The influence of genetics on intracranial aneurysm formation and rupture: current knowledge and its possible impact on future treatment

B. KRISCHEK and M. TATAGIBA

Department of Neurosurgery, University of Tuebingen, Tuebingen, Germany

Contents

Abstract

The etiology of intracranial aneurysm formation and rupture remains mostly unknown, but lately several studies have increasingly supported the role of genetic factors. In reports so far, genome-wide linkage studies suggest several susceptibility loci that may contain one or more predisposing genes. Depending on the examined ethnic population, several different non-matching chromosomal regions have been found. Studies of several candidate genes report association with intracranial aneurysms. To date, no single gene has been identified as responsible for intracranial aneurysm formation or rupture.

In addition to the well-published environmental factors, such as alcohol intake, hypertension and smoking, only the recent progress in molecular genetics enables us to investigate the possible genetic determinants of this disease. Although a familial predisposition is the strongest risk factor for the development of intracranial aneurysms, the mode of Mendelian inheritance is uncertain in most families. Therefore, multiple genetic susceptibilities in conjunction with the environmental factors are considered to act together in the disease's etiology. Accordingly, researchers performed linkage studies and case-control association studies for the genetic analysis and have identified several genes to be susceptible to intracranial aneurysms. The identification of susceptible genes may lead to the understanding of the mechanism of formation and rupture and possibly lead to the development of a pharmacological therapy. Furthermore, should it be possible to identify a genetic marker associated with an increased risk of formation and rupture of an intracranial aneurysm, the necessity for screening and urgency of treatment could be determined more easily.

In this review we summarize the current knowledge of intracranial aneurysm genetics and also discuss the method to detect the causalities. In view of the recent advances made in this field, we also give an outlook on possible future genetically engineered therapies, whose development are well underway.

Keywords: Cerebral aneurysms; subarachnoid hemorrhage; genetic; intracerebral hemorrhage.

Introduction

Although the incidence of other kinds of stroke has declined in the last three decades the frequency of subarachnoid hemorrhage due to a ruptured intracranial aneurysm has remained the same. The peak incidence of suffering from a subarachnoid hemorrhage is in persons 55–60 years of age [26], whereas there is a preponderance of the female ratio of around 2:1 [76, 83]. Recent technical advances have changed intracranial aneurysm (IA) treatment dramatically. According to availability, coiling has taken over a large part of IA treatment in industrialized countries [75]. Nevertheless, mortality of individuals remains around 50% of patients who had suffered an intracranial aneurysm [44], while the survivors have a 30% danger of developing a persisting neurological deficit. Environmental factors associated with intracranial aneurysms such as hypertension, alcohol intake and smoking have all been well documented [20] but they alone can not be held responsible for IA formation and rupture. It has been shown that the risk of ruptured IA in first-degree relatives of patients with aneurysmal SAH is four times higher, and the relative risk in siblings is six times higher, than that in the general population [77, 81]. According to the period of follow-up the risk of rupture is between 0.6 and 1.3% per year [63, 95]. As the relative risk (RR) for rupture is 2.0 in patients

above the age of 60, it is also increased in the female gender (RR1.6) and in patients of Japanese and Finnish descent (RR3.4) [95].

Different epidemiology in different countries

Rupture rates in highly industrialized countries have been determined to be between 11/100,000 in the USA and 96/100,000 in distinct regions in Japan, which may again reflect the genetic component that plays a role in formation and/or rupture [39, 50, 63]. Even within Japan the incidence varies from 20 to 96/100,000 [36, 50, 90]. As of now, still little is known about the rates in highly populated countries such as India and China [38]. The increasingly wide-spread use of digital communication between IA treating departments that are in different locations allows easy access to each others documentation within different countries and will hopefully lead to further insight into the globally differing epidemiological data of IA.

A widely cited figure for the prevalence for asymptomatic unruptured intracranial aneurysms is 5% although the prevalence in the general population is unclear. The prevalence for all cerebral aneurysms according to autopsy procedures ranges from 0.2 to 9%, with a prevalence of 0.6 to 4.2% for unruptured aneurysms alone [96]. As an example, one of the largest autopsy studies of 1230 Japanese cases over a period of 30 years revealed a prevalance of incidental intracranial aneurysms to be 4.6% [41].

The increasingly sophisticated and susceptible means of non-invasive diagnostic imaging will further change early treatment modalities. Nowadays 3 Tesla MRIs can detect IAs with diameters as small as 2–3 mm. With health care systems providing extensive check-ups, e.g. – prophylactic brain MR scan in Japan [63, 101], the very early detection and treatment of IAs is bound to change.

