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Molecular Genetics of Cerebral Aneurysm

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ABSTRACT

Subarachnoid hemorrhage (SAH) secondary to ruptured cerebral aneurysm is a complex trait, with both genetic and environmental risk factors playing an important part. The 30-day mortality rate of patients with SAH is 40% to 44%, with survivors suffering from major disability. Despite the high incidence and catastrophic consequences of a ruptured cerebral aneurysm and the fact that there is considerable evidence that predisposition to cerebral aneurysm has a strong genetic component, very little is understood with regard to the pathology and pathogenesis of this disease. Recent advances in molecular genetics provide evidence that genetic variants of different candidate genes are associated with the occurrence of cerebral aneurysm. This article reviews the evidence supporting a genetic predisposition to SAH from cerebral aneurysm, the conditions commonly associated with cerebral aneurysm, and the search for genetic risk factors. **(Kor J Cerebrovascular Surgery 5:12-6 2003)**

KEY WORDS : Cerebral aneurysm · Pathogenesis · Molecular genetics · Risk factors · Genes.

Introduction

Rupture of cerebral aneurysms followed by subarachnoid hemorrhage(SAH) is a serious disease associated with high mortality and morbidity. Treatment modalities comprise microsurgery and endovascular techniques, eg, Guglielmi detachable coils or intraluminal stents. Optimization of surgical procedures and anesthesia as well as advances in emergency and intensive-care medicine and radiological diagnostics have all resulted in a better clinical course and outcome for SAH patients. However, SAH continues to represent a serious health problem and the cause of major annual costs.

While the incidence of other types of stroke has declined in recent decades, primarily because of improved detection and management of hypertension, the incidence of SAH has remained relatively constant. Despite the high incidence of cerebral aneurysm and the catastrophic consequences of rupture, relatively little is understood with respect to molecular pathology and pathogenesis. This lack of information has severely hampered efforts to identify individuals at risk of a cerebral aneurysm and to define novel points of therapeutic intervention and is largely due to the difficulty in obtaining suitable aneurysmal tissue specimens for use in these analyses. The few studies that have attempted to investigate the molecular basis of cerebral aneurysm have focused on a limited number of biological markers that have been studied in isolation.[1)7)] Also, several candidate genes have been analyzed which, because of the function of the encoded proteins, might contribute to the pathogenesis of cerebral aneurysm. Local genetic variants in the vessel wall may affect endothelial cells or repair or remodeling processes in deeper layers. In this article, we review current models for the genesis of cerebral aneurysms.

1. Studies of candidate genes for cerebral aneurysms

1) Collagen type III

Given the absence of collagen type III and the great destruction of elastin fibers and collagen fibers seen within the aneurysmal wall, research efforts have focused on these elements as well as the regulation and degradation of these elements. Although several instances of abnormal collagen type III production and procollagen production appear in the literature,[3)11)19)23)] a sequence analysis of the collagen type III gene performed on spontaneous cerebral aneurysm failed to

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find a mutation that could explain the decreased and abnormal production of collagen type III.[9] Kuivaniemi et al[9] performed detailed DNA sequencing analyses on 58 patients with cerebral aneurysms or cervical artery dissections. In an analysis of 3,232 nucleotides per patient, mutations in the coding sequences were excluded in 40 individuals with aneurysm. In fact, only 2 nucleotide variations were found in the cohort of 58 individuals, but procollagen synthesis did not differ among these patients. This study strongly suggests that abnormalities of type III collagen are not likely to be a common cause of cerebral aneurysm.

2) Matrix methalloproteinases

Matrix methalloproteinases(MMP) are a family of enzymes that includes collagenase, gelatinase, and elastases. Vessel wall destruction would be increased if an abnormally high level or potent form of the MMPs were present or if there were a decrease in the tissue inhibitors of the MMPs(TIMP). Much of the literature is focused on the presence of MMPs and TIMPs in aortic aneurysm. Yet a few studies regarding the importance of MMPs and TIMPs in cerebral aneurysm are also available. MMP-2 and MMP-9 are also known as gelatinase A and B because of their strong activity against extracellular matrix gelatin(Fig. 1).

