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Single nucleotide polymorphisms associated with sporadic brain arteriovenous malformations: where do we stand?

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Brain arteriovenous malformations are characterized by a tangle of abnormal vessels directly shunting blood from the arterial to venous circulation. They are known to occur either sporadically or in the context of well-defined genetic disorders. Haemorrhage represents the most severe clinical manifestation, whereas other common symptoms include headache, seizures and neurological deficits. Although sporadic forms do not recognize a specific genetic cause, in recent years, it has been hypothesized that genes involved in angiogenesis and inflammation or coding for proteins, such as fibronectins, laminins and integrins, may play a role in the pathophysiology of brain arteriovenous malformations. More recently, a new trend of genetic studies has investigated the association between sporadic arteriovenous malformations and single nucleotide polymorphisms, single base variations between genomes within members of a biological species or between paired chromosomes in an individual, which may determine the susceptibility to develop complex diseases and influence their natural history. Several polymorphisms in two different families of genes have been associated with disease susceptibility and increased haemorrhagic risk. These genes are mainly involved in the inflammatory cascade and in the regulation of angiogenesis. However, most of the investigated polymorphisms have been selected on the basis of candidate genes because of their potential functional role in the pathogenesis of brain arteriovenous malformations or in other cerebrovascular diseases. Only one hypothesis-free genome-wide association study in a small number of patients has been performed so far, but it was unable to identify significant associations between brain arteriovenous malformations and specific genetic loci. In this article, we review and analyse the polymorphisms investigated to date in association with sporadic brain arteriovenous malformations in the medical literature. We discuss the biological, pathophysiological and clinical implications of these studies, with particular attention to the prediction of haemorrhagic risk and the possibility of building genetic profiles capable of defining the architectural features of the malformations and predict their evolution and natural history. We also present a joint analysis of the risk estimates found by the studies in literature that have evaluated the association between single nucleotide polymorphisms and brain arteriovenous malformation susceptibility and risk of bleeding. This analysis shows a statistically significant association between the interleukin $6 \rightarrow 174G \rightarrow C$ (odds ratio $= 1.97; 95\%$ confidence interval: $1.15–3.38$) and the tumour necrosis factor $\alpha \rightarrow 238G \rightarrow A$ (odds ratio $= 2.19; 95\%$ confidence interval: $1.25–3.83$) gene polymorphisms and risk of intracranial haemorrhage and between the activin-like kinase 1 (also known as...
Introduction

Brain arteriovenous malformations are characterized by a tangle of abnormal vessels, not clearly differentiated, directly shunting blood from the arterial to venous circulation without an interposed capillary bed. They are known to occur either sporadically or in the context of genetic disorders such as Osler–Weber–Rendu syndrome (hereditary haemorrhagic telangiectasia), an autosomal dominant disease characterized by severe and recurrent nose bleeds, muco-cutaneous telangiectasias and visceral arteriovenous malformations affecting lung, liver and CNS (Moftakhar et al., 2009). Brain arteriovenous malformations also characterize certain congenital, non-hereditary conditions such as the Wyburn–Mason syndrome, in which brain arteriovenous malformations are associated with retinal angiomas (Schmidt et al., 2008), and Sturge–Weber syndrome, a neurocutaneous disorder in which brain arteriovenous malformations are associated with a large port-wine stain birthmark on the forehead (Di Rocco and Tamburrini, 2006).

Brain arteriovenous malformation rate is ~1.1 per 100,000 adults per year (Brown et al., 1996). The medical literature does not recognize a specific genetic cause for sporadic brain arteriovenous malformations; thus, they are to date considered a multigenic/multifactorial disease. However, in recent years, there has been increasing appreciation of the molecular mechanisms underlying their aetiology. All the genetic and molecular factors that have so far been identified as potentially involved in brain arteriovenous malformation pathogenesis play a role in cerebral vasculogenesis, and their deregulation seems to create a hyperangiogenic environment, resulting in proliferation of abnormal vessels and impaired arteriovenous specification (Hashimoto et al., 2004; Moftakhar et al., 2009). Recently, a new trend of genetic studies has investigated the association between single nucleotide polymorphisms (SNPs) and sporadic brain arteriovenous malformations with the aim to evaluate the potential ability of certain SNPs to influence their pathogenesis and clinical course. SNPs are point variations in DNA sequence occurring when a single nucleotide (A, T, C or G) in the genome differs between members of a biological species or paired chromosomes in an individual (Manolio, 2010). Their genetic influence in biomedical research is in comparing genomic regions between cohorts and testing their association with a disease. Indeed, SNPs can affect how humans develop diseases and respond to pathogens, chemical agents and drugs. SNP analysis has revolutionized the research of the genetic influences in complex traits, which, in contrast with single-gene disorders, are caused by many genetic and environmental factors working together, each having a relatively small effect and few, if any, being absolutely required for the occurrence of the disease (Manolio, 2010). Associations between SNPs and diseases are consistent with the common disease–common variant hypothesis, which posits that genetic influences on susceptibility to common diseases are attributable to a limited number of variants present in >1–5% of the population. However, despite their value in locating the vicinity of genomic variants that may cause the diseases, only a few among the identified SNPs seem to have clear functional implications in the pathophysiological mechanisms that influence expression or function of the codified proteins (Hindorff et al., 2009). The studies conducted in patients affected by brain arteriovenous malformations have identified two main families of polymorphisms concerning genes involved in the angiogenic and inflammatory cascades. Other SNPs associated with increased risk of intracranial bleeding in patients with brain arteriovenous malformation have been found in the apolipoprotein E (APOE) and EPH receptor B4 (EPHB4) genes: the first seems to have a role in several signalling cascades influencing inflammatory pathways; the latter is known to be involved in arteriovenous determination during embryogenesis.

The identification of SNPs associated with brain arteriovenous malformation might help to understand the biological mechanisms underlying the pathogenesis and natural history of this disease, to facilitate risk-based stratification and to define personalized therapeutic approaches.

This article focuses on the SNPs that have been identified in association with brain arteriovenous malformation in the medical literature. We distinguish SNPs associated with disease susceptibility and SNPs associated with increased risk of bleeding and discuss the potential pathophysiological, clinical and therapeutic implications of these studies. Also, we present the results of a joint analysis of the measures of risk of the same polymorphism reported by different authors in literature, to identify the SNPs more strongly associated to brain arteriovenous malformation susceptibility and risk of bleeding, thus providing novel and crucial information to better understand the role of gene variations in this pathological condition.

Materials and methods

PubMed (http://www.ncbi.nlm.nih.gov/pubmed/) was used to identify the original research studies and the review articles that were included in this article. To maximize the research power for pertinent literature, we used the general search terms ‘polymorphism’ and ‘arteriovenous’. This yielded 66 results. Among these studies, 45 articles were excluded because they focused on other arteriovenous malformations (such as brain cavernous malformations, non-sporadic brain arteriovenous malformation, visceral arteriovenous malformations and...
peripheral arteriovenous fistulae in haemodialysis patients), rather than sporadic brain arteriovenous malformations. The remaining 21 articles focused on sporadic brain arteriovenous malformations and were then selected for further analysis. All of these studies were published between 2004 and 2012. Among them were three review articles (Young and Yang, 2004; Kim et al., 2008a, 2009b) and a Letter to the Editor (Young et al., 2007). All SNPs significantly associated with brain arteriovenous malformation susceptibility and increased risk of bleeding were selected and reported in two different tables along with the data regarding the study design adopted, the sample size collected and the significance of association found at the univariate and multivariate (with the measure of risk observed) analyses.

Finally, all SNPs analysed in more than one manuscript were selected to conduct a joint analysis of their odd ratios and identify a total measure of risk. The Mantel–Haenszel method was used to compare case–control studies with dichotomous outcomes. The DerSimonian–Laird method was used when the preliminary test of heterogeneity yielded different results for the two studies. Missing data regarding SNP references, polymorphism characteristics and information regarding their functional activity were drawn from the online SNPs archives (http://www.ncbi.nlm.nih.gov/snp/ and http://mutdb.org/cgi-bin/mutdb.pl).

A polymorphism was considered functional when data were available regarding its ability to alter the transcriptional profile of the corresponding gene or to produce an abnormal amino acid. Vice versa, it was considered non-functional when it determines synonymous substitutions that do not lead to the production of an abnormal amino acid or when it is located in an intergenic region that does not have a documented regulatory activity.