Etiology of intracranial aneurysm formation and rupture

The detailed causes for intracranial aneurysm formation and rupture have not been elucidated, but there have been several studies on possible etiological causes. Inflammatory mechanisms [12], hypertension [37] and hormonal influence in the female gender [31, 42, 43] have all been connected to IA formation and rupture.

The typical intracranial artery is made up of three histological layers: 1) the inner layer (tunica intima) consisting of an endothelial layer and smooth muscle cells, 2) the muscular layer (tunica media) made up of the internal elastic lamina and SMCs and 3) the outer loose connective tissue layer (tunica adventitia) [78]. At bifurcation sites they have a gap in the continuity of the muscular media layer which are called medial gaps or raphés. This particular gap has often been

cited as a predisposing weakness to the formation of intracranial aneurysms due to a possible decrease in tensile strength. But ultrastructural examination at these sites has revealed a tendon-like organization of collagen fibers increasing the resistency to mechanical stretching [22, 85, 86]. In intracranial aneurysms the layers are often not clearly defined [24]. A lack of elastic lamina, disorganized smooth muscle cells, neointimal and myointimal hyperplasia as well as early atherosclerotic changes are common features [45, 51]. Structural abnormalities in structural proteins of the extracellular matrix have been identified in the arterial wall at a distance from the aneurysm itself. Reticular fibers were significantly decreased in the Tunica media of intracranial aneurysms as compared to those of control arteries [13].

Continuous pressure exerted at points of bifurcation around the circle of Willis are subject to aneurysm formation [18]. This leads to the conclusion that a vessel wall weakness predisposes an outpouching. Furthermore, structural weaknesses seen in connective tissue disorders are associated with the presence of IA and their rupture. Among them are diseases such as the autosomal dominant polycystic kidney disease, Ehlers-Danlos Syndrome, pseudoxanthoma elasticum and fibromuscular dysplasia [80]. Hereditary hemorrhagic telangiectasia (HHT) or Osler-Weber-Rendu syndrome is an autosomal dominant vascular disorder characterized by telangiectases, internal arteriovenous malformations and intracranial aneurysms. Endoglin gene mutations are responsible for HHT type 1 and ACVRL1 (activin receptor like kinase 1) mutations cause HHT type 2 [21]. A polymorphism of the endoglin gene has been correlated with intracranial aneurysm formation in a Japanese population but could not be replicated by others [54, 69, 89]. Several genes of the extracellular matrix have been examined regarding their immediate role in the vessel wall formation. Most recent reports have shown that irregularities in the elastin gene [2], the collagen gene [100] and the lysl oxidase like family gene 2 (LOXL 2), which cross links collagen and elastin [1], play a role in IA formation although replication studies in patients of different ethnic origin did not always reach the same conclusions [56].

Whether or not genetic susceptibilities play a role in the rupture of IA in combination with the well-known environmental factors, remains to be seen. In an article comparing a group of ruptured and unruptured intracranial aneurysm patients of Caucasian ethnicity it was found that three polymorphisms in the endothelial nitric oxide synthase gene (eNOS) could possibly indicate an enheightened risk of rupture [47]. In a larger study of Japanese patients none of the aforementioned polymorphisms of the eNOS gene could be verified [57].

Aside from the strucutral differences several findings of infiltrating inflammatory cells have been reported [12, 45, 92]. Macrophages and T-cells infiltrate all layers of the aneurysm vessel wall. Comparison between ruptured and unruptured aneurysm tissue has demonstrated similar histological findings in-

dicating a restrucuring process to have begun before the aneurysm's rupture [12, 15, 25, 51].

Vascular and cerebrovascular diseases associated with a genetic component

Family history studies and the results from studies of twins have shown a tendency for different types of stroke to cluster within families. Several mendelian and mitochondrial disorders cause cerebrovascular malformations, ischemic stroke as well as hemorrhagic stroke [62]. As an example, among the most intensively genetically researched cerebral vascular malformations are the cerebral cavernous malformations (CCM). Three linkage regions have been described: CCM1 on chromosome locus 7q21–q22, CCM2 on 7p13–15 and CCM3 on 3q25.2–q27. The genetic defects for CCM 1 are due to various mutations in the gene Krit1, which encodes for the Krev interaction Trapped 1 (Krit1) protein. CCM2 encodes the MGC4607 protein, also called malcavernin, and CCM3 the programmed cell death 10 (PDCD10) protein [60]. Other cerebrovascular malformations in which multiple genetic components are likely to play a role include brain arteriovenous malformations and Moyamoya disease [66]. Recently there have been reports on the association of polymorphisms of Interleukin-6 [8] and ACVRL1 [82] with arteriovenous malformations of the central nervous system.

