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Biology, Physics and Genetics of Intracranial Aneurysm Formation: A Review

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Abstract:
Intracranial aneurysms (IAs) are persistent, localised dilatations of the arterial wall that are found in approximately 3% of the general population. The most severe complication of IAs is rupture, which results in devastating consequences such as subarachnoid haemorrhage and brain damage with serious neurological sequelae. Numerous studies have characterised the mechanisms underlying IA development and growth and identified a number of environmental modifiable (smoking, hypertension) and nonmodifiable risk factors (related to the histology of cerebral arteries and genetic factors) in its pathogenesis. Haemodynamic stress also likely plays a crucial role in the formation of IAs and is conditioned by the geometry and morphology of the vessel tree, but its role in the natural history of unruptured IAs remains poorly understood; it is believed that changes in blood flow might generate the haemodynamic forces that are responsible for damage to the vascular wall and vessel remodelling that lead to IA formation. This review summarises the most relevant data on the current theories on the formation of IAs, with particular emphasis on the roles of special conditions resulting from the microscopic anatomy of intracranial arteries, haemodynamic factors, bifurcation morphometry, inflammatory pathways, and the genetic factors involved in IA formation.

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Biology, Physics and Genetics of Intracranial Aneurysm Formation: A Review

1. Introduction

Intracranial aneurysms (IAs) are persistent, localised dilatations of the arterial wall. The prevalence of IAs has been estimated to be approximately 3% of the general population [1]; however, this is difficult to estimate since they are mostly clinically silent and are therefore mainly diagnosed fortuitously due to routine computed tomography angiography (CTA) and magnetic resonance angiography (MRA) or in postmortem examinations. IAs are reported three times more often in adults over the age of 40 years than in patients under 40 years [1]. They are rarely found in children or adolescents under the age of 19 [2] and are diagnosed more often in women than in men [1, 3-6]. The prevalence of IAs in women is approximately 6%, and the female-to-male ratio is 1.57 [1].

In addition to female sex, other nonmodifiable risk factors for IAs include a family history of stroke (ischaemic, haemorrhagic stroke and subarachnoid haemorrhage) [1, 7] or unruptured IAs [1] and autosomal-dominant polycystic kidney disease (ADPKD) [1, 7, 8]. The most frequent modifiable factors leading to the formation of IAs include arterial hypertension and smoking [7]. Other modifiable risk factors for IA formation include ischaemic heart disease, hypercholesterolaemia, diabetes, body mass index, and alcohol consumption, but data on their specific role in the disease remain limited and inconclusive [1, 7].

IAs are most often found in the anterior part of the circle of Willis (approximately 85–95%), whereas 5–10% of IAs are located in the posterior part of the circle [1, 4-6]. Approximately 20% of patients present with multiple intracranial aneurysms (MIA) [9].

Despite the large amount of research that has been conducted to date, the precise way in which IAs form has been a matter of debate for decades and remains poorly understood. In this review, the authors discuss the current theories on the formation of IAs, with particular emphasis on

the role of special conditions resulting from the microscopic anatomy of intracranial arteries, haemodynamic factors, bifurcation morphometry, and the genetic factors involved in IA formation.

2. Anatomy and ultrastructure of the intracranial arteries: The role of medial defects in the aetiology of IAs

The vascular wall of intracranial arteries consists of three layers: the intima, consisting of a single layer of endothelial cells (ECs); the media, mainly composed of circumferentially oriented smooth muscle cells (SMCs) embedded in a dense network of collagen (mostly types I and III), elastin fibres and proteoglycans; and the adventitia, the outer connective tissue layer that is composed of collagen fibres and fibroblasts [10, 11]. Beneath the EC layer is a subendothelial layer of connective tissue containing a small number of SMCs. The intima and media are separated by a membrane of elastic fibres – the internal elastic lamina (IEL). The IEL is the key structure involved in the formation of IAs at the structural level [12, 13]. Unlike extracranial arteries of comparable size, which are muscular in type, intracranial arteries have a less developed media and a well-developed IEL but no external elastic lamina (EEL). Moreover, in contrast to extracranial arteries, whose media and adventitia contain abundant elastic fibres, in intracranial arteries, the vast majority of elastic fibres are localised in the IEL [14]. The small amount of connective tissue in the subarachnoid space along with the thin media and adventitia make intracranial arteries more vulnerable to haemodynamic stress and aneurysm development [11, 15].

In 1930, Forbus first reported the presence of gaps within the media, particularly at the main arterial junctions or at the apices of vessel bifurcations. These so-called medial defects, most likely of an innate nature, were considered to be *locus minoris resistentiae*, a place of decreased resistance, and regarded as the primary causal factor in the genesis of most IAs [16]. The key importance of medial defects in the formation of IAs was questioned by Glynn, who argued that medial defects affect the muscular coat alone and do not impair local resistance to internal pressure

due to the well-developed IEL, which contains an adequate number of elastic fibres and is solely responsible for limiting undue expansion [17].

A number of other studies have found that the location of medial defects does not correlate with the most common locations of IAs and that aneurysms are also found in human extracranial arteries (e.g., the renal, splenic, meningeal and mesenteric arteries) [18-20] and in animals, while saccular IAs are found almost exclusively in humans [21]. As first proposed in 1981, medial defects are still considered to act as a raphe, which can provide a degree of structural support necessary to maintain blood flow during the vasoconstriction of the parent vessel [22].

Elastic tissue loss and fragmentation of the IEL are among the first symptoms of aneurysm initiation [23, 24]. In 1950, Carmichael was one of the first to report that IEL disintegration may be caused by an atheromatous degeneration process, noting that IEL disintegration coexisted with the presence of a medial defect and that the absence of support from the muscular coat played a large role in the final disruption of the IEL [25]. Together, these observations suggested that the formation of IAs is not caused by the mere presence of a medial defect or atheromatous degeneration itself but by a combination of the two [25, 26]. Crawford suggested that in addition to the two main factors weakening the vascular wall, namely, the presence of developmental deficiency and atherosclerosis, a third factor, haemodynamic in nature (e.g., arterial hypertension), is necessary. He argued that only a sufficiently high blood pressure could lead to a bulging of the arterial wall at the site of a medial defect and initiate the formation of an IA [27].

Studies have on morphological changes in the cerebral arteries of experimental animals have also provided different theories on their possible relationship to the development of IAs. Hassler observed that the defects in arteries exposed to increased haemodynamic stress and caused by augmented blood flow were also larger and showed aneurysmal bulging. In his opinion, the streaming blood shapes the arterial wall and thus determines the location and size of a medial defect [28]. Kim et al. demonstrated that damage to the endothelium around the apex of a bifurcation and IEL disintegration in monkeys initiate the formation of an aneurysm, and the aneurysm enlarges

because the arterial wall at a medial defect is more prone to depression [24]. According to an overwhelming number of authors, however, thinning of the media and disintegration of the IEL at the apex on the distal side of a major branch medial to the intimal pad are acquired changes and should be distinguished from innate, medial defects [23, 29, 30].

