ADAMTS genes and the risk of cerebral aneurysm

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OBJECTIVE Cerebral aneurysms (CAs) affect 2%–5% of the population, and familial predisposition plays a significant role in CA pathogenesis. Several lines of evidence suggest that genetic variations in matrix metalloproteinase genes (*MMP*) are involved in the etiopathology of CAs. The authors performed a case-control study to investigate the effect of 4 *MMP* variants from the *ADAMTS* family on the pathogenesis of CAs.

METHODS To identify susceptible genetic variants, the authors investigated 8 single nucleotide polymorphisms (SNPs) in 4 genes from the *ADAMTS* family (*ADAMTS2*, -7, -12, and -13) known to be associated with vascular diseases. The study included 353 patients with CAs and 1055 healthy adults.

RESULTS The authors found significant associations between CA susceptibility and genetic variations in 3 members of the *ADAMTS* family. The largest risk for CA (OR 1.32, $p = 0.006$) was observed in carriers of the *ADAMTS2* variant rs11750568, which has been previously associated with pediatric stroke. Three SNPs under investigation are associated with a protective effect in CA pathogenesis (*ADAMTS12* variant rs1364044: OR 0.65, $p = 0.0001$; and *ADAMTS13* variants rs739469 and rs4962153: OR 0.77 and 0.63, $p = 0.02$ and 0.0006 , respectively), while 2 other *ADAMTS13* variants may confer a significant risk (rs2301612: OR 1.26, $p = 0.011$; rs2285489: OR 1.24, $p = 0.02$).

CONCLUSIONS These results suggest that reduced integrity of the endothelial wall, as conferred by *ADAMTS* variants, together with inflammatory processes and defective vascular remodeling plays an important role in CA pathogenesis, although the mechanism of action remains unknown. The authors' findings may lead to specific screening of at-risk populations in the future.

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KEY WORDS cerebral aneurysm; *ADAMTS*; genes; vascular disorders

CEREBRAL aneurysms (CAs) are dilations of cerebral arteries that affect 2%–5% of the population.³⁵ Although most of these lesions are clinically unapparent, CA rupture and subarachnoid hemorrhage usually occur between 40 and 60 years of age. Therefore, early identification of a CA is essential for preventing subsequent bleeding events, which often lead to substantial morbidity and mortality.^{4,16}

The pathogenesis of CA formation and rupture is complex and seems to arise from multiple factors. A familial predisposition is the strongest risk factor for the development of a CA; siblings of patients with CA are at a 4-fold increased risk of hemorrhage,⁴⁰ suggesting a significant genetic component to CA pathogenesis.³⁷ Environmental factors, such as smoking, hypertension, and extensive alcohol intake, appear to play a more important role in the

ABBREVIATIONS CA = cerebral aneurysm; FDR = false discovery rate; GWAS = genome-wide association study; HWE = Hardy-Weinberg equilibrium; ICB = intracerebral bleeding; IS = ischemic stroke; MI = myocardial infarction; MMP = matrix metalloproteinase; PS = pediatric stroke; SAB = subarachnoid bleeding; SNP = single nucleotide polymorphism; TSR = thrombospondin Type 1 sequence repeat motif.

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rupture of CAs.^{10,11,17,26,37} The development and rupture of aneurysms involve local degradation of vascular structural proteins and remodeling of the diseased blood vessels. Elastin and collagens are major structural components of the vascular wall, and it has been suggested that elastinolysis is particularly pertinent to vessel dilation, while collagenolysis relates to aneurysm rupture.⁴⁵

In recent years, compelling evidence has indicated that a number of matrix metalloproteinases (MMPs), which possess proteolytic activity against extracellular matrix proteins such as elastin and collagens, play an important role in the disease etiopathology of various types of aneurysms.⁴⁵

Although the genetic basis of CA is poorly understood, the identification of susceptible genetic variants may increase our understanding of CA formation and rupture mechanisms. Extensive genetic association studies are urgently needed to elucidate the molecular pathogenesis of CAs.⁴⁶

