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REVIEW PAPER

Intracranial Aneurysms: Pathology, Genetics, and Molecular Mechanisms

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Abstract

Intracranial aneurysms (IA) are local dilatations in cerebral arteries that predominantly affect the circle of Willis. Occurring in approximately 2–5% of adults, these weakened areas are susceptible to rupture, leading to subarachnoid hemorrhage (SAH), a type of hemorrhagic stroke. Due to its early age of onset and poor prognosis, SAH accounts for > 25% of years lost for all stroke victims under the age of 65. In this review, we describe the cerebrovascular pathology associated with intracranial aneurysms. To understand IA genetics, we summarize syndromes with elevated incidence, genome-wide association studies (GWAS), whole exome studies on IA-affected families, and recent research that established definitive roles for Thsd1 (Thrombospondin Type 1 Domain Containing Protein 1) and Sox17 (SRY-box 17) in IA using genetically engineered mouse models. Lastly, we discuss the underlying molecular mechanisms of IA, including defects in vascular endothelial and smooth muscle cells caused by dysfunction in mechanotransduction, Thsd1/FAK (Focal Adhesion Kinase) signaling, and the Transforming Growth Factor β (TGF-β) pathway. As illustrated by THSD1 research, cell adhesion may play a significant role in IA.

Keywords Intracranial aneurysm · Subarachnoid hemorrhage · Etiology · Genetics · Animal models · THSD1

Introduction

An intracranial aneurysm (IA) is a dilatation in the wall of a cerebral artery that occurs predominantly in the circle of Willis. A ruptured IA is the most common cause of subarachnoid hemorrhage (SAH), a devastating condition that can lead to death or permanent disability (Brain Aneurysm Foundation 2017). Due to early age of onset and high mortality, SAH accounts for > 25% of years lost for all stroke victims under the age of 65 years (Johnston et al. 1998). Despite treatment advances, the SAH mortality rate is ~ 40%, and only half of survivors return to independent life. The IA prevalence in the adult population is approximately 2–5% with an annual rupture rate of 8–10 per 100,000 (Hop et al. 1997; Tromp et al. 2014; Zacharia et al. 2010; Linn et al. 1996; Korja et al. 2013; Andreasen et al. 2013; Wiebers et al. 2003, 2004). Prior to rupture, IAs are usually asymptomatic, and population-wide IA screening is unrealistic due to brain imaging costs. Currently, unruptured IAs are primarily discovered as an incidental finding or in affected families. When treated before rupture, survival rates improve dramatically (International Study of Unruptured Intracranial Aneurysms 1998).

IAs usually develop in adulthood. Structurally, there are two types of intracranial aneurysms: saccular and fusiform. Saccular (aka berry) aneurysms are sac-like pocket that arises from a cerebral wall, while the less-common fusiform aneurysms are dilations that affect a short length of vessel where the entire vessel diameter is increased. Female sex, smoking, hypertension, and alcohol consumption are risk factors, but a positive family history confers the greatest risk (Andreasen et al. 2013; Korja et al. 2013). It is known that 7–20% of patients have a family history, and first-degree relatives are at increased risk, regardless of ethnic background.(Norrgard et al. 1987; Ronkainen et al. 1993; Schievink et al. 1994, 1995; Bromberg et al. 1995; Teasdale

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et al. 2005; Ronkainen et al. 1997; Kim et al. 2003) The Nordic Twin Study reports a SAH heritability estimate of 41% [95% CI 23.7% to 55.5%], while the odds ratio for SAH is 51.0 [95% CI 8.56–1117] when ≥ 2 affected first-degree relatives have SAH (Korja et al. 2010; Bor et al. 2008). In a recent study of 21 twin pairs (Mackey et al. 2015), 11 of 12 monozygotic twins developed IA, irrespective of smoking or hypertension, while only 5 of 9 dizygotic twins were both affected. Increased IA incidence is a prominent phenotype in Autosomal Dominant Polycystic Kidney Disease (ADPKD); however, the vast majority of IA occurs in nonsyndromic families or sporadic cases. In this review, we present our current understanding of intracranial aneurysms, highlighting pathological features, several syndromes with elevated IA incidence, and genetic studies that elucidate molecular mechanisms that contribute to disease. We will also describe the discovery and functional significance of THSD1 (Thrombospondin type I Domain containing 1), a gene whose deleterious rare variants cause a subset of human IA/SAH cases.

Methods

The medical literature on intracranial aneurysms was reviewed up to March 25, 2019 using PubMed searches using combinations of the following terms: intracranial, cerebral, brain, aneurysm, syndrome, genetics, exome sequencing, pathology, physiology, biology, histology, vascular, endothelial, smooth muscle, inflammatory, cell signaling, molecular mechanism, animal model, mouse, and zebrafish. For whole exome sequencing results of IA families, both the data in the paper and supplementary information are summarized in Table 3. In several subsections, we refer to review articles that provide additional information on a particular topic. Nonetheless, we added newly discovered facts and discussed their significances as well as referred the previous reviews, so as to maintain the overall integrity and continuity.

Results

Cerebrovascular Pathology of Intracranial Aneurysms

Pathological analyses on human IA samples provide critical insights into the molecular mechanism of IA formation and rupture. A common feature of IA is disintegration of the internal elastic lamina (IEL), a subendothelial connective tissue that separate intima from media. Other characteristics may include irregular luminal surface, myointimal hyperplasia, disorganization of the muscular media, hypocellularization, and infiltration of inflammatory cells (reviewed in Santiago-Sim (2011)).

Normal Intracranial Artery in Human

An intracranial artery consists of three layers: the intima, media, and adventitia. The intima is the innermost layer that faces the luminal side and has direct contact with blood flow. It is composed of a monolayer of endothelial cells and a subendothelial extracellular matrix. Glycoproteins, proteoglycans, and elastin can be deposited into the extracellular matrix that forms the internal elastic lamina (IEL) that separates intima from media. The media is mainly composed of smooth muscle cells with an extracellular matrix that contains predominantly type III collagen (Canham et al. 1991). The adventitia is the outmost layer and consists of a complex network of type I collagen fibers, elastin, nerves, and fibroblasts (Finlay et al. 1995). It is notable that the external elastic lamina (EEL) that separates media from adventitia in aortic arteries does not exist in intracranial arteries, potentially rendering intracranial arteries more vulnerable to hemodynamic stress.

IA occurs in high frequency at a bifurcation of the circle of Willis, a ring-like arterial structure located at the base of the brain which supplies blood to the brain and surrounding structures (Williams and Brown 2013). It is well documented that these bifurcations are characterized by high wall shear stress due to the impingement of blood flow that may contribute to IA formation (Sforza et al. 2009). In 1930, Forbus proposed that the smooth muscle layer is lacking at bifurcation apexes in the circle of Willis, resulting in inherent IA susceptibility (Forbus 1930). Subsequently, the "media gap" was shown to be the physiological junction between two smooth muscle layers that primarily contain tendon-like collagen that provides strength and stability (Finlay et al. 1998). Meng and colleagues later designed elegant rabbit experiments that showed that IA does not originate from the apex but from the proximal region, where both high wall shear stress and positive shear stress gradient apply (Meng et al. 2007, 2014).

