

REVIEW ARTICLE

Molecular basis and genetic predisposition to intracranial aneurysm

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Intracranial aneurysms, also called cerebral aneurysms, are dilations in the arteries that supply blood to the brain. Rupture of an intracranial aneurysm leads to a subarachnoid hemorrhage, which is fatal in about 50% of the cases. Intracranial aneurysms can be repaired surgically or endovascularly, or by combining these two treatment modalities. They are relatively common with an estimated prevalence of unruptured aneurysms of 2%–6% in the adult population, and are considered a complex disease with both genetic and environmental risk factors. Known risk factors include smoking, hypertension, increasing age, and positive family history for intracranial aneurysms. Identifying the molecular mechanisms underlying the pathogenesis of intracranial aneurysms is complex. Genome-wide approaches such as DNA linkage and genetic association studies, as well as microarray-based mRNA expression studies, provide unbiased approaches to identify genetic risk factors and dissecting the molecular pathobiology of intracranial aneurysms. The ultimate goal of these studies is to use the information in clinical practice to predict an individual's risk for developing an aneurysm or monitor its growth or rupture risk. Another important goal is to design new therapies based on the information on mechanisms of disease processes to prevent the development or halt the progression of intracranial aneurysms.

Key words: Berry aneurysm, candidate gene analyses, genetic association studies, intracranial aneurysms, linkage mapping, microarray analysis, subarachnoid hemorrhage

An intracranial aneurysm (IA), also called a cerebral aneurysm, is a dilatation of an artery that supplies blood to the brain (1). The shape of a dilatation can vary from a local sac-like pouch to long and tortuous enlargement of the diameter of a vessel, called a fusiform aneurysm. The most common form of IA is a berry, or saccular, form; consequently most research is about the saccular or berry IA (Mendelian Inheritance in Man, MIM number: 105800) unless specifically indicated otherwise. IAs form a complex disease with both genetic and environmental risk factors, and a poorly understood molecular pathology.

Key messages

- Intracranial aneurysms, with an estimated prevalence of unruptured aneurysms of 2%–6% in the adult population, are a complex disease with both genetic and environmental risk factors.
- Family members of patients with intracranial aneurysms are at increased risk for developing an intracranial aneurysm, and genome-wide DNA linkage and genetic association studies have identified a large number of genetic risk factors for intracranial aneurysms.
- Genome-wide microarray-based mRNA and microRNA expression studies provide unbiased information about molecular mechanisms of intracranial aneurysm and the foundation for functional studies.

In this review we will summarize the current knowledge on the pathogenesis of IA with special emphasis on molecular and genetic studies in humans. We will concentrate on genome-wide DNA linkage and genetic association studies as well as microarray-based mRNA expression studies, and only discuss candidate gene studies that were of sufficient size to provide reliable data.

Intracranial aneurysm is a complex disease: clinical characteristics and risk factors

IAs are relatively common among human populations with an estimated 2%–6% prevalence of unruptured IAs in the adult population (2–6). Unruptured IAs are mainly asymptomatic and are most often found either through screening high-risk patients or as purely incidental findings of magnetic resonance imaging (MRI) or computerized tomography (CT) studies for other neurological symptoms. The danger posed by IAs is the possibility of rupture leading to life-threatening subarachnoid hemorrhage (SAH) (4). Many clinical studies have assessed the risk for

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rupture of unruptured aneurysms; however, most estimates are from retrospective studies with the concomitant biases. Retrospective series estimate the risk of rupture to be between 1% and 3% (1 to 3 per 100 patient years), whereas the prospective series estimate it to be between 0.78% and 1.4% (7). The risk of rupture increases with aneurysm size, location, specifically in the posterior circulation, irregular shape, and history of previous aneurysmal SAH (8,9).

The most common clinical symptoms of aneurysmal SAH are a sudden onset of severe headache with stiff neck, vomiting, and photophobia. The severity of aneurysmal SAH can range in a spectrum from relatively mild and atypical, with few of the common symptoms, to sudden death. The incidence of aneurysmal SAH varies between different populations; the highest number of 22.5–32 per 100,000/y reported in Finland and Japan, while worldwide the incidence of SAH is 9.1 per 100,000/y (10).

The overall mortality rate of aneurysmal SAH has not changed much during the last decades, being nearly 50% of all ruptured cases (11–14). In the USA alone aneurysmal SAH is estimated to affect about 30,000 individuals each year (15). Also the greatest impact of the aneurysm rupture is in the age group younger than 65 years (16). The most important factor affecting the outcome is the severity of the initial aneurysm rupture. Patients with poor medical condition after aneurysmal SAH have less favorable outcome than patients with only minor neurological symptoms.

Diagnosis of SAH is primarily by CT, CT angiography (CTA), or catheter angiography. During the acute onset of symptoms CT has nearly 100% sensitivity for SAH (17), and a lumbar puncture to confirm the diagnosis is seldom needed. CTA yielding high-quality images of the intracranial vascular structures is easily and rapidly available in tertiary care emergency rooms. Even though CTA is also less invasive than angiography, catheter angiography remains the standard for diagnosing aneurysm in all locations in the cranium. MRI is used only as a screening method for unruptured IAs (18).

Aneurysms, whether ruptured or unruptured, can be treated either by open surgery or endovascularly, or by combining these two treatment modalities. The occlusion of the aneurysm neck prevents the risk of possible rebleeding of the aneurysm and can save the patients from secondary complications. It also enables effective prevention and treatment of possible delayed complications related to aneurysmal SAH. The treatment modality of IAs is highly dependent on the institution, some institutions favoring microsurgery and others endovascular treatment. The treatment results of IAs seem to improve with the increasing number of treated patients at the institution (19,20). The long-term outcomes between different treatment modalities do not differ significantly (21–23). Advances in clinical management of SAH during the last two decades have substantially improved the overall outcome of aneurysmal SAH patients surviving to reach hospital even as the incidence of SAH is decreasing (24).