Kim et al[7] reported that levels of MMP-9 as well as TIMP are markedly increased in the aneurysmal wall compared with extracranial arteries. This finding suggests that abnormalities in MMPs are a local rather than a systemic phenomenon and that the increase in destruction is not secondary to low levels of TIMP. Bruno et al[1] compared aneurysmal tissue with the basilar artery of control autopsy subjects and found that MMP-9 was heterogeneously distributed in 40% of cases of aneurysm, whereas it was diffusely distributed in the control patients. They also reported that 64% of patients had MMP-2 activity identified, compared with only 14% of controls.

From the same study group, Todor et al[32] reported that increased serum levels of pro-MMP2 occur in some patients with cerebral aneurysm. This increase may be secondary to

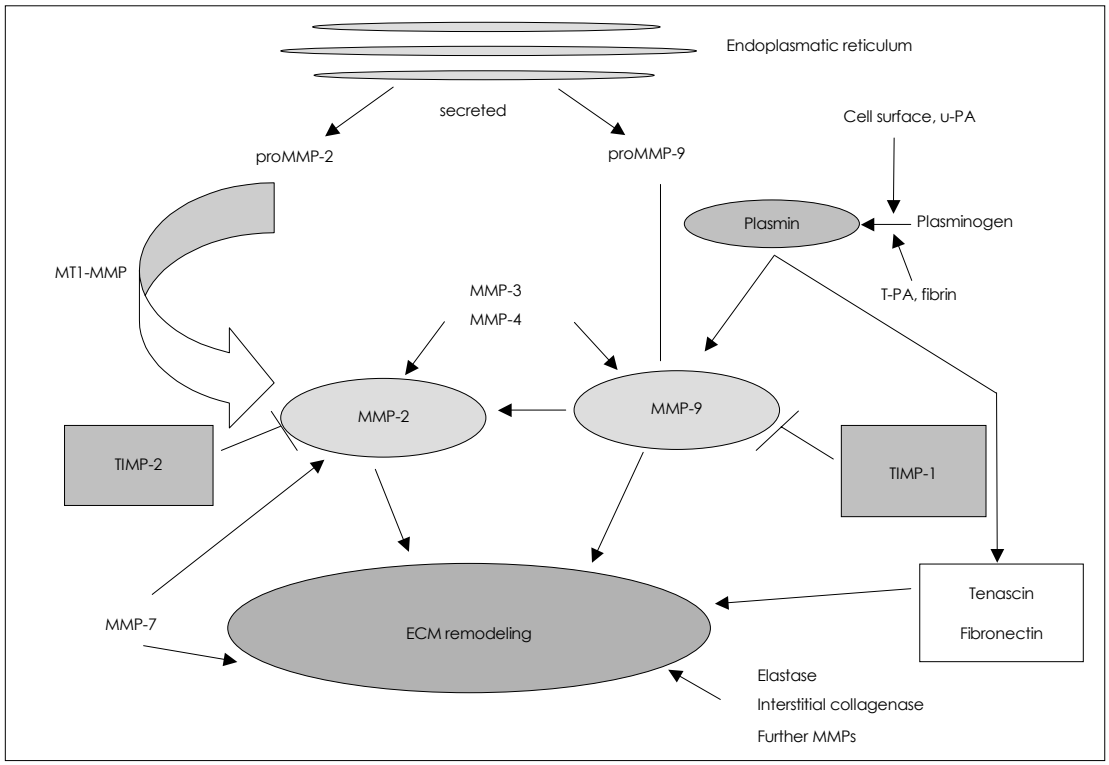

Fig. 1. Interaction of matrix metalloproteinases (MMP), especially MMP-2 and MMP-9, which might contribute ot deg-radation of a vessel wall by remodeling of the extracellular matrix (ECM). After processing in endoplasmic reticulum, the pro-enzyme is secreted in an inactivated form (zymogen) by different cell types like endothelial cells, smooth muscle cells or fibroblasts. In the activation of pro MMP-2 tow pathway are probably involved. Binding to transmembrane MMPs (membrane-type-1 MMP and MT2-MMP) and the plasmin activator system leads to a final active enzyme. However, plasmin does not seem to act directly on MMP-2 but by means of other MMPs, especially MMP-9. By destruction of glycoprotein like fibronectin and tenascin, plasmin may contribute to ECM remodeling more directly. U-PA : urokinase-type plasminogen activator, t-PA : tissue-type plasminogen activator, TIMP : tissue inhibitor of metalloproteinases.

other conditions that are associated with aneurysm. For example, smokers are known to develop emphysema, atherosclerosis, and cerebral aneurysm, and it is possible that smoking behavior itself may induce expression of pro-MMPs.