Abdominal aortic aneurysms have been examined extensively, revealing similar results of a multigenic origin [67]. Comparative studies between aortic and intracranial aneurysms have yet to yield identical findings [49], but similar mechanisms of formation seem fathomable.

Approaches to genetic research of intracranial aneurysms

There are two major approaches for the identification of possible intracranial aneurysm genes. They are not mutually exclusive, but more complementary. One is to perform a **linkage** analysis which locates the locus of disease using DNA and clinical information of families (including more than one member). The other is the **association** approach that may comprise the whole genome or single candidate genes. It identifies potential disease alleles in a case-control design.

While linkage analysis is arguably the most powerful method for identifying a locus involving rare, high-risk alleles in Mendelian diseases, as was the case for the Krit1 gene in cerebral cavernous malformation [30] and Notch3 for CADASIL (cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy) [91], many consider genetic association analyses to be the best method for identifying genetic variants related to common and

complex diseases, such as for IA. The HapMap project, in particular, has made genomewide association studies the most powerful tool for identification of common alleles to common diseases. The recently emerged hypothesis, the so called "rare variant-common disease hypothesis", implies that several rare variants in a gene may be involved in the causality of a common disease [14]. If this is the case, an association study (even one that is genome-wide with high-density genotyping of single nucleotide polymorphisms) may not be able to detect the disease gene because most of the SNPs in the database are common SNPs designed to map common alleles. Therefore, both family-based genetic linkage studies as well as association studies are required for the full understanding of the genetics of IA.

Linkage analyses reveal chromosomal loci

Although the molecular basis of the disorder is not known, family studies strongly support genetic factors in the formation of IA [77]. Several studies of familial aneurysms have identified chromosomal loci showing suggestive evidence of linkage [56]. The mode of transmission for harboring an IA is not clear, and the genetics of the disorder appear to be complex, involving multiple loci and interaction of multiple genes [70]. In accordance with this, several genomewide scans and linkage studies have identified multiple chromosomal regions that may contain one or more susceptible genes. However, in some cases, results could not be replicated, even when examining patients of the same ethnic background [58, 70, 98, 99]. Onda *et al.* observed positive evidence of linkage on chromosome 5q22–31 (MLS 2.24), 7q11 (MLS 3.22) and 14q22 (MLS 2.31) with 104 affected sib-pairs. Yamada *et al.* observed positive evidence of linkage on chromosome 17cen (MLS 3.00), 19q13 (MLS 2.15) and Xp22 (MLS 2.16) with 29 extended families [98]. The inconsistency must be interpreted with caution. Discrepancies are possibly due to genetic heterogeneity and differences of patient cohorts (affected sib-pairs vs. extensive nuclear families). Further studies comprising larger sample sizes are undoubtedly needed, as multiple interacting genes and environmental factors contribute to the phenotype. Three regions that were confirmed in samples of patients of different ethnic origin are on chromosome 7q [19, 68, 70], 19q [19, 68, 98] and 14q [70, 72]. All regions were verified once using affected sib-pairs and once using extended pedigrees.

Alternatively, the rare Mendelian forms of disease might lead to the identification of genes or pathways that play a key role in the pathogenesis of the common form of the disease. Nahed *et al.* identified a large family of IA (six living patients and four deceased) showing autosomal dominant inheritance and detected a single locus with a LOD score of 4.2 at chromosome 1p34.3–36.13 [65]. Positional (candidate) cloning might be underway in the locus.

Candidate gene association analyses: positional and functional

Association studies and the selection of target genes and sequence variants have often been limited to the investigation of candidate genes selected because of a priori hypotheses about their etiological role in a disease. These studies depended on the ability to predict functional candidate genes and polymorphisms [88]. Positional candidate genes can be selected from regions that have been identified by linkage analyses or genome wide scans [27, 28, 33].

