p = 0.56). Funnel plots
were symmetrical and the Egger test for the dominant
(p = 0.149) and recessive (p = 0.670) models showed no sig-
ificance, suggesting little evidence of publication bias.

For the APOE gene, 5 studies were identified that
evaluated its association with ruptured/unruptured IA.13,25,
29,38,61 We assessed both ε2 and ε4 as risk factors in domi-
nant and recessive models. In the case of ε4, there was
no significant association found in either the dominant
model (OR 1.13, 95% CI 0.78–1.63; p = 0.52) or the reces-
sive model (OR 1.57, 95% CI 0.71–3.45; p = 0.26). Funnel
plots were symmetrical, and the Egger test for the domi-
nant (p = 0.987) and recessive (p = 0.947) models showed
no significance, suggesting little evidence of publication
bias.

Similarly, for APOE ε2 there was no association with
ruptured/unruptured IA in either the dominant model (OR
1.05, 95% CI 0.77–1.44; p = 0.77) or the recessive model
(OR 1.41, 95% CI 0.48–4.12; p = 0.53). Funnel plots were
symmetrical and the Egger test for both dominant (p = 0.694) and recessive (p = 0.781) models showed no significance, suggesting little evidence of publication bias.

Two studies were identified that evaluated the intron 20 T to C mutation on the elastin gene and its association with ruptured/unruptured IA. No association with the dominant CT/CC model (OR 0.91, 95% CI 0.56–1.48; p = 0.69) or recessive CC model (OR 1.72, 95% CI 0.73–4.03; p = 0.22) was observed.

Neither of the 2 studies in intron 23 T to C polymorphism found in either the dominant model (OR 0.79, 95% CI 0.53–1.18; p = 0.25) or recessive model (OR 1.04, 95% CI 0.73–1.51; p = 0.81) showed a significant association.

Two studies were identified evaluating the MMP-3 nt-1612 polymorphism and its association with ruptured/unruptured IA. No significant association was found in either the dominant model (OR 0.81, 95% CI 0.49–1.33; p = 0.40) or the recessive model (OR 1.14, 95% CI 0.74–1.77; p = 0.55).

Two studies for the alanine/threonine polymorphism of the α-1-antichymotrypsin SERPINA3 gene were identified. No significant association in the dominant AT/TT model (OR 1.35, 95% CI 0.73–2.49; p = 0.34) or the recessive TT model (OR 1.01, 95% CI 0.80–1.28; p = 0.93) was found.

**Discussion**

In this comprehensive meta-analysis, 3 (eNOS T786C, IL-6 G572C, and IL-6 G174C) of the polymorphisms analyzed in 2 genes were significantly associated with ruptured/unruptured IAs. The eNOS gene SNPs increased the risk of IA while IL-6 G174C seemed protective. The mean number of participants for these polymorphisms was > 2800, allowing more precise estimates to be made of the effect of these genes than could be estimated from any single study. The individual risk provided by these polymorphisms was moderate except in the case of IL-6 G572C (OR 7.21). The risk provided by eNOS T786C (OR 1.24) was in agreement with previous studies in IAs and other complex diseases that are thought to have a polygenic basis such as myocardial infarction. Previous meta-analyses from our group have shown the ACE/D polymorphism to have an OR of 1.21 in ischemic stroke, an OR of 1.90 in ischemic stroke in patients of non-European descent, and the ACE/I polymorphism...
to have an OR of 1.48 when assessed in all hemorrhagic stroke.45 However, no significant association was found with the ACE gene and IA.

