Are Cerebral Aneurysms Atherosclerotic?

Jane Adamson, MSc; S.E. Humphries, PhD; J.R. Ostergaard, MA; B. Voldby, PhD; P. Richards, FRCS; J.T. Powell, MD

Background and Purpose The aim of our study was to investigate plasma and genetic risk factors for rupture of cerebral aneurysms.

Methods In London, a case-control study was made of 56 consecutive patients admitted to a regional neurological service for treatment of ruptured cerebral aneurysm and of 93 control subjects. A further 40 consecutive patients admitted in Aarhus with ruptured cerebral aneurysm also were studied.

Results The British case-control study showed that smoking was associated with an increased risk of ruptured cerebral aneurysm (odds ratio, 9.1; 95% confidence interval [CI], 3.4 to 23.8; P<.001 for a history of >10 pack years). After age and sex adjustment, factors associated with ruptured cerebral aneurysm included a cholesterol concentration in the highest tertile (>6.3 mmol/L; odds ratio, 10.2; 95% CI, 3.9 to 26.7; P<.001), an apolipoprotein B concentration in the highest tertile (a0.84 g/L; odds ratio, 6.4; 95% CI, 2.5 to 16.3; I²<.001), and concentrations of HDL cholesterol in the lowest tertile (<1.1 mmol/L; odds ratio, 3.6; 95% CI, 1.4 to 8.2; P<.01). History of hypertension was of less importance (odds ratio, 4.0; 95% CI, 1.41 to 11.7; I²<.01). Smoking history (I²<.001) and increased concentrations of cholesterol (P<.0001) were the most important independent risk factors associated with ruptured cerebral aneurysm on multivariate analysis. The histories of hypertension and smoking, together with apolipoprotein B levels, in the Danish patients were similar to those in the British patients. In the entire patient group, the frequencies of two polymorphic variations in the type III collagen gene and polymorphisms at the apolipoprotein B, apolipoprotein C-III, and haptoglobin gene loci were not different from control subjects or the normal population; allele frequencies in British and Danish patients were similar.

Conclusions An atherosclerotic profile including increased total cholesterol concentration and a long smoking history may contribute to the rupture of cerebral aneurysms. This study provides no support for the hypothesis that inherited abnormalities of type III collagen are a common cause of cerebral aneurysms. (Stroke. 1994;25:963-966.)

Key Words • atherosclerosis • cerebral aneurysm • genetics • risk factors • smoking • subarachnoid hemorrhage

Rupture of a cerebral aneurysm is the most common cause of subarachnoid hemorrhage (SAH), particularly in persons over 30 years of age in whom it accounts for 70% to 90% of all SAH.1-2 The incidence of SAH resulting from ruptured cerebral aneurysm increases with age and is more common among women than men.1-2 The reasons for the development and rupture of cerebral aneurysms are not clear, but three main risk factors have been considered: congenital or inherited defects weakening the arterial wall, hypertension, and atherosclerosis.3

Inherited abnormalities in type III collagen have been suggested as an important factor contributing to an altered vascular extensibility and weakening of the cerebral artery wall.4-5 The widespread recognition and treatment of hypertension and improved neurosurgical management may have had an impact on the apparent decline in mortality from SAH, which has been reported in Sweden and elsewhere.5-6 Atherosclerosis is a widespread disorder in Western societies that results from complex interactions between environmental and genetic risk factors. We have investigated a cohort of 96 patients for plasma and genetic risk factors that may predispose to the weakening of cerebral artery walls. The genetic risk factors include polymorphic variation in the type III collagen gene and polymorphic variations in apolipoprotein genes.

Subjects and Methods

Consecutive patients with ruptured cerebral aneurysms proven by angiography were recruited from Charing Cross Hospital (n=56) between October 1989 and December 1991. Consecutive patients from Aarhus Kommunehospital (n=40) were recruited during 1991 to increase the number of patients for the genetic studies. The patients or their relatives were interviewed about medical, family, and social history and a single anticoagulated (ethylenediaminetetraacetic acid) blood sample obtained on admission. The patients included 43 men and 53 women (median age, 48 years; range, 25 to 70 years); none were diabetic. No patient was taking lipid-lowering drugs. Among the women, 18 (34%) were postmenopausal; none were taking hormone replacement therapy, and of the remainder only 5 (9%) were taking oral contraceptives. Healthy control subjects, 56 men and 37 women, were recruited consecutively from an occupational cardiovascular screening program (1989 to 1990) and had a mean age of 44 years (range, 25 to 64 years); the screening procedure for carotid, coronary, and peripheral arterial disease was as described previously.7

From the blood sample, plasma was separated for the determination of cholesterol, high-density lipoprotein (HDL) cholesterol by Mg2+ dextran precipitation, apolipoprotein (apo) B by end-point immunonephelometry, cotinine by gas-liquid chromatography, and haptoglobin and α1-antitrypsin phenotype by polyacrylamide gel electrophoresis. Genomic DNA was isolated from the peripheral leukocytes. Polymorphic variation at the Ava II
site in the 3' noncoding region of the type III collagen gene (COL3A1) was determined by Southern blotting, and variation at the Ala I site in exon 31 was determined after amplification of the relevant portion of genomic DNA using the polymerase chain reaction. The polymerase chain reaction was used also to amplify DNA surrounding the variable Pvu II site in the apo C-III gene and the variable Xba I site in the apo B gene. All the biochemical and genetic marker analyses were performed at Charing Cross and Westminster Medical School.