Current smoking, hypertension, and heavy alcohol consumption are independent risk factors for aneurysmal SAH (25–27). Increasing age, female sex, familial occurrence of IAs, and the use of sympathomimetic drugs are also known risk factors for aneurysmal SAH (28). Patients with certain genetic disorders, such as autosomal dominant polycystic kidney disease and vascular Ehlers–Danlos syndrome (also known as type IV EDS) (29), are at increased risk for aneurysmal SAH. Recently, the PHASES study found that geographical location, e.g. Finnish or Japanese origin, was also a strong risk factor for aneurysmal rupture, possibly supporting a genetic influence on rupture risk (30). Of the different risk factors, only smoking and hypertension can be controlled and treated.

Family studies in intracranial aneurysms

Family history of IA is an important risk factor for IA. The largest collections of families with at least two affected members include a study by Wills et al. (31) with 346 Finnish IA families and a study by the International FIA Consortium with 542 families (32). A follow-up study on the Finnish IA families in which the clinical characteristics of the familial IA cases were compared to sporadic IA cases demonstrated that the familial IA group was slightly younger (46 versus 51 years in men; 50 versus 57 years in women), and had fewer females (49% versus 54%), whereas there was no difference in the number of ruptured IAs (33).

Several genome-wide DNA linkage studies have been carried out on IA (Table I). Many of these studies concentrated on single, large families and used parametric statistical approaches (34–38). The results were often restricted to the family under study and could not be extended to a larger IA population. Larger studies by three groups, the Finnish (39,40), the Japanese (41–43), and the FIA Consortium (44,45), have concentrated on non-parametric statistical approaches using an affected relative pair design (Table I). Details on the DNA linkage studies and the 13 genomic loci identified are shown in Table I. Six of the genomic regions (1p34–36, 4q32, 7q11, 14q22, 19q13, and Xp22) were discovered in two or more independent studies, thus providing strong evidence that they represent true linkage.

The challenge for DNA linkage studies comes when fine-mapping to narrow the linkage interval and find the gene(s) harboring genetic variants contributing to the IA susceptibility. This usually requires a large number of new families. Recent technological developments in next-generation sequencing are expected to ameliorate this limitation. Interpretation of the results will remain a challenge, since incomplete penetrance and genetic heterogeneity are likely to be present.

Genetic association studies in intracranial aneurysms: from candidate gene studies to genome-wide association studies

Genome-wide linkage studies of IA families, some of which were large, have identified several susceptibility loci for IA (Table I), but it is unknown if these same variants contribute to IA disease risk in individuals without a family history of IA. Another major approach used to identify genetic risk factors for IA is to genotype polymorphisms in unrelated (sporadic) cases and controls to determine if there is an association between the genetic marker and IA. The most widely used genetic markers in these studies are the so-called single nucleotide polymorphisms (SNPs). A genetic association study can be performed either as a candidate gene study or as a genome-wide association study (GWAS). In a candidate gene study specific, common polymorphisms are selected in candidate genes chosen for testing based on biological evidence that they may be functionally relevant to the development of IAs (i.e. functional candidate genes). Candidate genes for IA have also been selected from genetic studies on connective tissue disorders (e.g. vascular Ehlers–Danlos syndrome), known genetic diseases with IA as a phenotype (e.g. polycystic kidney disease), and gene expression studies (see below).

A recent meta-analysis of all genetic association studies (candidate gene and GWAS) of sporadic IA was conducted by Alg et al. (46) and included 116,000 study participants (although there may be overlap of subjects between some studies). They identified 19 SNPs that were associated with IA in at least one genetic model. The strongest associations were from the GWAS reports (summarized in the GWAS section below), while eight SNPs from

Table I. DNA linkage studies on intracranial aneurysms.

Chromosomal region	Study design ^a	Study population	LOD score ^c	Genetic marker	OMIM locus and phenotype IDs	Comments	PMID (ref.)
1p34.3 – p36.13	Family, AD	North American	4.2		ANIB3; 609122	1 family: 10 IA cases	15540160 (34)
1p36.21 – p36.13	Non-parametric	Dutch	3.18	D1S2826 – D1S234	ANIB3; 609122	1 family: same family also linked to X	18309175 (37)
4q32.2	Non-parametric	Multiple	2.5	rs1458149		192 families; LOD = 3.5 in gene × smoking interaction	18323491 (44)
4q32.3	Parametric	Multiple	2.6			333 families	9144135 (45)
5p15.2 – p14.3	Family, AD	French Canadian	3.57	D5S1954	ANIB4; 610213	1 family: 12 IA cases; results confirmed in a second family	16740915 (36)
5q22 – q31	Affected sib pair	Japanese	2.24	D5S471 – D5S2010		85 families	11536080 (41)
7q11	Affected sib pair	Japanese	3.22	D7S2415 – D7S657	ANIB1; 105800	85 families	11536080 (41)
7q11	Affecteds, AR	North American	2.34	D7S2421	ANIB1; 105800	13 families: 39 IA cases	14605871 (97)
8p22	Family, AD	Korean	3.61	D8S552	ANIB11; 614252	5 families: 9 members with and 22 without IA	21451530 (98)
11q24 – q25	Family	Colombian	4.3	rs618176 – rs1940033	ANIB7; 612161	1 family: 7 IA cases	16497978 (35)
12p12.3	Parametric	Multiple	3.1			333 families	9144135 (45)
13q14.12 – q21.1	Family	French Canadian	4.56	rs7983420 – rs17054625		1 family: 10 IA cases	19064780 (38)
14q22	Affected sib pair	Japanese	2.31	D14S258 – D14S74		85 families	11536080 (41)
14q23 – q31	Family	North American	3.0	rs235991 – rs2373098	ANIB8; 612162	1 family: 9 IA cases	16497978 (35)
17cen	Non-parametric	Japanese	3.0	D17S921 – D17S1800		29 families	15569837 (42)
19q13	Affected sib pair with covariates ^b	Finnish	5.70	D19S178 – D19S545	ANIB2; 608542		12153705 (39), 14872410 (40)
19q13	Non-parametric	Japanese	2.15	D19S198 – D19S596	ANIB2; 608542	29 families	15569837 (42)
19q13.3	Affecteds only, parametric ^d	Japanese	4.10	D19S198 – D19S902	ANIB2; 608542		17322081 (43)
Xp22	Affected sib pair	Finnish	2.08	DXS987	ANIB5; 300870	48 affected sib pairs	12153705 (39)
Xp22	Non-parametric	Japanese	2.16	DXS987 – DXS7593	ANIB5; 300870	29 families	15569837 (42)
Xp22.32 – p22.2	Non-parametric	Dutch	4.54	DXS6807 – DXS1224	ANIB5; 300870	1 family: 10 IA cases; same family linked to ANIB3	18309175 (37)