## Results

The SNPs significantly associated with sporadic brain arteriovenous malformation susceptibility are shown in Table 1, whereas those associated with increased risk of intracranial haemorrhage are shown in Table 2. In both cases, SNPs located in genes related to the inflammatory pathways are distinguished from genes related to the angiogenesis.

### Single nucleotide polymorphisms in genes involved in inflammation

Molecular studies showed that the expression of genes involved in inflammation is increased in brain arteriovenous malformation tissue compared with normal tissue (Hashimoto et al., 2004; Xu et al., 2004). Based on these data, Pawlikowska et al. (2004) hypothesized that SNPs in these genes could influence the clinical course of patients with brain arteriovenous malformations. These authors genotyped 180 patients with brain arteriovenous malformations and found that the risk of haemorrhagic presentation was 3-fold higher in those carrying the −174GG genotype of the interleukin 6 (IL6) gene, compared with subjects carrying the CC and GC genotypes (Pawlikowska et al., 2004).

Achrol et al. (2006) tested the association between two SNPs in the promoter region of the IL6 gene (−174G>C and −572G>C) and two SNPs of the tumour necrosis factor α (TNFα) gene (−238G>A and −308G>A) with the risk of new intracranial haemorrhage after diagnosis in 280 patients with brain arteriovenous malformations. The −238G>A polymorphism of the TNFα gene was associated with increased risk of bleeding during the natural course of the disease. Indeed, a new intracranial haemorrhage after diagnosis occurred in 6.4% of patients carrying the TNFα −238 AG genotype (Achrol et al., 2006).

The same polymorphism (−174G>C) of the IL6 gene was associated with Kim et al. (2008a), with ~2-fold increased risk of brain arteriovenous malformation susceptibility among Latinos after accounting for differences in ancestral background. In fact, as the risk of the disease varies with ancestry, case–control studies in admixed populations might be susceptible to genetic confounders by population stratification. Therefore, the authors of this study tested 83 ancestry informative markers with large absolute allele frequency differences (δ > 0.5) that were known to not be associated with brain arteriovenous malformation in Africans, Europeans and Native Americans. They also compared 79 patients affected by brain arteriovenous malformations and 215 unaffected individuals, all of self-reported Latino race/ethnicity, finding a significant difference in the frequency of this polymorphism between case and control subjects. Finally, they found that this SNP was also associated with a different frequency in genetic ancestry (Kim et al., 2008a).

The role of inflammatory cytokines was further investigated by Kim et al. (2009a) by studying 410 subjects with brain arteriovenous malformations and testing the association between three SNPs of the IL1β gene (−511C>T, −31T>C and +3953C>T) and the occurrence of intracranial haemorrhage. They found that patients with the IL1β −31CC and −511TT genotypes had a higher rate of intracranial haemorrhage after diagnosis compared with reference genotypes. Restricting the analysis to subjects with Caucasian background, the IL1β −31CC, −511TT and +3953CC genotypes were still significantly more frequent in patients with brain arteriovenous malformations than in control subjects, even after adjustment for age and sex, suggesting that this gene influences the risk of bleeding and the susceptibility to the disease (Kim et al., 2009a).

The role of functional polymorphisms of pro-inflammatory cytokine genes in brain arteriovenous malformation pathophysiology was again emphasized by Fontanella et al. (2012), who studied 101 unrelated patients with brain arteriovenous malformations and 210 healthy subjects. They found a significant association between the IL1α −889C>T gene polymorphism and brain arteriovenous malformation susceptibility; subjects carrying the T allele displayed a 2.47-fold greater risk of acquiring the disease than the CC genotype. In the same study, the allelic and genotypic frequencies of interleukin 1 receptor antagonist gene (IL1RN) were also analysed. IL1RN contains an 86-base pair variable number (2–6) tandem repeat polymorphism in intron 3, and allele 2 of that polymorphism is the shortest of the five alleles, with only two repeats. The authors found increased (~2-fold) disease susceptibility in subjects carrying allele 1. Additionally, the −511C>T polymorphism of the IL1α gene was investigated, but no significant differences were found in the genotypic or allelic distribution of this SNP between patients and control subjects (Fontanella et al., 2012).

In a recent study by Jiang et al. (2011), conducted with a Chinese population of subjects affected by sporadic brain arteriovenous malformations, the G allele of the −197G>A
Table 1 SNPs associated with sporadic brain arteriovenous malformation susceptibility

<table>
<thead>
<tr>
<th>Genes related to the inflm pathway</th>
<th>Functional rationale for protein</th>
<th>Chromosome position and SN variant</th>
<th>dbSNP ID</th>
<th>Functional rationale for SNP</th>
<th>Study design</th>
<th>Sample size (cases versus control subjects)</th>
<th>Univariate model</th>
<th>Multivariate model</th>
<th>Author and year</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL6</td>
<td>Inflm cytokine</td>
<td>Chr 7: 22766645</td>
<td>rs1800795</td>
<td>Promoter variant</td>
<td>Case-control</td>
<td>79 versus 215</td>
<td>0.023*</td>
<td>–</td>
<td>0.039 OR 1.96 1.03–3.72</td>
</tr>
<tr>
<td>IL18</td>
<td>Inflm cytokine</td>
<td>Chr 2: 11359467</td>
<td>rs169444</td>
<td>Promoter variant</td>
<td>Cohort, case-control and meta-analyses</td>
<td>110 (cohort analysis); 235 (case-control analysis)</td>
<td>–</td>
<td>–</td>
<td>&lt;0.001 OR 3.40 1.72–6.71</td>
</tr>
<tr>
<td>CC</td>
<td>IL18</td>
<td>Chr 2: 113594387</td>
<td>rs1143672</td>
<td>Promoter variant</td>
<td>Exon 5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;0.001 OR 3.35 1.71–6.57</td>
</tr>
<tr>
<td>CT</td>
<td>IL18</td>
<td>Chr 2: 113590390</td>
<td>rs1143634</td>
<td>Promoter variant</td>
<td>Exon 5</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>&lt;0.001 OR 3.35 1.71–6.57</td>
</tr>
<tr>
<td>TT</td>
<td>Inflm cytokine</td>
<td>Chr 3: 30647160</td>
<td>rs3087465</td>
<td>Promoter variant</td>
<td>Case-control</td>
<td>53 versus 120</td>
<td>&lt;0.01*</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Cytokine receptor</td>
<td>Chr 3: 22658341</td>
<td>rs1730589</td>
<td>Promoter variant</td>
<td>Case-control</td>
<td>101 versus 210</td>
<td>&lt;0.001*</td>
<td>OR: 2.47 1.72–3.56</td>
<td>–</td>
</tr>
<tr>
<td>IL1R</td>
<td>Cytokine receptor</td>
<td>Allele 1</td>
<td>rs1794068</td>
<td>Inton 2</td>
<td>Case-control</td>
<td>177 versus 129</td>
<td>&lt;0.001</td>
<td>OR: 2.68 1.64–4.39</td>
<td>–</td>
</tr>
<tr>
<td>MMP3</td>
<td>Proteolytic enzyme</td>
<td>Chr 11: 10271504</td>
<td>rs522616</td>
<td>Promoter variant</td>
<td>Case-control</td>
<td>319 versus 333</td>
<td>–</td>
<td>–</td>
<td>0.006 OR 0.615 0.436–0.868</td>
</tr>
<tr>
<td>Genes related to the angiogenic pathway</td>
<td>Surface receptor + surface receptor</td>
<td>Chr 12: 52307308</td>
<td>r2071219</td>
<td>Intron 3 + exon 2</td>
<td>Case-control</td>
<td>177 versus 129</td>
<td>&lt;0.01</td>
<td>OR: 2.68 1.64–4.39</td>
<td>–</td>
</tr>
<tr>
<td>ALK1 (ACVR1L1) + ENG</td>
<td>Surface receptor + surface receptor</td>
<td>Chr 12: 52307308</td>
<td>r2071219</td>
<td>Intron 3 + exon 2</td>
<td>Case-control</td>
<td>177 versus 129</td>
<td>0.002</td>
<td>OR: 2.47 1.38–4.44</td>
<td>0.002 –</td>
</tr>
<tr>
<td>ANG PTL4</td>
<td>Serum glycoprotein</td>
<td>Chr 19: 8344716</td>
<td>rs1167243</td>
<td>Exon 7</td>
<td>Case-control</td>
<td>216 versus 246</td>
<td>0.046</td>
<td>OR: 1.56 1.01–2.41</td>
<td>–</td>
</tr>
<tr>
<td>VEGFA</td>
<td>Angiogenic growth factor</td>
<td>Chr 6: 43845555</td>
<td>rs3025010</td>
<td>Intron 5</td>
<td>Case-control</td>
<td>319 versus 333</td>
<td>0.05*</td>
<td>–</td>
<td>0.017 OR 0.48 0.26–0.88</td>
</tr>
<tr>
<td>Genes related to the angiogenic pathway</td>
<td>Surface receptor + surface receptor</td>
<td>Chr 12: 52307308</td>
<td>r2071219</td>
<td>Intron 3 + exon 2</td>
<td>Case-control</td>
<td>177 versus 129</td>
<td>0.002</td>
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<td>0.002 –</td>
</tr>
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<td>ALK1 (ACVR1L1) + ENG</td>
<td>Surface receptor + surface receptor</td>
<td>Chr 12: 52307308</td>
<td>r2071219</td>
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<td>Case-control</td>
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<td>VEGFA</td>
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<td>Chr 6: 43845555</td>
<td>rs3025010</td>
<td>Intron 5</td>
<td>Case-control</td>
<td>319 versus 333</td>
<td>0.05*</td>
<td>–</td>
<td>0.017 OR 0.48 0.26–0.88</td>
</tr>
<tr>
<td>Intergenic regions with unknown functional significance</td>
<td>Surface receptor + surface receptor</td>
<td>Chr 12: 52307308</td>
<td>r2071219</td>
<td>Intron 3 + exon 2</td>
<td>Case-control</td>
<td>177 versus 129</td>
<td>0.002</td>
<td>OR: 2.47 1.38–4.44</td>
<td>0.002 –</td>
</tr>
<tr>
<td>3p12</td>
<td>–</td>
<td>Chr 3: 8484209</td>
<td>rs1384090</td>
<td>Intergenic region</td>
<td>Genome-wide linkage and association</td>
<td>26 versus 30</td>
<td>0.0000895 –</td>
<td>– – – –</td>
<td>Inoue et al., 2007.</td>
</tr>
<tr>
<td>11q22</td>
<td>–</td>
<td>Chr 11: 9676780</td>
<td>rs1938887</td>
<td>Intergenic region</td>
<td>Intergenic region</td>
<td>26 versus 30</td>
<td>0.0000149 –</td>
<td>– – – –</td>
<td>Inoue et al., 2007.</td>
</tr>
<tr>
<td>18q22</td>
<td>–</td>
<td>Chr 18: 6794085</td>
<td>rs728714</td>
<td>Intergenic region</td>
<td>Intergenic region</td>
<td>26 versus 30</td>
<td>0.0000539 –</td>
<td>– – – –</td>
<td>Inoue et al., 2007.</td>
</tr>
<tr>
<td>Xp21</td>
<td>–</td>
<td>Chr X: 33950550</td>
<td>rs953009</td>
<td>Intergenic region</td>
<td>Intergenic region</td>
<td>26 versus 30</td>
<td>0.0000399 –</td>
<td>– – – –</td>
<td>Inoue et al., 2007.</td>
</tr>
</tbody>
</table>

