ALTERED ARTERIAL HOMEOSTASIS AND CEREBRAL ANEURYSMS: A REVIEW OF THE LITERATURE AND JUSTIFICATION FOR A SEARCH OF MOLECULAR BIOMARKERS

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DESPITE THE CATASTROPHIC consequence of ruptured intracranial aneurysms, very little is understood regarding their pathogenesis, and there are no reliable predictive markers for identifying at-risk individuals. Given that intracranial aneurysms have a strong but complex genetic component and well-characterized modifiable risk factors, it seems likely that the most valuable approach to developing minimally invasive diagnostic and prognostic tools will involve a multifactorial model that includes both genetic and environmental risk factors. Unfortunately, the genetic basis of intracranial aneurysms is poorly described, and reports describing the association of nonrandom deoxyribonucleic acid sequence variation with intracranial aneurysms have been limited to a handful of ad hoc studies that have focused on a variety of markers in small populations. One reason for this lack of coordinated analysis of the genetic basis of intracranial aneurysms is that the molecular pathogenesis and pathobiological characteristics of the disease are poorly described, so candidate marker selection has been problematic.

Few studies have addressed the molecular pathological basis of intracranial aneurysms or the possible mechanisms of intracranial aneurysm formation. In this regard, candidate gene selection strategies have relied almost exclusively on limited knowledge of monogenic disorders such as Ehlers-Danlos syndrome and Marfan's syndrome, in which intracranial aneurysm is a feature of a spectrum of syndromic phenotypes. Without exception, these approaches have not affected the clinical identification and/or management of intracranial aneurysms significantly. Therefore, it is imperative that coordinated large-scale efforts in genetics, molecular biology, and genetic epidemiology are implemented to overcome these obstacles and drive developments in the field. In this review, we summarize the current screening modalities for intracranial aneurysms, review the current state of understanding relating to the genetic basis of intracranial aneurysms, and suggest a broader theory of aneurysm pathogenesis to form the foundation of a coordinated molecular search for biological markers that may be associated with aneurysm formation and rupture.

KEY WORDS: Aneurysms, Literature review, Molecular pathogenesis

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Despite considerable advances in treatment, morbidity and mortality from cerebral aneurysms remain exceptionally high. In the case of unruptured aneurysms, operative mortality has been reported to be as low as 2.5% and morbidity to be less than 6% (28, 29, 35, 37, 67). Once an aneurysm ruptures, however, subarachnoid hemorrhage (SAH) (89) occurs, and the impact becomes much more devastating (21, 70). Aneurysmal SAH involves catastrophic intracranial bleeding with immediate and delayed sequelae. Between 30 and 50% of patients die after SAH, and 40 to 50% of survivors have significant neurological deficits resulting in severe disability.

To reduce morbidity and mortality rates, it is vital to identify a cost-effective means of effectively screening patients at

risk for the development of intracranial aneurysms. This is a necessary first step toward early intervention and, ideally, prevention. We think that this step probably will occur at a molecular biological level.

There is tremendous variability in the genetic makeup of each individual's organs. In this regard, vascular tissue is similar to organs and should be considered an organ rather than an inanimate conduit of blood. Thus, vascular tissue is subject to the same molecular control mechanisms as any other tissue in the body. Individuals who develop intracranial aneurysms may be genetically vulnerable because of an impaired ability to respond at a molecular level to the stress factors that affect their particular cerebrovasculature.

PREVALENCE, CLINICAL CONSEQUENCES, AND COST OF CEREBRAL ANEURYSMS

Intracranial aneurysms are relatively common, with a prevalence estimated to be as high as 6% on the basis of autopsy specimens (11, 81). Each year in the United States, more than 28,000 people have SAH from aneurysm rupture (89). Unfortunately, 50% of these patients die either at the moment of rupture or shortly thereafter. Another 25% have devastating neurological complications, resulting in loss of motor or cognitive function that necessitates institutionalization and imposes enormous costs on both the patients and the system that must deliver care for their chronic condition. The remaining 25% may experience protracted hospitalization and some degree of cognitive or neurological impairment. In contrast, intracranial aneurysms that are repaired before they rupture have a reported mortality as low as 2.5% and morbidity less than 6% (68).

From an economic perspective, the cost of acute care for patients with ruptured cerebral aneurysms is inordinately high. Hospital expenses to provide acute care for just 112 patients after SAH are estimated at $2.8 million. The majority of these costs are for intensive care (18). Again, the estimated costs involved in caring for a patient with a ruptured aneurysm far exceed those for the care of a patient whose aneurysm is treated before it ruptures ($38,000 versus $12,685) (18).

A patient's neurological condition at presentation is strongly associated with outcome (8). Because there is no predictable way to determine how an individual will respond neurologically to a rupture, clearly it would be advantageous to identify all intracranial aneurysms before rupture. To justify intervention before rupture, however, we must first understand the actual risk of rupture. If an intracranial aneurysm has been discovered incidentally, what is the true risk of rupture? The recent results of the International Study of Unruptured Aneurysms indicate that intracranial aneurysms less than 1 cm in diameter are associated with an exceptionally low risk of rupture, which suggests that they should be managed conservatively (33). This conclusion has been met with considerable controversy, however, and it has not been accepted uniformly. There is significant evidence to suggest that the majority of aneurysms that rupture are less than 1 cm in diameter (53, 69).

We think that it is naive to accept that a simple black-and-white image estimating size, such as an arteriogram, is an adequate means of predicting probability of rupture. The dynamic molecular environment of the vasculature, ultimately controlled by gene expression, may prove to be more predictive of aneurysms that are most likely to rupture. Furthermore, by understanding the molecular alterations within an aneurysm, we may elucidate the pathogenesis of aneurysm development and rupture. After the pathophysiology has been elucidated, physicians may develop novel therapies that genetically and chemically alter the vessel so that it can repair itself, thus avoiding more invasive therapy such as surgical clipping or endovascular embolization. We think intracranial aneurysms represent an ideal disease model for screening and prevention, in that effective screening and early intervention may avert the devastating consequences of rupture.

SCREENING

Current Concepts

Attempts to identify a high-risk patient group with intracranial aneurysms that would be amenable for screening began with the 1954 description by Chambers et al. (8) of a familial cluster. Several radiological studies have emerged to better estimate the risk of intracranial aneurysms in families within which at least one relative has a known aneurysm. Much of the defining work analyzed the Finnish population, whose limited migration and well-characterized genetics provide a relatively stable group that is ideal for study (70). In the most comprehensive study to date, Ronkainen et al. (68) used magnetic resonance angiography (MRA) to screen 400 patients who had two or more first-degree relatives (e.g., parents, siblings) with a documented cerebral aneurysm. MRA revealed aneurysms in 37 asymptomatic individuals, accounting for a 9.25% yield in this group (68). Although not ideal, this figure is thought to be the best estimate of familial cerebral aneurysms.