3. Haemodynamics in the aetiology of IAs

Given that IAs are frequently located at the bifurcations and sharp curves of the intracranial vessels, previous studies have also investigated the role of haemodynamics in their formation [12, 31]. According to the degenerative theory of IA formation, IA initiation occurs as a result of endothelial damage, elastic tissue loss and IEL fragmentation caused by haemodynamic forces [12, 13, 32, 33]. In particular, haemodynamic factor was identified as important for IAs located in arteries feeding arteriovenous malformation [34] and *de novo* IAs formed in the collateral circulation pathways in patients who have undergone ICA ligation [35] or suffered from atherosclerotic occlusion of one or more extracranial arteries [36]. It is also well known that asymmetry in the A1 segments of the anterior communicating artery (ACoA) complex, a relatively common anomaly of the circle of Willis, is associated with a significant risk for the formation of ACoA aneurysms [37-39], probably due to augmented haemodynamic stress on the dominant A1 segment of the anterior cerebral artery (ACA) [32].

These clinical observations regarding the influence of the hemodynamic factor on IA formation were further confirmed by experimental studies performed on IA animal models, including those in rats [40] and monkeys [41]. In these studies, IAs were induced through the ligation of one or both common carotid arteries. The animals were made hypertensive and treated with the lathyrogen β-aminopropionitrile, which inhibits the cross-linking of collagen and elastin fibres, thereby weakening the arterial walls. Within several months, the formation of IAs was observed in the large arterial bifurcations at the base of the brain (i.e., the anterior cerebral artery (ACA) - ACoA

complex and the internal carotid artery (ICA) - posterior communicating artery (PCoA) junction) subjected to the augmented haemodynamic stress associated with collateral flow [40, 41].

The haemodynamically generated forces produced by impingement of the central bloodstreams at the apices of bifurcations are key factors leading to the focal degeneration of the IEL [12, 13, 32, 33, 42]. According to Hassler [43] and Stehbens [44, 45], turbulence that arises due to the separation of blood flow at major arterial intracranial bifurcations creates vibrations within the vascular wall, leading to EC injury. A major contributor to this injury process is the fact that most of the elastic fibres in a subendothelial elastic lamina, which makes the vascular walls particularly vulnerable to the effects of vibrations.

Ferguson proposed a different theory on the role of haemodynamic factors in IA formation, suggesting that wall shear stress (WSS), impulse and pressure were vital to this process. He argued that impingement of the central bloodstream at the vessel bifurcation results in the formation of two types of haemodynamic forces. First, the kinetic energy of the blood is changed into pressure energy (stagnation pressure) at the apex, which, in addition to the transmural pressure, accounts for the total pressure that acts at the apex. Second, when the high velocity of the central bloodstream strikes the apex, the velocity gradient, and consequently the WSS, significantly increases [15].

The presence of IAs primarily at bifurcations and high-curvature vascular segments is associated with disturbed flow, characterised by large fluctuations in WSS [12, 13, 33, 42]. Findings from studies on glass models of arterial bifurcations [15, 46, 47], preclinical *in vivo* studies [48-50] and computational fluid dynamics (CFD) studies [51, 52] suggest that high WSS adjacent to the flow divider contributes to IA formation. Moreover, it has been shown that as the volume of blood flow and WSS increase, so too does the risk of destructive vascular remodelling, characterised by IEL fragmentation and EC and SMC proliferation [53]. Ferguson suggested that IA formation is related to the co-occurrence of haemodynamic forces and additional anatomical factors, including a thin media and adventitia; the location of the intracranial arteries within the subarachnoid space,

which does not provide them with adequate support; and environmental factors (e.g., hypertension and smoking) that can damage the ECs and hence weaken the vascular wall [15].

According to the results of relatively recent *in vitro* [54] and *in vivo* studies [48, 49], high WSS and a high positive WSS gradient (WSSG, a haemodynamic index characterised by the spatial derivative of the WSS along the flow direction with respect to the streamwise distance) are considered to play a key role in initiating IA formation. *In vitro* studies showed that high WSS and a positive WSSG exacerbate endothelial dysfunction, influencing EC alignment, proliferation, and apoptosis [54, 55]. Meng et al., through *in vivo* studies combined with CFD simulations, found that the combination of high WSS and a high WSSG could lead to local morphological changes in the arterial wall, promoting IA formation, and that the impingement, acceleration and recovery regions around the bifurcation apex exhibited different flow characteristics [48, 49]. The authors proposed that, the process of IA initiation occurs in the acceleration zone, where the WSS is high and the WSSG is high and positive [48, 49].

Several recently published image-based CFD studies have confirmed the above results, demonstrating a positive correlation between IA formation and WSS and/or the WSSG [56-60]; others, however, have identified a negative correlation between sidewall aneurysms and WSS and/or the WSSG [61-63]. For example, Chen et al., analysing sidewall aneurysms, showed a strong correlation between a local elevation in WSS and the location of IA formation, while that between the WSSG and the location of IA formation appeared to be low [64]. Mantha et al. proposed a new haemodynamic index, the aneurysm formation indicator (AFI), for the detection of stagnation zones —characterised by relatively low and rotating WSS—where sidewall IAs frequently occur [62].

However, Shimogonya et al. did not find a significant correlation between WSS, the WSSG, the AFI, or the oscillatory shear index (OSI; an index representing the cyclic departure of WSS from its predominant axial direction, as proposed by Ku et al. [65]) and the location of IA formation [61]. Both the OSI and AFI, which quantify the oscillatory nature of WSS vectors, have similar distribution patterns on the vascular wall and poorly correlate with the area of IA formation [61, 63,

64]. Consequently, Shimogonya et al. proposed the gradient oscillatory number (GON), which describes the fluctuation of the WSSG integrated over one pulse cycle and may predict IA initiation [61]; this new index demonstrates a significantly higher positive correlation with the location of IA formation when used for this purpose [61, 63, 64]. Chen et al. [64] and Ford et al. [63] then showed that the GON increases at different nonaneurysmal sites in the arteries, suggesting that it can be a sensitive but not specific marker for IA formation.

4. Geometry of vessel bifurcations and its importance in the aetiology of IAs

The bifurcation geometry, including the diameter of the bifurcation vessels and the bifurcation angle, plays an important role in the distribution of WSS [46, 47, 51, 66-68], suggesting its importance in considering the aetiology of IAs. For example, Roach et al., using a glass model of the arterial bifurcation, found that as the bifurcation angle increases, there is a significant increase in turbulence at the bifurcation apex, which subsequently increases the risk of endothelial damage [46]. Furthermore, CFD simulations performed on basilar artery (BA) parametric models revealed that widening of a narrow bifurcation angle may cause a significant spreading of the area, accelerating the WSS towards the daughter vessels [67]. Additionally, as the asymmetry of the A1 segments of ACAs in a glass model and a 3-dimensional CFD model of the ACoA complex or the flow in one of the two A1 segments in a symmetrical glass ACoA model increased, a notable increase in WSS (above 70 Pa in the glass models and above 30 Pa in the CFD model) on the ACoA occurred [47, 51].