Chr = chromosome; db = database; HR = hazard ratio; Inflm = inflammatory; OR = odds ratio; SN = single nucleotide; VNTR = variable number tandem repeats.

Indicated risk prediction was obtained by Cox proportional hazard estimates or logistic regression analysis in univariate and multivariate model. Statistical significance in case-control analysis was obtained by χ².

* P value obtained by χ².

Chromosome sequences were provided by the Genomic Reference Consortium (GRC37.p5).
Table 2 SNPs associated with risk of bleeding in patients with sporadic brain arteriovenous malformations

<table>
<thead>
<tr>
<th>Gene or locus</th>
<th>Functional rationale for protein</th>
<th>Genotype associated to the risk</th>
<th>Chromosome position and SNP variant</th>
<th>dbSNP ID</th>
<th>Functional rationale for SNP</th>
<th>Sample size (cases versus control subjects)</th>
<th>Study design</th>
<th>Univariate model</th>
<th>Multivariate model</th>
<th>Associated with year</th>
<th>Author and year</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chr: 7: 22766645 –174 G &gt; C</td>
<td>rs1800795</td>
<td>Promoter variant</td>
<td>180 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>P-value (OR)*</td>
<td>OR 2.62</td>
<td>1.38–4.98</td>
<td>0.039</td>
</tr>
<tr>
<td>IL6</td>
<td>Inflm cytokine</td>
<td>GG</td>
<td>Chr 7: 22766645 –174 G &gt; C</td>
<td>rs1800795</td>
<td>Promoter variant</td>
<td>180 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.003</td>
<td>OR: 2.62</td>
<td>1.38–4.98</td>
<td>0.039</td>
</tr>
<tr>
<td>TNFα</td>
<td>Inflm cytokine</td>
<td>AG</td>
<td>Chr 6: 31543101 –238 G &gt; A</td>
<td>rs361525</td>
<td>Promoter variant</td>
<td>280 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.071</td>
<td>HR: 2.53</td>
<td>0.92–6.90</td>
<td>0.015</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chr 6: 31543101 –238 G &gt; A</td>
<td>rs361525</td>
<td>Promoter variant</td>
<td>280 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.071</td>
<td>HR: 2.53</td>
<td>0.92–6.90</td>
<td>0.015</td>
</tr>
<tr>
<td>IL1β</td>
<td>Inflm cytokine</td>
<td>TT</td>
<td>Chr 2: 113594867 –511 C &gt; T</td>
<td>rs16944</td>
<td>Promoter variant</td>
<td>410 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.018</td>
<td>HR: 2.90</td>
<td>1.20–7.01</td>
<td>0.039</td>
</tr>
<tr>
<td>IL1β</td>
<td>Inflm cytokine</td>
<td>CC</td>
<td>Chr 2: 113594867 –511 C &gt; T</td>
<td>rs16944</td>
<td>Promoter variant</td>
<td>410 cases</td>
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<td>0.018</td>
<td>HR: 2.90</td>
<td>1.20–7.01</td>
<td>0.039</td>
</tr>
<tr>
<td>IL17A</td>
<td>Inflm cytokine</td>
<td>Any (AG + GG)</td>
<td>Chr 6: 52051033 –197 G &gt; A</td>
<td>rs2277913</td>
<td>Promoter variant</td>
<td>53 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>&lt;0.05*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>TGFBR2</td>
<td>Cytokine receptor</td>
<td>Any (AG + GG)</td>
<td>Chr 6: 52051033 –197 G &gt; A</td>
<td>rs2277913</td>
<td>Promoter variant</td>
<td>53 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>&lt;0.05*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>APOE</td>
<td>Apolipoprotein</td>
<td>Any C (CT + CC)</td>
<td>Chr 19: 50103781 –31 T &gt; C</td>
<td>rs429358</td>
<td>Exon 4</td>
<td>284 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.012</td>
<td>HR: 4.97</td>
<td>1.43–17.3</td>
<td>0.010</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chr 19: 50103781 –31 T &gt; C</td>
<td>rs429358</td>
<td>Exon 4</td>
<td>284 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>0.012</td>
<td>HR: 4.97</td>
<td>1.43–17.3</td>
<td>0.010</td>
</tr>
<tr>
<td>EPHB4</td>
<td>Tyrosine kinase receptor</td>
<td>Any C (CT + CC)</td>
<td>Chr 7: 100258814 –232 T &gt; C</td>
<td>rs314308</td>
<td>Intron 3</td>
<td>236 cases (II); 248 versus 225 (III)</td>
<td>Cross-sectional study (II), case-control (III)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.001</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Chr 7: 100258814 –232 T &gt; C</td>
<td>rs314308</td>
<td>Intron 3</td>
<td>236 cases (II); 248 versus 225 (III)</td>
<td>Cross-sectional study (II), case-control (III)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.001</td>
</tr>
<tr>
<td>VEGFA</td>
<td>Angiogenic growth factor</td>
<td>Any T (AT + TT)</td>
<td>Chr 6: 43838622 –232 T &gt; C</td>
<td>rs1547651</td>
<td>Promoter variant</td>
<td>311 cases</td>
<td>Cohort analysis (prospective study)</td>
<td>&lt;0.05*</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Apolipoprotein; chr = chromosome; db = database; HR = hazard ratio; ICH = intra-cranial haemorrhage; Inflm = inflammatory; OR = odds ratio; SN = single nucleotide.