AD = autosomal dominant; AR = autosomal recessive.

^aFamily study design refers to parametric analysis.

^bSex and number of affected first-degree relatives were used as covariates.

^cOnly studies with a LOD score of ≥ 2.0 are listed in the table.

^dRe-analyzed 41 affecteds from Yamada et al. 2004 (42).

candidate gene studies were also significantly associated with IA upon a meta-analysis (Table II).

Positional candidate genes identified from IA linkage studies have been prioritized for association testing based on additional knowledge that the genes may function in the development of IA. The results suggest that the genes associated with IA are biologically relevant as they are mainly involved in vascular endothelial maintenance, integrity of the extracellular cellular matrix (ECM), and inflammation. Six SNPs associated with IA are ECM gene variants: *COL1A2* (rs42524), *COL3A1* (rs1800255), *HSPG2* (rs3767137), *SERPINA3* (rs4934), and *VCAN* (previously *CSPG2*; rs251124 and rs173686) (Table II). Collagen types 1 and 3 are present in the adventitial and medial layers of cerebral arteries and affect vessel tensile strength (47). The versican (*VCAN*) gene maps to 5q13-14 and also has significant association with thoracic aortic dissections (48). *IL6* is a proinflammatory cytokine, and rs1800796 (g.572G>C) SNP is associated with a protective effect in IA (Table II). Vascular remodeling and inflammation contributes to the formation and progression of IAs (49). The exact role of *IL6* in the pathogenesis of IA is unknown as the timing of *IL6* expression may directly affect the vessel wall (*IL6* inhibits collagen synthesis) or expression may be due to systemic inflammation.

The 9p21 locus has been a target for IA association studies (and has subsequently been detected in GWAS) (Table III) because of the known sequence variant rs10757278, which contributes to many vascular diseases including myocardial infarction and abdominal aortic aneurysms (AAAs). While Helgadottir et al. (50) were the

first to report the association of the 9p21 locus (rs10757278) with IA susceptibility, rs10757278 and rs1333040 (which are in linkage disequilibrium (LD), i.e. correlated) were the SNPs with the strongest association with IA in the recent meta-analysis performed by Alg et al. (46). This variant is located in a non-coding RNA called *CDKN2BAS1* (also known as *ANRIL*). Altered *CDKN2BAS1* expression is known to affect two genes involved in cell proliferation and apoptosis, *CDKN2A* and *CDKN2B* (51,52). SNP rs10757278 is in strong LD with rs10757272, which showed a trend toward association with IA in two GWASs (53,54), but not significantly associated with IA upon meta-analysis. The biological relevance of these SNPs on IA is unclear (55); however, *CDKN2BAS1* may affect MMP3 levels and play a role in ECM repair (56).

A recent meta-analysis (57) on the *ACE* insertion/deletion (I/D) polymorphism, rs4646994, showed an association between the I allele and IA risk (17q23.3, odds ratio (OR) = 1.21, $P = 0.003$) (Table II). Individuals with *ACE* I/I and I/D genotypes had significantly increased risk for IA (OR = 1.27, $P = 0.03$). The mechanism by which the *ACE* I allele contributes to IA is unknown.

Some additional candidate genes associated with IA, but not included in any meta-analysis, are noteworthy (Table II). Genome-wide linkage studies identified a susceptibility locus for IA on chromosome 19q13 in the Finnish and Japanese populations (39,40,42,43), which includes the kallikrein gene cluster. In a follow-up study, 18 haplotype-tagging SNPs spanning the *KLK* gene cluster were tested for association in the Finnish and Russian populations (58). Two intronic SNPs, rs1722561 and rs1701946,

Table II. Most significant associations with IA in candidate gene studies.