Abnormal Intracranial Artery in IA Patients

Disintegration of Internal Elastic Lamina (IEL) Internal elastic lamina contains elastic fibers that provide flexibility to arteries. In a normal intracranial artery, IEL is well preserved and homogeneous. In IA, IEL becomes torn, fragmented, or disappears, especially in the fundus of the aneurysm. The disruption of IEL is considered as a hallmark for IA pathology (Krings et al. 2011).

Irregular Luminal Surface of Intima In IAs from human patients or induced in animal models, the luminal surface

of intima shows large evaginations and deep, narrow invaginations as detected by transmission electron microscopy (Draghia et al. 2008). In addition, small holes and enlarged gaps were detected at the junction of endothelial cells at the luminal surface which accordingly, attracts a thick layer of platelets and/or leukocytes. Overall, the luminal surface of intima becomes rugged in comparison to the well-preserved and smooth surface in the normal intracranial artery (Scanarini et al. 1978).

Myointimal Hyperplasia In normal intracranial arteries, IEL separates intima from media. When IEL becomes disintegrated, smooth muscle cells in media can migrate into the intima layer and proliferate, causing myointimal hyperplasia. This phenomenon results in intimal thickening that is frequently observed in IA samples (Fennell et al. 2016). It is unclear whether myotintimal hyperplasia contributes to IA formation or merely is a result of IEL degeneration.

Disorganization of the Muscular Media The smooth muscle cells in the media are organized onto the reticular fiber that mainly consists of type III collagen. In IA, the medial layer is disorganized, and smooth muscle cells often undergo a "phenotypic switch" from contractile to a synthetic type that is pro-inflammatory and pro-remodeling (Chalouhi et al. 2012). Morphologically, their appearance changes from their original spindle-like shape into a spider-like one (Song et al. 2018), resulting in media that is no longer arranged in tightly compacted bands. In comparison with unruptured IA, the media of ruptured IA is thin and often devoid of smooth muscle cells, probably due to increased apoptosis. A similar phenotype has also been observed in mouse models (Aoki and Nishimura 2011; Morimoto et al. 2002).

Hypocellularization Hypocellularization in IA walls occur commonly. Both apoptotic and necrotic cells have been observed in human IAs, especially in their necks and domes, while few have been described in control arteries (Pentimalli et al. 2004). Data from experimentally induced IA animal model support that apoptosis of smooth muscle cells is associated with IA formation (Kondo et al. 1998). Necrosis is in general considered an acute response to injury. Recently, necroptosis was identified as a new type of necrosis that could occur in a programmed manner similar to apoptosis. Given its link to aortic aneurysms via smooth muscle cell death (Wang et al. 2015a, b), necroptosis may also play a role in IA pathogenesis.

Infiltration of Inflammatory Cells Inflammatory cells are frequently found in aneurysmal tissues in both animal models and humans where they are thought to contribute to disease. These cell populations include macrophages, neutrophils, T cells, and B cells. Of note, macrophages can secret matrix-degrading enzymes such as MMP2 and MMP9, and cytokines that further recruit other inflammatory cells (reviewed in Chalouhi et al. (2012)). Macrophages are also thought to be important for aneurysmal rupture. Further descriptions of the roles of immune cells in IA is presented in section "Contribution of Inflammatory Cells".

Genetic Syndromes with Elevated Intracranial Aneurysm Incidence

In addition to environmental risk factors, genetics plays a significant role in disease, providing valuable clues on the molecular mechanisms of IA. The available data demonstrates that IAs can be caused by rare variants of major effect as illustrated by multiple genetic syndromes (Table 1) or common variants of minor effect as shown by GWAS. According to the American Heart Association (AHA) and American Stroke Association (ASA) guidelines (Thompson et al. 2015), IA screening is recommended for those affected by Autosomal Dominant Polycystic Kidney Disease (ADPKD), Type IV Ehlers–Danlos (vascular subtype),

Table 1 Genetic syndromes, underlying defective gene, and IA incidence

Syndrome	Major gene(s)	IA incidence	References
Autosomal Dominant Polycystic Kidney Disease (ADPKD)	PKD1, PKD2	11%	Cagnazzo et al. (2017)
Type IV Ehlers–Danlos Syndrome (Vascular Subtype)	Col3a1	17.5%	Kim et al. (2016)
Microcephalic/Majewski's Osteodysplastic Primordial Dwarfism, Type II (MOPD2)	PCNT	up to 50%	Teo et al. (2016), Bober et al. (2010), Brancati et al. (2005), and Li et al. (2015)
Loeys-Dietz Syndrome (LDS)	TGFBR1, TGFBR2, SMAD3, TGFB2	10–28%	Loeys et al. (2006), Rodrigues et al. (2009), Vanakker et al. (2011), and Kim et al. (2016)
Marfan Syndrome	FBN1	0–14%	van den Berg et al. (1996), Conway et al. (1999), and Kim et al. (2016)
Neurofibromatosis Type I	NF1	9%	Conway et al. (2001) and Schievink et al. (2005)

and Microcephalic Osteodysplastic Primordial Dwarfism (MOPD). The mechanisms by which the underlying syndromic genes as well as others contribute to IA will be discussed in section "Biology of Intracranial Aneurysms". Computed tomography (CT) or magnetic resonance angiography (MRA) based imaging is strongly encouraged for IA detection.

Autosomal Dominant Polycystic Kidney Disease (ADPKD)

Autosomal dominant polycystic kidney disease (ADPKD) has an IA prevalence of roughly 11% as determined by a systematic literature review (Cagnazzo et al. 2017). Caused by loss-of-function in polycystin 1 (PKD1) or polycystin 2 (PKD2), ADPKD is primarily characterized by polycystic kidney, leading to renal dysfunction and eventual failure (reviewed in Pirson (2010) and Bergmann et al. (2018)). In addition, other organs, most notably the liver, are adversely affected. ADPKD patients frequently have high blood pressure that is a risk factor for IA as well as other diseases. This disorder has reduced average life expectancy to 53 and 69 years for European PKD1 and PKD2 patients, respectively (Hateboer et al. 1999).

Type IV Ehlers–Danlos Syndrome (Vascular Subtype)

Affecting 1 in 50,000–200,000, vascular EDS is an autosomal dominant connective tissue disorder characterized by extreme vascular fragility that often leads to hemorrhage and death (reviewed in De Paepe and Malfait (2012) and Malfait (2018)). This disease is caused by variants/mutations in Col3a1 that encodes an abundant, extracellular matrix protein that is required to support and strengthen connective tissue (Tsipouras et al. 1986; Nicholls et al. 1988; Superti-Furga et al. 1988). In a retrospective study, 7 out of 40 (17.5%) individuals with vascular EDS had IA (Kim et al. 2016). Annual, noninvasive imaging (e.g., CT or MRA) of the vascular tree is strongly recommended. Unfortunately, the discovery of IA in these patients has little clinical utility as surgical intervention is highly risky in these patients.

Microcephalic/Majewski's Osteodysplastic Primordial Dwarfism, Type II (MOPD2)

MOPD2 is a rare, autosomal recessive disorder characterized by short stature with other skeletal abnormalities including a disproportionately small head size (reviewed in Rauch (2011)). Due to the extreme rarity of this disease, the precise incidence of IA in MOPD2 remains unknown, but the current data suggest that it may occur in up to half (Teo et al. 2016; Bober et al. 2010; Brancati et al. 2005; Li et al. 2015). This disease is caused by the inheritance of two defective copies of the Pericentrin 1 (PCNT) gene that encodes a centrosome-associated protein that is important for proper chromosomal segregation (Rauch et al. 2008; Willems et al. 2010). In addition, PCNT loss-of-function in epithelial cells disrupts cilia formation and PDK2 localization to basal bodies (Jurczyk et al. 2004). The significance of the PCNT-PDK2 interaction and potentially PCNT haploinsufficiency in IA is further suggested as PCNT rare missense variants were found in multiple IA families where several affected also had kidney cysts (Lorenzo-Betancor et al. 2018). In these particular families, it should be noted that other rare variants beyond PCNT exist where another gene variant may be responsible for disease.