Indicated risk prediction was obtained by Cox proportional hazard estimates or logistic regression analysis both in univariate and multivariate model.

Statistical significance in case–control analysis was obtained by χ². *P-value obtained by χ².

Chromosome sequences were provided by the Genomic Reference Consortium (GRC37.p5).
polymorphism in the IL17A gene and the G allele of the −875A>G polymorphism in transforming growth factor, beta receptor II (70/80kDa) (TGRBR2) genes were both associated with risk of intracranial haemorrhage. In addition, the frequency of the TGRBR2 −875AG genotype was significantly different between affected patients and control subjects, indicating that this genotype might also influence the brain arteriovenous malformation susceptibility (Jiang et al., 2011).

Zhao et al. (2009) first explored the involvement of the matrix metalloproteinase 3 (MMP3) gene in brain arteriovenous malformation pathophysiology. They carried out a case–control study on 319 Chinese patients affected by brain arteriovenous malformations and 333 healthy control subjects to evaluate the association between five common SNPs of the MMP3 gene and the disease. They found that the frequency of the AA genotype of the −707A>G polymorphism of the promoter region of the MMP3 gene was significantly different between case and control subjects, seeming to be associated with a significantly decreased risk of brain arteriovenous malformation susceptibility, even after adjustment for age and sex (Zhao et al., 2009).

Finally, a relationship between the APOE gene and an increased risk of intracranial haemorrhage in patients with brain arteriovenous malformations was observed by Pawlikowska et al. (2006). A statistically significant association between the APOE-ε2 allele and the risk of intracranial haemorrhage during the natural course of the disease was found in a series of 284 Caucasian individuals affected by sporadic brain arteriovenous malformations. In these subjects, a 5-fold increased risk of new intracranial haemorrhage was still present after adjustment of race and ethnicity. Importantly, this association was found for new intracranial haemorrhage over the course of the disease and not for intracranial haemorrhage as a presentation symptom. No significant effect was observed for the APOE-ε4 allele. In further multivariate analysis, APOE-ε2 allele and TNFα −238AG genotype remained significantly associated with new intracranial haemorrhage, thus seeming to be independent bleeding risk predictors (Pawlikowska et al., 2006).

The involvement of the APOE gene in brain arteriovenous malformation pathophysiology has been confirmed by Achrol et al. (2007). They found that the ε2 isoform of APOE gene and the AG genotype of the −238A>G polymorphism of TNFα were independent risk predictors of post-treatment intracranial haemorrhage in 215 Caucasian subjects with sporadic brain arteriovenous malformations, who had previously undergone open surgery (n = 138), radiosurgery (n = 54) or multiple treatments (n = 23). The risk of post-treatment intracranial haemorrhage was 3.2-fold higher in subjects carrying the APOE-ε2 allele and 3.5-fold higher in those with the AG genotype of the −238A>G polymorphism of the TNFα gene (Achrol et al., 2007).

**Single nucleotide polymorphisms in genes involved in angiogenesis**

Pawlikowska et al. (2004) first explored the possible association between SNPs in angiogenic genes and the risk of haemorrhagic onset in patients with sporadic brain arteriovenous malformations. They studied 180 subjects with brain arteriovenous malformations and determined the frequency of nine SNPs located in coding and promoter regions of prototypical angiogenic genes, such as vascular endothelial growth factor A (VEGFA) and its receptors Fms-related tyrosine kinase 4 (FLT4) and kinase insert domain receptor (KDR), and angiopoietin 2 (ANGPT2) and its receptor TIE2 (now known as TEK). However, none of the investigated SNPs seemed to be significantly associated with an increased risk of intracranial haemorrhage (Pawlikowska et al., 2004).

The same authors also tested the association between polymorphisms in the endoglin (ENG) and activin-like kinase 1 (ALK1—also known as ACVRL1) genes and sporadic brain arteriovenous malformation susceptibility. Comparing 177 affected patients and 129 healthy control subjects, they found a significant association between the AA and AG genotypes of the intervening sequence (IVS) 3 −35A>G polymorphism of the ALK1 gene and disease susceptibility. This association also seemed significant at multivariate analysis after correction for age and gender. They also investigated possible interactions between ENG and ALK1 gene polymorphisms and found that the association with brain arteriovenous malformation susceptibility was stronger in subjects concomitantly carrying the A allele of the IVS3 −35A>G polymorphism of the ALK1 gene and the GG genotype of the 207G>A polymorphism of ENG gene (Pawlikowska et al., 2005).

The association between SNPs in the ALK1 gene (ACVRL1) and sporadic brain arteriovenous malformation susceptibility was later investigated by Simon et al. (2006). They compared the following three groups of subjects: (i) individuals with arteriovenous shunts; (ii) subjects with cerebral aneurysms; and (iii) healthy control subjects. The IVS3 −35A allele was found to be significantly more frequent among patients with brain arteriovenous malformations than in control subjects, and this association remained significant after adjustments for age and sex. In contrast, no significant association was found with the IVS9 +45C>T polymorphism (Simon et al., 2006).

The role of angiogenic genes was further studied by Weinsheimer et al. (2009), who tested the association of eight haplotype-tagging SNPs in the EPHB4 gene and the risk of haemorrhagic onset in patients with brain arteriovenous malformations. The study was conducted in three phases. In Phase I, 146 affected subjects (56 with intracranial haemorrhage and 90 without intracranial haemorrhage) were genotyped for all eight SNPs. Significantly different frequencies were found between patients with and those without intracranial haemorrhage for the SNPs rs314353, rs314308 and rs314313. In particular, the alleles rs314313C and rs314308T were significantly associated with reduced risk of haemorrhagic presentation. To replicate these findings, in Phase II, the SNPs rs314313 and rs314308 were genotyped in an additional cohort of 102 patients with brain arteriovenous malformations (37 with intracranial haemorrhage and 65 without intracranial haemorrhage). Both polymorphisms seemed to be associated with haemorrhagic presentation in this cohort. Finally, in a joint analysis (Phase III) on 248 affected subjects (93 with intracranial haemorrhage and 155 without intracranial haemorrhage), the SNPs rs314313 and rs314308 still seemed to be significantly associated with intracranial haemorrhage, even...
after adjustment for age, gender, recruitment site, nidus size and deep venous drainage (Weinsheimer et al., 2009).

Recently, Mikhak et al. (2011) studied the association between SNPs in the angiopoietin-like 4 (ANGPTL4) gene and sporadic brain arteriovenous malformation susceptibility. They genotyped 216 affected Caucasian and 246 healthy control subjects for four tagging SNPs, finding that a greater proportion of affected patients (33.8%) carried the rs11672433A allele compared with control subjects (22.8%). After adjustment for age and sex, the investigated SNPs were significantly associated with increased risk of intracranial haemorrhage compared with non-carriers. None of the investigated SNPs were significantly associated with risk of haemorrhagic presentation (Mikhak et al., 2011).

Finally, Chen et al. (2011) reported an association between SNPs in the VEGFA gene and brain arteriovenous malformation susceptibility in 319 Chinese patients. They studied nine tagging SNPs potentially functional or with a high minor allele frequency, observing a significant difference of genotype distribution for the SNPs rs1547651A > T and rs3025010T > C between case and control subjects. At logistic regression analysis adjusted for age and sex, rs2010963, rs833069, rs1547651 and rs3025010 still showed significant association with brain arteriovenous malformation susceptibility. The patients who presented the AG/GG genotypes of the rs1547651 showed a higher risk of brain arteriovenous malformation compared with the AA genotype. A significantly protective effect was instead associated with the AT/TT genotypes of rs1547651 compared with AA genotype, the GG/GC genotype of rs2010963 compared with CC genotype, and TT/TC genotypes of rs3025010 compared with CC genotype (Chen et al., 2011). The AT/TT genotypes of the rs1547651 also seemed to be significantly associated with increased risk of intracranial haemorrhage when compared with AA genotype in another study by Gong et al. (2011).