Morphometric studies using patient-derived models of the circle of Willis with an IA have provided inconsistent results regarding vessel radius. For example, Can et al. reported that BA and middle cerebral artery (MCA) aneurysms were significantly associated with a smaller radius of the parent vessel [69, 70]. Therefore, they suggested that as the vessel radius decreases, the blood flow velocity in a vessel increases, leading to maximum haemodynamic stress at the bifurcation apex

[69-71]. However, Kaspera et al. found that an increased volume flow rate (VFR), which was associated with a larger MCA radius but not the blood velocity in the MCA parent vessel, was an independent risk factor for MCA aneurysm formation [72]

In addition to vascular dimensions, the symmetry of the bifurcation vessels was also found to be important in the pathogenesis of IAs, with higher asymmetry between the bifurcation branches leading to increased haemodynamic stress in the smaller branch and IA development [73-76]. However, the findings of studies published by several other groups [72, 77] have suggested that the degree of symmetry between bifurcating vessels for IA formation cannot be considered independently from the principle of minimum work (PMW) formulated by Murray [78], who used it to predict the vessel diameters and bifurcation angles in a vascular tree. The PMW establishes a functional relationship among flow, velocity, and vascular dimensions in a typical vascular tree without communication between the bifurcation branches. According to this principle, the vascular system utilises energy to maintain circulation at low energy expenditures while simultaneously avoiding increased WSS [78, 79]. The theoretical assumptions of the PMW define a strict mathematical relationship between the blood flow and the vessel radius, which is adjusted to the cube root of the volumetric flow. This means that to achieve the optimal connections within the arterial tree, the radius of the parent artery should be equal to the sum of the cubes of the volumetric flows of its branches. Thus, theoretically, the value of the junction exponent in this equation is a measure of the adjustment of the vascular system to the energetic optimum. There is a mathematical relationship among the junction exponent fulfilling the equation by Murray, the calibre ratio between the branch and the parent vessel (that is, the degree of asymmetry between the two), and the relative value of WSS acting on the vessels of the bifurcation [80]; specifically, the WSS increases when the junction exponent and the branch-to-parent-calibre ratio decrease. In cases where the dimensions of bifurcation branches follow Murray's formula with $n = 3$, the energy expenditure for circulation and WSS are the lowest, regardless of differences in the values of bifurcation radii. However, when the radii of the bifurcation vessels are not governed by Murray's

formula ($n \neq 3$), the WSS becomes significant at the bifurcation vessels. Building on Murray's theory, several groups have demonstrated that the dimensions of human blood vessels [81, 82], including the intracranial arteries [83], are in line with the theoretical assumptions of the PMW. Importantly, Rossitti found that deviations from the optimal values of vessel dimensions derived from the PMW result in higher WSS, which can lead to the formation of IAs [80]. His observations were confirmed by other authors who showed significant deviations in the values of the junction exponent in patients with MCA [72, 77] and ACoA aneurysms [68].

Relatedly, Ingebrigsten et al. found that other arteries in the circle of Willis (i.e., the ICA and BA) without IAs also have values for the junction exponent that deviate from the values derived from the PMW. In his opinion, as the infrastructure of the circle of Willis is a combination of three main arteries (i.e., both ICAs and the BA) and communicating arteries, its arterial bifurcations do not follow the PMW. Therefore, a yet unknown principle of optimality may be relevant to this junction [84].

In contrast to the vessel dimensions, the bifurcation angles, including those of the intracranial arteries, were found to be scattered considerably around the theoretical optimum derived from the PMW [72, 83-85]. However, imaging studies, including 3D rotational angiography, MRA, and CTA, have demonstrated that the differences between the predicted optimal and the observed values of the bifurcation angles were significantly higher in patients with IAs than in those without IAs [72, 84] and a wide bifurcation angle represented a risk factor for IA formation in the ACoA complex [37, 75, 86-89], the BA [67, 70, 75, 90], and the MCA [69, 72, 74, 75, 91]. Additionally, CFD-based studies showed that an increase in the asymmetry of the vascular dimensions of the bifurcations and a wide bifurcation angle may lead to enhanced haemodynamic stress and IA formation [67, 86]. To summarize the above, the dimensions related to vessel bifurcations seem to play a decisive role in predicting the formation of IAs.

5. Fluid shear stress modulation of gene expression in ECs

Blood flow plays a key role in initiating the process of IA formation by converting the mechanical stimuli generated by the bloodstream into biochemical signalling cascades [92, 93]. ECs are the primary sensors of shear and mechanical stretch stresses [13, 33, 92]. It has been shown that an intact endothelium and laminar flow guarantee vascular homeostasis through the associated secretion of several antithrombotic and anti-inflammatory factors [13, 92-94]. Alterations in blood flow and haemodynamic stress lead to changes in the transcription of endothelial genes, which ultimately affect the endothelial phenotype, causing physiological or pathological vascular remodelling [55, 95].

Several endothelial cell-matrix and cell–cell junction molecules and membrane structures have been suggested to act as shear stress receptors or transducers within these ECs. [96-98]. These structures are located in different compartments of the endothelium [96, 97], triggering the activation of specific signalling cascades, including protein phosphorylation, cytoskeleton rearrangement, and nitric oxide (NO) and reactive oxygen species (ROS) production [96, 98]. Following these signalling cascades, many transcription factors, such as AP-1, nuclear factor kappa B (NF-κB), Sp1 and early growth response protein 1 (EGR-1), are activated, leading to the modulation of several genes encoding growth factors, cytokines and chemokines, oxidising enzymes, and extracellular matrix (ECM) and angiogenic molecules [96-99]. Regulation of the transcription and expression of endothelial genes is mediated by so-called shear stress-responsive promoter elements (SSREs) that bind transcription factors [96, 97].

ECs located in straight segments of the vascular tree are exposed to laminar shear stress (LSS), while those located at vessel curvatures or bifurcations are subjected to both spatial and temporal shear stress disturbances [42, 96, 97]. The findings from studies that compared the expression profile of hundreds of endothelial genes under disturbed and LSS conditions suggest that many genes differ in their expression levels depending on the pattern of shear stress and its magnitude [97].