It should be noted that the excellent study design of Ronkainen et al. (68) revealed many of the potential limitations of MRA, demonstrating it to be far from an optimal screening modality even in this high-risk group. They used a prospective model with three independent observers to obtain inter- and intrarater reliability with cross-correlation with digital subtraction angiography for all the MRA-positive lesions. On this basis, they identified four patients with MRA evidence of aneurysms that were revealed as vessel loops by digital subtraction angiography, representing a false-positive rate of approximately 10%. This rate is very similar to that obtained in a study by Blatter et al. (3a), who were able to confirm with conventional angiography only 14 of 18 aneurysms that had been identified by MRA. Digital subtraction angiography identified four aneurysms that MRA failed to disclose. This 10% false-negative rate is probably an underestimation, be-

cause not all of the MRA-negative individuals were subjected to the "gold standard," invasive angiography. Therefore, some of the MRA-negative patients may have harbored an undetected aneurysm. Furthermore, although the inter-rater reliability was acceptable, ranging from a κ value of 0.59 to 0.80 among the three readers, Ronkainen et al. astutely recognized that one reader consistently labeled MRA-questionable lesions as negative, thus reporting fewer positives overall. This illustrates the subjective nature implicit in MRA.

Even in the case of familial associations (high-risk population), controversy exists regarding screening candidates and timetables. Current recommendations are that individuals be screened if they have two immediate family members known to have cerebral aneurysms. However, several studies suggest that screening be broadened to include patients with only a single afflicted family member (71). To further complicate the matter, on the basis of an estimated incidence of 2% de novo aneurysm formation (39), there is a question of how often the screening should be repeated in initially negative patients. Recommendations range from every 6 months to every 5 years for those with a strong family history (71). Furthermore, data from the International Study of Unruptured Aneurysms indicate that it may not be sufficient to identify a patient with MRA evidence of an intracranial aneurysm; the group most at risk of rupture also must be known.

Aside from these concerns associated with screening, even in this selected high-risk population with a family history of intracranial aneurysm, the high costs and limited availability of MRA make it an unrealistic modality for mass screening of the general population. Therefore, the normative cost-benefit analysis mandates a screening program that is convenient, inexpensive, objective, repeatable, and safe. A biological marker seems ideal in this context.

Despite many shortcomings, familial screening studies provide the important first step of definitively demonstrating that the disease has an increased tendency to cluster in families, and findings clearly implicate molecular factors in the development of intracranial aneurysms. The exact pattern of inheritance is unclear. The consanguinity studies reported from the Saguenay region in Quebec suggest an autosomal dominant mode of transmission, although this has been disputed (15). On the basis of the different models of inheritance, the cause for this predisposition probably is genetically heterogeneous. This heterogeneity is manifest in the various syndromes associated with increased prevalence of intracranial aneurysms. An analysis of the genetic and molecular biology of these conditions can narrow the search for the specific abnormalities associated with intracranial aneurysms. This approach has identified the extracellular matrix of the arterial wall as the most likely site of molecular alterations implicated in aneurysm formation.

Epidemiological and Environmental Risk Factors

The identified epidemiological risk factors for the development of intracranial aneurysms include increasing age, cigarette smoking, alcohol consumption, sex hormones, and presence of systolic hypertension. Cigarette smoking is the most consistent, modifiable risk factor. The risk among current smokers is 3- to 10-fold higher than among those who never smoked, with the risk increasing in a dose-dependent manner (5, 38, 45). The mechanism by which smoking increases risk is not known, but it may be related to cigarette oxidant inactivation of α-1-antitrypsin, a major circulating inhibitor of serine proteases (64, 65). An imbalance between proteases and antiproteases may lead to increased proteolysis of connective tissue elements of the arterial wall and extracellular matrix elements supporting the architecture of the cerebral arteries, thus disrupting the normal homeostatic mechanism controlling vascular repair. Several studies support this theory (71–73), including our own (57, 78), which demonstrated an increased frequency of genetically determined α-1-antitrypsin deficiency among patients with SAH. Furthermore, Gaetani et al. (22, 23) observed that although immunologically determined levels of α-1-antitrypsin are elevated in patients with SAH, the collagenase inhibitory capacity in such patients is low, indicating a functional deficiency of α-1-antitrypsin.

Heavy alcohol use also is associated with SAH. Like smoking, heavy alcohol consumption acts in a dose-dependent manner (86), with a relative risk (RR) of 2.8 (95% confidence interval [CI], 2.1–3.6) for consumption of less than 150 g per week and RR of 4.7 (95% CI, 2.1–10.5) for consumption of 150 g per week or more. No mechanism for the increased incidence of SAH among drinkers is clear, but a significant association between smoking and alcohol use is known, and most studies fail to adjust for cigarette smoking.

The age-specific rate of SAH is higher in men than in premenopausal women, but it is higher in postmenopausal women than in men of the same age. Hormone replacement therapy modestly reduces the risk among postmenopausal women (RR range, 0.5–0.6), and oral contraceptive use modestly increases the risk (RR range, 1.4–1.5) (36, 46, 58, 79, 87, 90).

Hypertension is perhaps the most commonly studied risk factor for the development and rupture of intracranial aneurysms. Longitudinal studies have demonstrated that hypertension increases the risk of SAH approximately 2.8-fold (95% CI, 2.1–3.6) (41, 75, 91), and RR is 2.9 (95% CI, 2.4–3.7) in population-based case-control studies (5, 46, 85). The risk of occurrence or rupture of an intracranial aneurysm associated with hypertension seems to be less than that associated with smoking.

None of the known epidemiological factors provides the predictive power to identify individuals with sufficiently elevated risk to make presymptomatic screening by current imaging techniques cost effective (21, 68). Specifically, it would not be cost effective to use MRA to screen every patient who has hypertension or who smokes. It is possible that epidemiological factors are implicated in the pathogenesis of intracranial aneurysms via their impact on the preprogrammed molecular environment of vascular tissue.

Theoretical Impact of Hypertension on Molecular Arterial Homeostasis

An example of the interaction between environmental factors and genetic activity is provided by the potential impact of hypertension on the local cerebrovasculature and the response at a molecular level. Both Schneider et al. (74) and Sorteberg et al. (77) independently demonstrated that carotid artery closure led to an instantaneous drop in ipsilateral intracranial blood flow velocities, whereas contralateral velocities increased. We suggest that this regional state of hypertension will initiate a molecular response, on the basis of the individual's genetic programming, to maintain cerebrovascular homeostasis. The response probably involves the activation of NO pathways in the endothelium and their effects on smooth muscle cell relaxation through hyperpolarization via potassium channel activation (74). As illustrated in *Figure 1*, changes in flow velocity (secondary to hypertension) cause changes in shear stress, which in turn deform the endothelium, leading to an increase in intracellular calcium and activation of nitric oxide synthase and phospholipase A_2.