More than 25 different candidate genes have been examined in case-control studies by different groups using the DNA of patients with different ethnic backgrounds. Selection of these genes were usually either of functional or positional nature. After identifying several susceptibility loci on different chromosomes, many **positional candidate genes** have been looked at that are located in the found regions. On the other hand, many of the examined **functional candidate genes** play a role in connective tissue formation, such as the collagen gene [100], the elastin gene [2], the matrix metalloproteinases and their tissue inhibitor genes [52, 53] and the endoglin gene [89]. Only a few have shown moderate positive association. Considering the genetic role in the formation of IAs, some of the examined candidate genes potentially possess both attributes of function and position (e.g., the elastin gene [2] which makes up part of the extracellular matrix of the vessel wall and is located in the linkage region of chromosome 7q11 found in a Japanese sibpair linkage study and replicated in a group of white patients in Utah). Detailed contemporary descriptions and tables of all examined candidate genes and chromosomal loci have been published in recent articles [56, 64, 79]. Conflicting results have been obtained, and no single gene has been consistently identified as a candidate gene. Possible reasons for these inconsistencies are false-positive studies, false-negative studies and differences between populations. Inadequate sample size is a major cause for false-positive and false-negative results [40]. Other causes include population stratification, misclassification (genotyping or phenotyping errors), and inappropriate statistical methods.

Variability in the association between different populations may be due to the frequency of disease-causing alleles, the pattern of association between disease causing alleles or interacting genetic or environmental factors. Therefore, failure to replicate does not necessarily mean lack of causality but possibly point to the need for additional studies [17]. As mentioned most of the association studies to date have focussed on single polymorphisms, but recently joint effects of single markers on a haplotype level have been examined [2]. Haplotypes are a set of markers that are physically close to each other on the DNA strand and are therefore inherited as a unit (a set of alleles in strong linkage disequilibrium (LD)). Association between a polymorphism and a trait, such as intracranial aneurysm, does not necessarily imply causality. Instead, the polymorphism/haplotype under investigation may be in LD with the causative

sequence variant, requiring more detailed studies to identify the causative sequence alteration. Thus far, numerous association studies have been performed, some showing positive associations, some negative. But the majority of studies only examined small sample sizes and thus are mostly preliminary.

Many tested variants are single-nucleotide polymorphisms (SNPs) that change an amino acid and are therefore more likely to have a functional consequence. However, based on the successful positional cloning of disease genes for several common diseases, including schizophrenia [84], osteoporosis [87], myocardial infarction [33], ischemic stroke [28] and asthma [3], the most important variants are noncoding variants that affect the expression and/or efficiency of splicing. A large percentage of many organisms' total genome sizes is comprised of noncoding DNA. Some noncoding DNA is involved in regulating the activity of coding regions. However, much of this DNA has no known function and is sometimes referred to as "junk DNA". Recent evidence suggests that "junk DNA" may in fact be employed by proteins created from coding DNA [6].

Gene expression microarray analyses

One of the newer genetic research techniques is the microarray which allows examination of several thousand genes at once. Although its deployment is related to substantial costs, its efficiency can hardly be beaten. No other methodological approach has transformed molecular biology more in recent years than the use of microarrays. Microarray technology has led the way from studies of the individual biological functions of a few related genes, proteins or, at best, pathways towards more global investigations of cellular activity. The development of this technology immediately yielded new and interesting information, and has produced more data than can be currently dealt with. To many, the term microarray analysis is equivalent to transcript analysis. Although transcriptional profiling is unquestionably the most widely used application at present, it might become less important in future because it focuses on a biological intermediate. Currently a whole battery of sophisticated applications for this technology are being developed, e.g. for epigenetic studies, expanding RNA studies and probing with genomic sequences [35].

Concerning intracranial aneurysms there are several studies with this technique that are being completed worldwide. Analysis of the results of those genes differentially expressed within IA tissue will again shift focus towards hitherto unexamined targets.

Application of genetic findings to novel diagnostic tests and future therapies

Nowadays, there is a wide array of over-the-counter commercially available tests which, for example, lets users find out whether they are prone to develop

cardiovascular disease or are the carriers of Factor V Leiden, a single mutation that raises the risk for thrombophilia, or abnormal blood clotting. The results of these kinds of tests have to be interpreted with caution, as in some cases they could lead to false behaviour after negative results [16, 97]. Nevertheless, with increasing availability of such non-invasive diagnostic procedures, a wider spectrum of people can be screened at a relative cost-efficient basis, which in turn, in the case of intracranial aneurysms, could lead to timely prophylactic measures.