### Genome-wide linkage and association studies in patients with brain arteriovenous malformations

To date, only a genome-wide association study has been conducted among patients with sporadic brain arteriovenous malformations. It is the study conducted by Inoue et al. (2007) in the community of Takayama in Japan, where a particularly high incidence of brain arteriovenous malformations (from 2.4 to 3.1/100,000 subjects/yr) has been reported, along with multiple familial cases. The genome-wide association study, which was carried out in a small population of 26 unrelated affected patients and 30 healthy control subjects, analysed the association data for 10,240 SNPs. Because of the high frequency of familial cases, the genome-wide association study was preceded by a preliminary linkage analysis to evaluate the tendency of certain loci or alleles to be inherited together. Indeed, neighbouring genes on the

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**Table 3** Joint analysis of the risk estimates reported in the literature about the association between the same SNPs and sporadic brain arteriovenous malformations

<table>
<thead>
<tr>
<th>SNP</th>
<th>Authors and year</th>
<th>Risk/referent genotype</th>
<th>Crude association</th>
<th>Summary</th>
<th>Method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymorphisms associated with risk of bleeding</td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>IL6 –174G &gt; C</td>
<td>Pawlikowska et al., 2004. Achrol et al., 2006.</td>
<td>GG/(CC; CG)</td>
<td>OR: 2.62 (1.38; 4.98) OR: 0.99 (0.37; 2.70)</td>
<td>OR: 1.97 (1.15; 3.38)</td>
<td>Fixed effects (Mantel–Haenszel test)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(CC; CG)/GG</td>
<td>OR: 1.34 (0.7; 2.56) OR: 1.42 (0.51; 3.97)</td>
<td>OR: 1.36 (0.79; 2.35)</td>
<td>Fixed effects (Mantel–Haenszel test)</td>
</tr>
<tr>
<td>TNFα –238G &gt; A</td>
<td>Achrol et al., 2006. Achrol et al., 2007. Pawlikowska et al., 2004.</td>
<td>AG/GG</td>
<td>OR: 4.54 (1.57; 13.10) OR: 2.70 (1.07; 6.85)</td>
<td>OR: 2.19 (1.25; 3.83)</td>
<td>Fixed effects (Mantel–Haenszel test)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(AA; AG)/GG</td>
<td>OR: 1.28 (0.52; 3.14) OR: 1.03 (0.33; 3.27)</td>
<td>OR: 1.18 (0.58; 2.39)</td>
<td>Fixed effects (Mantel–Haenszel test)</td>
</tr>
<tr>
<td>APOE ε2</td>
<td>Achrol et al., 2007. Pawlikowska et al., 2006.</td>
<td>ε2/not ε2</td>
<td>RR: 1.28 (0.54; 3.01) RR: 1.97 (0.69; 5.65)</td>
<td>RR: 1.51 (0.78; 2.93)</td>
<td>Fixed effects (Mantel–Haenszel test)</td>
</tr>
</tbody>
</table>

| Polymorphisms associated with disease susceptibility |                                |                        |                   |         |                             |
| IL1β –511C>T              | Kim et al., 2009a Fontanella et al., 2012. | TT/(CC; GG)            | OR: 2.93 (1.61; 5.33) OR: 0.83 (0.39; 1.75) | OR: 1.59 (0.46; 5.48) | Random effect (DerSimonian–Laird test) |
| ALK1 (ACVRL1) IVS3 –35A > G | Pawlikowska et al., 2004. Simon et al., 2006. | (AA; AG)/GG            | OR: 2.47 (1.38; 4.44) OR: 2.35 (1.15; 4.76) | OR: 2.42 (1.54; 3.8) | Fixed effects (Mantel–Haenszel test) |

OR = odds ratio; RR = risk ratio.
chromosomes have a tendency to stick together when passed to offspring. Therefore, if a disease is often passed to offspring along with specific marker genes, it can be concluded that the genes that are responsible for the disease are located on the chromosome close to these markers. With a dominant model, the preliminary linkage analysis revealed seven candidate regions in different chromosomes (3q27, 4q34, 6q25, 7p21, 13q32-33, 16p13-12 and 20q11-13). Contrariwise, with a recessive model, a statistical significance was only obtained at the 6q25 locus. Through the association analysis, the authors identified four SNPs (rs1384309 on 3p12, rs1938887 on 11q22, rs728714 on 18q22 and rs953009 on Xp21) and two haplotypes (both on Xp21) with statistically significant associations with the disease. However, none were localized on the chromosomal regions suggested by the linkage analysis. Also, the subsequent direct sequencing of the genes EPHB3 in chromosome 3q27, EFNB2 in chromosome 13q32-33 and POFUT1 in chromosome 20q11 did not reveal any causal variants. Accordingly, this genome-wide association study was unable to identify any genetic determinants of sporadic brain arteriovenous malformations, although the low statistical power could have influenced these results (Inoue et al., 2007).

A similar genome-wide linkage and haplotype study was performed by Oikawa et al. (2010) in a Japanese family in which several members from four successive generations were affected by brain arteriovenous malformations without any associated disorders, such as hereditary haemorrhagic telangiectasia. After a two-step linkage analysis, four loci on different chromosomes (5p13.2q14.1, 15q11.2q13.1, 18p11.32p11.22 and 19q13.33q13.42) were identified as potential candidate regions, and three of them were further confirmed at the subsequent genotyping with microsatellite markers. They included a 48-Mb region between markers rs1366265 and rs1373965 at 5p13.2q14.1, a 6-Mb region between rs850819 and rs8110889 at 15q11.2q13.1 and a 9-Mb region between rs486633 and rs1942150 at 18p11.32p11.22. Chromosome 19 was ruled out because a possible disease-associated haplotype on 19q13.33q13.42 was transmitted to two definitively unaffected individuals. Ten genes within the 48-Mb region at 5p13.2q14.1 (MAP3K1, DAB2, OCLN, FGF10, ESM1, ITGA1, ITGA2, EGFAM, ERBB2IP and PIK3R1) were selected as candidates for brain arteriovenous malformation, as they concern development and maintenance of vessels and are associated with other heritable vascular disorders, such as hereditary haemorrhagic telangiectasia. However, mutation analyses in these 10 genes revealed no pathological mutation in the probands (Oikawa et al., 2010). In conclusion, the three regions identified neither overlapped with candidate locus of familial brain arteriovenous malformation at 6p25, previously reported by Inoue et al. (2007), nor contained genes responsible for syndromic brain arteriovenous malformations, such as ENG and ALK1.

Joint analysis of the risk estimates reported in the literature

From our analysis of the medical literature, we found that five SNPs (APOE -e2, TNFα -238G>A, IL6 -174G>C, TNFα -308G>A, IL6 -572G>C) were studied in more than one manuscript for their association with risk of intracranial haemorrhage and two SNPs (IL1β -511C>T, ALK1 IVS3 -35A>G) for their association with disease susceptibility. We thus performed a joint analysis of the risk estimates reported by the different authors for the same SNPs, and we found that IL6 -174G>C and TNFα -238G>A were associated with an increased risk of intracranial haemorrhage of 1.97-fold (95% confidence interval (CI): 1.15–3.8) and 2.19-fold (95% CI: 1.25–3.83), respectively. Likewise, ALK1 (ACVR1L) IVS3 -35A>G was associated with disease susceptibility, with an odds ratio (OR) of 2.42 (95% CI: 1.54–3.8) (Table 3 and Fig. 1).