Loeys-Dietz Syndrome (LDS)

Similar to Type IV Ehlers–Danlos, LDS is an autosomal dominant, connective tissue disease. Characterized by mutations in the Tgf-β pathway genes (predominantly TGFBR1, TGFBR2, SMAD3, and TGFB2), these patients have severe vascular phenotypes where arterial aneurysm dissection and bleeding are common that often manifest early in life. Roughly one-third of LDS deaths are caused by cerebrovascular bleeding with an overall IA incidence of 10–28% (Loeys et al. 2006; Rodrigues et al. 2009; Vanakker et al. 2011; Kim et al. 2016). Given the widespread defects in the vasculature, biennial screening of the entire vascular tree is recommended, including brain CTs or MRAs (MacCarrick et al. 2014).

Other Disease with Putative IA Association: Marfan Syndrome and Neurofibromatosis Type I

Marfan syndrome (MFS) is an autosomal dominant connective disorder that leads to skeletal, ocular, and cardiovascular malformations due to mutations in the fibrillin-1 (FBN1) gene that leads to increased Tgf-β signaling (Dietz et al. 1992; Hayward et al. 1992). The initial association of MFS with IA was largely based on numerous case reports (Finney et al. 1976; Matsuda et al. 1979; Ohtsuki et al. 1984; Higashida et al. 1988; Stehbens et al. 1989; Hainsworth and Mendelow 1991; Schievink et al. 1997). Subsequent analysis of 129 Marfan patients with > 3400 observation years found no evidence for symptomatic IA in any patient (van den Berg et al. 1996), while an autopsy study of 25 Marfan patients revealed only one case with a 2-mm unruptured aneurysm (Conway et al. 1999). In contrast, a recent study found that 14% (8/59) Marfan individuals were characterized by IA (Kim et al. 2016). Further research will be required to reconcile these results.

Like Marfan, Neurofibromatosis Type 1 (NF1) is an autosomal dominant disease. Caused by pathogenic variants in the NF1 gene, this disease is characterized by cutaneous neurofibromas (typically benign), café-au-lait spots, iris

Lisch nodules, and vasculopathy. The association of NF1 with IA remains controversial. Intracranial aneurysms have been reported in 9% (2/22) of NF1 cases as detected by MRI (Schievink et al. 2005). An autopsy study of 25 NF1 individuals found none with an intracranial aneurysm nor were there any NF1-affected individuals among 925 intracranial aneurysm patients at Johns Hopkins Medical Institutions from 1990 to 2000 (Conway et al. 2001).

Genetic Studies on Nonsyndromic Intracranial Aneurysms

Genetic studies on nonsyndromic IA can be broadly classified into four categories: linkage analyses, genome-wide association studies (GWAS), candidate gene approaches, and whole exome sequencing research.

Linkage Analysis

In IA, linkage analyses on aneurysm families or sib-pairs have mapped 15 IA susceptibility loci (reviewed in Hitchcock and Gibson (2017) and Zhou et al. (2018)) (Nahed et al. 2005; Ruigrok et al. 2008; Roos et al. 2004; Foroud et al. 2008; Foroud et al. 2009; Verlaan et al. 2006; Onda et al. 2001; Farnham et al. 2004; Kim et al. 2011; Ozturk et al. 2006; Yamada et al. 2004; Olson et al. 2002; van der Voet et al. 2004; Mineharu et al. 2007; Santiago-Sim et al. 2009a). These studies suggest that a subset of IAs may be caused by rare mutations/variants with major effect. The identified IA linkage peaks are often quite broad encompassing tens or hundreds of genes and the definitive causative gene(s) remain largely unidentified. The one exception is a linkage peak at 13q14-21 that we identified in a large family with 9 IA-affected and 13 unaffected members who provided DNA (Santiago-Sim et al. 2009a). Subsequently, we discovered that this chromosomal region harbored a nonsense THSD1 variant that co-segregates exclusively with disease as determined by whole exome and targeted sequencing. Furthermore, we demonstrated Thsd1 heterozygous and null mice develop IA and SAH, validating a direct disease role (unpublished observations and Santiago-Sim et al. (2016)).

Genome-Wide Association Studies (GWAS)

To date, many groups have performed GWAS on those affected by IA and controls, identifying more than 20 IA-candidate loci (reviewed in Hitchcock and Gibson (2017) and Zhou et al. (2018)). An overarching conclusion is that there is no common genetic variant that has a large effect on IA. Of note, only two loci have been replicated by more than two studies: CDKN2A/CDKN2B/CDKN2B-AS on chromosome 9p21.3 (Bilguvar et al. 2008; Yasuno et al. 2010; Deka et al. 2010; Foroud et al. 2012, 2014; Low et al. 2012; Abrantes et al. 2015; Akiyama et al. 2010; Kurki et al. 2014) and SOX17 on 8q11.23 (Bilguvar et al. 2008; Yasuno et al. 2010; Deka et al. 2010; Foroud et al. 2012). A recent meta-analysis of 116,000 IA cases reported an odds ratio of 1.29 (95% CI 1.21–1.38) for rs10757278 in CDKN2B-AS and 1.21 (95% CI 1.15–1.27) for rs9298506 near SOX17.(Alg et al. 2013) These loci have been implicated in diverse populations that include North American, Finnish, Dutch, Japanese, New Zealanders, and Australians. SOX17 was recently implicated as a *bona fide* IA-causing gene as endothelial-specific deletion of Sox17 in mice induces IA formation under hypertension stress.(Lee et al. 2015) Functional validation of CDKN2A/B in IA pathogenesis remains elusive and is complicated by the structural complexity of this locus. Specifically, CDKN2A undergoes alternative promoter utilization that yields transcripts encoding either p14ARF or p16/INK4A. The promoter for P14ARF in the divergent orientation directs expression of CDKN2B-AS that is also known as long noncoding RNA *Anril*. The CDKN2B gene lies within this lncRNA gene in the reverse orientation.