In recent years, candidate gene association studies have been the most common approach to identify susceptibility genes for complex diseases such as CA. However, our ability to identify relevant candidates is generally restricted by our understanding of disease pathophysiology, which in many cases is limited. For CA, several physiological pathways, including inflammation,^{5,15,33,36} disruption of the extracellular matrix surrounding the arterial wall,³⁸ and numerous heritable connective tissue disorders,³⁹ have been implicated in disease occurrence. However, the conventional candidate gene approach has been widely disappointing for CA, and most findings have not been replicated.^{21,29} Once MMP participation in vascular remodeling became apparent, researchers proposed that variations in *MMP* may play a role in CA pathogenesis.^{12,21,24} In addition, subsequent results from a genome-wide association study (GWAS) for pediatric stroke patients have highlighted a crucial role for various *ADAMTS* (A-disintegrin and metalloproteinase with thrombospondin motifs) metalloproteinases in disease pathophysiology.¹

The *ADAMTS* proteins are members of a metalloprotease family that contain the thrombospondin type 1 sequence repeat motifs common to extracellular matrix proteins.²⁷

Members of the *ADAMTS* family play a role in extracellular matrix degradation and are involved in several other biological processes, including coagulation, angiogenesis, and cell migration.¹³ Variations within several *ADAMTS* genes have been implicated in prothrombotic,¹³ inflammatory,^{22,28} and connective tissue diseases,⁴² suggesting a potential role in CA pathogenesis.¹⁸ Together, these findings suggest that *ADAMTS* genes may be excellent screening candidates for CA pathogenesis; however, to our knowledge, it remains unknown if and where these genes act on disease susceptibility.

To address whether variations in *ADAMTS* genes are genetic determinants of CAs, we analyzed 8 variants of 4 *ADAMTS* genes associated with diverse vascular phenotypes in 353 patients with CAs and 1055 healthy adults, all from Northwest Germany.

Methods

Patients

Our study included 258 patients harboring a CA who were treated at the Department of Neurosurgery, University Hospital Muenster. From these 258 patients, we identified 222 probands (79.4% with ruptured aneurysms and 20.6% with unruptured aneurysms) who were considered eligible for genotyping after assessment of DNA quality. All study participants were investigated with respect to clinical parameters. To enhance the power of our study, we also included in our analysis 131 CA patients who experienced either subarachnoidal bleeding (SAB) or intracerebral bleeding (ICB) from a 2010 independent genetic association study initiated at the Institute of Epidemiology and Social Medicine in Muenster. In all patients (9.07% of whom had multiple aneurysms), the diagnosis of CA was made using cerebral digital subtraction angiography and confirmed at surgery. Healthy adults served as the control group (n = 1055) and were recruited from the population-based Dortmund Health Study, which was conducted in the same region.^{31,41,43} All recruitment and research protocols were reviewed and approved by the institutional review board of the University of Muenster, and written informed consent was obtained from all study participants.

Single Nucleotide Polymorphism Selection, Genotyping, and Quality Control

DNA was extracted from peripheral blood using standard methods. For genotyping, we selected 8 single nucleotide polymorphisms (SNPs) recently shown to be associated with pediatric stroke (rs469568, rs11750568, rs1364044),¹ ischemic stroke (rs2285489, rs739469, rs2301612, rs4962153),¹³ and myocardial infarction (rs4380028; Table 1).³⁴

For genotyping, we used 2 ng genomic DNA and Taqman SNP Genotyping Assays (Applied Biosystems, Inc.), following the manufacturers' instructions.

Genotyping efficiency was > 98%, and allele identification was performed manually using SDS2.3 software. All laboratory analyses were performed at the Institute of Arteriosclerosis Research in Muenster.

All patients in the control group were already genotyped using the Infinium HD 2.5 M Omni Chip (Illumina, Inc.) and analyzed with GenomeStudio. For SNPs with no directly genotyped data available, we inputted genotypes using the genome-analysis software PLINK V 1.06.³²

To ensure the comparability of our results, we replicated the genotyping of all variants and showed a significant association with CA using Taqman SNP Genotyping Assays, following the manufacturers' instructions.

Statistical Analysis

We compared allele frequencies of the risk-associated variants in patients with CA and in the control group by using the chi-squared test, as analyzed in PLINK V1.06.³² We used Haploview 3.0² to verify that the observed genotype distributions were in Hardy-Weinberg equilibrium (HWE).