Discussion

Role of the interleukins in the pathophysiology of brain arteriovenous malformations

Inflammation contributes to the pathogenesis of several vascular malformations including cerebral cavernous malformations (Shenkar et al., 2007), intracranial aneurysms (Hashimoto et al., 2006) and abdominal aortic aneurysms (Lenk et al., 2007). Recent studies have also demonstrated the presence of inflammatory cells in brain arteriovenous malformation tissue, suggesting a role of the inflammation in their pathophysiology and rupture (Chen et al., 2008). The pro-inflammatory cytokine IL6 is produced by many tissues, including the endothelium. It is a key mediator of the acute phase of the inflammatory response, and it is known to play a role in dysplasia and rupture of intracranial vessels (Kim et al., 2008a). IL6 expression is largely regulated at the level of transcription (Heinrich et al., 2003). The −174G>C polymorphism of the IL6 (especially the GG genotype) seems to play a functional role, being located in a regulatory region of the gene. Indeed, promoter constructs containing the −174G>C polymorphism showed higher stimulated activity in vitro (Fishman et al., 1998), although in vivo studies on systemic levels of IL6 and inflammatory phenotypes yielded controversial results. Patients affected by abdominal aortic aneurysms who carried the −174GG genotype showed lower plasmatic levels of IL6 and reduced cardiovascular mortality from small aneurysms (Jones et al., 2001). Similarly, peak levels of IL6 after coronary bypass surgery were lower in patients who carried the GG genotype than in those with the C allele (Brull et al., 2001). The IL6 −174C allele also seemed to be associated with higher susceptibility of myocardial infarction (Georges et al., 2001) and impaired endothelial function (Brull et al., 2002). On the other hand, some studies found that the −174GG genotype was associated with higher postoperative serum levels of IL6 and poor outcome after coronary bypass (Gaudino et al., 2003), increased intima-media thickness of the carotid arterial wall (Rundek et al., 2002), occlusive diseases of peripheral arteries (Flex et al., 2002) and history of ischaemic stroke (Pola et al., 2003). Among patients with a history of stroke, haplotypes containing the −174G allele were associated with higher serum levels and in vitro stimulated transcription of IL6 (Acalovschi et al., 2003). Although the relationship between
inflammatory cascade and brain arteriovenous malformation pathophysiology is controversial, the association between the −174GG genotype and the 3-fold increased risk of haemorrhagic presentation suggested that this cytokine could play a role in the mechanisms underlying brain arteriovenous malformation rupture (Pawlikowska et al., 2004). This would be consistent with the increased risk of subarachnoid haemorrhage among Caucasians that harbour this polymorphism (Morgan et al., 2006). The hypothesis that the −174G>C polymorphism of the IL6 gene plays a role in brain arteriovenous malformation is further strengthened by our joint analysis, which, taking into account the risk estimates reported by Pawlikowska et al. (2004) and Achrol et al. (2006), has found a 1.97-fold increased risk of bleeding in subjects carrying the GG genotype (Table 3). The molecular mechanisms underlying such an effect remain to be elucidated, although some authors have proposed that IL6 could also act indirectly, as it stimulates the induction of MMP9 (Fig. 2), which is a prototypical metalloproteinase that degrades the extracellular matrix around blood vessels and can damage endothelial cells predisposing them to rupture (Rosenberg, 2002). Alternatively, if the −174G allele is associated with a less vigorous response to IL6 as suggested by some studies, these results raise the intriguing possibility that brain arteriovenous malformation rupture might be related to the inability to mount a successful inflammatory response to an inciting event that challenges the vascular wall (Pawlikowska et al., 2004).

In contrast with the −174G>C polymorphism, the −572G>C polymorphism of the IL6 gene did not show any significant association with risk of bleeding in patients with sporadic brain arteriovenous malformations neither in the two series reported in literature (Pawlikowska et al., 2004; Achrol et al., 2006) nor after our joint analysis (Table 3). This finding is representative of the fact that, within a gene, only certain SNPs play a role in disease susceptibility, depending on their functional activity and ability to affect transcription or production of a protein with altered biological activity.

A similar concept may be applied to the polymorphisms of the IL1 gene, which have previously been associated with ischaemic...
stroke (Iacoviello et al., 2005) and subarachnoid haemorrhage from brain aneurysm rupture (Slowik et al., 2006). The −511C>T and +3953C>T polymorphisms of the IL1 gene are able to affect the production of IL1β in vitro (Kimura et al., 2004). Haplotypes containing the −511TT and −31CC genotypes seemed to be correlated with increased gene transcriptional activity (Chen et al., 2006). This is consistent with the fact that these two SNPs are located in the promoter region (TATA box) of the gene. In contrast, the +3953C>T polymorphism is located in exon 5 of the gene and seems unable to affect the expression of IL1β.

Regarding these SNPs and brain arteriovenous malformations, Kim et al. (2009a) found an association between the IL1β −511C>T polymorphism and brain arteriovenous malformation susceptibility, but Fontanella et al. (2012) were unable to replicate this findings in their series. Our joint analysis also found a lack of association between this SNP and brain arteriovenous malformation (Table 3).

Possible explanations for this discrepancy include the larger number of patients analysed by Kim et al. (2009a) compared with that studied by Fontanella et al. (2012) (231 versus 101), the different geographic area (USA and Italy) and the slightly higher frequency of the mutated T allele in the brain arteriovenous malformation group evaluated in the first study compared with that analysed in the second group (39.4% versus 36.1%).

Experimental studies also suggested that the IL1RN genotypes could play an important role in endothelial cell proliferation because they regulate the activity of proteins involved in the cellular cycle. In fact, in the second intron of the IL1RN gene, there is a functional polymorphism of a variable number of tandem repeats,
which plays an important role in regulating the serum IL1RN levels (Fontanella et al., 2012). Regarding the −889C>T polymorphism of the IL1α gene, it is located in a regulatory region of the gene and has been reported to be able to influence the transcriptional activity of the gene in vitro (Fontanella et al., 2012).

Finally, the −197G>A polymorphism in the IL17 gene, which has been associated with increased risk of intracranial haemorrhage in brain arteriovenous malformations, has been described as being able to increase gene transcriptional activity in some studies. IL17 is a recently identified pro-inflammatory cytokine involved in the occurrence and development of several inflammatory, autoimmune and cerebrovascular diseases and, as shown in Fig. 2, it acts as a main modulator of the inflammatory response, inducing the secretion of other pro-inflammatory cytokines (including IL6, IL1 and TNFα) and prostaglandins, MMPs, chemokines and complement components. In addition, IL17 seems to trigger the expression of inducible nitric oxide synthase, an enzyme responsible for the generation of cytotoxic and immunoregulatory free radicals, whose uncontrolled release is known to affect tissue inflammation and destruction (Miljkovic and Trajkovic, 2004).

The role of TNFα in the pathophysiology of brain arteriovenous malformations

TNFα is a pro-inflammatory cytokine, with crucial immunomodulatory properties and proteolytic processes (Lawton et al., 2005). The discovery of the association between the TNFα −238G>A polymorphism and a 4-fold increased risk of bleeding in patients with brain arteriovenous malformations strengthens the hypothesis that inflammation may play an important role in the pathophysiology of brain arteriovenous malformations (Achrol et al., 2006). The −238AG genotype is functionally important, it influences the transcriptional activity of the gene and has been associated with increased expression of TNFα. The overexpression of TNFα indirectly enhances the pathway of MMP9, as it induces IL6 (Fig. 2) (Hashimoto et al., 2004). However, it remains unclear why the IL6 −174GG genotype is associated with haemorrhagic presentation of brain arteriovenous malformations, whereas the TNFα −238AG genotype is only associated with increased risk of intracranial haemorrhage during the natural course of disease. In fact, the absence of haemorrhagic presentation was recently reported as an under-recognized risk factor in predicting morbidity after brain arteriovenous malformation microsurgical resection (Reich et al., 2002). Thus, factors that may predict this risk seem to be of particular importance. After our joint analysis of the risk estimates reported by Pawlikowska et al. (2004) and Achrol et al. (2006, 2007), the −238G>A polymorphism of TNFα was still associated with 2.19-fold increased risk of bleeding in patients with brain arteriovenous malformations. Different from the −238G>A gene polymorphism, the TNFα −308G>C SNP did not show a significant association with risk of bleeding in patients with brain arteriovenous malformations, in the series reported in literature (Pawlikowska et al., 2004; Achrol et al., 2006) or in our joint analysis (Table 3).

Regarding the molecular mechanisms that are able to explain the potential role of TNFα in brain arteriovenous malformations, many authors believe that TNFα may lead to the rupture of the brain arteriovenous malformation nidus, by triggering a proteolytic process through stimulation of the inflammatory cascade (Fig. 2) in the capillary endothelial cells and the expression of pro-adhesive molecules on the endothelium, resulting in the accumulation of leucocytes and their adhesion and migration from capillaries to the brain parenchyma (Feuerstein et al., 1994; Hashimoto et al., 2003). In addition, the inflammatory pathway could activate glial cells and stimulate remodelling, gliosis and scar formation (Selmaj et al., 1990; Gaetani et al., 1999).