Gene symbol	Polymorphism	Region	Context	Genetic model	Sample size (n)		OR [95% CI]	P value ^b	Multiple testing correction	Potential biological mechanism	PMID (ref.)
					Cases ^a	Controls ^a					
ACE	rs4646994	17q23.3	intron	Recessive	854	1,280	1.27 [1.03–1.57]	0.03	Bonferroni	Vascular endothelium	23860151 (57)
COL1A2	rs42524	7q21	missense (p.549P>A)	Dominant	812	806	1.77 [1.14–2.75]	0.0009	Bonferroni	ECM	23733552 (46)
COL3A1	rs1800255	2q32	missense (p.698A>T)	Dominant	546	2,235	1.55 [1.21–2.00]	<0.00001	Bonferroni	ECM	23733552 (46)
ELN	rs8326	7q11.2	3'-UTR	Allelic	404	458	3.11 [1.96–5.45]	0.000001	Permutation	Vascular endothelium	16611674 (59)
HSPG2	rs3767137	1p36.1	intron	Additive	1,316	1,742	1.22 [1.08–1.39]	0.002	Bonferroni	ECM	23733552 (46)
IL6	rs1800796	7p15	intergenic	Recessive	912	3,410	0.47 [0.34–0.65]	<0.00001	Bonferroni	Inflammatory mediator	23733552 (46)
JDP2	rs175646	14q24	intron	Allelic	403	412	1.44 [1.15–1.81]	0.0042	Bonferroni	Apoptosis	20452405 (62)
KLK8	rs1722561	19q13.3	intron	Additive	524	578	1.35 [1.14–1.60]	0.008	Sidak	ECM	17761919 (58)
LIMK1	rs6460071	7q11.2	intergenic	Dominant	2,050	1,835	1.31 [1.12–1.53]	0.026	Bonferroni	Actin depolymerization	21228795 (60)
SERPINA3	rs4934	14q32	missense (p.9A>T)	Dominant	892	1,029	2.22 [1.68–2.94]	<0.00001	Bonferroni	ECM	23733552 (46)
TCN2	rs1801198	22q12	missense (p.259R>P)	Genotypic	255	348	0.48 [0.30–0.77]	0.002	Step-down Sidak–Holm	Methionine metabolism	18799873 (63)
TNFRSF13B	rs4985754	17p11.2	promoter	Haplotype	304	332	0.69 [0.52–0.92]	0.048	Bonferroni	Immunity	16618819 (61)
	rs2274892	17p11.2	intron								
	rs34562254	17p11.2	missense (p.251P>L)								
VCAN	rs11078355	17p11.2	synonymous	Additive	857	879	1.23 [1.05–1.43]	0.008	Bonferroni	ECM	23733552 (46)
VCAN	rs173686	5q14	intron	Additive	1,489	1,687	1.25 [1.1–1.41]	0.0007	Bonferroni	ECM	23733552 (46)
VCAN	rs251124	5q14	intron								

^a Only studies with a minimum of 250 cases and 250 controls, and a replication sample and correction for multiple testing were included in the table.
^b P values are taken from either a meta-analysis or the report with largest sample size demonstrating association with IA.

mapping to *KLK8* and in high LD were associated with IA (Table II). *KLK8* is a serine protease that can cleave the ECM protein fibronectin. It is thus plausible to hypothesize that dysregulated kallikrein may contribute to the pathogenesis of IA (58).

Another plausible candidate gene for IA is the elastin (*ELN*) gene. SNP rs8326 located in the 3' untranslated region of the *ELN* gene (+659 G>C) is associated with IA and is in an at-risk haplotype for IA that includes two functional SNPs that lead to decreased transcript levels of *ELN* and *LIMK1* (Table II) (59). Elastin and *LIMK1* proteins are involved in the actin depolymerization pathway and may affect the stability and synthesis of vascular walls, thus contributing to the pathogenesis of IA (59). Another study (60) subsequently reported an association of SNP rs6460071 in *LIMK1* with increased risk of IA, but was unable to replicate the association between the *ELN* gene and IA.

In a follow-up study to the IA linkage region on the chromosome 17 centromere, a haplotype in the *TNFRSF13B* gene was found to be protective for IA in the Japanese population (Table II) (61). *TNFRSF13B* is involved in immunity as it mediates isotype switching in B cells. This association supports a role for immunologic mechanisms in the IA pathogenesis.

An intronic SNP, rs175646, in *JDP2*, a positional candidate gene that maps to chromosome 14q22, was associated with IA in a Japanese cohort, and this association was replicated in a Korean IA cohort (Table II) (62). *JDP2* encodes Jun dimerization protein 2, a repressor of transcription activator protein 1 (*AP1*), which is involved in apoptosis. Hence, *JDP2* may contribute to vascular remodeling in IA via apoptotic cell death.

In addition, a functional polymorphism in *TCN2* p.259R>P (rs1801198; c.1025C>G; previously reported as c.776C>G), is associated with IA (63). *TCN2* encodes transcobalamin II, which is involved in methionine metabolism, and the protective effect in IA may be due to more effective inhibition of the nitric oxide synthase (NOS).

Unfortunately, many of the genes thought to be strong functional candidates for IA have not shown significant association with IA upon meta-analysis including: *ENG*, *NOS3*, *APOE*, and *MMP3* (46). The major limitation of genetic association studies in IA has been small sample size and failure to replicate positive associations, especially given the necessary multiple testing correction for large candidate SNP and GWAS studies (64–67). Studies with fewer than 250 cases provide unreliable OR (effect) estimates (68). IA association findings have been inconsistent between studies, and some candidate genes could be population-specific. Hence, in the absence of a reliable candidate gene for IA, it is premature to move this research forward to the stage of developing transgenic animal models that assess the impact of such functional candidate genes on the formation of IAs.

Genome-wide association studies

A hypothesis-free discovery approach to examine the genetic determinants of IA is GWAS by genotyping unrelated individuals (69). To date there have been a total of six GWAS studies of IA, some of which have identified novel genes for IA, recently reviewed by Hussain et al (70). Four of the studies included sufficient numbers of individuals to power the studies to identify associated SNPs at the genome-wide significant threshold (1×10^{-7}). Here we review the most significant findings from the studies and summarize them in Table III.

The first GWAS of IA was published in 2008 (71). The discovery cohort included Finnish and Dutch cases and controls. Significant SNPs were subsequently genotyped in two pooled

Table III. Most significant associations from genome-wide association studies.