The activation of nitric oxide synthase leads to the synthesis of NO, which diffuses to the smooth muscle cell and activates the calcium-dependent K^+ pump and cyclic guanosine monophosphate production. This process also leads to K^+ pump activation and stimulation of cyclic adenosine monophosphate production, resulting in adenosine triphosphate-dependent and delayed-rectifier K^+ channels. The increase in the permeability of K^+ channels causes hyperpolarization of the smooth muscle cell and relaxation. As a result of activation of phospholipase A_2, hydrolysis of phospholipids, specifically, phosphatidylinositol, occurs. Consequently, arachidonic acid accumulates, which leads to the generation of prostacyclin via the action of cyclooxygenase and prostacyclin synthase. Prostacyclin diffuses to a smooth muscle cell receptor, which activates cyclic adenosine monophosphate generation, thereby activating adenosine triphosphate-dependent and delayed-rectifier K^+ channels, and thus contributing to the hyperpolarization and relaxation of the smooth muscle cell.

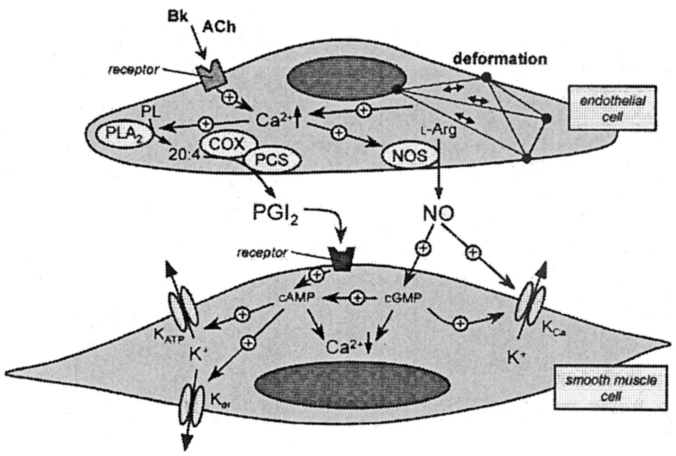

FIGURE 1. *Schematic view of pathways leading to NO and prostacyclin* (PGI_2) *formation in endothelial cells. This diagram depicts these pathways and their effects on smooth muscle cell tone.* K_{CA}, K_{ATP}, *and* K_{dr}, Ca^{2+}*-dependent, adenosine triphosphate-dependent, and delayed-rectifier* K^+ *channels, respectively;* 20:4, *arachidonic acid;* ACh, *acetylcholine;* Bk, *bradykinin;* COX, *cyclooxygenase;* NOS, *NO synthase;* PCS, *prostacyclin synthase;* PL, *phospholipid;* PLA_2, *phospholipase* A_2*;* L-Arg, *L-arginine (*from, *Hashimoto N, Kim C, Kikuchi H, Kojima M, Kang Y, Hazama F: Experimental induction of cerebral aneurysms in monkeys.* ***J Neurosurg*** *67:903–905, 1987 [25]).*

The previous discussion exemplifies the potential interaction between the molecular environment of the vasculature and physical factors that can affect this environment. In addition to hypertension, all of the previously described epidemiological risk factors identified from prior studies must be considered in assessing any potential relationship between molecular alterations and the development of intracranial aneurysms. On the basis of our review of the literature and biological considerations, the key epidemiological factors identified are smoking, hypertension, and sex.

The current literature does not support the isolated use of any of these epidemiological risk factors as a basis for screening aneurysm candidates. These factors probably do not act alone, but they probably influence the molecular environment of genetically predisposed patients. The consequent impaired arterial homeostasis may result in aneurysm formation. It is hoped that identification of relevant molecular alterations of homeostasis will provide a series of candidate genetic markers. These will have to be tested for their predictive value in identifying individuals at risk for the development and rupture of cerebral aneurysms.

BIOLOGICAL RATIONALE FOR SELECTING CANDIDATE MOLECULAR MARKERS

Before embarking on a search for genetic markers, a genetic basis for the disease must be established. The next step is to use associated genetic syndromes to identify structural abnormalities that could help guide the search for potential markers. The validity of selected screening markers then can be tested in a prospective controlled fashion. To date, the approach to understanding the molecular pathogenesis of intracranial aneurysms has focused on individual markers on the basis of presumed structural relationships. Markers have been considered candidates because of structural abnormalities in genetic syndromes known to be associated with intracranial aneurysms. As discussed below, most of the structural alterations

associated with these syndromes are at the level of the extracellular matrix. This has led to a relatively uncoordinated effort to examine the molecular structure of the extracellular matrix. This in turn has created a confusing body of literature yielding conflicting reports, in which the same marker is associated with intracranial aneurysms in one study and discounted in another (*Table 1*).

Aneurysms and Associated Conditions

The strongest association between cerebral aneurysms and other syndromes is with polycystic kidney disease. As in those who have spontaneous intracranial aneurysms, patients with polycystic kidney disease have been reported to develop de novo aneurysms (9, 10, 32). The formation of a new aneurysm in these predisposed patients further suggests the possibility of altered molecular response to some environmental factors. That is, genetic programming of the cerebrovasculature may impair the ability to respond to hemodynamic stressors. An underlying structural defect thus develops in the cerebral arteries, and new aneurysms form over time. The known structural defect in polycystic kidney disease is in polycystin, which is a membrane protein responsible for maintaining the structural integrity of extracellular matrices of connective tissue (24, 76). At least two separate genes, *PKD1* and *PKD2*, have been identified, and they are located on separate chromosomes, which suggests a multigenic process.

Neurofibromatosis Type 1 is another associated condition that provides further evidence of altered arterial structure. The defect in this condition is thought to be in the protein neurofibrin, which contains a central guanosine triphosphatase-activating protein domain. In the mouse model, this protein is important for cytoplasmic microtubules, and defects lead to arterial thinning and rupture (30).

In Marfan's syndrome, a defect in the glycoprotein fibrillin-1 forms microfibrils. This protein is an important constituent of the extracellular matrix and is distributed throughout elastic tissue.

Fifty percent of individuals with intracranial aneurysms reportedly have morphological derangements in the structural proteins that provide mechanical integrity to the arterial walls (81). From the discussion above, alterations in syndromes associated with intracranial aneurysms seem to be the result of a multigenic process, thus suggesting genetic heterogeneity. Furthermore, the molecular alterations associated with aneurysm formation in these syndromic patients suggest a defect of the structural matrix proteins that form the framework of the arterial wall. The next question is: what within the arterial wall should be the focus?