In response to the physiological levels of WSS, the gene expression profile of ECs becomes atheroprotective, resulting in resistance to oxidative stress, apoptosis and inflammation [97]. Arterial bifurcations or sinuses opposite the apices are considered to be the preferred sites for the formation of flow separation areas. Several studies performed using *in vitro* and *in vivo* experimental model systems suggest that the values of shear stress in the flow separation regions range from negative to zero dyn/cm^2 [42, 97]. For example, in straight sections of vessels, under physiological conditions, the shear stress values range from 10 to 40 dyn/cm^2 [97]. ECs in these regions exhibit an increase in lipid accumulation and monocyte adhesion, accompanied by a more pronounced increase in vascular cell adhesion molecule-1 (VCAM-1), platelet-derived growth factor subunit A (PDGFA), connexin-43 and EGR-1, resulting in an atherogenic phenotype [96, 97, 100-102]. Although the relationship between low WSS and the expression of genes associated with atherosclerosis is quite evident, different studies have yielded different findings regarding the relationship between EC gene expression in response to high WSS and IA formation.

Aoki et al., using a rodent model of IAs, found increased expression of a large group of genes influenced by high WSS associated with proteinases (for example, matrix metalloproteinase (MMP-2) and MMP3 (encoded by the *Mmp2* and *Mmp3* genes, respectively) and cathepsin Z (encoded by *Ctsz*), ROS (including superoxide producers such as nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (*Nox1*) and superoxide eliminators such as superoxide dismutase-2 (SOD-2) (*Sod2*)), growth factors, chemokines (monocyte chemoattractant protein-1 (MCP-1) and CC motif chemokine ligand 5 (CCL5)) and complement adhesion molecules (complement C3 and C6) in both the intima and media of the aneurysmal wall. In addition, several genes encoding members of the claudin and cadherin families, as well as VCAM-1, and proapoptotic genes encoding Bcl-2 (*Bcl2* gene) protein family members (Bok, Bik, and Bmf), caspases (caspase-1 and -7; the *Casp1* and *Casp7* genes, respectively) and the Fas ligand (the *Fas* gene) were also found to be upregulated in the intima under very high WSS [103]. The expression patterns of gene groups related to inflammation, ECM degradation and apoptosis promoted expansive arterial remodelling

and were quite distinct from the expression patterns observed under physiologically normal WSS and those of gene groups expressed under low WSS and associated with atherosclerotic changes [55, 103]. Furthermore, some genes demonstrate an inverse expression pattern between the intima and media, which may indicate that ECs and vascular SMCs have a different role in the formation and progression of cerebral aneurysms [103].

Furthermore, Dolan et al., using cultured bovine aortic ECs, reported that high WSS together with a high and positive WSSG induced gene expression that may trigger EC proliferation, apoptosis, and extracellular signalling, leading to aneurysmal remodelling of the vessel wall [55, 95]. They also demonstrated that a positive WSSG downregulates the *RPRM* and *BMP4* genes, which affects cell cycle arrest, cell growth and differentiation, and genes that inhibit apoptosis (e.g., *MCP1, CSF2, BMP4, and THBS1*) [95]. In addition, a positive WSSG markedly increases the expression of ADAMTS1 (an ECM protease that degrades aggrecan, versican and thrombospondin) in ECs and several genes involved in cell division (e.g., *CKAP2, SMC2, TOP2A, and CENPF*). However, a positive WSSG may also downregulate TAGLN (a repressor of MMP-9 expression), further increasing ECM degradation [95]. These processes all make the arterial wall more susceptible to the destructive impact of haemodynamic stress [95]. Interestingly, Dolan et al. suggested that a positive WSSG may amplify the effect of high WSS on gene expression, in contrast to a negative WSSG, which may antagonise the impact of high WSS on gene expression [95].

6. Molecular mechanisms induced by the haemodynamic factors involved in IA formation

The endothelium, which is an important centre of vascular homeostasis, plays a key role in initiating the formation of an aneurysm [12, 13, 33, 92, 104]. The main task of the vascular endothelium is to prevent atherosclerotic (inflammatory) and thrombotic changes in the vessel wall [12, 13, 92, 105]. While the specific molecular mechanisms induced by the haemodynamic factors involved in IA formation remain under investigation, it has been suggested that the high WSS-

stimulated EC injury and inflammatory cell recruitment mediated by NF-κB are initial events in the pathogenesis of IAs [13, 98, 105, 106].

The inflammatory cells infiltrating the vessel wall mainly include macrophages, although neutrophils, T lymphocytes and mast cells have also been detected [12, 13, 33, 98, 104-111]. Numerous experimental animal model studies have demonstrated the presence of inflammatory cells (primarily macrophages) infiltrating the wall of the forming aneurysm, confirming their key role in this process, especially during the initial stages [112-117]. In contrast, Tulamo et al., using models of hypertension-induced IAs, reported that the infiltration of inflammatory cells occurs in rather well-formed IAs in the later stage of IA formation [13]. Other groups have also observed that macrophage infiltration caused by chemotaxis from IA walls follows endothelial dysfunction and IEL degradation [112, 113, 116].

Magnetic resonance imaging of vessel walls with IAs allows the detection of aneurysm wall enhancement associated with inflammation and neovascularization [118-120]. Histological analyses of surgically resected human IA specimens demonstrating enhancement have revealed the association this vessel wall enhancement with the presence of abundant macrophage infiltration [120]. Aneurysm enhancement was more frequently observed in unstable aneurysms than in stable aneurysms; this enhancement might therefore reflect the potential vulnerability of the aneurysmal wall and could be used to estimate the risk of aneurysm rupture [118, 119].

The method by which macrophages migrate to the vascular wall remains unclear. MCP-1 plays a key role in macrophage recruitment in the initial stage of aneurysm formation [13, 33, 98, 104-106, 108, 109, 111]. ECs are the main source of MCP-1 in these early stages, although MCP-1 expression has also been found in medial SMCs [13, 33, 104, 106, 109, 111]. VCAM-1, which mediates the strong adhesion of monocytes to ECs, also plays a central role in monocyte and macrophage recruitment [13, 105, 106, 108, 109, 111]. Evidence for the key role of VCAM-1 in the pathogenesis of aneurysms was provided by immunohistochemical analysis of aneurysmal wall fragments obtained during surgical clipping of an aneurysm, which showed increased VCAM-1

expression [110]. Additionally, studies of gene expression using the DNA microarray method from experimentally induced aneurysm samples showed increased expression of the *VCAM-1* gene [121]. On the other hand, an *in vitro* study on the effects of WSS on ECs showed that shear stress decreases tumour necrosis factor-alpha (TNF-α)-induced VCAM-1 expression [122]. Despite these promising findings, the precise involvement of VCAM-1 in the pathogenesis of IA development continues to be debated.

The release of proinflammatory cytokines and proteinases by macrophages leads to inflammation and degenerative changes in aneurysmal walls [12, 13, 33, 98, 104-106, 108, 109, 111]. One theory states that the arterial wall is affected directly by a homologous group of zinc- and calcium-dependent neutral proteinases known as MMPs, which are responsible for the degradation of vascular ECM, and indirectly by cytokines such as MCP-1, TNF-α, stromal cell-derived factor 1a (SDF-1a), interleukin-1β (IL-1β), and IL-6 and adhesive molecules [12, 13, 33, 98, 104-106, 108, 109, 111]. Among MMPs, particularly important are MMP-1, MMP-2 and MMP-9, which are known to induce macrophage migration and infiltration through the ECM [13, 105, 109].