Gene symbol	Polymorphism	Region	Context	OR	95% CI	P value ^a	Sample size (n)		
							Cases	Controls	PMID (ref.)
<i>CDKN2B-AS1 (ANRIL)</i>	rs1333040	9p21	intron	1.24	[1.2–1.29]	9.83×10^{-08}	11,949	29,014	23733552 (46)
<i>CDKN2B-AS1 (ANRIL)</i>	rs10757278	9p21	intergenic	1.29	[1.21–1.38]	1.59×10^{-13}	3,394	17,075	23733552 (46)
<i>CDKN2B-AS1 (ANRIL)</i>	rs6475606	9p21	intron	1.35	NR	3.59×10^{-08}	1,483	1,683	22961961 (53)
<i>CNNM2</i>	rs12413409	10q24.3	intron	1.29	[1.19–1.40]	1.20×10^{-09}	2,780	12,515	20364137 (72)
<i>EDNRA</i>	rs6841581	4q31.23	intergenic	1.22	[1.14–1.31]	1.95×10^{-08}	4,370	14,181	23733552 (46)
<i>FGD6</i>	rs6538595	12q22	intron	1.16	[1.10–1.23]	1.12×10^{-07}	4,370	14,181	23733552 (46)
<i>RRBP1</i>	rs1132274	20p12.1	missense (p.891R>L)	1.19	[1.11–1.28]	8.29×10^{-07}	4,370	14,181	23733552 (46)
<i>SOX17</i>	rs10958409	8q11.23	intergenic (5')	1.20	[1.15–1.26]	1.78×10^{-15}	9,873	27,029	23733552 (46)
<i>SOX17</i>	rs9298506	8q11.23	intergenic (3')	1.21	[1.15–1.27]	1.55×10^{-13}	9,246	26,331	23733552 (46)
<i>STARD13</i>	rs9315204	13q13	intron	1.20	[1.13–1.28]	2.50×10^{-09}	2,780	12,515	20364137 (72)

NR = not reported.

^aOnly results reaching genome-wide significance ($\times 10^{-7}$) are listed in the table.

Japanese replication cohorts. Combination of the data sets including a total of 2196 IA cases and 8085 controls identified SNPs in three loci that passed the genome-wide significance threshold: *BOLL/PLCL1* (2q33.1, OR = 1.24, $P = 4.4 \times 10^{-8}$, rs700651), *SOX17* (8q11.23, OR = 1.36, $P = 1.4 \times 10^{-10}$, rs10958409), and *CDKN2BAS* (9p23.1, OR = 1.29, $P = 1.4 \times 10^{-10}$, rs1333040). In addition, rs9298506 located near *SOX17*, but not in LD with rs10958409, was also associated with IA (8q11.23, OR = 1.35, $P = 1.8 \times 10^{-9}$, rs9298506).

The same group (72) added more cohorts to increase the sample population (5891 cases, 14,181 controls) and identified a total of five IA loci. Two of the loci were previously identified and had strengthened association with IA: *SOX17* (8q11.23, OR = 1.28, $P = 1.3 \times 10^{-12}$, rs9298506) and *CDKN2BAS1* (9p21, OR = 1.32, $P = 1.5 \times 10^{-22}$, rs1333040). In this study (72), SNP rs10958409 was not associated (8q11.23, OR = 1.17, $P = 9.0 \times 10^{-7}$, rs10958409); however, three new associations with IA were found: *CNNM2* (10q24.32, OR = 1.29, $P = 1.2 \times 10^{-9}$, rs12413409), *STARD13* (13q13.1, OR = 1.20, $P = 2.5 \times 10^{-9}$, rs9315204), and *RRBP8* (18q11.2, OR = 1.22, $P = 1.1 \times 10^{-12}$, rs11661542). It should be noted that some SNPs that reached genome-wide significance, e.g. rs700651 and rs11661542, failed to replicate and are not significant in meta-analyses.

In 2011, Yasuno et al. (73) reported the results of their expanded analysis in which they genotyped 25 SNPs in 14 loci that had previously demonstrated a posterior probability of association of 0.1 to 0.5 in the discovery cohort from their 2010 study (72) in Japanese replication cohorts. After combining the results from the discovery and replication cohorts they found significant association of IA with rs6841581 on chromosome 4q31.23 located 5' of the endothelin receptor type A (*EDNRA*) gene (4q31.23, OR = 1.22, $P = 2.2 \times 10^{-8}$). They also reported trends toward association for two additional regions: *FGD6* (12q22, OR = 1.16, $P = 1.1 \times 10^{-7}$, rs6538595) and *RRBP1* (20p12.1, OR = 1.20, $P = 6.9 \times 10^{-7}$, rs1132274). SNPs with strong or suggestive association with IA identified in the Yasuno et al. GWAS reports (72,73) were also tested for association with blood pressure, an important risk factor for IA (74). The suggestive IA locus at 5q23.2 (rs2287696) in *PRDM6* was significantly associated with increased systolic blood pressure (5q23.2, $P = 8.13 \times 10^{-7}$, rs2287696). The authors (74) hypothesized that variants in *PRDM6* may contribute to alterations in vascular wall structure that lead to increases in systolic blood pressure and predispose an individual to IA.

Low et al. (54) reported their GWAS in another Japanese cohort (1383 IA-SAH cases, 5484 controls). No SNP met genome-wide significance in the stage 1 analysis. They selected 36 unlinked SNPs with suggestive associations ($P < 1 \times 10^{-4}$) and seven previously reported SNPs associated with IA (nominal $P < 0.05$

in this study) to genotype in a replication cohort of 1048 cases and 7212 controls. They identified a genome-wide significant SNP in *EDNRA* (4q31.2, OR = 1.25, $P = 9.6 \times 10^{-9}$, rs6842241) that was more significantly associated with IA than reported in the Yasuno 2011 study (73). *EDNRA* is an endothelin-1 receptor, which activates a G-protein(s) and their second messenger system. It is located predominantly in the vascular smooth muscle cells of the cerebrovascular system and mediates vasoconstriction and proliferation (54). This SNP is located in a regulatory region of the *EDNRA* gene on 4q31.22. Functional promoter analysis using an electrophoretic mobility shift assay identified two alleles of rs6841581 (upstream of *EDNRA*, and in tight LD ($r^2 > 0.8$) with rs6842241) that had different binding affinities to a nuclear protein(s) from human embryonic kidney cells (HEK293). Reporter assays suggested the 5' flanking region including SNP rs6841581 might function as a transcriptional repressor and be a functional variant conferring IA susceptibility.