Meta-analysis of molecular variants in the 5 remaining genes, endoglin, APOE, Elastin, MMP-3, and SERPINA3 have thus far failed to provide evidence of increased susceptibility to IA formation and rupture. When assessed in all cases of IA and SAH, the previous study by our group showed a significant association with the endoglin intron 7 insertion polymorphism in the recessive model (OR 3.47, 95% CI 0.53–8.30; p = 0.01), but only 3 studies were included in that meta-analysis. In the same study, APOE and SERPINA3 failed to show any significant association when assessed in all hemorrhagic stroke; thus these findings are consistent with our present study.45

The PARs for gene variants with significant associations for aneurysm in this study were 6.4% for eNOS 786C and 3.89% for IL-6 G572C. These values are far lower than those reported for well-established risk factors for IA such as smoking and hypertension.4 This low level of PAR is not surprising given that the genetic contribution of any single gene toward a complex disease is unlikely to act in a simple mendelian fashion but rather with epistatic (gene-gene or gene–environmental interaction) effects. Nevertheless, given the high incidence of IA rupture causing SAH (5000 patients per year in the UK28), if these estimates hold true, they suggest that common variants in the genes may alone contribute to between 180 and 320 aneurysms in the United Kingdom alone each year.

Endothelial nitric oxide synthase catalyses the synthesis of NO, a regulator of basal vascular tone, cerebral blood flow, and smooth-muscle cell proliferation in the arterial endothelium.10,39 A number of studies have shown that the NO pathway is impaired and that NO levels in CSF are raised in animals following experimentally induced SAH.63 The NO levels have also been shown to increase in humans following aneurysmal SAH.54 Furthermore NO knock-out mice are prone to various vascular diseases such as aortic aneurysm.53 The precise molecular effects of polymorphisms in the eNOS gene have not yet been established, but there is biochemical evidence for decreased eNOS gene promoter activation, reduced protein expression, and reduced enzymatic activation in individuals with the T786C polymorphism.57,62 Our results showed significant association with the T786C polymorphism for carriers of the C allele (OR 1.24, 95% CI 1.0–1.54; p = 0.05). It is possible that this variant may convey a predisposition for the development and rupture of IA through aberrant NO signaling.15,21,39 A previous study by our group failed to show a significant association between SAH and the T786C polymorphism; however, the meta-analysis was limited to 4 studies.45

The strongest association with IA was observed in the IL-6 polymorphism G572C; however, IL-6 G172C was shown to be protective for IA. The IL-6 gene is located in the short arm of chromosome 7.13 Interleukin-6 is a proinflammatory cytokine19 and is involved in a number of diseases such as myocardial infarction14 and juvenile chronic arthritis.11 The polymorphisms G174C and G572C have both been shown to alter plasma expression of IL-6 in healthy individuals.5,11 Carriers of the -572C allele have been shown to have significantly higher plasma IL-6 levels than those of the -572GG genotype.1 In regard to G172C, results have been conflicting, with 1 study reporting the C allele to be significantly associated with lower plasma levels of IL-6 while another study associated it with a raised level of plasma IL-6.5 Our results showed a significant association with IA for IL-6 G572C with those homozygous for the mutant allele (OR 0.49, 95% CI 0.25–0.95; p = 0.04). With regard to the G174C polymorphism, it exerted a protective effect from IA, with a significant association in those homozygous for the mutant allele (OR 1.54; p = 0.05). It is possible that this variant may convey a predisposition for the development and rupture of IA through aberrant NO signaling.15,21,39