TNF-α is a multifunctional proinflammatory cytokine secreted primarily by macrophages in response to damage to the arterial wall, which initiates proinflammatory and proapoptotic signalling pathways [12, 13, 105, 106, 108, 109]. TNF-α-mediated ROS production leads to endothelial dysfunction and stimulates NF-κB activation and subsequent expression of MCP-1 and cycloexygenase-2 (COX-2) [123]. Furthermore, TNF-α-mediated ROS production stimulates the infiltration of macrophages into the vascular wall [124] and promotes phenotypic changes in SMCs, reducing the release of contractile proteins but increasing the secretion of proinflammatory mediators such as MCP-1, IL-1β and MMPs [125]. In addition, TNF-α enhances the activity of adhesion molecules such as intercellular adhesion molecule 1 (ICAM-1), VCAM-1 and E-selectin in ECs, fibroblasts and SMCs [13, 105, 108, 109, 126]. The expression of TNF-α is correlated with increased levels of toll-like receptor (TLR). The activation of TLR4, a member of the TLR family, stimulates the intracellular NF-κB signalling cascade, leading to the production of proinflammatory

cytokines responsible for the stimulation of the immune system [127]. This TNF-α/TLR4/NF-κB pathway has been demonstrated to be involved in IA development [124, 127].

IL-1β is another proinflammatory cytokine that acts on a wide range of normal and pathological inflammatory conditions, such as the proliferation, differentiation and apoptosis of ECs and SMCs [13, 105, 106, 108, 109, 111]. Recent preclinical studies using IA models have shown that the upregulation of IL-1β and NF-κB pathway activation may significantly inhibit collagen biosynthesis at the transcription level or during posttranscriptional enzymatic modification within IA walls [128, 129]. Furthermore, Moriwaki et al. revealed that in IL-1β-deficient mice, induced IAs were smaller in size than in wild-type mice, suggesting that IL-1β expression in SMCs promotes SMC apoptosis, which leads to a thinning of the media [130]. Consequently, collagen biosynthesis disorders and SMC apoptosis render the vascular wall more vulnerable to the effects of blood flow, thus promoting the formation of a cerebral aneurysm.

In a recent study, the tissue plasminogen activator (tPA) - plasmin axis was shown to play a major role in the promotion of the inflammatory process and the MMP-dependent degradation of the ECM during IA formation. Labeyrie et al. performed immunohistochemistry analysis and confirmed tPA and plasminogen/plasmin overexpression in the aneurysmal wall of murine and human samples. tPA is involved in the process of IA formation through direct or indirect plasmin-dependent activation of MMPs. In addition, tPA upregulates the expression of MCP-1 and then stimulates diapedesis, participating in the process of macrophage infiltration within the arterial wall [131].

It is well recognised that the transcription factor NF-κB upregulates the expression of a number of proinflammatory genes encoding inflammatory mediators, such as the chemokines MCP-1, IL-1β, inducible nitric oxide synthase (iNOS), and MMPs involved in aneurysm formation and progression [105, 106, 109, 111, 114, 132-137]. Preclinical experiments on IA models showed that excessive haemodynamic stress induces NF-κB activation in the intima, mainly in ECs, in the early stage of IA formation [114].

One of the key mechanisms of NF-κB activation is a positive feedback loop between COX-2 and NF-κB via the shear stress-induced prostaglandin E2 (PGE2)-PGE2 receptor 2 (EP2) signalling pathway. Aoki et al. demonstrated that haemodynamic stress increases the expression of COX-2, microsomal PGE synthase (mPGES1) and EP2 in ECs at the initial stages of aneurysm formation. They also showed that increased COX-2 expression in the aneurysmal wall is correlated with the activation of NF-κB, which is consistent with the fact that COX-2 is transcriptionally regulated by NF-κB. Importantly, the stimulation of the PGE2-EP2 signalling pathway may also lead to an increase in MCP-1 expression in the endothelium via an NF-κB-dependent pathway, significantly enhancing macrophage infiltration. Aoki et al. also observed the presence of COX-2, mPGES1 and EP2 over time in other regions of the aneurysmal wall, such as in SMCs, which indicated an increase in PGE2 production during the progression of aneurysm formation. Taken together, these data suggest that the PGE2-EP2 signalling pathway induces NF-κB activation and an increase in the production of other proinflammatory mediators, such as IL-1β and iNOS, in the media and SMCs in the late stage of cerebral aneurysm formation, ultimately resulting in a thinning of the aneurysmal wall and its subsequent enlargement and rupture [132].

NO plays a central role in vascular biology and pathobiology [138, 139]. NO is the final product of the conversion of L-arginine to L-citrulline by one of three members of the NO synthase (NOS) family: endothelial NOS (eNOS), neuronal NOS (nNOS), and iNOS. Among them, the one with the most important role in IA is eNOS, which is expressed in the ECs of arterial walls and contributes to arterial dilatation via NO production. Under physiological conditions, low concentrations of NO produced by eNOS limit low-density lipoprotein (LDL) oxidation, leukocyte and platelet activation and adhesion, SMC proliferation and migration into the intima, which together maintain the anticoagulant and anti-inflammatory properties of the vascular wall [138, 140]. Both eNOS and nNOS play a protective role in preventing inflammation of intracranial arteries through NO production and shear stress regulation [138].

Subendothelial reduced expression of eNOS is a characteristic feature of endothelial dysfunction and plays a crucial role in the formation and progression of the inflammatory zone in the aneurysmal wall [115, 116, 141, 142]. In particular, using *in vivo* IA models, Jamous et al. demonstrated that one of the first steps preceding the formation of an aneurysmal vascular wall bulge was partial loss of eNOS expression at the apical intimal pad. The authors observed swelling of the luminal side of the apical intimal pad in line with a loss of eNOS endothelial expression, suboptimal migration of vascular SMCs and macrophage infiltration of the vascular wall [116]. In contrast, Aoki et al. demonstrated that the frequency of IA formation in male eNOS-deficient mice was similar to that in wild-type mice and that nNOS was upregulated in a compensatory manner in the IA wall of eNOS-deficient mice; however, interestingly, eNOS and nNOS double-knockout mice exhibited an increased frequency of IA formation accompanied by notable macrophage infiltration [115].