Case–Control Studies for Candidate Gene Association

Based on linkage analysis, expression studies, or biological function, multiple case–control studies have interrogated whether specific polymorphisms are IA-associated (Table 2). The implicated genes include: ACE (Cun et al. 2017), ADAMTS2 (Arning et al. 2016), ARHGEF (Zholdybayeva et al. 2018), COL1A2 (Joo et al. 2009; Glasker et al. 2014; Gan et al. 2017), COL3A1 (Zholdybayeva et al. 2018), CSPG2 (Ruigrok et al. 2009; Zholdybayeva et al. 2018), ELN (Akagawa et al. 2006; Paterakis et al. 2017), ENG (Joo et al. 2008; Hu et al. 2015; Zholdybayeva et al. 2018), HSPG2 (Ruigrok et al. 2009), IL6 (Sun et al. 2008; Zhang et al. 2011; Zheng et al. 2013), JDP2 (Krischek et al. 2010; Zholdybayeva et al. 2018), KLK5-10 (Weinsheimer et al. 2007; Suo et al. 2014), let-7a-1/let-7f-1/let-7d (Sima et al. 2015), LIMK1 (Akagawa et al. 2006; Low et al. 2011; Zholdybayeva et al. 2018), LOX (Hong et al. 2017), MLL2 (Zholdybayeva et al. 2018), MMP2 (Low et al. 2011; Alg et al. 2018), NOS3 (Paschoal et al. 2018), PRDM9 (Zholdybayeva et al. 2018), RNF213 (Zhou et al. 2016), SERPINA3 (Zholdybayeva et al. 2018), SOX17 (Zholdybayeva et al. 2018), StAR (Zholdybayeva et al. 2018), TNF-α (Low et al. 2011; Hu et al. 2017), TSLC2A9 (Zhang et al. 2015), UBR3 (Zholdybayeva et al. 2018), VCAN (Ruigrok et al. 2006; Sathyan et al. 2014), and WWOX (Fan et al. 2016). Further research using mouse models will be required to determine which of the aforementioned genes contribute directly to IA.

Table 2 Intracranial aneurysm candidate gene studies

Gene	Reference(s)
ACE	Cun et al. (2017)
ADAMTS2	Arning et al. (2016)
ARHGEF	Zholdybayeva et al. (2018)
COL1A2	Joo et al. (2009), Glasker et al. (2014), and Gan et al. (2017)
COL3A1	Zholdybayeva et al. (2018)
CSPG2	Ruigrok et al. (2009) and Zholdybayeva et al. (2018)
ELN	Akagawa et al. (2006) and Paterakis et al. (2017)
ENG	Joo et al. (2008), Hu et al. (2015), and Zholdybayeva et al. (2018)
HSPG2	Ruigrok et al. (2009)
IL6	Sun et al. (2008), Zhang et al. (2011), and Zheng et al. 2013
JDP2	Krischek et al. (2010) and Zholdybayeva et al. (2018)
KLK5-10	Weinsheimer et al. (2007) and Suo et al. (2014)
let-7a-1/let-7f-1/let-7d	Sima et al. (2015)
LIMK1	Akagawa et al. (2006), Low et al. (2011), and Zholdybayeva et al. (2018)
LOX	Hong et al. (2017)
MLL2	Zholdybayeva et al. (2018)
MMP2	Low et al. (2011) and Alg et al. (2018)
NOS3	Paschoal et al. (2018)
PRDM9	Zholdybayeva et al. (2018)
RNF213	Zhou et al. (2016)
SERPINA3	Zholdybayeva et al. (2018)
SOX17	Zholdybayeva et al. (2018)
StAR	Zholdybayeva et al. (2018)
TNF-α	Low et al. (2011) and Hu et al. (2017)
TSLC2A9	Zhang et al. (2015)
UBR3	Zholdybayeva et al. (2018)
VCAN	Ruigrok et al. (2006) and Sathyan et al. (2014)
WWOX	Fan et al. (2016)

Whole Exome Sequencing IA Studies

With advances in high-throughput sequencing that have significantly reduced costs, whole exome sequencing has recently been applied to identify candidate disease-causing genes in affected families. Using relative stringent filtering criteria, first cousins who share 12.5% of their genomic DNA have in common an average of 27 rare coding variants with a minor allele frequency (MAF) < 0.2%. This reality highlights the inherent limitations of many familial IA studies. Table 3 highlights published studies that have used whole exome sequencing (and often subsequent targeted sequencing) to identify candidate IA genes. As illustrated in Table 3, many candidate genes have been identified on a per family basis in these studies. Of note, most families selected for study appear to have an autosomal dominant pattern of inheritance, and a single gene defect is most likely responsible. In addition, the number of candidate genes are less than what is predicted in several cases due to the difference in how variants were filtered and reported. To date, only a single family has yielded a definitive IA gene. Specifically, our group identified multiple rare variants in thrombospondin type I domain containing 1 (THSD1) from IA patients by combining linkage analysis, whole exome sequencing, and targeted sequencing (Santiago-Sim et al. 2009a; Santiago-Sim et al. 2016). The THSD1 nonsense variant R450X was identified in a large IA family where it co-segregated in nine affected members and was absent in the 13 unaffected family members who provided DNA. Our loss-of-function studies in zebrafish and mice validated a direct role for Thsd1 in intracranial hemorrhage.

Biology of Intracranial Aneurysms

Intracranial arteries are mainly composed of two types of cells, endothelial cells forming intima and smooth muscle cells forming media. When IA occurs, infiltration of inflammatory cells such as macrophages are inevitable as well as neutrophils, T cells, and B cells. Each type of cells has different biological characteristics and contributes to IA via different molecular and cellular mechanisms.

Table 3 Candidate IA genes implicated by whole exome sequencing studies

Study	Family	Total (affected/unaffected)	Sequenced (affected/unaffected)	Candidate gene(s)
Santiago-Sim et al. (2016)	IA001	(9/20)	(9/13)	THSD1 (Nonsense variant in family, Missense mutations were found in 8/507 probands in our IA/SAH cohort and each variant affected protein function)
Farlow et al. (2015) Familial Intracranial Aneurysm (FIA) Study	A	(6/2)	(5/1)	C1orf35, CALHM1, CCDC39, CTNND2, KLF11, PPIL4, ROBO3, SLK, TRMT1, WDR96, ZNF653
	B	(4/2)	(4/2)	ABCC3, DUSP16, MCM10, NME4, SNX21, TANC2, TSC2, TXNDC11, UNC93A, ZNF362
	C	(7/2)	(5/2)	C11orf65, SEC16B
	D	(4/0)	(4/0)	ALMS1, ARHGEF17, DGKA, FOXM1, HAL, IFNA21, MAP7D1, NLRP1, TAS1R1, *TMEM132B*
	E	(4/0)	(4/0)	HTRA2, NDST1, SMEK2
	F	(5/2)	(5/2)	AGMAT, CYP1A1, FOXRED1, LMBR1L, RFC4, SOX30, UNC13B
	G	(4/2)	(4/2)	ACSM3, ASPM, C1orf38, C10orf58, CCDC37, CHD9, COL17A1, DIAPH1, FAM71A, GSTCD, ITGB6, MLLT4, MTRF1L, OVGP1, PCDHGA11, PIK3C2B, PKP3, PPYR1, PTAFR, RSPH3, TBC1D7, TET2, TRPA1, ZNF264, ZNF835
Bourcier et al. (2018)	A	(5/26)	(2/0)	*ANGPTL6*, GSTA5, UNC5B, BATF2, DPF2, SART1, KEAP1, C19orf57
	B	(2/2)	(2/2)#	ANGPTL6 (p.Glu131Val)
	C	(2/8)	(2/8)#	ANGPTL6 (p.Glu131Val)
	D	(2/1)	(1/1)#	ANGPTL6 (p.Leu348Phe)
	E	(3/1)	(2/1)#	ANGPTL6 (p.Ala153ValfsTer66)
	F	(2/7)	(2/7)#	ANGPTL6 (p.Ala153ValfsTer66)
Wu et al. (2017)	A	(5/4)	(3/1)	MASP2, DHRS3*, OR2G3*, OR2T11, VWA3B, PHF3, *LOXL2**, TBC1D31, CAAP1, TTC16, SH3GLB2, LSM14A, MIA, PPP1R37, RIPK4, FGL1*, RRP12, KLC3*
Zhou et al. % (2016)	10	(6/10)	(5/0)	GPATCH8, *RNF213*, OR11H1, ZNF335
	60	(5/9)	(5/0)	ABCA10, HELZ2, *RNF213*, PLEC, ZNF335
	89	(9/11)	(6/0)	AIM1, CDAN1, OR11H1, PLEC, RTTN
	94	(5/8)	(4/0)	ABCA10, AIM1, *RNF213*, RTTN, SF3A2
	28	(8/9)	(4/0)	CDAN1, GPATCH8, HELZ2, *RNF213*, SF3A2
	9	(4/7)	(2/0)	Unknown
Yan et al. (2015)	P1	(5/8)	(4/0)	CFTR, KCNH3, MLL2, PDE11A, TMEM146, ZNF222, ZNF233
	P2	(5/6)	(4/0)	C5orf42, CYC1, IL10RA, KNTC1, PNKD
	P3	(5/1)	(3/0)	BPIFB3, BPIFB4, C10orf122, KCNV2, RDH16, RSU1, SGK223, TG
	P4	(5/3)	(5/0)	ABCA12, ADAMTS15, DEFB132, DNAH9, ZNF224
	P5	(4/2)	(3/0)	AMPD1, JMJD1C
	P6	(3/5)	(3/0)	CBLL1, DLG1, GLB1L2, LIMCH1, LRP5, MTA3, PLAU, SLC22A11, XAB2
	P7	(4/1)	(4/0)	C4orf45, CYP1B1, FILIP1L, FOXN1, KIAA1244, MYBBP1A, SGSM3
	P8	(3/1)	(3/0)	C2orf62, FCRL1, IQGAP3, KIF20A, SPAG17, STON1, THBD, USP4, VPS13B
	P9	(3/1)	(3/0)	C17orf53, CD320, DSG1, MLL2, MYO7B, RNF10
	P10	(3/9)	(3/0)	IL11RA, ITGB6, NEB, ZNF292
	P11	(6/5)	(4/0)	GPR63, VIPR1, ZXDC
	P12	(3/1)	(3/0)	ASTN2, CRAT, CRELD1, CYP4F11, FAM59A, GPR63, LRP4, MYCBPAP, PAFAH2, PEG3, PVRL1, TBX4, TTN