In a sequence analysis of the MMP-9 promotor region among 36 individuals with aneurysms, Peters et al[21] report that a single polymorphic site was present(the remainder of the genetic code being identical to controls). At this site, a variable number of cytosine-adenine(CA) repeats were seen with 23 repeats associated with internal carotid artery formation. This difference, however, was somewhat small, with 18% of cases having the 23 repeat alleles, compared with 9% of controls. In addition, having 22 repeats, a difference of only 1 repeat, showed the opposite relationship(9% of cases compared with 19% of controls having 22 CA repeats).

Increased number of CA repeats has been associated with increased MMP-9 expression. However, 2 subsequent studies have failed to find an association with higher CA repeats and cerebral aneurysm. Yoon et al[33] examined a Finnish population of 57 cases and 174 controls and found no significant association between these repeats and cerebral aneurysm. In their study, all cases of aneurysms had a family member with a history of cerebral aneurysm. Zhang et al[35] recently reported on 92 cases of SAH compared with 158 controls and also did not find an increased association of CA repeats. It may be possible that increased MMP-9 levels are secondary to environmental risk factors such as smoking or hyper-tension and not attributable to abnormalities in the MMp-9 gene.

3) Alpha-1-antitrypsin

Alpha-1-antitrypsin(AT) is a glycoprotein that inhibits proteases. AT deficiency is known to cause emphysema and liver disease. AT is also an elastase inhibitor, and mutations may lead to aneurysm formation. The normal variants of the AT gene are commonly referred to as PiM1, PiM2, and PiM3, and PiZ and PiS are the abnormal variants. The PiZ variant is associated with very low levels of AT and very low functional activity in he homozygous state(approximately 15% of normal). PiS is associated with less protein and less functional activity in the homozygous state(approximately 60% of normal).

Schievink et al[27] examined 362 patients with AT deficiency, and found 3 cases of ruptured cerebral aneurysm and 1 case of spontaneous dissection. They reported that 3 patients were heterozygous for the PiZ mutation and one was homozygous (PiZZ). This led to a study of 100 consecutive patients with cerebral aneurysm, which found that 16% of cases were heterozygous for either the PiS or PiZ alleles, compared with only 7% of 904 people from Minnesota(OR=2.56 ; 95% CI, 1.32 to 4.75).[25]

However, several studies have been unable to confirm these associations. St. Jean et al[28] reported that among 72 cases of cerebral aneurysm(26 from Pittsburgh and 46 from London), 2 patients carried the PiS allele and 4 carried the PiZ allele(includes 1 patient with PiSZ). These results were not found to be significantly increased compared with population-based controls from Pittsburgh. Elzouki et al[4] reported that of 30 consecutive individuals with known homozygous PiZZ AT deficiency, none of the cases were found at autopsy to have had a cerebral aneurysm or cervical dissection.

4) Angiotensin I-converting enzyme

Angiotensin I-converting enzyme(ACE) converts angiotensin I to angiotensin II, which is a potent vasoconstrictor as well as a regulator of aldosterone and catecholamine synthesis.[10][20][34] The ACE locus(17q23) has two major forms. The D or deletion allele has been associated with higher circulating ACE levels[13] and has been associated with cardiovascular disease and left ventricular hypertrophy.[2][5] Curiously, several studies have found that the D allele is actually less common among cases of ruptured aneurysm compared with controls. Takenaka et al[31] examined 83 patients with cerebral aneurysm compared with 104 control subjects. They found that the D/D genotype was slightly increased among the aneurysm group(46%) compared with the control group (41%). A separate group in Cambridge, England, identified a similar phenomenon. In their group, the I allele was associated with aneurysm risk over the D allele(OR=1.3 ; 95% CI, 1.02 to 1.65).[6] It is unclear whether ACE alleles are directly associated with SAH as a candidate gene or whether the alleles are in linkage disequilibrium with a causative gene.