Both eNOS and nNOS produce small amounts of NO [143, 144]. In contrast, iNOS, mainly expressed in macrophages and SMCs [145, 146], the expression of which is activated by a variety of proinflammatory cytokines or shear stresses resulting from blood flow [138, 143, 144], produces large amounts of NO in response to inflammatory stimuli [143, 144] and leads to ECM degradation and SMC apoptosis, causing a thinning of the aneurysmal wall [147]. Moreover, although several mechanisms of NO-induced cytotoxicity have been suggested, including mitochondrial function disruption, p53 activation, Fas upregulation, c-Jun N-terminal kinase activation and ceramide synthesis induction [148], the specific mechanism of NO-induced SMC apoptosis has not been defined.

iNOS is also involved in the production of ROS in IA walls, which may result in the degradation of the tight junction proteins occludin and ZO-1 and the subsequent migration of macrophages into the aneurysmal wall [149]. The disruption of tight junctions in the vascular wall may also be regulated by the high expression of MMP-9 and MCP-1 [149].

A summary of the role of haemodynamic factors in the formation of IAs–including the molecular and genetic changes that have been proposed to be induced by these factors–is shown in Figure 1. Specifically, data from preclinical and human IA studies show that IAs may be formed and grow in response to haemodynamic stress and inflammation in the vascular wall. The inflammatory response in the vascular wall leads to IEL disruption and proteolytic destruction of the ECM by MMPs, followed by IA formation. The inflammatory response also stimulates the process of phenotypic modulation of SMCs from a contractile to proinflammatory phenotype and their subsequent degeneration, which is crucial for IA formation and progression. The phenotypic modulation of SMCs is associated with the expression of proinflammatory and pro-matrix remodelling genes. Subsequent changes in the media involve the loss of SMCs due to apoptosis and the disturbance of collagen biosynthesis, leading to a thinning of the vascular wall. Presumably as a compensatory mechanism for IA formation, SMCs proliferate under the influence of several growth factors. However, enhanced cytokine levels, oxidative stress, and the release of proteolytic enzymes that degrade the ECM promote the fragmentation of IA wall structures and induce cell death.

7. Genetic factors in the development of IAs

ADPKD is the only genetic disorder correlated with a high incidence of IAs, with an estimated prevalence of 11% [8]. ADPKD is caused by mutations in *PKD1* and *PKD2*, which encode polycystin-1 and polycystin-2 (PC1 and PC2, respectively), components of a multicomponent membrane-spanning complex that is involved in cell–cell or cell-matrix interactions [150, 151]. Reduced PKD1 expression levels and abnormal intracellular Ca(2+) regulation related to PKD2 haploinsufficiency are directly associated with vascular endothelial defects, which could result in IA formation [150].

A group of hereditary connective tissue disorders, such as Marfan's syndrome, pseudoxanthoma elasticum (PXE), and Ehlers–Danlos syndrome (EDS) type IV, are also associated

with a high risk of IAs [152-156]. These diseases are related to a genetically determined defect in the structure of connective tissue proteins (i.e., collagen, elastin or fibrillin), resulting in increased susceptibility of the vascular wall to the destructive impact of blood flow, which increasing the risk of IA formation. For example, pathological studies of the affected vessels in a group of patients with Marfan's syndrome have revealed widespread changes in the arterial walls, consisting of intimal proliferation, medial degeneration, and IEL fragmentation, which could lead to aneurysmal formation [156]. However, an analysis of a large cohort of patients with inherited connective tissue disorders revealed no relationship between IAs and these connective tissue diseases [157-159].

Furthermore, a growing body of evidence suggests that genetic factors have a significant impact on the aetiology of nonsyndromic IAs, which has been confirmed particularly by cases with a family history of IAs. The prevalence of incidental IAs among first-degree relatives over 30 years of age is reported to be between 9.2% and 9.8%, which is approximately 2–3 times higher than the prevalence of IAs in the general population [160, 161]. Studies on genetic factors that predispose individual to IAs are hindered by the late onset and low penetrance of the condition and the high mortality rates of the patients [162]. Additionally, familial aggregation of IAs may be correlated with drinking and smoking behaviour and other environmental factors [163]. Therefore, changes in environmental factors that occur over time may influence patient and IA characteristics [164]. Table 1 summarizes the selected risk loci and candidate genes significantly associated with IA reported in genetic analyses.

Genome-wide linkage (GWL) studies of families and sibling pairs affected by IAs identified numerous loci on chromosomes suggesting evidence of linkage [163, 165]; of these, five (1p34.3–36.13, 4q32.3, 7q11, 19q13, and Xp22) seem to be particularly promising [166-169]. The perlecan (*HSPG2*) gene is a plausible candidate gene in the 1p locus; it encodes a heparan sulphate proteoglycan that is involved in maintaining the ECM of the arterial wall. For loci on 7q11, an association with the elastin gene and the collagen type 1 A2 (*COL1A2*) gene has been reported [170]. Both elastin and collagen are significant components responsible for the reversible

extensibility and the strength of the arterial wall, respectively [171]. A possible linkage on chromosome 5q22-31 has also been suggested [168]; the versican (*CSPG2*) gene, located in the vicinity of the IA locus on 5q22-31, was demonstrated to play a crucial role in ECM formation [170]. An association between *CSPG2*, *HSPG2*, fibrillin 2 (*FBN2*) and collagen type 4 A1 (*COL4A1*) gene variants and IAs has been confirmed in candidate gene association studies in which two independent Dutch populations were analysed [172, 173]. Further studies conducted in the Japanese population showed an association between single-nucleotide polymorphisms (SNPs) in the *CSPG2* and *HSPG2* genes, but the association of *FBN2* and *COL4A1* could not be replicated [174]. These data suggest that the genes involved in the maintenance of the integrity of the ECM of the arterial wall are important risk markers for IA formation.

Genome-wide association studies (GWASs) represent an alternative to candidate gene studies in IA research; these studies are performed to examine the genome in terms of statistically significant relationships between SNPs and IAs based on a large cohort of patients with and without the condition [175]. One of the first GWASs related to IAs using large cohorts from Dutch, Finnish and Japanese populations identified common SNPs on chromosomes 2q (*PLCL1*), 8q (*SOX17*), and 9p (*CDKN2A* and *CDKN2B*) that showed a significant association with IAs [176, 177]. Notably, sequence variations in *CDKN2A* and *CDKN2B*, which encode cyclin-dependent kinase inhibitors, were recently reported to associated with coronary artery disease [178, 179], myocardial infarction [180] and the progression of atherosclerosis [181]. These proteins act as tumour suppressors by regulating the cell cycle, thereby affecting cell proliferation, senescence, and apoptosis. There is a well-established relationship between somatic and germline mutations of the *CDKN2A* and *CDKN2B* genes and most human cancers, including melanoma, glioblastoma, pancreatic adenocarcinoma, lung carcinoma and prostate cancer [182]. Additionally, *SOX17*, which encodes SRY-box transcription factor 17 involved in the regulation of cell cycle progression, may be of interest as it affects the proliferation and senescence of progenitor cell populations [177].