Table 3 (continued)

Study	Family	Total (affected/unaffected)	Sequenced (affected/unaffected)	Candidate gene(s)
Yang et al. (2018)	P1	(3/2)	(3/0)	NCF1, SRGAP2D, CLEC18B, CCDC88C, UNC79, ARHGEF17, PLEK, KIAA0922, MKI67, PPARGC1B
	P2	(2/4)	(1/0)	NCF1, IL17F, ARHGEF17
	P3	(1/5)	(1/0)	SRGAP2D, IL17F, ZNF175, PLEK, KIAA0922, MLH1, ATXN7
	P4	(2/18)	(2/0)	*ARHGEF17*, GIPR, FAM47E
	P5	(2/11)	(2/0)	AOX1, RANBP3, PLEK, KIAA0922, ADAM15, XRCC1, SIGLEC1, CSMD2, UBE3B, ISOC2, PLTP
	P6	(2/18)	(2/0)	AOX1, ARHGEF17, AKAP13, MLH1, ATXN7, FAM47E, SCAF11, PPARGC1B
	P7	(2/3)	(1/0)	NCF1, TMED3, PLEK, AKAP13, ACSM5, GIPR, PLTP
	P8	(2/3)	(2/0)	NCF1, CLEC18B, ZNF175, MKI67, ACSM5, ISOC2
	P9	(2/20)	(1/0)	IL17F, TMED3, KIAA0922, ADAM15, TNRC6A
	P10	(2/20)	(2/0)	SRGAP2D, CLEC18B, UNC79, TMED3, RANBP3, PLEK, XRCC1, SIGLEC1, TNRC6A, CSMD2, UBE3B
	P11	(2/2)	(2/0)	SRGAP2D, CLEC18B, CCDC88C, TMED3, AKAP13, XRCC1, ACSM5, SCAF11
	P12	(2/4)	(1/0)	NCF1, SRGAP2D, AOX1

Genes highlighted in italics were the authors' preferred candidate gene

By targeted sequencing only

% In the four families with RNF213 variants, the RNF213 did not segregate with disease in 7 of 18 IA cases

*LOXL2 contained the only genetic variant that segregated with disease among the five candidates tested as denoted by an asterisk

Contribution of Vascular Endothelial Cells

Vascular endothelial cells organize into a polarized monolayer that surrounds the vessel lumen. The apical side of endothelial cells is in direct contact with and senses blood flow through mechanosensory pathways. At the lateral side, three different cell–cell adhesions exist: adherens junctions, tight junctions, and gap junctions. The basal side of endothelial cells contains focal adhesion that mediates the interaction between the cell and extracellular matrix. Figure 1 depicts some of the genes and pathways in endothelial cells dysfunction of which contributes to IA.

Shear Stress-Related Mechanosensory Defects in Endothelial Cells The luminal endothelium experiences blood flow with associated shear stress. Shear stress plays a critical role in intracranial aneurysm formation and growth. High shear stress may induce IA formation while low shear stress can promote IA growth and rupture.(Diagbouga et al. 2018) Shear stress mechanosensors have been identified in disease (reviewed in Deng et al. (2014) and Givens and Tzima (2016)). These include different transmembrane proteins such as G-protein coupled receptors. Moreover, plasma membrane microdomains such as lipid raft and cilia are likely critical. Several IA-predisposing genetic syndromes, most notably ADPKD and MOPD2 that were discussed previously, implicate mechanotransduction in IA. For ADPKD, the two responsible genes (PKD1 and PKD2) serve as mechanosensors that transduce blood flow-induced signaling via cilia (Nauli et al. 2008; AbouAlaiwi et al. 2009). In MOPD2 where between 25 and 50% of cases have IA (Lorenzo-Betancor et al. 2018), autosomal recessive variants in PCNT are disease-causing, and it is now known that PCNT loss inhibits cilia formation and leads to mislocalization of PKD2 (Jurczyk et al. 2004). When taken together, these results suggest mechanosensors including PKD1 and PKD2 can contribute to IA, likely by perturbing downstream signaling.