An early diagnosis through a genetic test, such as available for BRCA [4], could potentially spare patients from needless dangerous invasive diagnostic procedures or treatment. Furthermore, a precise diagnosis of an underlying genetic component could permit rational family counseling. Genetic markers showing an increased risk of rupture in patients harboring intracranial aneurysms that are of a smaller size (<7 mm) could facilitate the decision for immediate treatment. Genes, such as the above mentioned eNOS, could be possible candidates. Such kinds of genetic tests could easily be performed during a visit to the outpatient clinic during which a simple blood withdrawal for the extraction of DNA could take place.

Although a few publications have reported on ultrastructural findings of skin biopsies possibly being able to indicate the risk of developing an intracranial aneurysm this hypothesis has not been further substantiated. Mostly these findings were linked to connective tissue disease such as Ehlers Danlos Syndrome Type IV [29, 46, 55, 59, 93].

The goal of gene therapy is either to introduce a gene that is deficient in patients, to overexpress a therapeutic gene, or to silence a gene that is detrimental. Several studies have reported the feasibility of transferring genes to blood vessels to alter vascular function [10]. An alternative is to transfer the genes to the liver or the skeletal muscle so that the released protein from the transgene binds to blood vessels to alter vascular function [32]. The gene transfer, either direct or indirect by vector, is achieved with DNA or RNA. The transgene then expresses RNA or a protein.

Naked DNA, for direct transfection, is the safest approach but inefficient for transduction of cells and tissues [11]. Several recombinant viruses are used as vectors: Adenoviruses are efficient but the transfection period is short, as the viruses also induce an inflammatory response. Retroviruses provide long-term expression but may lead to insertional mutagenesis. Leukemia has been induced in children during retroviral transfer. Adeno-associated viruses provide long-term expression without inflammation, however it is difficult to produce large amounts of recombinant viruses [23]. So widespread use of gene therapy is being held back by the fact that a safe and efficient vector for delivery of genes has not been developed yet [32]. Cerebrovascular diseases have been the target of experimental gene therapy in animal models. Such as cerebral

vasospasm, chronic cerebral ischaemia with poor collateral circulation and restenosis of the extracranial artery.

Although lately drug eluting stents have been strongly criticized for a possible increase of restenosis after deployment [5], they or coils may be an effective method of administering genetically engineered treatment to the site of an intracranial aneurysm. For this kind of topically administered gene therapy, newer developments such as endovascular devices carrying vectors [74] and techniques of delivering genetically modified autologous fibroblasts are being pursued [61, 74].

A further approach and challenge is the direct administration of vectors into the carotid artery which unfortunately still requires an interruption of the blood flow for 10–30 min so that the virus can infect the endothelium [94]. Further techniques being studied are perivascular approaches, e.g., by administering adenoviral vectors into the CSF [7, 71] and by paintbrush technique [48]. An intravenous application could lead to the entrapment of the virus in the liver [34] where a secretable protein could then be released into the circulation. Similarly subcutaneous/intramuscular injections could deploy the same mechanism [9, 73].

Even though intracranial aneurysms may be considered as an irreversible process possibly genetic therapy can lead to a regression. A recent publication of an abdominal aortic aneurysm (AAA) mouse model has shown that an intraperitoneal application of a JNK inhibitor led to the regression of the aneurysm's diameter. In the case of AAA the diseased aorta seems to have the potential to regress if exacerbating factors are eliminated and/or the tissue repair is reinforced [102].

Conclusion and proposals for the future

Several reports have substantiated the fact that intracranial aneurysms and their rupture are associated with a strong genetic component. Overall, the results point to a multigenic disease in which environmental factors interact in the etiology.

It will be necessary to perform multicenter studies to 1) substantially increase the number of affected patients, 2) substratisfy the different regions and ethnicities of the patients and 3) to approach the genetic research from several angles. Positive results in candidate gene association studies as well as positive linkage regions need to be compared among cohorts of different ethnicity. The publications so far have shown the different influence in different countries. With the advent of ever increasing sophistication of computational analyses programs gene-gene interaction and gene-environment action will be scrutinized, leading to possible novel therapeutic approaches.