LITERATURE-BASED GENETIC/ MOLECULAR MARKERS

Type III Collagen

Ehlers-Danlos syndrome Type IV is a connective tissue disorder associated with an increased incidence of intracranial aneurysms. This condition involves a deficiency of Type III collagen (COL3), which is a critical component of distensible tissues including blood vessels (3, 7, 60, 83). The finding that this disease is associated with intracranial aneurysms initiated a flurry of studies to determine whether COL3 is diminished in aneurysmal patients.

Neil-Dwyer et al. (52) compared Type III/Type I collagen ratio in 17 patients with Ehlers-Danlos syndrome Type IV and intracranial aneurysms to that in 6 controls. They demonstrated a ratio reduction in 11 of the 17 patients, all of whom were older than the rest of the participants. These findings led to the notion that the Type III collagen loss results from degradation attributable to external factors (e.g., sustained hypertension or smoking) rather than inadequate synthesis. We suggest that this may reflect a process wherein older patients with a predetermined molecular predisposition, secondary to genetic programming, have an impaired ability to maintain arterial homeostasis. When such patients are exposed chronically to known environmental factors that stress and degrade the arterial system within the cerebrovasculature, the system becomes overwhelmed and the result is failure of the normal arterial remodeling process, leading to formation of an aneurysm.

In 1996, Brega et al. (6) took this theory one step further by treating the gene responsible for COL3 with the restriction fragment endonuclease *Ava*II, thus separating it into two alleles: a larger *A* allele (5.7 kilobases) and a smaller *B* allele (4.3 kilobases) (52). They then assessed the relative frequency of each of these alleles in 19 consecutive patients and 15 controls. They reported a *B* allele frequency of 0.34 in patients with aneurysms as compared with 0.10 in controls. They also noted that both of the patients with multiple aneurysms were homozygotic (*B/B*) for this allele. This finding seemed to correlate with the investigations by Powell et al. (63), which also demonstrated an increased incidence of the smaller *B* allele in patients with abdominal aortic aneurysms.

Many other investigators have studied Type III/Type I ratio abnormalities, with some conflicting results (1, 17, 54, 61). The results reported by Østergaard and Oxlund (54) were similar to those of Brega et al. (6): 6 of 14 patients with intracranial aneurysms had decreased ratios and increased distensibility of middle cerebral arteries. In contrast, Leblanc et al. (43) demonstrated no such decrease.

Persons with Ehlers-Danlos syndrome Type IV have numerous mutations in the proα 1 (III) chain (COL3A1) that codes for COL3. Kuivaniemi et al. (42) attempted to test whether this abnormality also applied to aneurysm patients without connective tissue disorder. However, they detected a mutation in the *COL3A1* gene in only 2 of 58 patients with intracranial aneurysms or cervical dissection, and these changes involved a minor amino acid substitution that was considered functionally insignificant. They concluded that the ratio of collagen Type I to COL3 may be decreased in patients with aneurysms, but that the decrease is not caused by mutations in the genetic coding as is the case with Ehlers-Danlos syndrome Type IV.

TABLE 1. Key literature examining molecular alterations associated with cerebral aneurysms[a]

Series (ref. no.)	Marker	Description	Analysis	Findings	Strengths/weaknesses
Neil-Dwyer et al., 1983 (52)	Type III collagen	*Cases:* 17 patients with ruptured aneurysms *Controls:* 6 age- and sex-matched controls (3 gliomas and 3 meningiomas) *Tissue source:* fibroblasts derived from skin/temporal artery biopsies	Protein analysis Expression of collagen by fibroblasts Radioactive labeling Compare Type I/III ratio deficiencies	Relative Type III collagen deficiency in 11 of 17 patients Not all or none; a graded response with partial loss in some patients	First systematic study to analyze molecular alterations Did not examine aneurysmal tissue Molecular alterations in skin and superficial temporal artery may have nothing to do with regional changes in cerebrovasculature adjacent to the aneurysm or in the dome Controls had brain tumors, which are known to have molecular alterations with altered genetic expression
Brega et al., 1996 (6)	Type III collagen	*Cases:* 19 patients with aneurysms *Controls:* 15 from DNA bank Analyzed differences in expression of Type III collagen *Source:* DNA from whole blood	DNA analysis *Cases:* DNA extracted from whole blood *Controls:* DNA from a known bank Using restriction fragment endonuclease *Ava*II, separated Type III collagen into a larger *A* allele and smaller *B* allele	*B* allele frequency of 0.34 (cases) versus 0.10 (controls) Both patients with multiple aneurysms were homozygotic (*B/B*) for this allele	First effective study at the DNA level Controls had cystic fibrosis or muscular dystrophy; no other medical information known No distinction between rupture and unruptured or adjustment in the analysis No adjustments or description for epidemiological risk factors Blood-based DNA study assumes that the regional molecular alterations at the dome and adjacent vasculature are reflected in the blood
Østergaard and Oxlund, 1987 (54)	Type III collagen	*Cases:* 14 patients with fatal ruptured aneurysms *Controls:* 14 age- and sex-matched subjects who died of other causes *Tissue source:* postmortem tissue from the MCA	Mechanical distensibility and protein analysis Protein analysis using electrophoresis for quantitative expression of type III collagen Mechanical analysis using distension of the tissue to correlate with forces equivalent to blood pressure between 100 and 200 mm Hg	*Protein analysis:* 6 of 14 patients with deficiency of Type III collagen compared with MCA tissue of controls *Mechanical analysis:* Significant increase in the extensibility of the MCA tissue compared with controls, but not associated with alterations in mechanical strength	First study to demonstrate alterations in mechanical distensibility No demographic considerations or adjustments for epidemiological factors (e.g., hypertension) No consideration of unruptured aneurysms Postmortem study; therefore subject to secondary artifacts associated with death; tissue examined was not viable MCA tissue was harvested from the proximal vessel, with hemodynamic properties very different from those at an arterial bifurcation
Leblanc et al., 1989 (43)	Type I/III collagen	*Patient:* 1 with positive family history *Tissue source:* fibroblast cultures	Fibroblast cultures Quantitative protein analysis using electrophoresis of Type I/III collagen production Comparison group was control cell lines	No difference in ratio	Single-patient study Strong family history; therefore may not be generalizable Regulatory factors not taken into account
Kuivaniemi et al., 1993 (42)	Type III collagen	Cases only; no controls 40 patients with aneurysms and 18 patients with cervical carotid dissection *Tissue source:* skin biopsies, fibroblast cultures, DNA from blood	Skin biopsies, fibroblast cultures, RNA extraction, reverse transcriptase cDNA, then PCR analysis Genomic DNA also isolated from blood Analysis of DNA polymorphisms Protein analysis	No evidence of polymorphisms or mutations affecting Type III collagen in patients with aneurysms or dissections	Extremely thorough molecular analysis Skin fibroblast and blood-based DNA analysis may not represent the local changes at the circle of Willis or in the dome No stratification or adjustment to consider rupture status or epidemiological data No controls