The most recent GWAS of IA was performed by Foroud et al. (53). The study design was unique from the other GWAS of IA in that the discovery cohort included two samples: Sample 1 was 388 European cases with a strong family history of IA and 387 controls; Sample 2 was 1095 IA cases without family history of IA and 1286 controls. There were no genome-wide significant SNPs in either discovery cohort. Meta-analysis (1483 cases, 1683 controls) confirmed association with IA for two previously reported SNPs: one SNP in *CDKN2BAS1* (9p21, OR = 1.36, $P = 3.6 \times 10^{-8}$, rs6475606) and one SNP nearby *SOX17* (8q11.23, OR = 1.25, $P = 8.7 \times 10^{-5}$, rs1072737). In addition, they studied the effect of smoking and these two SNP genotypes on IA risk. Their results suggest that smoking acts multiplicatively with the SNP genotype, and smoking has a greater effect on risk than SNP genotype.

Gene expression studies in intracranial aneurysms

Microarray-based mRNA expression profiling provides an unbiased approach for defining the molecular signature of each organ in disease and health (75). Arteries have distinct mRNA expression profiles from other tissues, and the same artery (e.g. aorta) from different individuals is more similar than other arteries from the same person, demonstrating the need for a careful selection of control tissues for expression studies (75). In Table IV all published microarray-based expression studies on IA are summarized. In these nine studies (76–84) genome-wide expression profiles were generated on 80 IA and 77 control tissue samples (6 intracranial control arteries, 34 superficial temporal arteries, 4 arteriovenous malformation feeder (AVMf) cell samples, 33 middle meningeal arteries), as well as on blood samples from 43 IA patients and 18 controls.

Table IV. Microarray-based mRNA and microRNA expression studies on intracranial aneurysms.

PMID of study	Samples for microarray study ^a		Study design	Microarray platform (Vendor)	Analysis methods	Public data ID ^b
	IA	Controls				
17878320 (76)	Pool of 5 RIA (F) Pool of 3 RIA (M) Pool of 4 UIA (M)	Pool of 5 (F) ^c Pool of 3 (M) ^c 4 non-IA M	Establish expression profile of intracranial arteries to aid in selecting candidate genes for IA	HG-U133 Plus 2.0 (Affymetrix); Sentrix Human WG-6 (Illumina)	Genespring (Silicon Genetics); R and Bioconductor; Beadstudio (Illumina); GO; KEGG	GSE6551
18538937 (77)	6 RIA 4 UIA	4 AVMf	Compare mRNA expression levels between a) ruptured and unruptured IAs b) IA and control artery	Human 1A.v2 Oligo Microarray (Agilent)	GeneSpring GX 7.3 (Agilent); FDR; GO; Network analysis (IPA)	
19228845 (79)	3 RIA 3 UIA	6 STA	Compare mRNA expression levels between ruptured and unruptured IAs	Sentrix Human WG-6 v2 (Illumina)	Bioconductor; FDR; GO; KEGG; Network analysis (IPA)	
19752560 (78) ^d	3 UIA	3 STA	Compare mRNA expression levels between unruptured IA and control arteries	HG-U133 Plus 2.0 (Affymetrix)	GeneSpring 9.05 (Agilent); GO; KEGG; DAVID	GSE26969
20487632 (80)	12 RIA 10 UIA	4 control STA ^e 4 control MMA 9 SAH-STA 9 SAH-MMA 12 UIA-STA 12 UIA-MMA	Compare mRNA expression levels between: a) ruptured and unruptured IAs b) IA and control artery	HG-U133A (Affymetrix)	GeneSpring GX 7.3 (Agilent); FDR	
20044533 (81)	8 RIA 6 UIA	5 control MMA	Compare mRNA expression levels between: a) ruptured and unruptured IAs b) IA and control artery	Human Gene ST 1.0 (Affymetrix)	dChip; FDR; GO	GSE15629
21336216 (82) ^f	11 RIA 8 UIA		Compare mRNA expression levels between ruptured and unruptured IAs	HG-U133 Plus 2.0 (Affymetrix)	Bioconductor; R; FDR; GO; KEGG; DAVID; Whole Genome rVISTA	GSE13353
24079748 (83)	3 RIA ^g	3 control MMA	Compare mRNA and miRNA expression levels between IA and control arteries. Investigate role of miRNAs in the control of gene regulation in IA	microRNA array 16.0 (Agilent); Whole Human Genome Oligo Microarray (Agilent)	Network analysis (IPA); miRNA target analysis (IPA)	GSE46338
23512133 (84)	43 RIA	18	Compare mRNA expression levels in blood between ruptured IA and controls	HumanHT-12 BeadChip (Illumina)	R; FDR; GO; KEGG	GSE36791

^aWeinsheimer et al. (76) study used autopsy samples, Pera et al. 2013 (84) used blood samples, and all other studies used aortic tissue samples obtained in operations.

^bData are available at <http://www.ncbi.nlm.nih.gov/geo>.

^cThese controls were from the non-aneurysmal contralateral artery.

^dThese data have been re-analyzed in another study (99).

^eControl artery samples were collected from the STA and MMA of SAH and UIA patients as well as from patients without IA.

^fThese data have been re-analyzed in another study (100).

^gOnly 2 IA and 2 control samples were used for miRNA microarray study, whereas 3 IA samples and 3 controls were used for mRNA expression study.