References

1. Akagawa H, Narita A, Yamada H, Tajima A, Krischek B, Kasuya H, Hori T, Kubota M, Saeki N, Hata A, Mizutani T, Inoue I (2007) Systematic screening of lysyl oxidase-like (LOXL) family genes demonstrates that LOXL2 is a susceptibility gene to intracranial aneurysms. Hum Genet 121(3–4): 377–387
2. Akagawa H, Tajima A, Sakamoto Y, Krischek B, Yoneyama T, Kasuya H, Onda H, Hori T, Kubota M, Machida T, Saeki N, Hata A, Hashiguchi K, Kimura E, Kim CJ, Yang TK, Lee JY, Kimm K, Inoue I (2006) A haplotype spanning two genes, ELN and LIMK1, decreases their transcripts and confers susceptibility to intracranial aneurysms. Hum Mol Genet 15: 1722–1734
3. Allen M, Heinzmann A, Noguchi E, Abecasis G, Broxholme J, Ponting CP, Bhattacharyya S, Tinsley J, Zhang Y, Holt R, Jones EY, Lench N, Carey A, Jones H, Dickens NJ, Dimon C, Nicholls R, Baker C, Xue L, Townsend E, Kabesch M, Weiland SK, Carr D, von Mutius E, Adcock IM, Barnes PJ, Lathrop GM, Edwards M, Moffatt MF, Cookson WO (2003) Positional cloning of a novel gene influencing asthma from chromosome 2q14. Nat Genet 35: 258–263
4. Armstrong K, Eisen A, Weber B (2000) Assessing the risk of breast cancer. N Engl J Med 342: 564–571
5. Camenzind E, Steg PG, Wijns W (2007) Stent thrombosis late after implantation of first-generation drug-eluting stents: a cause for concern. Circulation 115: 1440–1455
6. Castillo-Davis CI (2005) The evolution of noncoding DNA: how much junk, how much func? Trends Genet 21: 533–536
7. Chen AF, Jiang SW, Crotty TB, Tsutsui M, Smith LA, O'Brien T, Katusic ZS (1997) Effects of in vivo adventitial expression of recombinant endothelial nitric oxide synthase gene in cerebral arteries. Proc Natl Acad Sci USA 94: 12568–12573
8. Chen Y, Pawlikowska L, Yao JS, Shen F, Zhai W, Achrol AS, Lawton MT, Kwok PY, Yang GY, Young WL (2006) Interleukin-6 involvement in brain arteriovenous malformations. Ann Neurol 59: 72–80
9. Chu Y, Iida S, Lund DD, Weiss RM, DiBona GF, Watanabe Y, Faraci FM, Heistad DD (2003) Gene transfer of extracellular superoxide dismutase reduces arterial pressure in spontaneously hypertensive rats: role of heparin-binding domain. Circ Res 92: 461–468
10. Chu Y, Miller JD, Heistad DD (2007) Gene therapy for stroke: 2006 overview. Curr Hypertens Rep 9: 19–24
11. Chu Y, Weintraub N, Heistad DD (2005) Gene therapy and cardiovascular diseases. In: Runge M, Patterson C (eds) Principles of molecular cardiology. Humana Press, Totowa, New Jersey
12. Chyatte D, Bruno G, Desai S, Todor DR (1999) Inflammation and intracranial aneurysms. Neurosurgery 45: 1137–1146
13. Chyatte D, Reilly J, Tilson MD (1990) Morphometric analysis of reticular and elastin fibers in the cerebral arteries of patients with intracranial aneurysms. Neurosurgery 26: 939–943
14. Cohen JC, Kiss RS, Pertsemlidis A, Marcel YL, McPherson R, Hobbs HH (2004) Multiple rare alleles contribute to low plasma levels of HDL cholesterol. Science 305: 869–872
15. Crompton MR (1966) The comparative pathology of cerebral aneurysms. Brain 89: 789–796
16. De Francesco L (2006) Genetic profiteering. Nat Biotechnol 24: 888–890