TABLE 1. Continued

Series (ref. no.)	Marker	Description	Analysis	Findings	Strengths/weaknesses
Majamaa et al., 1992 (47)	Type I/III collagen	*Cases:* 11 patients with ruptured aneurysms (6 with family history) *Controls:* 9 patients; no details *Tissue source:* skin-derived fibroblasts	Protein analysis using cell cultures from fibroblasts from skin RNA extracted, Type I and III collagen isolated Thermal stability assessed using enzymatic assays and digestion	No difference in Type III/I ratios 2 of 11 patients had increased thermal instability in Type III collagen (both had family history) None of the controls had this increase No differences in thermal stability of Type I	Study looking beyond ratios and possibility of structural performance alterations Very contaminated study with family history in some and not in others No consideration of epidemiological factors or adjustments for them No information on controls Poor consideration of statistical analysis techniques
Chyatte and Lewis, 1997 (12)	Type III collagen	*Cases:* 31 patients with aneurysms *Controls:* 14 craniotomies with MRA evidence of no aneurysm *Tissue source:* skin fibroblasts	Skin fibroblasts and serum-derived RNA reverse transcription to isolate Type III collagen gene Translation to produce Type III collagen protein Collagen metabolism estimated using gelatinase activity	Type III collagen synthesis same in cases and controls Aneurysm patients had 3× increase in serum gelatinase activity, suggesting increased metabolism of Type III collagen	Excellent study showing possible increase in collagen degradation Not reflective of the vascular tissue in question, i.e., dome/adjacent vessels in the circle of Willis
Peters et al., 1999 (57)	MMP-9	*Cases:* 76 patients with aneurysms *Controls:* pooled genomic bank, representative of the study population based on demographics *Source:* DNA derived from peripheral blood	DNA analysis Patients were genotyped for MMP-9 expression Polymorphisms were noted based on CA repeat sequences in the promoter sequence proximal to the transcriptional site of the gene Varying expressed sequences were spliced into a vector reporter system using luciferase assays to reflect the promoter activity	Constructs bearing the long (CA) repeat sequence had the greatest association with cerebral aneurysms, suggesting that this functional polymorphism was associated with cerebral aneurysms	Another study suggesting possible increased degradation of structural matrix proteins, this time based on a polymorphism in a regulatory sequence found more commonly in aneurysm patients Very little known about the controls (presence of asymptomatic aneurysms) Blood-based DNA study and may not be reflective of local changes in the circle of Willis or at the aneurysm dome
Skirgaudas et al., 1996 (76)		*Cases:* 10 patients with aneurysms of various types *Controls:* 3 postmortem circle of Willis samples	Protein analysis Immunostaining VEGF, bFGF, fibronectin, Type IV collagen, α-smooth muscle actin	9 of 10 aneurysms positive for bFGF and 10 of 10 for VEGF None of the controls were positive for either growth factor Domes displayed disorganized staining for the structural matrix proteins	Study extended beyond structural matrix proteins to include growth factors Limited sample size Mixed population of aneurysms included berry, giant, and mycotic aneurysms Controls represented nonviable postmortem tissues

[a] DNA, deoxyribonucleic acid; MCA, middle cerebral artery; RNA, ribonucleic acid; cDNA, complementary DNA; MMP-9, matrix metalloproteinase-9; PCR, polymerase chain reaction; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; MRA, magnetic resonance angiography.

Brega et al. (6), however, noted that Kuivaniemi et al. (42) analyzed only the gene's central domain and did not effectively assess related modulating sequences, a concept that formed the basis of our initial preliminary work, as discussed below. Therefore, although the structural domain of the *COL3A1* gene did not demonstrate significant changes in the study by Kuivaniemi et al., mutations in regulatory genes still may lead to increased collagen degradation. Several studies have demonstrated that the increased susceptibility of COL3 to proteases results in increased collagen degradation (47).

Majamaa et al. (47) demonstrated no difference in the Type III/Type I ratio in 11 patients with intracranial aneurysms, but they showed that the COL3 in two of these patients had decreased thermal stability with increased degradation. Ana-

lyzing extracranial vessels, Dobrin et al. (17a) demonstrated that human iliac arteries treated with elastase dilated but did not rupture, whereas those pretreated with collagenase dilated further to the point of rupture. Armed with this information, Chyatte and Lewis (12) attempted to determine whether patients with cerebral aneurysms had decreased production or increased destruction of COL3. They demonstrated normal gene expression in all 31 aneurysm patients examined. However, with their use of gelatinase activity as a marker of collagen degradation, they demonstrated that patients with aneurysms had a threefold increase in enzymatic activity compared with controls.

Building on this foundation, we conducted a case-control study, which demonstrated that a specific-length polymorphism [(CA)23) in the promoter of Type IV collagenase (matrix metalloproteinase [MMP]-9) was nonrandomly associated with the occurrence of intracranial aneurysm (χ^2; $P = 0.02$) (57). Variation in the length of this repetitive element modulated promoter activity in an in vitro reporter assay, with the highest promoter activity occurring in constructs bearing the longest (CA)23 element. Further in vitro reporter analyses used MMP-9 promoter constructs in which the (CA) element was deleted or replaced with a nonrepetitive sequence. These studies verified that this repetitive element is important for regulation of the promoter (57). Long MMP-9 alleles [(CA)23] resulted in higher levels of MMP-9 promoter activity and were present in greater frequency in patients with an intracranial aneurysm compared with healthy controls. This increased frequency may result in subtle differences in MMP-9 activity and consequent degradation of Type IV collagen within the cerebral vasculature, thereby increasing susceptibility to intracranial aneurysm formation. To our knowledge, our report was the first to describe a functional polymorphism in a regulatory molecule as associated with aneurysm formation.

Finally, when tissue from mice with collagen deficiency (secondary to dietary-induced lathyrism) was subjected in vitro to hemodynamic stressors, aneurysm formation reliably resulted (27, 62). This finding also supports the concept that a genetically heterogeneous process degrades key structural proteins, thereby potentially predisposing vascular connective tissue to become aneurysmal when subjected to hemodynamic forces. We think that the alterations observed in these extracellular matrix proteins are the result of a complex process of arterial homeostasis and that they represent the net effect of this process. This leads to the question: what are the factors that regulate arterial homeostasis and result in the degradation of these matrix proteins?