Statistical Analyses
Results for the patient and control groups were compared using logistic regression analysis, correcting for age and sex. Odds ratios with 95% confidence interval (CI) were used to judge the strength of association between each variable and disease, with continuous variables categorized into tertiles. Categorical variables (eg, sex) were compared using the x² test with Yates' correction. Multiple logistic regression analysis was used to identify variables independently associated with disease, adjusted for age and sex.

Results
Demographic Characteristics
The demographic characteristics of the 40 Arhus patients, 56 London patients, and 93 London control subjects are given in Table 1. The distribution of cerebral aneurysms was internal carotid artery, 30%; anterior communicating artery, 26%; middle cerebral artery, 30%; and other or multiple arteries, 14%. Sixty-eight patients (71%) were current smokers before admission (serum cotinine >200 nmol/L), and only 9 (9%) had never smoked. The plasma concentrations of cholesterol, HDL cholesterol, apo B, and cotinine also are given in Table 1. Both the total cholesterol and the HDL cholesterol concentrations of the Arhus patients were significantly lower than those of the London patients, but the ratios of HDL cholesterol to cholesterol and apo B concentrations were similar. Other demographic features of the British and Danish patients were similar.

Case-Control Study: British Patients Versus British Control Subjects
There was no significant difference in age or sex between the patients and control subjects (Table 1). The risk of ruptured cerebral aneurysm was associated with a history of smoking (odds ratio, 9.1 for >10 pack-year history; 95% CI, 3.4 to 23.8; P<.001) and with a history of hypertension (odds ratio, 4.0 for previous usage of antihypertensive medication; 95% CI, 1.4 to 11.7; P=.009). The risk of ruptured cerebral aneurysm with increasing concentrations of total cholesterol and apo B and decreasing concentrations of HDL cholesterol is shown in the Figure. The age- and sex-adjusted odds ratio increased very significantly for the highest tertile of cholesterol (≥6.3 mmol/L) to 10.2 (95% CI, 3.9 to 26.7) and for the highest tertile of apo B (≥0.84 g/L) to 6.4 (95% CI, 2.5 to 16.3) (Figure).

When all the above factors were included in a multiple regression analysis, the two most important independent predictors of ruptured cerebral aneurysm were history of smoking (P<.001) and increased concentrations of plasma cholesterol (P<.001). Apo B also remained an independent risk factor (P<.02) (Table 2).

Genetic Factors
Among the 96 patients, 7 (7%) reported having at least 1 first-degree relative with a previously ruptured cerebral aneurysm (3 mothers, 2 fathers, 2 brothers, 1 sister). Genetic variation at loci in the type III collagen gene and the apo B and C-III genes in the 96 patients is reported in Table 3. Allele frequencies in British and Danish patients were closely similar. Therefore, results for the two groups have not been reported separately. All genotypes were in Hardy-Weinberg equilibrium, and no difference in frequency compared with the frequencies of these polymorphisms either in the control group or those previously reported in healthy British populations was observed (Table 2). In a previous study it was observed that variation at the apo C-III locus was associated with differences in triglyceride levels. Triglyceride levels were not measured in this study because fasting blood samples were not available, but variation at the apo C-III locus was associated with ratios of apo B to cholesterol, patients with the rare allele having the highest ratios. When cholesterol levels
were adjusted for age and sex, these differences failed to reach conventional levels of statistical significance (P=.08). Haptoglobin phenotypes also were normally distributed among the patients. Only the British patients were phenotyped for α1-antitrypsin: the frequency of the Z allele was 0.04, but none of the patients carrying the Z allele reported having a first-degree relative with cerebral aneurysm.