17. Dichgans M, Markus HS (2005) Genetic association studies in stroke: methodological issues and proposed standard criteria. Stroke 36: 2027–2031
18. Ellegala DB, Day AL (2005) Ruptured cerebral aneurysms. N Engl J Med 352: 121–124
19. Farnham JM, Camp NJ, Neuhausen SL, Tsuruda J, Parker D, MacDonald J, Cannon-Albright LA (2004) Confirmation of chromosome 7q11 locus for predisposition to intracranial aneurysm. Hum Genet 114: 250–255
20. Feigin VL, Rinkel GJ, Lawes CM, Algra A, Bennett DA, van Gijn J, Anderson CS (2005) Risk factors for subarachnoid hemorrhage: an updated systematic review of epidemiological studies. Stroke 36: 2773–2780
21. Fernandez-Lopez A, Garrido-Martin EM, Sanz-Rodriguez F, Pericacho M, Rodriguez-Barbero A, Eleno N, Lopez-Novoa JM, Duwell A, Vega MA, Bernabeu C, Botella LM (2007) Gene expression fingerprinting for human hereditary hemorrhagic telangiectasia. Hum Mol Gen 16: 1515–1533
22. Finlay HM, Whittaker P, Canham PB (1998) Collagen organization in the branching region of human brain arteries. Stroke 29: 1595–1601
23. Flotte TR, Carter BJ (1995) Adeno-associated virus vectors for gene therapy. Gene Ther 2: 357–362
24. Frosch M, Anthony D, De Girolami U (2004) The central nervous system. In: Kumar V, Fausto N, Abbas A (eds) Robbins & Cotran pathologic basis of disease. Elsevier Saunders, Philadelphia
25. Frosen J, Piippo A, Paetau A, Kangasniemi M, Niemela M, Hernesniemi J, Jaaskelainen J (2004) Remodeling of saccular cerebral artery aneurysm wall is associated with rupture: histological analysis of 24 unruptured and 42 ruptured cases. Stroke 35: 2287–2293
26. Greenberg M (2000) SAH and aneurysms. In: Greenberg M (ed) Handbook of neurosurgery. Thieme Medical, New York
27. Gretarsdottir S, Sveinbjornsdottir S, Jonsson HH, Jakobsson F, Einarsdottir E, Agnarsson U, Shkolny D, Einarsson G, Gudjonsdottir HM, Valdimarsson EM, Einarsson OB, Thorgeirsson G, Hadzic R, Jonsdottir S, Reynisdottir ST, Bjarnadottir SM, Gudmundsdottir T, Gudlaugsdottir GJ, Gill R, Lindpaintner K, Sainz J, Hannesson HH, Sigurdsson GT, Frigge ML, Kong A, Gudnason V, Stefansson K, Gulcher JR (2002) Localization of a susceptibility gene for common forms of stroke to 5q12. Am J Hum Genet 70: 593–603
28. Gretarsdottir S, Thorleifsson G, Reynisdottir ST, Manolescu A, Jonsdottir S, Jonsdottir T, Gudmundsdottir T, Bjarnadottir SM, Einarsson OB, Gudjonsdottir HM, Hawkins M, Gudmundsson G, Gudmundsdottir H, Andrason H, Gudmundsdottir AS, Sigurdardottir M, Chou TT, Nahmias J, Goss S, Sveinbjornsdottir S, Valdimarsson EM, Jakobsson F, Agnarsson U, Gudnason V, Thorgeirsson G, Fingerle J, Gurney M, Gudbjartsson D, Frigge ML, Kong A, Stefansson K, Gulcher JR (2003) The gene encoding phosphodiesterase 4D confers risk of ischemic stroke. Nat Genet 35: 131–138
29. Grond-Ginsbach C, Schnippering H, Hausser I, Weber R, Werner I, Steiner HH, Luttgen N, Busse O, Grau A, Brandt T (2002) Ultrastructural connective tissue aberrations in patients with intracranial aneurysms. Stroke 33: 2192–2196
30. Gunel M, Awad IA, Anson J, Lifton RP (1995) Mapping a gene causing cerebral cavernous malformation to 7q11.2-q21. Proc Natl Acad Sci USA 92: 6620–6624
31. Harrod CG, Batjer HH, Bendok BR (2005) Deficiencies in estrogen-mediated regulation of cerebrovascular homeostasis may contribute to an increased risk of cerebral aneurysm