We applied logistic regression models to examine the

TABLE 1. Overview of genetic variants and their associated diseases

Chromosome	Gene	SNP	Associated Disease	Reference
5	<i>ADAMTS12</i>	rs1364044	Pediatric stroke	Arning et al., 2012
5	<i>ADAMTS2</i>	rs11750568	Pediatric stroke	Arning et al., 2012
5	<i>ADAMTS2</i>	rs469568	Pediatric stroke	Arning et al., 2012
9	<i>ADAMTS13</i>	rs2285489	Ischemic stroke	Hanson et al., 2009
9	<i>ADAMTS13</i>	rs739469	Ischemic stroke	Hanson et al., 2009
9	<i>ADAMTS13</i>	rs2301612	Ischemic stroke	Hanson et al., 2009
9	<i>ADAMTS13</i>	rs4962153	Ischemic stroke	Hanson et al., 2009
15	<i>ADAMTS7</i>	rs4380028	Myocardial infarction	Reilly et al., 2011

association between each SNP and the occurrence of a CA in an additive model of inheritance that included testing trends across SNP categories. All models were adjusted for age and sex. Since 8 SNPs were genotyped, we used the false discovery rate control method to correct for multiple testing, which was analyzed with PLINK V1.06.³²

Results

To determine if genetic variations within the ADAMTS family could affect a patient's susceptibility for developing a CA, we analyzed 8 polymorphisms within 4 *ADAMTS* genes in 353 patients with CA and in 1055 adults in the control group.

Women represented the majority of patients (67%), and we found no significant differences in mean age (56.7 vs 54.5 years) between the CA group and the control group.

We genotyped 8 candidate SNPs previously associated with ischemic stroke,¹³ myocardial infarction,³⁴ or pediatric stroke.¹ The allele frequencies for each SNP and the odds ratio (OR) for each genotype are reported in Table 2. No deviation from the HWE was observed for any of the 8 SNPs.

Five of the 8 SNPs under investigation were significantly associated with CA after adjusting for age and sex. The ORs ranged from 0.65 to 1.31 and were similar in magnitude to those previously reported for pediatric¹ and ischemic¹³ stroke. The remaining 2 SNPs failed to demon-

strate a statistically significant association with CA. If the results were adjusted for multiple testing, we would lose 1 of the 6 significant findings.

The largest risk for CA (OR 1.32, $p = 0.006$) was observed in carriers of the *ADAMTS2* variant rs11750568, which was previously associated with pediatric stroke.¹

Discussion

In this study we evaluated the relationship between an elevated risk of CA development and the presence of 8 *ADAMTS* metalloproteinase gene variants already known to be associated with vascular diseases.

Once MMP participation in vascular remodeling became apparent, researchers proposed that MMPs might contribute to CA pathogenesis.^{12,21,24} The role of MMPs in normal tissue remodeling processes and connective tissue turnover has been well established,^{19,23} and increased MMP levels have been associated with several pathological states. To our knowledge, it remains unknown how MMPs act on CA susceptibility.^{20,30}

Previous studies found that *ADAMTS* gene variants were significantly associated with several related phenotypes,^{1,13,34} resulting in this gene family becoming an attractive target for genetic screening in patients with a CA.

In the current study, the largest CA risk associated with a single variant (OR 1.31, $p = 0.006$), was observed for carriers of the *ADAMTS2* variant rs11750568, an allele

TABLE 2. Summary of association results

CHR	SNP	NMISS	Minor Allele	MAF	CHISQ	OR	p Value*	Model 1†	FDR_BH‡
5	rs1364044	1360	T	0.2068	13.32	0.6524	0.0001889§	0.0003635§	0.001511§
5	rs11750568	1325	A	0.3704	7.785	1.318	0.005919§	0.07472	0.01578§
5	rs469568	1401	A	0.5014	0.8393	1.086	0.3517	0.958	0.4019
9	rs2285489	1382	T	0.4056	4.955	1.241	0.02158§	0.03018§	0.02923§
9	rs739469	1405	C	0.3821	3.091	0.7672	0.02192§	0.05004§	0.02923§
9	rs2301612	1384	C	0.506	6.458	1.257	0.01088§	0.04544§	0.02177§
9	rs4962153	1390	A	0.1209	11.62	0.6323	0.0006033§	0.00412§	0.002413§
15	rs4380028	1366	T	0.3841	0.4571	0.9391	0.4998	0.6065	0.4998

CHISQ = chi-squared; CHR = chromosome; MAF = minor allele frequency; NMISS = null or missing SAS values.