### Discussion

The autopsy incidence of cerebral aneurysms is about 5%. The rupture of these aneurysms is the principal cause of SAH. The only large prospective study of SAH has indicated that both hypertension and smoking were important risk factors. Adverse effects of smoking on SAH also have been shown in other studies. Current smoking of >20 cigarettes per day was particularly strongly associated with the risk of SAH. Our study revealed a history of heavy smoking in both British and Danish patients; in particular, 71% of patients with ruptured cerebral aneurysm were smoking before admission (plasma cotinine concentration >200 nmol/L). Smoking, and hypercholesterolemia and hypertension, are the major risk factors for atherosclerosis. Hypercholesterolemia may be evidenced by increased concentrations of apo B and decreased concentrations of HDL cholesterol, both of which are considered important risk factors for atherosclerosis. Both genetic and environmental factors, together with complex interactions between these factors, contribute to the development of atherosclerosis. A similar situation could prevail for cerebral aneurysms, and the investigations reported here were initiated to assess some possible genetic factors contributing to the development and rupture of cerebral aneurysms. The background environmental factor of smoking was similar in the two patient populations studied, British and Danish (Table 1). However, other factors strongly influenced by the environment (eg, plasma cholesterol) were different in the two populations. Therefore, the healthy subjects taken from a cardiovascular screening program endorsed by employers in the London area can function as control subjects for the British patients only, particularly with respect to factors influenced by the environment. When British patients were compared with British control subjects, smoking together with increased concentrations of cholesterol and apo B emerged as the important independent risk factors associated with rupture of a cerebral aneurysm. Both smoking and increased cholesterol concentrations were more potent risk predictors than hypertension or HDL cholesterol concentrations. However, many patients may have had unrecognized hypertension before the sudden rupture of a cerebral aneurysm. For this reason, together with the relatively small numbers in the case-control study, our conclusions must be tempered with caution. A high prevalence of smoking and high apo B concentrations also were observed in the Danish patients. These patients with ruptured cerebral aneurysm seem at increased risk of atherosclerosis: 7 patients already had suffered a myocardial infarction, and another had had an aortic aneurysm repaired.

Earlier this century atherosclerosis was considered a risk factor for cerebral aneurysm, but more recently the consideration that weakness of the cerebral arterial wall caused by inherited abnormalities in type III collagen has been both favored and disputed. For a disease with an autopsy incidence of 5%, it was perhaps not surprising that 7% of patients with cerebral aneurysm reported having first-degree relatives who were affected similarly. However, this study was not designed to investigate familial aggregation of cerebral aneurysms but rather to assess...
TABLE 3.  Genetic Variation In Patients With Ruptured Cerebral Aneurysm

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphic Restriction Site</th>
<th>No. Typed</th>
<th>Genotype</th>
<th>Rare Allele Frequency (95% CI)</th>
<th>Frequency of Rare Allele in Control Subjects and (Healthy Population)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type III collagen</td>
<td>Alu I</td>
<td>96</td>
<td>1-1</td>
<td>0.281 (0.222-0.352)</td>
<td>0.29</td>
</tr>
<tr>
<td>Apolipoprotein B</td>
<td>Xba I</td>
<td>94</td>
<td>1-2</td>
<td>0.489 (0.418-0.560)</td>
<td>0.48 (0.51)</td>
</tr>
<tr>
<td>Apolipoprotein C-III</td>
<td>Pvu II</td>
<td>91</td>
<td>2-2</td>
<td>0.284 (0.200-0.330)</td>
<td>0.24 (0.26)</td>
</tr>
<tr>
<td>Haptoglobin</td>
<td></td>
<td>96</td>
<td></td>
<td>0.359 (0.291-0.427)</td>
<td>0.34 (0.35)</td>
</tr>
</tbody>
</table>

Table and coordinated text:

the contribution of genetic factors to the risk of rupture of cerebral aneurysms through a study of relevant polymorphic genetic markers. Because the frequency of polymorphic genetic markers was closely similar in the British and Danish patients, the results have been aggregated. The similar frequency of two widely spaced polymorphic markers in the type III collagen gene in the patients, control subjects, and normal population provides no support for the hypothesis that a common mutation in this gene predisposes to weakness in the cerebral arteries and hence aneurysm rupture.

Apolipoproteins, the protein components of lipoproteins, control lipid metabolism. Apo B is the protein component of low-density lipoprotein particles whereas apo C-III, an inhibitor of lipoprotein lipase, is found in HDL and very-low-density lipoprotein particles. Polymorphic variation in these genes has provided indications of the genetic component to atherosclerosis and hyperlipidemia.19 In particular, associations have been reported between the Xba I polymorphism in the apo B gene and coronary artery disease and between the Pvu II polymorphism of the apo C-III gene and hypertriglyceridemia.20,21 Polymorphic variants of haptoglobin have long been used for disease linkage studies, particularly for the disordered lipid metabolism resulting from mutation in lecithin:cholesterol acyltransferase and for aortic aneurysms. However, the distribution of apo B and C-III genotypes and haptoglobin phenotypes provides no evidence for a genetic predisposition to cerebral aneurysm caused by variation at these loci. Interestingly, there was an unexpectedly high frequency of the α1-antitrypsin Z allele, present in 4 of 50 patients phenotyped. Any disturbance of protease/antiprotease balance could potentiate the damaging effects of smoking or atherosclerosis on the weakening of connective tissue in the arterial wall.21

Atherosclerosis is often evident in cerebral aneurysms. While such atherosclerosis may be aggravated by flow disturbance, it also will be aggravated by smoking and increased cholesterol concentrations. Our study indicates that both current smoking and dyslipidemia should be considered as more important factors than mutations of type III collagen in contributing to the rupture of cerebral aneurysms.

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References

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