Other Candidate Molecular Markers

There is evidence of a continuous homeostatic mechanism that repairs ongoing arterial wall (wear-and-tear) degradation and further evidence that disruption of this process of vascular remodeling results in aneurysm formation (16, 19, 31). Skirgaudas et al. (76) attempted to characterize some of the factors involved in this process and identify potential markers. All 10 of their patients with aneurysms had increased levels of vascular endothelial growth factor, and nine had increases in basic fibroblast growth factor; none of the three controls had increased immunoreactivity for either of these factors. Both factors are known to be involved in the sprouting of new vessels from preexisting vascular beds (4, 14, 20). In addition to mediating vascular proliferation, these factors also affect migration and adhesions to endothelial cells.

Vascular endothelial growth factor is produced by astrocytes and is an important regulator of vascular permeability through activation of pathways that cause the enzymatic breakdown of matrix proteins forming the vessel wall (2, 20, 44, 66, 88). Fibrocytes and myocytes release basic fibroblast growth factor, which acts synergistically with vascular endothelial growth factor to further degrade matrix proteins. These two angiogenic factors may play an important role in vascular remodeling and maintaining structural integrity in response to stress and injury. Although the increased levels may simply be an epiphenomenon reflecting a general stress response, the implications of matrix wall degeneration may provide an important clue to understanding aneurysm pathogenesis.

On the basis of this information, Skirgaudas et al. (76) proceeded to the next step of characterizing the changes in the structural wall proteins in aneurysm patients. They noted a regular band of Type IV collagen in the media and subendothelium of all control patients. This feature was absent in patients with aneurysms. Instead, they saw faint and diffuse expression of this protein within the media of the patients with aneurysms, perhaps as a function of increased Type IV collagenase activity. Fibronectin, another constituent of extracellular matrix, also had a disorganized arrangement in the aneurysm wall (76). Disruption of both of these basement membrane proteins likewise has been described in a rat aneurysm model (77). This latter finding further suggests the possibility of an enzymatic degradation process, perhaps associated with increased angiogenesis factors and induction of proteolysis. The resulting alteration within the matrix wall compromises structural integrity and may culminate in aneurysm formation. If one accepts that there is constant flux of repair and degradation, the question arises: what mediates this homeostatic process?

MMPs

An important clue may come from the study of MMPs, which are endopeptidases with selective and specific activities against the extracellular matrix of basement membranes (66). These have been extensively studied in the dissemination of metastatic carcinomas, and three subgroups have been characterized: interstitial collagenase, stromelysin, and gelatinase (Type IV collagenase). On the basis of tumor invasion models, the metastatic cascade involves the following: 1) attachment to the extracellular matrix; 2) creation of a proteolytic defect in the extracellular matrix; and 3) migration through the proteolytically modified matrix.

There may be similarities in these processes leading to metastatic spread and aneurysm formation. Specifically, the critical early event in metastatic dissemination seems to be vascular basement membrane degradation, which is also the case in aneurysm formation (66). Examining the factors involved with this process may provide insight into the mediators of the homeostatic mechanism responsible for arterial wall degradation and repair.

Type IV collagen is a basement membrane component that forms the scaffolding on which laminin and heparan, as well as other minor components, assemble (92). In the metastatic cascade, the disruption of Type IV collagen seems essential in creating a defect in the basement membranes that allows for cellular invasion (48, 84). The gelatinase subgroups of the MMPs are vital for initiating this defect. As discussed earlier, Chyatte and Lewis (12) demonstrated an association between collagen degradation and increased gelatinase activity, and we have confirmed functional polymorphic changes in Type IV collagenase (MMP-9) in patients with aneurysms (57). Understanding this process may be critical in finding the factors that lead to disruption in the structural matrix proteins. Specifically, persons vulnerable to aneurysm formation may have accelerated collagenase activity (MMP-9) on the basis of a functional polymorphism, such as the previously described long (CA)23 repeat sequence. These individuals would have normal Type IV collagen expression but develop a functional deficiency secondary to polymorphism-induced accelerated degradation. Therefore, understanding the dynamic process of arterial wall tear and repair, partially regulated by the MMPs, is important in understanding altered arterial homeostasis that is possibly implicated in aneurysm formation.

This mechanism becomes even further complicated by the presence of tissue inhibitors of the MMPs (TIMPs), as there exists a balance between the activated MMPs and TIMPs that results in the net degradation of arterial wall basement membranes. The TIMPs have three subgroups: 1) TIMP 1, a glycoprotein that interacts with gelatinase, resulting in protease inhibition; 2) TIMP 2, nonglycosylated, which inhibits Type IV collagenolytic activity; and 3) TIMP 3, a protein localized within the extracellular matrix that binds with its components.

Separate genes located on different chromosomes encode each of these proteins. Their expression is independently regulated, again suggesting genetic heterogeneity. The next question: can any of the above putative disruptions in the homeostatic mechanisms be correlated with known histopathological and ultrastructural changes observed in aneurysmal lesions?

Ultrastructural Correlation of Literature-based Markers

In a seminal 1975 article, Stehbens (82) reported that the subendothelial space adjacent to the aneurysm wall contained bare patches of endothelium where the basement membrane had separated from it. This separation was common, and the membrane often contained amorphous fibrillar material.

Stehbens (82) further noted that the sac wall had widely separated cells with abundant intercellular space. A more interesting finding was that although the collagen fibers were somewhat variable in caliber with haphazard arrangement, the actual structure of the collagen was not abnormal. This again suggests that the collagen loss associated with aneurysms is attributable not to impaired collagen synthesis but to accelerated degradation of existing collagen. Stehbens did not study other structural matrix proteins.

It has been established that the internal elastic lamina is consistently absent at the base of the aneurysm and is thin and fragmented at its periphery (81). Kim et al. (40) induced aneurysm formation in rats by exposing them to hemodynamic stressors in conjunction with a lathyrism-rich diet. In this model, they noted a defect in the internal elastic lamina and the media and therefore postulated remodeling whereby the internal elastic lamina is catabolized on the endoluminal side and synthesized on the media side. This homeostasis normally maintains a thick internal elastic lamina. The investigators demonstrated that the catabolic effects dominated at the apex of the aneurysm (weakest portion), resulting in degradation of the internal elastic lamina, which may be related to hemodynamic stressors activating predisposed bifurcation. They were unable to identify the specific factors that mediate this process to result in an altered structural matrix.

SUMMARY OF THE LITERATURE-BASED MARKERS

By examining the molecular alterations and the mediators responsible for arterial wall degradation and repair (arterial homeostasis), it may be possible to identify molecular markers to distinguish individuals at high risk for cerebral aneurysms. Furthermore, understanding this dynamic environment may provide a means to identify patients with aneurysms at greatest risk for rupture. *Table 1* summarizes the key literature to date for investigations of the molecular alterations associated with cerebral aneurysms.