* Analyzed by logistic regression.

† p value adjusted for age and sex.

‡ p value after adjustment using the false discovery rate as described by Benjamini-Hochberg.

§ Statistically significant.

previously associated with pediatric stroke.¹ Mutations in the *ADAMTS2* gene have been also connected to the Ehlers-Danlos syndrome, a severe connective tissue disorder.^{43,43} Schievink and colleagues proposed over 20 years ago that this kind of tissue disease enhances the potential for developing a CA.³⁹ This hypothesis has been supported by more current findings, which indicate that mutations in genes that affect the integrity of the extracellular matrix surrounding the arterial wall play an important role in CA vulnerability.^{3,38} In addition, several biomarkers for connective tissue disorders have been identified that are clearly linked to CA pathogenesis.⁴⁴

Moreover, we have identified significant associations between CA susceptibility and genetic variants for 2 other members of the ADAMTS family. The *ADAMTS2* variant rs1364044 seems to offer a protective effect against CA pathogenesis, while the *ADAMTS13* variants rs2285489 and rs2301612 confer a significant risk for developing CAs.

Interestingly, *ADAMTS12* seems to be involved in the degradation of cartilage oligomeric matrix proteins and has been shown to possess antiangiogenic and tumor-suppressive properties⁹ as well as a modest association with heart failure.²⁵ Furthermore, subsequent analysis suggested an important role for *ADAMTS12* in inflammatory processes.²²

Inflammation has been frequently connected to CA pathogenesis.^{3,8,15,33} This is primarily because extensive inflammatory and immunological reactions are common in unruptured CAs and may be related to aneurysm formation and rupture.⁸ These observations have been further supported in findings by Hashimoto and colleagues, who identified abnormal vascular remodeling mediated by inflammatory cells to be a key pathologic component of various vascular diseases. They postulated that CAs evolve from a combination of inflammatory processes, hemodynamics, and vascular remodeling.¹⁵

ADAMTS13, also known as von Willebrand factor cleaving protease, is already established as a pivotal component of blood coagulation,⁷ prothrombotic diseases,¹³ and vascular inflammation.⁶

Recent findings by Hanson et al. confirm an association between the *ADAMTS13* variant rs2285489 and CA susceptibility;¹⁴ in a previous study, the same authors reported an association between this variant and a decreased risk of ischemic stroke.¹³

Taken together, our findings emphasize the theory that genetic variants often confer susceptibility to more than 1 disease and that different diseases share common pathological pathways.

Nevertheless, we are aware that our study has several limitations. First, the limited number of individuals included in our study (353 patients with CA and 1055 healthy controls) limits the statistical power and may increase the risk of Type I (false positive) and Type II statistical errors (missed association). This is particularly relevant given the lack of replication of our findings in an independent cohort. However, all genetic variants analyzed in the current study originated from GWAS for related phenotypes. Second, we acknowledge that reproducing previous findings for pediatric¹ and ischemic¹³ stroke and myocardial

infarction³⁴ in our study may be considered “pseudoreplication.” A recent study reported an association between *ADAMTS13* genetic variants and aneurysmal subarachnoid hemorrhage in 183 patients and 680 control patients, which may be considered an independent association in a comparable clinical phenotype.¹⁴ However, additional studies with larger sample sizes are warranted to generalize our findings. Ideally, these studies should also include additional clinical subphenotypes, i.e., number of aneurysms, the location thereof, and relevant risk factors such as smoking habits, to allow for more detailed, stratified analysis and to refine the observed associations.

Conclusions

The mechanisms through which *ADAMTS* gene variants lead to the development of CAs remain unknown. However, our results give rise to the hypothesis that reduced integrity of the endothelial wall, as conferred by *ADAMTS* gene defects, together with inflammatory processes and defective vascular remodeling, plays an important role in CA pathogenesis and underscores the findings from previous analyses to CA susceptibility.^{3,8,13,15,38}

A better understanding of how these genetic variants modulate CA vulnerability may help to implicate underlying pathways, simplify disease prediction, and prevent resultant bleeding events by specific screening of at-risk populations.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Conception and design: Holling, Arning, Jeibmann, Köhnemann, Berger, Wellmann, Nowak-Göttl, Stummer, Stoll. Acquisition of data: Holling, Arning, Köhnemann, Brokinkel, Ewelt. Analysis

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