Role of the matrix metalloproteinases in the pathophysiology of brain arteriovenous malformations

MMPs are a family of proteolytic enzymes that degrade the proteins of the extracellular matrix, the surface molecules of the cells and other peri-cellular substances. Excessive degradation of the vascular matrix by MMPs may result in vessel destabilization, wall weakening and passive dilation (Gaetani et al., 1999; Fujimura et al., 2007). Therefore, a balanced activity of the MMPs represents a critical step in angiogenesis and vascular remodelling (Vu and Web, 2000). MMPs are also important in determining the histological phenotype of the brain arteriovenous malformation nidus, which is characterized by tangles of incompletely vessels fed by enlarged arteries and drained by dilated arterialized veins (Tu et al., 2009). An altered expression of MMPs and tissue inhibitors of metalloproteinases was demonstrated in the wall of these vessels. Compared with healthy brain tissue, brain arteriovenous malformation samples have higher levels of total MMP9, active MMP9, pro-MMP9, tissue inhibitors of metalloproteinases 1 and tissue inhibitors of metalloproteinases 3 (Hashimoto et al., 2003). In particular, MMP9 overexpression was found in plasma and in the nidus of patients with brain arteriovenous malformations (Hashimoto et al., 2004), and its activity may cause an excessive turnover of the extracellular matrix that predisposes to brain arteriovenous malformation rupture (Dasu et al., 2003). On the other hand, as summarized in Fig. 2, MMP3 seems to activate certain pro-MMPs (Suzuki et al., 1990), and polymorphisms of the MMP3 gene also seemed associated with diseases characterized by the presence of an unstable extracellular scaffold (Armstrong et al., 2007; Deguara et al., 2007). Although there is no current evidence of abnormal expression of MMP3 in brain arteriovenous malformation tissue, the association between the SNPs −707A>G and brain arteriovenous malformation susceptibility demonstrated by Zhao et al. (2009) indicates that this gene may also play a role in the aetiology of the disease (perhaps also by inducing the activation of MMP9 as was highlighted in some studies (Suzuki et al., 1990)). This promoter polymorphism is located in the 5′-untranslated region −709 bases upstream of the transcription start site, and it does not seem to be in a known conserved regulatory element. However, it is in a string of four bases that are conserved across several species.
Role of APOE in the pathophysiology of brain arteriovenous malformations

APOE is involved in several signalling cascades, including cholesterol transport, lipoprotein metabolism and neuronal sprouting (Koistinaho and Koistinaho, 2005). Numerous studies have associated the APOE-ε4 allele with poorer neurological outcome after intracranial haemorrhage (Leung et al., 2002; McCarron et al., 2003). ε2 and ε4 isoforms are functional polymorphisms involving exon regions associated with increased risk of recurrent intracranial haemorrhage in lobar amyloid angiopathy and subarachnoid haemorrhage (O’Donnell et al., 2000). Recently, as shown in Fig. 2, a role of APOE variants has been demonstrated in biological processes not directly related to lipoproteins, such as suppression of T cell proliferation, regulation of macrophage functioning, facilitation of lipid antigen presentation to natural killer T cells and modulation of inflammation and oxidation (Zhang et al., 2010). In addition, APOE interacts with the plasminogen activation cascade in an allele-specific manner. Indeed, the addition of exogenous APOE-ε2 enhances tissue plasminogen activator-induced thrombolysis (Clark et al., 2002), whereas ε4 decreases such a phenomenon and ε3 has no effect (Broderick et al., 2001). Supplementary APOE-ε2 reduces thrombus formation even in the absence of tissue plasminogen activator. Finally, tissue plasminogen activator and ε2 form a tight quaternary structure, different from the looser complex formed by tissue plasminogen activator and ε4 and the non-specific complex formed by tissue plasminogen activator and ε3 (Biehle et al., 2004). It seems obvious that these interactions have profound effects on the proteolytic activity of tissue plasminogen activator. Thus, an enhanced proteolytic activity in APOE-ε2 carriers might contribute to increase in the risk of brain arteriovenous malformation bleeding. Through its effects on the plasminogen activation system, APOE-ε2 could also influence activation of the MMP cascade, resulting in increased MMP9 activity (Tu et al., 2009). However, the association between the APOE-ε2 allele and increased risk of bleeding was not found to be significant after our joint analysis of the risk estimates reported by Pawlikowska et al. (2006) and Achrol et al. (2007).

Role of the ALK1 and ENG in the pathophysiology of brain arteriovenous malformations

Serine/threonine-protein kinase receptor R3 is an enzyme that in humans is encoded by the ACVR1L (also known as ALK1) gene. As shown in Fig. 2, ACVR1L receptor acts in the TGFβ signalling cascade forming a complex with activin-receptor type 2 (ACVR2), which can bind TGFβ. The response to TGFβ critically depends on the functional interaction between ACVR1L and ENG, which also acts as a TGFβ binding protein in the cellular membrane of endothelial cells. Although the molecular basis of this pathway is only partially known, it seems to be instrumental in the development of distinct arterial and venous vascular beds, vascular remodelling and vascular smooth-muscle and endothelial cell differentiation. Its disruption is thought to be responsible for the formation of arteriovenous dysplasia in patients with hereditary haemorrhagic telangiectasia. In this Mendelian disorder, defects in either ENG or ALK1 affect a common pathway that also includes bone morphogenetic protein 9 (now known as GDF2) (Kim et al., 2008b). In addition to the direct effects of impaired ENG and ALK1 signalling that result in abnormal angiogenic function, insufficient ENG might also affect local haemodynamics through its interaction with nitric oxide signalling.

The IVS3 –35A>G polymorphism of the ALK1 gene is located in an intronic region that seems to play a functional role as a transcriptional regulator. The ENG 207G>A is instead located in an exonic region, but the substitution did not show how to alter the final function of the encoded protein. Pawlikoska et al. (2005) and Simon et al. (2006) independently demonstrated that the IVS3 –35A>G polymorphism of the ALK1 gene is associated with sporadic brain arteriovenous malformations. In addition, after our joint analysis of the reported risk estimates, patients with AG and AA genotypes showed a risk of brain arteriovenous malformation susceptibility of 1.52-fold (95% CI: 1.19–1.96) and 2.33-fold higher (95% CI: 1.43–3.85), respectively, than subjects homozygous for the G allele (Young et al., 2007).

Consistent with this, the association between the IVS3 –35A>G gene polymorphism was significantly associated with disease susceptibility after our joint analysis of risk estimates reported in the literature (Table 3).

The link between syndromic and sporadic brain arteriovenous malformations is consistent with certain clinical, genetic and experimental observations. For instance, the cerebral arteriovenous dysplasia observed in transgenic mice lacking one copy of the ENG gene and in patients with hereditary haemorrhagic telangiectasia and sporadic brain arteriovenous malformations is morphologically indistinguishable. On the other hand, it was demonstrated that polymorphisms in genes linked to hereditary haemorrhagic telangiectasia also play a role in the development of sporadic intracranial aneurysms and spontaneous intracranial haemorrhage (Pawlikowska et al., 2005; Simon et al., 2006).

Role of the ephrin receptors in the pathophysiology of brain arteriovenous malformations

The EPHB4 gene encodes the erythropoietin-producing hepatocellular (EPH) receptor B4, a tyrosine-kinase receptor expressed in venous endothelial cells. As the cognate receptor for the arterial endothelial cell ligand Ephrin B2 (encoded by EFNB2), EPHB4 plays an important role in arterial-venous determination during the embryogenesis. These receptors are also abundantly expressed in endothelial and epithelial cells in the adult, where they may play a role in the inflammation cascade by regulating their permeability. Rat models have shown that during the later stages of inflammation, there is a decreased expression of several EPH receptors (including EPHB4) on leucocytes and endothelial cells, which results in an increased ability to mutual adhesion (Ivanov and Romanovsky, 2006). Deregulated function of EPHB4, caused either by structural changes in the gene that alter protein expression or in altered receptor signalling, could result in intracranial
vessel abnormalities that increase the risk of brain arteriovenous malformation bleeding.

The two SNPs of EPHB4 gene that have been associated with brain arteriovenous malformation haemorrhagic risk by Weinsheimer et al. (2009) are located in intronic regions, not well conserved with no known function. Hence, they are not likely to be causal alleles, but could be surrogate markers in linkage disequilibrium with functional polymorphisms located elsewhere in the EPHB4 gene or a neighbouring gene (Weinsheimer et al., 2009).