Cell–Cell Adhesion Defects in Endothelial Cells In some IA patients, red blood cells are detected in the intima or media, and this dissection could be caused by defects in endothelial cell–cell adhesion and integrity involving adherens, tight, and gap junctions. In mouse models, loss of Sox17 in endothelial cells lead to IA formation and disruption of VE-cadherin, a major player in adherens junctions, supporting that cell–cell adhesion can contribute to IA formation. It is possible that Sox17 as a transcription factor regulates a plethora of downstream targets including VE-cadherin and its modulators (Nakajima-Takagi et al. 2013). Interestingly, another IA-causing gene THSD1 regulates adherens junction assembly since VE-cadherin was reduced or mis-

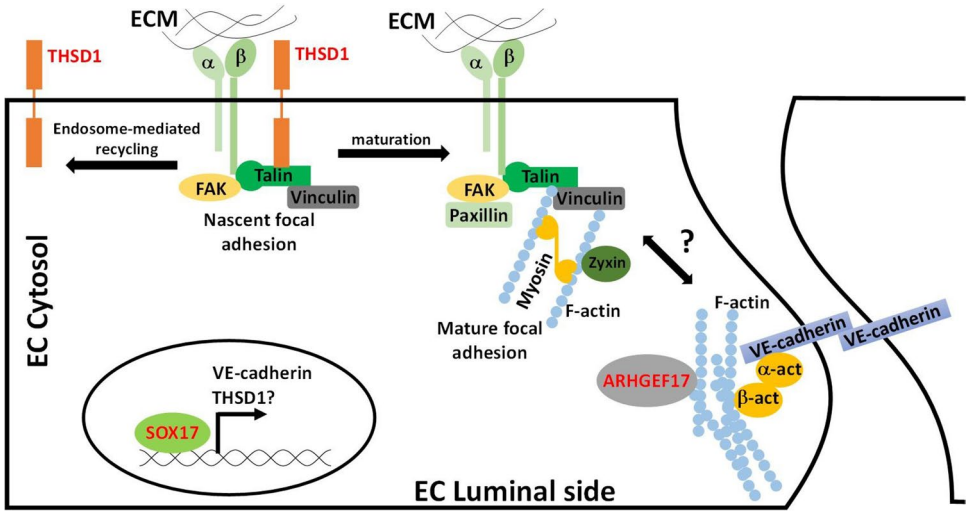

Fig. 1 Schematic of IA genes in vascular endothelial cells. Three IA genes including THSD1, SOX17, and ARHGEF17 are highlighted in red. THSD1 physically interacts with integrin complex through talin at nascent focal adhesion. When nascent focal adhesion matures, THSD1 leaves for next nascent focal adhesion site via endosome-mediated recycling process. Loss of THSD1 leads to defects in focal adhesion, a key determinant of the actin cytoskeleton, and modulator of several downstream pathways. Sox17 functions as a transcriptional factor and modulates VE-cadherin expression. Loss of VE-cadherin decreases cell–cell adhesion and increases permeability. ARHGEF17 is guanidine exchange factor that potentially regulates the remodeling of actin cytoskeleton

localized in endothelial cells that lack THSD1 (Haasdijk et al. 2016). In the future studies, it will be interesting to see if THSD1 is also a downstream target of SOX17 and adds an extra layer of regulation on VE-cadherin activity in endothelial cells.

Role of TGF-β Pathway in Endothelial Cells TGF-β pathway plays a pleotropic role in different cell types within the cerebrovasculature, and its disease significance is confirmed by Loeys-Dietz syndrome. Interestingly, loss of TGF-β pathway genes in mouse endothelial cells via either inactivation of Smad2/3 (Itoh et al. 2012) or Smad4 (Crist et al. 2018) cause vascular defects, highlighting a pivotal role in endothelial cells. Importantly, rare variants of endoglin and beta-glycan, both of which are type III receptors for TGF-β pathway, were found in IA patients, suggesting the potential role of TGF-β signaling transduction in IA pathogenesis (Santiago-Sim et al. 2009b).

Focal Adhesion is Essential for Cerebrovascular Integrity in Endothelial Cells In comparison with adherens junctions, focal adhesions are composed of superamolecular complexes that mediate the interaction between cell and extracellular matrix (reviewed in Wozniak et al. (2004)). Integrin is a major protein involved in focal adhesion and transduces signaling in bidirectional manner. When extracellular matrix proteins such as fibronectin binds to its receptor integrin, an outside-in signaling can be illicit through integrin and eventually activate cytoskeleton remodeling (Fig. 2). In addition, an inside-out signaling pathway exists where the intracellular adaptor protein talin binds to integrin, leading to a conformational change and its activation (Harburger and Calderwood 2009). Focal adhesions are known to maintain cerebrovascular integrity (Iida et al. 2018; Liu et al. 2012). For instance, inactivation of integrin αv and β1 leads to intracranial hemorrhage in zebrafish. Furthermore, depletion of talin, an integrin-binding protein that is essential for focal adhesion, also causes intracranial hemorrhage in zebrafish (Wu et al. 2015). Focal adhesion kinase (FAK) is a master kinase to regulate the dynamics of integrin-mediated focal adhesion, and inactivation of FAK in mouse endothelium causes brain hemorrhage (Shen et al. 2005).

THSD1 and Focal Adhesions THSD1 is a single-span transmembrane protein that is functionally linked to FAK signaling and cell adhesion (Fig. 2). In terms of protein partners, Thsd1 was initially identified as a putative talin-interactor based on a mass spectrometry of talin immunoprecipitation (de Hoog et al. 2004). Subsequently, we validated this interaction using reciprocal IPs in endothelial cells (Santiago-Sim et al. 2016). Protein–protein interaction databases do not provide further candidates for Thsd1 interactions in part due to biases in cell types used as THSD1 since it is predominantly present in endothelial cells. Since Talin is a key protein for integrin-mediated focal adhesion, we hypothesized that THSD1 might affect endothelial focal adhesion and FAK signaling. Functional studies in human umbilical vein endothelial cells suggest that THSD1 regulates focal adhesion stability. Knockdown of THSD1 by small interfering RNA reduces the

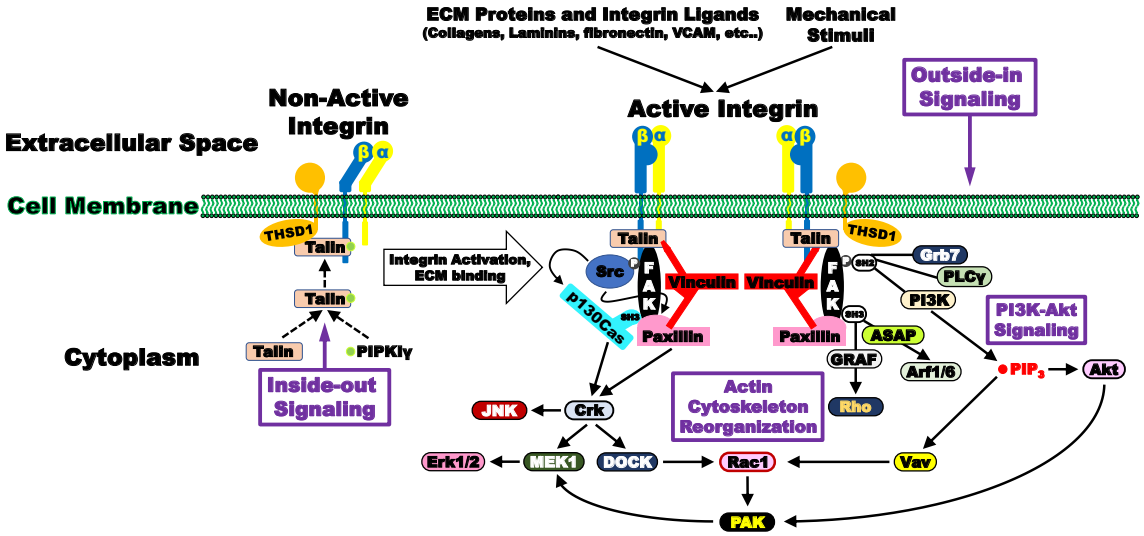

Fig. 2 THSD1/FAK signaling with its downstream targets. THSD1 is required for normal levels of focal adhesions and THSD1 loss reduces the overall amount of focal adhesions, FAK, and active phosphorylated FAK in endothelial cells. As a result, several pathways may be implicated including SRC, PI3K/AKT, Rho, and Rac1 signaling that affects actin cytoskeleton organization and cell adhesion mediated in part through integrins and mechanosensors

focal adhesion number, concomitant with reduced ability in cell attachment onto collagen but not fibronectin. Interestingly, a rescue experiment demonstrated that most of THSD1 variants showed impaired ability in focal adhesion and cell attachment.