AVMf = arteriovenous malformation feeder; F = female; FDR = false discovery rate; GO = Gene Ontology; KEGG = Kyoto Encyclopedia of Genes and Genomes; M = male; MMA = middle meningeal artery; RIA = ruptured intracranial aneurysm; SAH = subarachnoid hemorrhage; STA = superficial temporal artery; UIA = unruptured intracranial aneurysm.

One of the studies used mRNA expression profiles as a guide to select positional candidate genes for IA and combine the expression data with DNA linkage study results (76). To define the molecular signature of an intracranial artery, RNA samples isolated from both IA and non-IA intracranial arteries, and two different microarray platforms were used (76). The results revealed that approximately half of the genes represented on the microarray platforms were expressed in the intracranial arteries, and the IA loci identified in DNA linkage studies harbored approximately 800 different genes expressed in intracranial arteries. Pathway analysis using Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) bioinformatics tools showed enrichment of several biological pathways including the Notch and MAPK signaling pathways.

Seven of the studies listed in Table IV were designed to compare the expression profiles of either IA to non-IA tissue (77–81,83) or unruptured to ruptured IA (82); most studies addressed both of these goals. A recent meta-analysis (85) combined the gene lists reported in five of the microarray-based expression studies

on IA (77–81) and produced a list of 507 differentially expressed genes when comparing IA (ruptured or unruptured) to control tissue, 57 of which had altered expression levels in more than two of the five studies. Only seven genes (*BCL2*, *COL1A2*, *COL3A1*, *COL5A2*, *CXCL12*, *TIMP4*, and *TNC*) were found in more than three studies. It is of interest to note that all but two (*BCL2* and *CXCL12*) of these seven genes are ECM genes.

So far only one study (84) has analyzed peripheral blood samples to detect differences in gene expression between ruptured IA and non-IA controls using blood expression profiles. If successful, this systemic approach would allow assessment of mRNA expression profiles at different stages of the IA development and correlation of the molecular findings to imaging studies. A total of 57 genes were downregulated and 78 genes upregulated in the blood samples taken from patients with ruptured IA. These 135 genes were classified as genes of immune function and hematopoiesis. Quantitative reverse transcription PCR (qRT-PCR) was used to validate the findings on 16 genes in an independent set of IA patients and controls. No correlation was found between the time

of blood draw related to the SAH and the gene expression. Interestingly, there was an overlap of 29 genes identified in this study and in those on stroke (86) and AVM (87), when analyzing gene expression profiles of blood samples.

MicroRNAs (miRNA) have been recognized as important modulators of gene expression (88). The first miRNA analysis in IA revealed 18 miRNAs with decreased levels in IA tissue (Table IV) (83). Follow-up studies included analyzing mRNA expression profiles of IA and control samples to investigate the 681 target genes of the 18 miRNAs. Functional classification of the target genes showed enrichment in 'migration of phagocytes' and proliferation of many cell types.

In summary, genome-wide mRNA and miRNA expression studies provide an unbiased approach to understand IA pathophysiology. The use of GO and KEGG terms for a comprehensive functional classification of gene expression, utilizing annotated pathways rather than isolated genes, as well as the interconnectedness of the pathways, gives a more global picture of the pathology involved in IA.

Conclusions and future direction of research

The results summarized above have contributed to improved understanding of the genetics, pathophysiology, and etiology of IA. It is, however, important to understand that all studies have some limitations. One of the biggest challenges common to all human studies (DNA linkage, genetic association, and gene expression studies) is the number of samples in each study and how to re-analyze previously published data and carry out meta-analyses. In order to do this effectively, raw data from the studies should be publicly available. For example, in the case of the IA gene expression studies, six of nine studies have deposited the data to gene expression databases (Table IV) thereby making a valuable contribution to science.

Another challenge, particularly in the expression studies, is the availability of appropriate control samples. In most IA gene expression studies, samples from IA were compared to samples taken from other arteries as controls. This is problematic, since it is known that arteries at different locations in the body differ from each other in structure, embryologic origin, and disease susceptibility. Using tissues originating from autopsies as controls can also be problematic, since it is possible that post-mortem changes in RNA occur leading to decreased mRNA integrity (89). Nevertheless, degradation is both tissue- and time-dependent and can be minimal in the IA tissue. Yet another challenge is that IA samples in all gene expression studies were taken from IA tissue at the very late stage of the disease, when the aneurysm was large enough for surgical intervention or had already ruptured. Lack of studies from the early stages of human IA development makes it difficult to understand what is important for the initiation of vascular injury leading to IA.

The results from the genome-wide expression studies on IA have provided the foundation for many future studies. The evidence that miRNAs have an important regulatory function in vascular remodeling could be important in discovering targets for a potential therapy to stabilize or slow down IA growth. It would also be important to find the transcription factors responsible for the transcriptional control of gene expression (82). Levels of mRNA and miRNA in the blood of IA patients could provide diagnostic tests by serving as biomarkers. This information could help in identifying markers predictive of rupture; furthermore, use of methods such as laser-capture microdissection to isolate specific cell populations, present in IA wall, would allow for a detailed study of their contribution to IA pathobiology.

The genetic map of IA includes risk loci on almost every human chromosome (Figure 1). The results summarized in this review were derived from discoveries made using different approaches including DNA linkage (Table I), GWAS (Table III), and candidate gene studies (Tables II; Figure 1). It should be noted that many IA studies have limited power due to small sample size, and replication is unlikely (64,68). The next steps are then to investigate the molecular mechanisms by which the identified genetic risk factors contribute to the disease. As shown in Tables II and III, many of the SNPs associated with IA are not in the coding regions of proteins, thus necessitating further studies to find the actual functional variants or defining the regulatory functions of the non-coding SNPs (90,91). These studies are also likely to include epigenetic studies such as investigating the role of DNA methylation (92). Other genetic effects such as various forms of epistasis and modifier genes will also need to be considered (93).