Several other candidate genes have appeared in the literature, but thus far, they have not been confirmed by subsequent studies.[8][12][14][17-19][24-26][29-31] Recently, Peters et al[22] used a global gene expression analysis approach(SAGE-Lite) in combination with novel data-mining approach to perform a high resolution transcript analysis of a single cerebral aneurysm. The significant overexpression of genes involved in extracellular matrix components(eg, COL3A1, COL1A1, COL1A2, COL6A1, COL6A2, elastin) and genes

Table 1. Exmaples of genes that are overexpressed in cerebral aneurysm and are of biological significance and putative function of specific gene products

Gene	Ratio, ICA : STA	Putative function
Fibronectin	443 : 4	ECM constituent
Collagen type III α-1	34 : 7	ECM constituent
Collagen type I α-2	20 : 1	ECM constituent
Collagen type I α-1	33 : 1	ECM constituent
Collagen type VI α-1	13 : 1	ECM constituent
Collagen type VI α-2	9 : 1	ECM constituent
Collagen type IV α-1	5 : 0	ECM constituent
Elastin	5 : 0	ECM constituent
TIMP-3	20 : 0	ECM constituent
OSF-2	13 : 0	MMP activation/ECM remodeling
βig-h3	6 : 0	Collagen bridging/ECM
CTGF	6 : 0	TGF-β-induced collagen synthesis/ECM
SPARC	29 : 0	Antiadhesive glycoprotein/ECM remodeling
Hevin	6 : 0	Antiadhesion
β-Gal-binding lectin	26 : 7	Cell adhesion, proliferation/migration, immunomodulation
cdc-rel2a/PNUTL2	14 : 0	Cytokinesis
Tetraspanin-5	5 : 0	Cell proliferation/Cell migration
Cathepsin B	13 : 1	Lysosomal protease
Cathepsin D	4 : 0	Lysosomal protease
Vinculin	5 : 1	Adhesion plaques
c-Abl	7 : 0	Adhesion plaques

involved in extracellular matrix turnover(TIMP-3, OSF-2), cell adhesion and antiadhesion(SPARC, hevin), cytokinesis (PNUTL2), and cell migration(tetraspanin-5)(Table 1).

2. Linkage studies for cerebral aneurysm

Linkages studies use polymorphic markers(genes that are variable within the population) and the presence or absence of disease among affected and unaffected relatives to determine whether a specific marker occurs more often than expected by chance among affected individuals. Given the high mortality rate of SAH, the acquired nature of the disease, and the strong environmental covariants, a linkage study faces tremendous challenges both in data collection and in analysis.

In 1998, Olson et al[15] reported a genome-wide screen among 48 affected sibling pairs taken from 264 siblings of 85 cerebral aneurysms probands in Finland. Two regions had multipoint maximum LOD scores(MMLS) greater than 2.0 : chromosome 19(MMLS=2.63) and chromosome X(MMLS =2.08). Four additional regions had the highest MMLS, and this region is one in which numerous cerebrovascular and cardiovascular gees are located. Recently Onda et al[16] reported a genome-wide linkage study among 104 Japanese affected siblings pairs. They identified evidence of linkage on chromosome 5q22-31(maximum LOD score [MLS], 2.24), 7q11(MLS, 3.22) and 14q22(MLS, 2.31). The best evidence of linkage was detected at marker D7S2472 in the vicinity of the elastin gene(within 400 kb). Notably, linkage was not apparent in the regions reported by Olson et al.[15] Although no single polymorphism was found to have a clear association with cerebral aneurysm, patients homozygous for the major allele of Intron 20(M) and the minor allele for intron 23(m) were at high risk(OR for Mm=4.39, 95% CI, 2.6 to 12) for IA. Several other associations were also found. The association of mutations within the elastin gene is a compelling one given that elastin and its destruction are key elements of aneurysm formation. This study did not report analysis may occur, and confirmation of these findings in other populations is required.

Conclusions

Future investigations will focus on the genetic background of aneurysm pathogenesis. It is unlikely that there is only one gene affected since the development of an aneurysm is clearly a complex multifactorial event. Linkage analysis is a suitable method for investigating the genetic origin of a disease.

However, this requires a large number of affected families. Another method is the analysis of candidate genes. The genes of interest encode for proteins or enzymes, which are essentially involved in biochemical pathways known to be part of aneurysm pathogenesis like arteriosclerosis or extracellular matrix remodeling.

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