These studies represent the initial critical investigations delineating the molecular alterations associated with cerebral aneurysms. The focus on collagen reflects the alterations observed in the genetic syndromes associated with intracranial aneurysms and indicates that extracellular matrix proteins were the first to be considered probable candidates. It is only recently that Chyatte and Lewis (12), Peters and Kassam (56), and Skirgaudas et al. (76) broadened the search to include molecules associated with arterial homeostasis. A major limitation of the studies cited above is that each focused on a single molecular marker. The process of arterial repair and tear is surely a complex system, involving multiple molecules that respond to regional hemodynamic flow patterns. The function of these molecules is to maintain the appropriate vascular tone in this dynamic system. This system also involves a compensatory process of repair in response to the stressors leading to degradation. The net result of the extracellular matrix construct probably reflects the balance of all of these factors—arterial homeostasis.

During the past decade, researchers have increasingly considered molecules other than collagen as putative molecules critical in aneurysm formation. We have considered the possibility that the ability of the cerebrovasculature to repair itself is impaired after moment-to-moment microinjury. An important mediator in this process is secreted protein rich in cysteines (SPARC) (osteonectin), which is a counteradhesive glycoprotein expressed in a variety of tissues, including vascular endothelium, smooth muscle, and fibroblasts. SPARC is known to inhibit endothelial cell adhesion and proliferation. SPARC and messenger ribonucleic acid levels are increased in renal vascular injury (59). It has been proposed that extracellular matrix remodeling is regulated in part by an interaction between SPARC and Type I collagen (34). We previously reported a differential expression of SPARC within aneurysmal tissue as compared with pericranial vascular control tissue (56).

Another notable messenger ribonucleic acid in which elevated expression may be characteristic of intracranial aneurysms is cdc-rel2a/PNUTL2 (55). This factor belongs to an expanding family of guanosine triphosphate-binding proteins, called septins, which are thought to be involved in cytokinesis (13). Vinculin, an adhesion plaque protein, and c-Abl, a nonreceptor tyrosine kinase recruited from the nucleus, both migrate to focal adhesion sites. Through their interaction with fibronectin, these proteins are implicated in the phosphorylation of the protein paxillin. It is noteworthy that paxillin, an integrin, is a substrate for SPARC-induced tyrosine phosphorylation (56).

A search for molecular markers that can be used for screening purposes must take into account the entire spectrum of arterial homeostasis that leads to the architectural failure evidenced by the well-studied alterations in extracellular matrix proteins. *Table 2* summarizes these additional key markers and suggests some novel markers on the basis of factors critical to maintaining arterial homeostasis.

CONCLUSION

The need for screening markers that can predict the likelihood of aneurysm formation or rupture is obvious. We are at

TABLE 2. Key molecular markers, series, and justification[a]

Molecular marker	Type of molecular (role in homeostasis)	Series (ref. no.)	Comments/justification
Type III collagen (COL 3A1)—allele *A* and allele *B*	Structural matrix protein (arterial integrity)	Neil-Dwyer et al., 1983 (52) Brega et al., 1996 (6) Østergaard and Oxlund, 1987 (54) Leblanc et al., 1989 (43) Kuivaniemi et al., 1993 (42) Majamaa et al., 1992 (47) Chyatte and Lewis, 1997 (12)	Initial molecule of interest based on structural alterations in genetic syndromes associated with cerebral aneurysms Controversial and conflicting results
Fibronectin, laminin, and heparan	Structural matrix proteins (arterial integrity)	Skirgaudas et al., 1996 (76) Stehbens, 1961 (80)	Poorly studied and implicated based on structural matrix function
Elastase, α-1-antitrypsin	Proteases (arterial degradation)	Schievink et al., 1994 (73) Peters et al., 1999 (57) St. Jean et al., 1996 (78)	Association studies linking smoking with aneurysm formation
Type IV collagenase (MMP-9)	Metalloprotease (arterial degradation)	Peters et al., 1999 (57)	Polymorphism with increased activity
VEGF and bFGF	Angiogenesis factors (flow responsive and arterial repair)	Skirgaudas et al., 1996 (76)	Regulators of arterial homeostasis
PSF	Flow-responsive molecule	Mizushima et al., 1996 (49) Hata et al., 2000 (26) Moncada et al., 1976 (51) Moncada et al., 1978 (50)	Role in flow-dependent vascular tone relaxation Not yet directly studied in patients with aneurysm
RAI, PNUT, SPARC	Tissue-reparative molecules	Paavola et al., 1999 (55) Cooper and Kiehart, 1996 (13) Iruela-Arispe et al., 1996 (34) Pichler et al., 1996 (59)	Role in homeostasis by repairing arterial wall injuries Not yet directly studied in patients with aneurysms

[a] MMP-9, matrix metalloproteinase-9; VEGF, vascular endothelial growth factor; bFGF, basic fibroblast growth factor; PSF, prostacyclin-stimulating factor; SPARC, secreted protein rich in cysteines.

a relatively early but critical stage in defining these markers. The progression has been to develop epidemiological risk factors and then radiological predictors of aneurysm rupture; so far, this has proven to be unreliable. The answer probably will come from an analysis of the dynamic molecular milieu that surrounds intracranial aneurysms.

The molecular search has focused predominantly on the extracellular matrix, which is a reflection of the collagen vascular syndromes associated with aneurysm formation. We suggest a broader search of the entire process of arterial homeostasis. There are at least three critical components to homeostasis: 1) flow modulation; 2) arterial wall degradation and repair; and 3) the extracellular matrix that results. In this review, we have discussed the current screening options; presented the literature regarding the extracellular matrix, which often is confusing; and introduced the concept of arterial homeostasis. A focus on molecular markers associated with arterial homeostasis is critically needed, and it is our hope that this review further fosters this pursuit.

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COMMENTS

In this extensive review of the literature, the authors make a case for a systematic review of molecular markers for the causes of cerebral intracranial aneurysms. The article includes a summary of the present state of epidemiological knowledge and the relationship to known risk factors such as smoking, hypertension, and sex. The authors further observe the relatively nonspecific nature of these risk factors. Therefore, they attempt to develop an epidemiological approach leading to a mechanical theory regarding the presence of developing aneurysms as a defect in the repair and remodeling that occurs with flow in all vessels. They then discuss target proteins first derived from known inherited disorders. The authors reinforce the theory that this is a heterogeneous polymorphic change, possibly in regulatory molecules, and possibly with different causes and different circumstances. All seem to lead to disruption of the basement membrane or the extracellular matrix.