In a follow-up GWAS of a larger European and Japanese patient cohort, three new loci were identified, namely, 18q11.2 (*RBBP8*), 13q13.1 (*STARD13*), and 10q24.32 (*CNNM2*), and previously discovered associations of 8q11.23-12.1 containing *SOX17* and 9p21.3, including *CDKN2A-CDKN2B,* were replicated [177]. One of the strongest new associations was discovered at rs12413409 on 10q24.32 in the *CNNM2* gene, which encodes cyclin M2, a protein important for magnesium homeostasis whose exact function is unknown. Sequence variants in the *CNNM2* gene identified by GWASs have been associated with common complex diseases such as hypertension and coronary artery disease, which suggests a vital role of the *CNNM2* gene in the cardiovascular system [183]. Both the remaining *RBBP8* and *STARD13* genes, located on chromosomes 18 and 13, respectively, encode proteins involved in cell proliferation [177].

A comprehensive systematic review and meta-analysis of 66 genetic association studies (including GWASs) demonstrated strong associations for SNPs on chromosome 9 in *CDKN2B*, on chromosome 8 near the *SOX17* gene, and on chromosome 4 near the endothelin receptor A (*EDNRA*) gene [184]. Interestingly, the loci recognised by the GWASs had a relatively small effect size on the risk of IA development, contrary to the postulated high-penetrance Mendelian loci in familial IAs. For example, SNPs at the strongest associated loci on 8q11, 4q31.23 and 9p21.3 had odds ratios of 1.22–1.36 [176, 177, 185].

Unlike GWASs, but similar to linkage analyses, whole-exome sequencing (WES) can allow the identification of variations in the protein-coding region of any genes that are related to a large effect size and cause a high risk of developing IAs. The first WES study on IAs was published in 2015; in this study, exome sequencing in 12 Japanese families with a history of multiple IAs, including as many as 42 individuals, revealed 78 candidate variants. Among these candidates, ten variants from nine genes (*GPR63, ADAMTS15, MLL2, IL10RA, PAFAH2, THBD, IL11RA*, *FILIP1L*, and *ZNF222*) were further analysed in replicate association studies in a separate cohort with familial and sporadic IAs due to their significance for angiogenesis, including ECM integrity, inflammatory mediators, blood coagulation, and the maintenance of the vascular endothelium. However, only the

variant in *ADAMTS15* (rs185269810, p.E133Q) demonstrated a significant association with familial IA (odds ratio=5.96), which indicated the possible antiangiogenic activity of this gene [186]. Furthermore, an independent WES study based on seven families of European-American ancestry identified 68 potential risk variants in 68 genes. Linkage analysis supported the potential of 23 variants, of which only 8 were associated with all IA phenotypes (*KLF11, ABCC3, TANC2, ALMS1, ARHGEF17, SMEK2, HTRA2, and NDST1*). RNA-sequencing analysis demonstrated that only the *TMEM132B* gene, encoding a protein of unknown function, was significantly overexpressed in IA tissue compared to control tissue [187]. Three other recent WES studies on multiple IA-affected families identified another three IA candidate genes (*RNF213, THSD1, and ARHGEF17*) [188-190]. It has been shown that *RNF213* deficiency leads to increased MMP-9 expression [191] and vascular fragility [192, 193], which make vessels more susceptible to haemodynamic stress. Furthermore, a mutation in *THSD1* (thrombospondin type-1 domain-containing protein) leads to disruption of EC focal adhesion to the basement membrane that favours IA formation [189]. Recent studies on *ARHGEF17* showed that ARHGEF17 localises to the actin cytoskeleton through sequences in the N-terminus and activates Rho GTPases at intercellular junctions [194], which in turn are critical for the structural and functional organisation of endothelial junctions [195].

8. Conclusions

As shown in Figure 2, the formation of IAs is complex and multifactorial, involving haemodynamic, environmental and genetic factors. Specifically, the aetiology of IAs is related to i) poorly developed intracranial arterial walls, which contain less elastin than the walls of muscular, extracranial arteries of comparable size and ii) the location of the intracranial arteries in the subarachnoid space, which does not provide them with adequate support and is influenced by environmental factors (e.g., hypertension and smoking) that can damage ECs and weaken the vascular wall. WSS is a direct factor that damages the vascular tissue, initiating the formation of

IAs, and is higher in cerebral arteries than in other vessels of the vascular bed because of their smaller diameter and relatively high blood flow. High WSS-stimulated endothelial injury leads to inflammation, which results in functional and morphological changes in the vascular wall leading to the formation of IAs. However, the question of why IAs are mostly formed at selected intracranial bifurcations (i.e., the ICA, MCA, and ACoA complex) still remains unanswered. One factor that determines the formation of IAs within the cerebral arteries is the structure of the circle of Willis, which is unique in terms of its geometry. The bifurcation geometry of the main arterial trunks of the circle of Willis (i.e., the ICA, BA, and ACA) is anatomically significantly different from the normal branching nature governed by the optimality principle. As a result, deviations of vascular dimensions and bifurcation angles of the intracranial arteries different from the values based on the PMW lead to an increase in haemodynamic stress at arterial bifurcations (especially WSS values) and initiate the formation of IAs.

Although many genetic associations have been identified, a clear link between a specific gene and the risk of developing an IA has not yet been established. The numerous selected loci on chromosomes identified in GWL studies of familial IAs related to IA formation can provide an answer only in terms of a small proportion of familial IAs. The discrepancies indicate a potential very strong impact of genetic and environmental factors. Therefore, other next-generation sequencing studies are needed to identify the causative genes in cases of nonfamilial IAs.

List of abbreviations

ACA - anterior cerebral artery

ACoA - anterior communicating artery

ADPKD – autosomal-dominant polycystic kidney disease

AFI - aneurysm formation indicator

BA - basilar artery

CCL5 - CC motif chemokine ligand 5

CFD - computational fluid dynamics

COX-2 - cycloexygenase-2

CTA - computed tomography angiography

EC - endothelial cell

ECM - extracellular matrix

EDNRA - endothelin receptor A

EDS - Ehlers–Danlos syndrome

EEL - external elastic lamina

EGR-1 - early growth response protein 1

eNOS - endothelial nitric oxide synthase

EP2 - prostaglandin receptor 2

GON - gradient oscillatory number

GWAS - genome-wide association study

GWL - genome-wide linkage

IA - intracranial aneurysm

ICA - internal carotid artery

ICAM-1 - intercellular adhesion molecule 1

IEL - internal elastic lamina

IL-1β - interleukin-1β

iNOS - inducible nitric oxide synthase

LSS - laminar shear stress

MCA - middle cerebral artery

MCP-1 - monocyte chemoattractant protein-1

MIA - multiple intracranial aneurysms

MMP - matrix metalloproteinase

MRA - magnetic resonance angiography

NADPH) oxidase - nicotinamide adenine dinucleotide phosphate oxidase

NF-κB - nuclear factor kappa B

nNOS - neuronal nitric oxide synthase

NO - nitric oxide

NOS - nitric oxide synthase

OSI - oscillatory shear index

PC1 - polycystin-1

PC2 - polycystin-2

PCoA - posterior communicating artery

PDGFA - platelet-derived growth factor subunit A

PGE2 - prostaglandin E2

PGES - prostaglandin synthase

PMW - principle of minimum work

PXE - pseudoxanthoma elasticum

ROS – reactive oxygen species

SDF-1a - stromal cell-derived factor 1a

SMC - smooth muscle cell

SNP - single-nucleotide polymorphism

SOD-2 - superoxide dismutase-2

SSRE - shear stress-responsive promoter element

THSD1 - thrombospondin type-1 domain-containing protein

TNF-α - tumour necrosis factor-alpha

TLR - Toll-like receptor

tPA - tissue plasminogen activator

VCAM-1 - vascular cell adhesion molecule-1

VFR - volume flow rate

WES - whole-exome sequencing

WSS - wall shear stress

WSSG - wall shear stress gradient

Conflict of Interest

None declared.