IA is considered a complex disease characterized by initiation, growth, and rupture, with multiple genetic and environmental risk factors that might contribute to one or more of the stages. When estimating the contribution of all the identified factors to the risk of a person to develop an IA, the growth of an IA and its rupture, the interactions of the various factors, including environmental risk factors such as smoking, should also be considered. Such analyses require large sample sizes with comprehensive data necessitating collaborative multi-center projects.

The ultimate goal of the molecular and genetic studies is to use the information to design new risk-scoring algorithms to predict an individual's risk for developing an IA or monitor its growth or rupture risk for use in clinical practice. To account for all the genetic variation contributing to IA, hundreds of genetic variants might have to be analyzed. For example, a recent meta-analysis on multiple sclerosis identified 110 non-major histocompatibility complex (MHC) susceptibility loci, which explained only about 20% of the sibling recurrence risk; this number was about 28% if including MHC loci (94).

Whole exome or whole genome sequencing approaches may prove an aid in the identification of genes underlying IA linkage peaks or to identify new genetic risk variants. With the help of the high-throughput next generation sequencing (NGS) approaches it is potentially feasible to identify sequence differences between affected and unaffected individuals. NGS has so far been most useful in finding mutations in highly penetrant Mendelian disorders (95). MacArthur et al. (95) emphasize the difficulty of distinguishing between pathogenic and non-pathogenic variants, concluding with: 'Objective, systematic and quantitative evaluation of the evidence for pathogenicity and sharing of these evaluations and data amongst research and clinical laboratories will maximize the chances that disease-causing genetic variants are correctly differentiated from the many rare non-pathogenic variants seen in all human genomes.' Other groups have emphasized similar challenges and included concerns about problems with incomplete coverage and low reproducibility of variant detection (96). Differentiating pathogenic variants from non-pathogenic variants under linkage peaks will remain the vexing problem it has been since the relatives in small families will share large portions (many centi-Morgans, usually many Mbp) of the chromosome identical-by-descent. For a complex disease such as IA case-control studies will require thousands of individuals. In spite of the aforementioned cautions, the ability to define substantially more variation by NGS will provide a far richer information base than we currently have.

Another important goal is to design new therapies based on the information on mechanisms of disease processes to prevent the development or halt the progression of IA. In addition, there

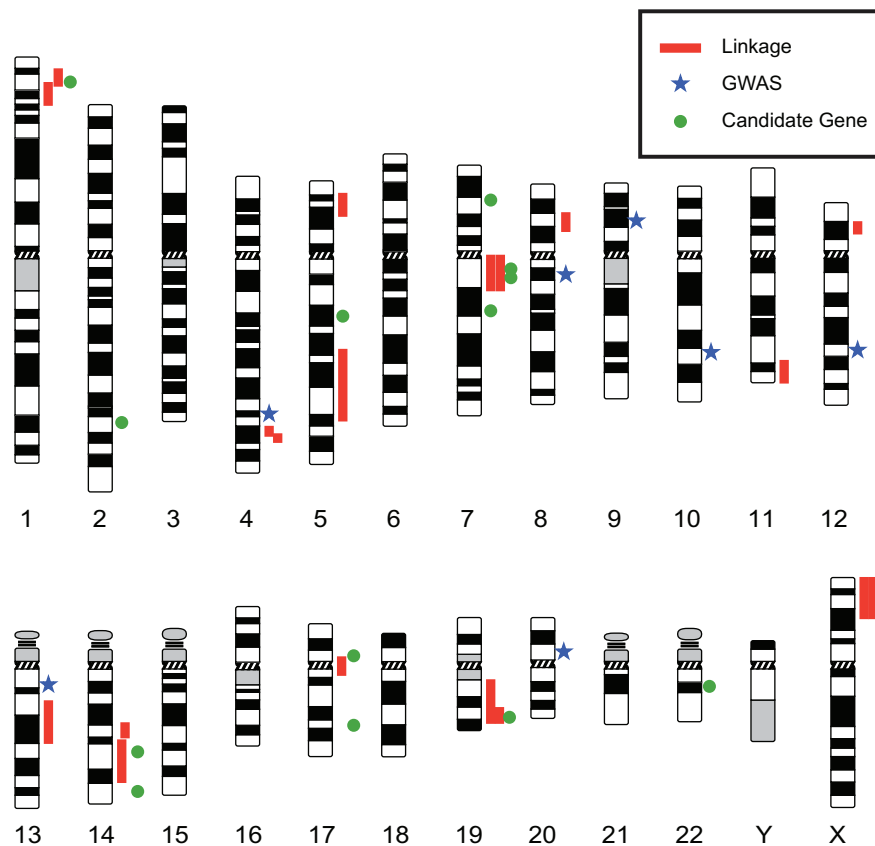


Figure 1. Genetic map of intracranial aneurysm loci. Red vertical lines adjacent to the chromosome ideograms indicate regions identified by DNA linkage studies (Table I), pale red vertical lines indicate support for a region that arises from a family linked to two loci, blue stars indicate locations of SNPs found in genome-wide association studies (Table III), and green round symbols indicate locations of SNPs found in candidate gene association studies (Table II). The ideograms can be obtained from 'Idiogram Album: Human' (copyright© 1994 David Adler, University of Washington, Department of Pathology) at <http://www.pathology.washington.edu/research/cytopages/idiograms/human/>.

is a potential genetic overlap among different types of aneurysms since the same sequence variant on 9p21 contributes to risk for myocardial infarction, AAA, and IA, apparently predisposing to an aberrant vascular injury response (50). In addition, future studies should aim to compare the genetics of different types of aneurysms (e.g. intracranial, extracranial, thoracic, aortic), which may aid in identifying potential treatments.

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