The role of ANGPT and ANGPTL in the pathophysiology of brain arteriovenous malformations

Normal angiogenesis is regulated by many factors including VEGF, ANGPT (Kim et al., 2000), the VEGF receptors family and the TIE receptors family (Davis et al., 1996). Although germ line and somatic mutations in TIE-2 were associated with venous malformations (Limaye et al., 2009), common polymorphisms in the TIE receptor family genes did not show any relationship with brain arteriovenous malformations (Pawlikowska et al., 2004). Likewise, other polymorphisms in genes with a key role in angiogenesis (i.e. ANGPT2, FLT4, KDR and VEGF) did not seem to be associated with brain arteriovenous malformations (Pawlikowska et al., 2004). However, one of the four SNPs studied in the ANGPTL4 gene by Mikhak et al. (2011) seemed to be associated with brain arteriovenous malformation susceptibility, but not with haemorrhagic risk. ANGPTL4 is well known for its role in lipid metabolism (Romeo et al., 2007; Folsom et al., 2008; Hato et al., 2008; Legry et al., 2009; Miida and Hirayama, 2010). It is also an important regulator of angiogenesis, displaying anti- and pro-angiogenic effects (Li, 2006; Chomel et al., 2009; Niki et al., 2009; Tian et al., 2009). ANGPTL4 was reported to inhibit vascular permeability (Fig. 2), tumour cell motility, invasiveness (Galaup et al., 2006) and sprouting (Cazes et al., 2006) as well as tubule-like structure formation (Ito et al., 2003; Cazes et al., 2006; Gealekman et al., 2008) and vascular leakiness (Ito et al., 2003). Unlike ANGPT, ANGPTL4 exerts its effect independently from the TIE receptor family (Kim et al., 2000). In addition, experimental models of ischaemia show that ANGPTL4 expression is increased in response to hypoxia at the level of messenger RNA transcription and protein synthesis; ANGPTL4-induced angiogenesis (Fig. 2) is independent of VEGF (Gealekman et al., 2008). However, although genetic mutations in the ANGPTL4 gene were shown to affect protein processing and function, the role of the polymorphism identified by Mikhak et al. (2011) is not clear. In fact, this SNP is located in an exonic region and has not shown the ability to alter the function of the translated protein.

The role of VEGF in the pathophysiology of brain arteriovenous malformations

VEGFA is the main isoform among the six subtypes of VEGF. A prominent feature of the brain arteriovenous malformation tissue is the relative overexpression of VEGFA, as a messenger RNA and a protein (Hashimoto et al., 2004). VEGF is the most important factor during vasculogenesis, representing the earliest vascular marker. The cellular titre of the VEGF family within the ventricular zone is higher during the early period of cerebral vascular development and is a key factor in the migration of the centripetally directed vessels. When haemangioblastic progenitor cells clamp together, they are the earliest cells that express VEGFA ligands and VEGFR2. They form angiocytes that migrate and fuse together forming a primitive capillary plexus. The completion of such vascular plexus, positive for VEGF/VEGFR complex, defines the end of vasculogenesis and is followed by a dramatic downregulation of VEGF expression.

In the setting of brain arteriovenous malformation, VEGF is expressed in the astroglia adjacent to the lesion, whereas VEGFC and -D are highly expressed in brain arteriovenous malformations with large nidi. VEGFC and -D in particular seem to contribute to brain arteriovenous malformation growth. In addition, VEGF is highly expressed in the endothelial layer and in the tunica media of vessels in children with recurrent brain arteriovenous malformations (Moftakhar et al., 2009). Although on one hand, the altered VEGF expression affects arterial differentiation, on the other the formation of arteriovenous shunts contributes to form a surrounding hypoxic environment that stimulates the secretion of all the aforementioned angiogenic factors, thus creating a pathological loop that can lead to further development and growth of the malformation (Fig. 2). All SNPs in the VEGF gene found to be associated with brain arteriovenous malformation susceptibility by Chen et al. (2011) are located in regulatory and intronic regions and are considered potentially functional in influencing the gene transcriptional activity.

Conclusions and perspectives

As outlined previously, several studies have investigated the association between SNPs and sporadic brain arteriovenous malformation susceptibility or their risk of bleeding. The question for researchers and clinicians is how to interpret such an increasing amount of data.

On one hand, it is important to point out that the vast majority of the studies available in the medical literature involve SNPs in candidate genes, which may play a plausible role in pathways potentially involved in the pathogenesis, development, growth and rupture of brain arteriovenous malformations. These pathways concern inflammatory cascade, angiogenesis, vascular remodelling and stabilization. However, we have learnt from previous studies in other medical and scientific fields that candidate gene association studies may produce many positive and negative findings, with few consistent replications. Indeed, a major problem with these studies is that relationships that seem to be significant may actually be an artefact of genetic differences between the case and control subjects because of population stratification (or admixture) because of ethnic variation or other confounding factors that can generate considerable population differences in marker allele frequencies. The chances of false-positive and false-negative findings in candidate gene association studies in
brain arteriovenous malformations are also high because of low previous odds of association and small samples size. It is rarely possible to define highly plausible candidates, and the previous odds against true associations are considerable. Also, many negative studies have little power to detect moderate or small effect sizes.

On the other hand, the many studies conducted to date have increased our understanding of the mechanisms potentially involved in the pathogenesis and clinical course of brain arteriovenous malformations. This knowledge may be a key step in designing effective strategies for risk assessment and treatment and building a genetic profile that is able to identify patients at high risk of bleeding.

To provide a better assessment of the role played by SNPs in brain arteriovenous malformation pathophysiology, we conducted a joint analysis of the risk estimates reported in the literature and found that only two SNPs continued to display a statistically significant association with increased risk of intracranial haemorrhage in patients with brain arteriovenous malformations. Likewise, our joint analysis demonstrated a significant association with brain arteriovenous malformation susceptibility for only one of the several SNPs that have been investigated in the medical literature.

Our analysis has some potential limitations. First, the polymorphisms that we analysed in this study have been investigated by a relatively limited number of researchers, thus selective publication bias may not be excluded. Also, the studies that have examined polymorphisms associated with risk of bleeding have been performed by the same group of researchers, thus it is possible that they include subsets of the same study population. In addition, as none of the analysed studies reported the estimates of genetic effect and the standard deviations of the estimate, the joint-analysis was conducted for the simple measures of association, such as risk ratio (for prospective studies) and OR (for case–control studies). Consequently, all the risk measures provided are crude results.

Based on these considerations, we expect the results of well-powered genome-wide association studies may be able to confirm a role for the SNPs investigated so far and might also lead to the identification of novel and unexpected genetic loci in the pathogenesis of brain arteriovenous malformations, thus disclosing new molecular mechanisms and new pathways in the field of the pathophysiology of these vascular malformations.

In fact, genome-wide association studies are ‘hypothesis free’ investigations exploring the entire genome, usually designed with a case–control setup to compare genetic variants (e.g. SNPs) between affected and unaffected subjects. The large samples and number of association tests required need a so-called ‘tiered design’ in which a subset of SNPs found to be significant in the first genome-wide association study (discovery set) is genotyped in a second tier (replication set), yielding a smaller subset of significantly associated SNPs that are then tested in a third tier (second replication set) and so on.

SNPs significantly replicated in association with a pathologic condition are then considered as markers of a genome region influencing the risk of the disease (Manolio, 2010). However, genome-wide association studies present problems and limitations, especially because of the fact that the large number of statistical tests performed presents an unprecedented potential for false-positive results. Also, one of the most important problems in performing a genome-wide association study in a disease as rare as sporadic brain arteriovenous malformation is how to collect a large sample of affected patients. In this regard, it is predictable that the lack of well-defined case and control groups and sufficient sample size may constitute a serious problem. Multicentre collaborative studies and the institution of national and international registries for the disease might be a useful way to overcome these issues and obtain a relevant number of homogeneous patients.

For the future, much attention should be paid to the human exome sequencing, an effective alternative to whole-genome sequencing, because it selectively identifies only the DNA coding regions translated into proteins (exons). In fact, large genome-wide association studies so far identified >250 common allelic variants associated with risk of a wide range of diseases, but most of them seem to impart only a small effect showing a low OR. Moreover, the vast majority of the genetic contributions to the pathophysiology of these diseases remain unexplained. Consistent with the Mendelian model, coding variants have proven to be prevalent sources of such rare variants. These considerations motivated the implementation of the identification of long coding regions through high-throughput sequence capture methods and next-generation sequencing technologies (exome arrays), which are able to identify the functional variations that may be responsible for Mendelian and common diseases. This approach could be clinically relevant in genetic diagnoses because of the current understanding of functional significance in sequence variation (Choi et al., 2009).

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