The authors make the strong argument that the associated conditions, multiple causes, and multiple locations suggest a multiplicity of sources with a common mechanism of disruption of vascular remodeling. They recommend a systematic review for molecular markers of disorders. Given the relatively increased access to vessels in this condition, it is suggested that such studies may be possible. We look forward to focused studies that translate information from the basic pathophysiology of disease to potential diagnostic and therapeutic interventions.

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In this article, the authors to summarize current screening modalities for intracranial aneurysms, review the current state of understanding of the genetic basis of intracranial aneurysms, and suggest a broader theory of aneurysm pathogenesis to form the foundation of a coordinated molecular search of biological markers that may be associated with aneurysm formation and rupture. The authors present an overview of the literature in detail, and they provide data regarding the prevalence of intracranial aneurysms and clinical and financial consequences of aneurysm rupture. Consequently, they elucidate current concepts for screening and review the evidence for the contribution of epidemiological and environmental risk factors. The core of the review, however, is the biological rationale for selecting candidate markers. The section regarding genetic/molecular markers that have been reported in the literature discusses contradictory published results of the role of Type III collagen and mutations of this gene in the pathogenesis of intracranial aneurysms. In addition, they discuss vascular endothelial growth factor, matrix metalloproteinases, and tissue inhibitors of metalloproteinases, which are genes in the center of interest.

This article provides a comprehensive review of these topics. In particular, it discusses and clarifies contradictory results published in the field. The identification of genetic markers associated with aneurysm predisposition is a highly ambitious goal. It would be of great interest to find genes and their variants involved in aneurysm formation and to use these for identification of individuals at risk. Furthermore, a better understanding of the molecular pathogenesis of aneurysm development is a prerequisite for targeted molecular prevention of the disease.

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This is an innovative article in a series by Kassam et al. This article, the first in the series, is a review of the literature supporting the authors' main hypothesis that alterations in vascular homeostasis dependent on genetic factors are important determinants in the development and eventual rupture of intracranial aneurysms. The article progresses from the obvious relationship among some genetically based connective tissue diseases and the incidence of intracranial aneurysms to a review of some of the initial genetic marker studies related to aneurysm formation. The authors state that the initial studies looking for genetic markers of aneurysm formation have concentrated on extracellular matrix proteins related to collagen; their hypothesis is that proteins associated with vascular homeostasis, i.e., reparative genetic products and those associated with response to shear stress and remodeling, are important in the pathophysiology of aneurysm formation. Their stated goal is to find markers that can predict aneurysm formation and allow preemptive treatment as well as to understand the biology of this disease. This article provides a thorough review of this topic and services as a useful repository of this information. Furthermore, the article serves as a

means by which the authors can present their hypotheses in a format that fully explains the context of their work.

The second article (1) provides an epidemiological approach to answering some of the questions posed in the first article. The authors evaluated the presence of a variety of vascular homeostatic and structural genes in aneurysm domes (ruptured and unruptured) and superficial temporal or occipital arteries from patients with and without aneurysms. They measured messenger ribonucleic acid (mRNA) associated with modulation of arterial flow (prostacyclin-stimulating factor [PSF]), vascular repair (*PNUT*, secreted protein rich in cysteines [SPARC], and *RAI*), and structural strength (fibronectin and collagen Type III). The authors demonstrated a decrease of prostacyclin-stimulating factor and *RAI* in aneurysm domes compared with superficial temporal arteries (STAs). There was no difference in the expression of mRNA in the STAs of patients with aneurysms versus those without aneurysms. Among the subgroup of smokers, there was no difference between gene expression in the aneurysm domes and the STAs. In nonsmokers, however, PSF was decreased in the domes compared with the STAs. Smoking significantly affected the odds ratios of the presence of prostacyclin-stimulating factor and *RAI*. This article gives hints at mechanisms associated with aneurysm formation and rupture, i.e., the alteration in genetic responses to flow and the ability to mount continuing tissue repair. That smoking influences these factors is, in a way, validation of the findings. It is unfortunate that there is no difference in the STAs of patients with and without aneurysms. Therefore, the idea that the tested mRNA forms the genetic basis for aneurysm formation is questionable. It is more likely that the anatomic and histological changes associated with aneurysm formation lead to the alterations in mRNA expression. This article provides significant insight into some of the biological mechanisms associated with aneurysm growth and rupture. This is clearly only the beginning of understanding the biology associated with this disease process.

These articles represent a basic biological approach to a devastating clinical problem. The initial results of some of the studies were disappointing; for instance, the authors discovered no difference in the STAs of patients with and without aneurysms. However, these articles provide value in two regards. First, this is a beginning to understanding the details of the biological basis of aneurysms. Second, and perhaps more important, is the contribution to development of an experimental technique template that can point the way toward study of other important brain diseases.

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1. Kassam AB, Horowitz M, Chang Y-F, Peters D: Altered arterial homeostasis and cerebral aneurysms: A molecular epidemiology study. **Neurosurgery** (in press).

In recent years, there has been an explosion of information regarding the biological basis of arterial wall formation and maintenance. There also has been a cogent characterization of molecular mechanisms of a number of monogenetic syndromes associated with vascular wall degeneration and intracranial aneurysms. However, the problem of berry aneurysms predisposing to subarachnoid hemorrhage remains the hallmark of a complex genetic disease. It is probable that a complex interplay of multiple genetic predispositions interact with host-related factors and other factors to affect the pathogenesis of these lesions. Hence, monomolecular approaches for markers of intracranial aneurysms have not yielded, and are not likely to yield, the simple answers desired by clinicians and by patients. Instead, aneurysmal disease is probably the result of a number of different pathways leading to arterial wall degeneration.

Kassam et al. have succeeded brilliantly at summarizing evolving concepts in this field. They have reviewed a number of approaches focused toward single genes or molecular substrates and synthesized this knowledge into a broader and more unifying scheme. Although the task seems highly complex, emerging bioinformatic and other methods are providing novel and powerful tools to tackle the problem. In a future article (1), the authors propose a molecular screening approach to try to dissect some of the complex factors at play. Other approaches, including genomics and proteomics, also will provide information on complex multigenetic and multimolecular factors operating simultaneously. A problem remains, however, that the arterial aneurysm is an end-stage phenotype, and host predisposition is only a pathogenetic trigger. Working on the lesion will reveal a complex set of secondary phenomena, all of which are important in the final disease phenotype. Working on the host predisposition may yield a purer link to the original pathogenetic triggers without revealing much information regarding subsequent critical contributing factors. Indeed, progress in this field will depend on a combination of integrational and reductionalist biological research.

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1. Kassam AB, Horowitz M, Chang Y-F, Peters D: Altered arterial homeostasis and cerebral aneurysms: A molecular epidemiology study. **Neurosurgery** (in press).