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Figure 1. Schematic of the inflammatory cascades induced by the haemodynamic factors involved in IA formation.

ECs: endothelial cells, IEL: internal elastic lamina, SMCs: smooth muscle cells, PGE2: prostaglandin E2, EP2: prostaglandin receptor 2, MCP-1: monocyte chemoattractant protein-1, VCAM-1: vascular cell adhesion molecule-1, COX-2: cyclooxygenase-2, NF-κB: nuclear factor kappa B, IκB: IκB kinase, ECM: extracellular matrix, ROS: reactive oxygen species, iNOS: inducible nitric oxide synthase, IL-1β: interleukin-1 beta, TNF-α: tumour necrosis factor-alpha, MMP: matrix metalloproteinase. Created with BioRender.com.

Figure 2. Summary of the main factors contributing to IA formation.

ECs: endothelial cells, IEL: internal elastic lamina, SMCs: smooth muscle cells, ECM: extracellular matrix, MMP: matrix metalloproteinase, ROS: reactive oxygen species, iNOS: inducible nitric oxide synthase, TNF-α: tumour necrosis factor-alpha, IL-1β: interleukin-1 beta, *MCP1*: monocyte chemoattractant protein-1, *CSF2*: colony-stimulating factor 2, *THBS1*: thrombospondin 1, *RPRM*: reprimo, TP53 dependent G2 arrest mediator homologue, *BMP4*: bone morphogenetic protein 4, *TAGLN*: transgelin, *ADAMTS1*: a disintegrin and metalloproteinase with thrombospondin motifs 1, *CTSZ*: cathepsin Z, *NOX1*: NADPH oxidase 1, *SOD2*: superoxide dismutase 2, *BCL2*: proapoptotic genes for Bcl-2, *CASP3*: caspase 3, *CASP7*: caspase 7, *FAS*: Fas ligand, *PLCL1*: phospholipase C like 1, *SOX17*: SRY-box transcription factor 17, *CDKN2A:* cyclin-dependent kinase inhibitor 2A, *CDKN2B*: *c*yclin-dependent kinase inhibitor 2B, DM: diabetes mellitus, BMI: body mass index. Created with BioRender.com.

Table 1. Risk loci and candidate genes significantly associated with IA as identified in genetic analyses (GWL, GWAS, and WES), the products of these genes and the potential biological mechanisms of association with IA.

Risk locus	Type of genetic analysis	Candidate gene	Protein/glycoprotein	Potential biological mechanism of association with IA	Reference/Year
1p34.3–36.13	GWL	*HSPG2*	perlecan	part of heparan sulfate proteoglican, maintaining the	Nahed et al. [167]/2005
4q32.3	GWL	none proposed	none proposed	none proposed	Foroud et al. [166]/2009
7q11	GWL	*COL1A2*	elastin and collagen type 1 A2	components of the vessel wall	Onda et al. [168]/2001
19q13	GWL	*APOE*	apolipoprotein E (arginine-rich glycoprotein)	surface component of chylomicrons, VLDL, HDL	Yamada et al. [169]/2004
Xp22	GWL	*ACE2*	angiotensin I-converting enzyme 2	lowers blood pressure, catalysing the hydrolysis of angiotensin II into angiotensin (1-7)	Yamada et al. [169]/2004
5q22-31	GWL	*CSPG2*	versican	involved in intercellular signalling in the ECM	Onda et al. [168]/2001
2q	GWAS	*PLCL1*	phospholipase C-like protein 1	regulation of synaptic transmission (GABAergic), related to VEGF signalling	Bilguvar et al. [176]/2008
8q11.23-12.1	GWAS	*SOX17*	SRY-box transcription factor 17	transcription factor, regulation of cell cycle progression,	Yasuno et al. [177]/2010
9p21.3	GWAS	*CDKN2A-CDKN2B*	cyclin-depending kinase inhibitors 2A and 2B also known as the INK4 family members, p16 (INK4A) and	tumour suppressor, regulating the cell cycle	Yasuno et al. [177]/2010
18q11.2	GWAS	*RBBP8*	retinoblastoma binding protein 8	regulates cell proliferation by interacting with BRCA1	Yasuno et al. [177]/2010
13q13.1	GWAS	*STARD13*	StAR-related lipid transfer domain containing 13	involved in the regulation of cytoskeletal reorganization and	Yasuno et al. [177]/2010
10q24.32	GWAS	*CNNM2*	cyclin M2	plays a role in magnesium homeostasis by mediating the epithelial transport and renal reabsorption of Mg2+	Yasuno et al. [177]/2010
4q31.23	GWAS	*EDNRA*	endothelin receptor A (a G protein-coupled receptor for endothelins)	involved in maintaining vasomotor control and vascular homeostasis	Yasuno et al. [185]/2011
11q24.3	WES	*ADAMTS15*	a disintegrin and metalloproteinase with thrombospondin motifs 15	probably antiangiogenic activity, important for ECM	Yan et al. [186]/2015
12q24.31	WES	*TMEM132B*	transmembrane protein 132B	none proposed	Farlow et al. [187]/2015
13q14.3	WES	*THSD1*	thrombospondin type-1 domain-containing protein 1 (a	tether endothelial cells to the underlying basement membrane	Santiago-Sim et al. [189]/2016
17q25.3	WES	*RNF213*	ring finger protein 213 (AAA-type ATPase with E3 ubiquitin ligase	involved in vascular remodelling processes	Zhou et al. [188]/2016
11q13.4	WES	*ARHGEF17*	Rho guanine nucleotide exchange factor	involved in cellular processes (cell shape, polarity, migration,	Yang et al. [190]/2018

GWL - genome wide-linkage studies, GWAS - genome-wide association studies, WES - whole exome sequencing, IAs - intracranial aneurysms, ECM - extracellular matrix, VLDL – very-low-density lipoprotein, HDL – high-density lipoprotein, GABA - gamma-aminobutyric acid, VEGF - vascular endothelial growth factor, BRCA1 - breast cancer type 1 susceptibility protein