### Table: Odds Ratios for eNOS 786C and IL-6 Polymorphisms

<table>
<thead>
<tr>
<th>Study or Subgroup</th>
<th>Case Events</th>
<th>Total</th>
<th>Control Events</th>
<th>Total</th>
<th>Weight</th>
<th>Odds Ratio M-H, Random, 95% CI</th>
<th>Test for overall effect: Z (P)</th>
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</thead>
<tbody>
<tr>
<td>Akagawa 2005</td>
<td>91</td>
<td>411</td>
<td>77</td>
<td>405</td>
<td>39.9%</td>
<td>1.21 [0.86, 1.70]</td>
<td></td>
</tr>
<tr>
<td>Khurana 2004</td>
<td>39</td>
<td>51</td>
<td>62</td>
<td>90</td>
<td>7.5%</td>
<td>1.47 [0.67, 3.22]</td>
<td></td>
</tr>
<tr>
<td>Krex 2006</td>
<td>87</td>
<td>135</td>
<td>113</td>
<td>184</td>
<td>21.8%</td>
<td>1.14 [0.72, 1.81]</td>
<td></td>
</tr>
<tr>
<td>Krischek 2006</td>
<td>79</td>
<td>405</td>
<td>31</td>
<td>176</td>
<td>21.9%</td>
<td>1.13 [0.72, 1.79]</td>
<td></td>
</tr>
<tr>
<td>Song 2006</td>
<td>26</td>
<td>132</td>
<td>13</td>
<td>113</td>
<td>8.9%</td>
<td>1.89 [0.92, 3.87]</td>
<td></td>
</tr>
<tr>
<td>Total (95% CI)</td>
<td>1134</td>
<td>968</td>
<td>1134</td>
<td></td>
<td></td>
<td>1.24 [1.00, 1.54]</td>
<td></td>
</tr>
</tbody>
</table>

**Fig. 2.** Forest plot of pooled OR of eNOS 786C in IAs. The 95% CIs are drawn with the size of the box reflecting the weighting given to each study. M-H = Mantel-Haenszel.
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The interpretation of any meta-analysis must be made within the context of its limitations, including study selection, publication bias, and variability in the methodological quality of the included studies. Egger asymmetry tests and funnel plots showed no substantial evidence of publication bias. Publication bias cannot be completely excluded, but it is an unlikely explanation for our findings. Moreover, rigorous selection criteria (use of cerebral angiography, MR imaging, MR angiography, and 3D CT scanning to diagnose IA; and a surgical, CT scanning or lumbar puncture to diagnose SAH) enriched the meta-analyses for the quality of the studies to be included. Only 2 of our meta-analyses included > 500 cases (eNOS T786C and IL-6 G572C) with the average number of cases and controls in the significant meta-analyses being 636 and 2284, respectively. More reliable assessment of attributable risk will only come from studies with a much larger number of individuals. Using cerebral angiography, MR imaging, MR angiography, and 3D CT scanning to diagnose IA and a surgical diagnosis, CT scanning, or lumbar puncture for SAH as a selection criterion may have helped to maintain comparable groups of cases; however, the selection of control groups varied considerably between studies.

We have combined the available data on ruptured and unruptured aneurysms. While it could be argued that different and distinct mechanisms may exist that account for the rupture of a preexisting aneurysm, the purpose of this study was to gain as much power as possible on the genetic influence in cerebral aneurysms rather than the subclassifying by eventual clinical outcome. This strategy had the additional benefit of increasing power of the overall pooled results and providing us with a more robust result. However, we accept that this could be considered a limitation, and readers should be aware of the strategy used. Statistical methods using marker genotype data may in the future permit the detection and control of confounding due to population stratification and selection bias in genetic association studies. This may reduce the impact of variability within control groups. Finally, there is evidence to suggest that allele frequencies for several candidate genes investigated in our study vary by ethnicity. However, we have recently found that, at least in the case of ischemic stroke, differences in genetic effects across different ethnic groups may be overstated. Notwithstanding this observation, the confounding effect of heterogeneity between different ancestral populations cannot be dismissed. Regrettably, sample sizes were insufficient to determine whether the development of the initial aneurysm is influenced by genetics beyond which other, more established factors come to the fore.

Conclusions

Our finding suggests that there exists a genetic etiology of sporadic IA with ≥ 3 polymorphisms (eNOS T786C, IL-6 G572C, and IL-6 G174C) in 2 genes, each with a moderate effect associated with these aneurysms; however, the evidence base is small when compared against other common disorders. Our results determine ORs with greater reliability and more robust CIs than has previously been assessed by any single study.

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