To characterize THSD1 further, Rui and coworkers utilized a novel precision tagging technique to epitope-tag THSD1 and tracked its subcellular localization.(Rui et al. 2017; Xu et al. 2017, 2018) Interestingly, THSD1 localizes at nascent focal adhesion that displays a dot-like appearance rather than mature focal adhesion that displays as streak-like patterns, suggesting that THSD1 may function at the early phase of focal adhesion assembly (Rui et al. 2017). Multiple THSD1 missense variants identified in IA patients encode proteins with reduced binding affinity for talin, which may contribute to their compromised function in focal adhesion stability. Surprisingly, THSD1 was also located at the endosome, in addition to nascent focal adhesions, suggesting that THSD1 may function at the interface between these two organelles and orchestrate the efficiency of focal adhesion assembly. On one hand, THSD1 facilitates nascent focal adhesion assembly by directly promoting integrin complex formation. On the other hand, THSD1 leaves mature focal adhesion for next nascent one via endosome-mediated protein trafficking. Recently, a novel mechanism was revealed that integrin can maintain an active conformation in endosomes via FAK/Talin/PIPK1gamma complex (Nader et al. 2016). It will be interesting to know if THSD1 is also a part of such protein complex to modulate integrin activity in endosomes.

Contribution of Vascular Smooth Muscle Cells

In IA, changes in the vascular endothelium often occur in concert with phenotypic changes in smooth muscle cells that provides structural support to vessel walls (reviewed in Starke et al. (2014)). Pathology demonstrated that smooth muscle cells can switch from contractile to synthetic type, which is pro-inflammatory, pro-remodeling, and de-differentiated (Fennell et al. 2016). This transition may lead to a morphological switch from spindle-like smooth muscle cells into spider-like cells that can migrate to and proliferate in the intima, causing myointimal hyperplasia. Moreover, smooth muscle cells with synthetic type can secret cytokines and metalloproteases that recruit inflammatory cells and degrades theextracellular matrix, respectively (Chalouhi et al. 2012). This will further compromise the integrity of media layer of vessel wall. In comparison with contractile type, synthetic smooth muscle cells have reduced collagen biosynthesis and often undergo increased cell death that further weakens the aneurysm wall and predispose it to IA rupture (Kondo et al. 1998; Pentimalli et al. 2004). It is worth noting that the critical role of smooth muscle cells in preserving vascular integrity was also highlighted by a clinical case where a child carrying a mutation in myosin heavy chain 11 (MYH11) developed rapidly progressing IAs at early age with one rupture (Ravindra et al. 2016).

Contribution of Inflammatory Cells

Macrophages Macrophages have been noted in numerous human IA samples. Their function in IA is twofold.

Macrophages secrete metalloproteases such as MMP2 and MMP9 that can degrade extracellular matrix in vessel wall and render IA prone to rupture. Also, macrophages release a variety of cytokines that further recruit other inflammatory cells such as neutrophils, T cells and mast cells. The causal role of macrophages in IA formation and rupture has been well established (Chalouhi et al. 2012; Signorelli et al. 2018). Kanematsu et al. found that clodronate liposome-induced macrophage depletion in mice have a much lower risk of developing IAs (Kanematsu et al. 2011). Monocyte Chemoattractant Protein-1 (MCP1) is one of the key chemokines that regulate infiltration and polarization of macrophages. MCP1-deficient mice exhibit a significant decrease in IA formation (Aoki et al. 2009). Furthermore, the incidence of IA in mice lacking NF-κB, a master transcription factor regulating the inflammatory function of macrophage, was significantly reduced (Aoki et al. 2007). Of note, this study utilized advanced imaging to detect macrophages in animal models and human patients, such as the application of an FDA-approved nanoparticle ferumoxytol that can be efficiently cleared off by macrophages while remaining intact in other non-phagocytic cells (Aoki et al. 2017). Despite the established link between macrophages and IA, more mechanistic studies are needed especially on understanding how imbalance of the two subtypes of macrophage with opposing functions (M1 vs. M2 macrophage) contributes to IA disease (Shao et al. 2017). It is worth mentioning that M2 macrophages can be subdivided into M2a, M2b, M2c, and M2d in response to different ligands stimulations, such as interleukins or TGF-beta (Roszer 2015). Identification of other new phenotypes of macrophages such hemorrhage-associated macrophages called Mhem continue to expand the list (Batra et al. 2018). It will be intriguing to see whether intracranial aneurysm formation or rupture can be preferentially associated with a specific subtype of macrophages, which may provide a novel therapeutic target.

Other Inflammatory Cells Other inflammatory cells such as neutrophils, T cells, B cells, and mast cells are also detected in the aneurysm wall in human samples and animal models (Chalouhi et al. 2012; Signorelli et al. 2018). Neutrophils and mast cells are regarded as functional players in IA pathogenesis (Ishibashi et al. 2010; Chu et al. 2015; Meng et al. 2014). Nonetheless, the role of T cells in IA remains controversial. Sawyer and colleagues found that in Rag1 knockout mice, which lacks T cells, the incidence of IA formation and rupture was reduced (Sawyer et al. 2016). Later, another research group induced IA, utilizing rats lacking T cells caused by a mutation in the *Whn* gene (Miyata et al. 2017). Surprisingly, they found T cells are not required for IA formation and progression. It is unclear why discordant results were found between rodent species on the requirement for T cells in IA. Since T cells have different subpopulations that exert different, even opposite functions, further dissection of their specific contribution might help resolve this discrepancy.

Animal Models of Intracranial Aneurysms

To interrogate candidate disease genes, animal models provide an invaluable genetic tool. Although mice are the most attractive model organism for mammalian IA, their cost and the limited availability of appropriate genetically engineered mouse strains are often prohibitive when encountering a list of candidate IA genes that warrant further evaluation. To overcome this limitation, we recently used zebrafish to determine if candidate IA genes play critical roles in early cerebrovasculature development as defined by cerebral hemorrhages. Afterward, we validated our results in a genetically engineered mouse strain.

Zebrafish Models

Intracranial aneurysm and hemorrhage are tightly associated with compromised cerebrovascular integrity. Recently, zebrafish have gained additional popularity as a vertebrate model organism for studying the cerebrovasculature. Since zebrafish embryos are transparent, intracranial hemorrhage can be directly observed using a standard microscope. Furthermore, zebrafish fecundity and rapid development permits rapid phenotypic evaluation as intracranial hemorrhage in zebrafish fry are detectable as early as 2–3 days post fertilization. Importantly, gain-of-function and loss-of-function approaches are well established in zebrafish that include morpholino and mRNA injections and more recently, through applications of CRISPR/Cas9 technology. A drawback of zebrafish studies is that there are no established protocols for identifying IA due in part to their small size.

Loss of Thsd1 Induces Intracranial Hemorrhage in Zebrafish Two research groups have independently observed intracranial hemorrhage in zebrafish deficient in Thsd1 caused by embryonic morpholino injection using two distinct, nonoverlapping antisense oligonucleotides in a concentration-dependent manner (Haasdijk et al. 2016; Santiago-Sim et al. 2016). Future zebrafish studies of genes involved in integrin and FAK signaling are clearly warranted given the significance of THSD1 in IA. Other pathways also contribute to cerebrovascular integrity in zebrafish. For example, intracranial hemorrhage was observed in zebrafish upon inactivation of VE-cadherin-mediated cell adhesion, Birc2-mediated cell death, fibrinogen-mediated thrombosis, ift81-related hedgehog signaling pathway (Montero-Balaguer et al. 2009; Santoro et al. 2007; Vo et al. 2013; Kallakuri et al. 2015). It remains unknown if Thsd1 is involved in these pathways or vice versa.

Loss of Arhgef17 Induces Intracranial Hemorrhage in Zebrafish Yang et al. identified rare variants in ARHGEF17 from intracranial aneurysm families. To validate its causal role, they tested two different splicing morpholinos against arhgef17 into zebrafish embryo and found a significant intracranial hemorrhage. Importantly, expression of human ARHGEF17 but not its rare variant forms can partially rescue the hemorrhage phenotype (Yang et al. 2018). Since ARHGEF17 encodes a protein's functions as guanine nucleotide exchange factor that regulates cytoskeleton remodeling, it is possible that loss of function in ARHGEF17 may lead to cell adhesion defects during IA pathogenesis.

Mouse IA Models

To dissect the molecular mechanisms of IA, several induced mouse models have been employed, and more recently, genetically engineered mice have demonstrated that two genes, Sox17 and Thsd1, play a direct role in disease. For induced IA models, systemic hypertension, local cerebrovascular hemodynamic stress, and/or extracellular matrix integrity are manipulated (reviewed in Wang et al. (2015a, b)). Seminal work in the induced IA field has been performed by Nobuo Hashimoto and Tomoki Hashimoto to develop and test these models (Morimoto et al. 2002; Nuki et al. 2009; Kanematsu et al. 2011; Tada et al. 2011). Systemic hypertension can be induced by surgery and/or medicine such as renal artery ligation, angiotensin II infusion, or a diet containing high salt and Nitic Oxide synthase inhibitor L-NAME. To alter the local cerebral blood flow, carotid artery ligation is often applied. To weaken cerebrovascular walls, mice are either injected with elastase, a matrix-degrading enzyme, to disrupt internal elastic lamina, or fed a diet containing BPAN, an irreversible inhibitor of lysyl oxidase that can prevent crosslinking between elastin and collagen fibers. These experimental manipulations have been applied to mice singly and in different combinations to generate IA models with distinct kinetic profiles. Since multiple stresses are often utilized in these mouse models, there are concerns about how accurately they reflect natural disease progression and may bypass key signaling pathways important for IA. This shortcoming is especially relevant to understanding IA development and progression.

Loss of Sox17 in Endothelial Cells Induces Intracranial Aneurysm and Subarachnoid Hemorrhage in an Angiotensin II-Induced Mouse Model SOX17 was identified as a genetic risk factor by genome-wide association studies in multiple cohorts and populations. Lee and colleagues found that endothelial-specific deletion of SOX17 in mice causes IA formation and its incidence was dramatically increased by hypertension stress such as infusion of angiotensin II together with injection of elastase (Lee et al. 2015). Subarachnoid hemorrhage was infrequently observed in mutant mice, while hypertension stress significantly increases it.

Loss of Thsd1 Causes Intracranial Aneurysm and Subarachnoid Hemorrhage Previously, we identified a large IA family where a nonsense THSD1 variant co-segregated in all 9 affected individuals and was absent in 13 unaffected family members as determined by whole exome and targeted sequencing (Santiago-Sim et al. 2016). Thsd1 missense variants were identified in 8 among 507 probands in our IA/SAH patient cohort. Each missense protein led to adhesion defects to collagen I endothelial cells in vitro, due in part to compromised ability to promote the FAK-talin interaction (Santiago-Sim et al. 2016; Rui et al. 2017). In the mouse cerebrovasculature, Thsd1 is strictly expressed in endothelial cells and not smooth muscle cells. We further demonstrated that Thsd1-deficient mice (+/− and −/−) had cerebral hemorrhage localized to the subarachnoid space. More recently, we found that Thsd1-deficient mice by 12 months of age are characterized by high incidence of IA as determined by cerebrovascular casting using Microfil (unpublished observations). Further experiments are needed to examine whether IA development is accelerated in response to modifiable risk factors such as hypertension; however, Thsd1-deficient mice are a unique resource as the only genetically engineered mouse IA model that develop IA at high rates without induction by additional factors.

Other Mammalian Models

Besides IA induction in mouse models, other animals such as rats, rabbits, dogs and pigs have also been used to assess pathological changes under IA-related stresses or evaluating the technical proficiency of endovascular devices, due to their larger vessels (Bouzeghrane et al. 2010). To dissect molecular mechanisms of IA formation and rupture, mouse models have noteworthy benefits due to their genetic tools and costs.

Conclusion and Future Perspectives

Although mechanotransduction, Thsd1/FAK signaling, and extracellular matrix integrity are implicated in IA, several noteworthy opportunities and challenges remain to understand more fully IA genetics and pathobiology. The available data suggest that many high-risk genes remain to be discovered and that these genes may have prominent roles in distinct pathways. Advances in high-throughput sequencing will facilitate future studies on IA-affected families and large patient cohorts to identify putative disease genes. As discussed previously, whole exome sequencing studies often

report numerous candidate rare variants per IA family with several affected individuals. After prioritization based on gene expression and the predicted impact of the variant, the ability to narrow the candidate list is difficult. A critical bottleneck exists as experimental evidence of causality using animal models is severely lacking. To illustrate this point, only Thsd1 and Sox17 are implicated directly in IA using mouse models. Specifically, IA and SAH occur in Thsd1 heterozygous and null mice with high incidence in comparison to wild-type controls (unpublished observations and Santiago-Sim et al. (2016)). Endothelial-specific deficiency of Sox17 increases IA incidence in an Angiotensin II infusion model (Lee et al. 2015). To evaluate the functional significance of a candidate IA gene in vertebrates, we and others have employed zebrafish, an increasingly popular model organism for studying cerebrovascular integrity in an efficient and economical way. Genetically engineered mouse models also will continue to have a prominent role. With recent advances in the CRISPR/Cas9 system, the generation of site-specific mouse knockins of candidate IA variants is becoming increasingly affordable to test directly if specific candidate variants are responsible for disease.

Currently, mouse studies on IA formation and rupture is an endpoint assay that requires sacrifice of the experimental animal. Therefore, new techniques that permit the noninvasive determination of IA growth over time would be advantageous. Micro-computerized tomography (micro-CT) might provide such an opportunity, since remodeling processes in mouse carotid arteries can be well documented by utilizing it together with AuroVist, a nontoxic X-ray contrast agent (Schurmann et al. 2015). Recently, a commercial company, Nanoprobes, utilized the same approach to successfully track aortic aneurysm growth over time in living mice. The innovations in imaging techniques will certainly bring paradigm shifts for understanding molecular mechanisms of intracranial aneurysm. In conclusion, past and future research hold promise for identification of high-risk patients and subsequent prevention of IA formation or therapeutic intervention prior to rupture.

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Compliance with Ethical Standards

Conflicts of interest There are no conflicts of interest to report.

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