pathogenesis and rupture in menopausal and postmenopausal women. Med Hypotheses 66: 736–756
32. Heistad DD (2006) Gene therapy for vascular disease. Vascul Pharmacol 45: 331–333
33. Helgadottir A, Manolescu A, Thorleifsson G, Gretarsdottir S, Jonsdottir H, Thorsteinsdottir U, Samani NJ, Gudmundsson G, Grant SF, Thorgeirsson G, Sveinbjornsdottir S, Valdimarsson EM, Matthiasson SE, Johannsson H, Gudmundsdottir O, Gurney ME, Sainz J, Thorhallsdottir M, Andresdottir M, Frigge ML, Topol EJ, Kong A, Gudnason V, Hakonarson H, Gulcher JR, Stefansson K (2004) The gene encoding 5-lipoxygenase activating protein confers risk of myocardial infarction and stroke. Nat Genet 36: 233–239
34. Herz J, Gerard RD (1993) Adenovirus-mediated transfer of low density lipoprotein receptor gene acutely accelerates cholesterol clearance in normal mice. Proc Natl Acad Sci USA 90: 2812–2816
35. Hoheisel JD (2006) Microarray technology: beyond transcript profiling and genotype analysis. Nat Rev 7: 200–210
36. Inagawa T, Ishikawa S, Aoki H, Takahashi M, Yoshimoto H (1988) Aneurysmal subarachnoid hemorrhage in Izumo City and Shimane Prefecture of Japan. Incidence. Stroke 19: 170–175
37. Inci S, Spetzler RF (2000) Intracranial aneurysms and arterial hypertension: a review and hypothesis. Surg Neurol 53: 530–540
38. Ingall T, Asplund K, Mahonen M, Bonita R (2000) A multinational comparison of subarachnoid hemorrhage epidemiology in the WHO MONICA stroke study. Stroke 31: 1054–1061
39. Ingall TJ, Whisnant JP, Wiebers DO, O'Fallon WM (1989) Has there been a decline in subarachnoid hemorrhage mortality? Stroke 20: 718–724
40. Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG (2001) Replication validity of genetic association studies. Nat Genet 29: 306–309
41. Iwamoto H, Kiyohara Y, Fujishima M, Kato I, Nakayama K, Sueishi K, Tsuneyoshi M (1999) Prevalence of intracranial saccular aneurysms in a Japanese community based on a consecutive autopsy series during a 30-year observation period. The Hisayama study. Stroke 30: 1390–1395
42. Jamous MA, Nagahiro S, Kitazato KT, Satomi J, Satoh K (2005a) Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part I: experimental study of the effect of oophorectomy in rats. J Neurosurg 103: 1046–1051
43. Jamous MA, Nagahiro S, Kitazato KT, Tamura T, Kuwayama K, Satoh K (2005b) Role of estrogen deficiency in the formation and progression of cerebral aneurysms. Part II: experimental study of the effects of hormone replacement therapy in rats. J Neurosurg 103: 1052–1057
44. Juvela S (2002) Natural history of unruptured intracranial aneurysms: risks for aneurysm formation, growth, and rupture. Acta Neurochir Suppl 82: 27–30
45. Kataoka K, Taneda M, Asai T, Kinoshita A, Ito M, Kuroda R (1999) Structural fragility and inflammatory response of ruptured cerebral aneurysms. A comparative study between ruptured and unruptured cerebral aneurysms. Stroke 30: 1396–1401
46. Kato T, Hattori H, Yorifuji T, Tashiro Y, Nakahata T (2001) Intracranial aneurysms in Ehlers-Danlos syndrome type IV in early childhood. Pediatr Neurol 25: 336–339
47. Khurana VG, Meissner I, Sohni YR, Bamlet WR, McClelland RL, Cunningham JM, Meyer FB (2005) The presence of tandem endothelial nitric oxide synthase gene polymorphisms identifying brain aneurysms more prone to rupture. J Neurosurg 102: 526–531

48. Khurana VG, Weiler DA, Witt TA, Smith LA, Kleppe LS, Parisi JE, Simari RD, O'Brien T, Russell SJ, Katusic ZS (2003) A direct mechanical method for accurate and efficient adenoviral vector delivery to tissues. Gene Ther 10: 443–452
49. Kim DH, Van Ginhoven G, Milewicz DM (2005) Familial aggregation of both aortic and cerebral aneurysms: evidence for a common genetic basis in a subset of families. Neurosurgery 56: 655–661
50. Kiyohara Y, Ueda K, Hasuo Y, Wada J, Kawano H, Kato I, Sinkawa A, Ohmura T, Iwamoto H, Omae T, *et al.* (1989) Incidence and prognosis of subarachnoid hemorrhage in a Japanese rural community. Stroke 20: 1150–1155
51. Kosierkiewicz TA, Factor SM, Dickson DW (1994) Immunocytochemical studies of atherosclerotic lesions of cerebral berry aneurysms. J Neuropathol Exp Neurol 53: 399–406
52. Krex D, Kotteck K, Konig IR, Ziegler A, Schackert HK, Schackert G (2004) Matrix metalloproteinase-9 coding sequence single-nucleotide polymorphisms in caucasians with intracranial aneurysms. Neurosurgery 55: 207–212
53. Krex D, Rohl H, Konig IR, Ziegler A, Schackert HK, Schackert G (2003) Tissue inhibitor of metalloproteinases-1, -2, and -3 polymorphisms in a white population with intracranial aneurysms. Stroke 34: 2817–2821
54. Krex D, Ziegler A, Schackert HK, Schackert G (2001) Lack of association between endoglin intron 7 insertion polymorphism and intracranial aneurysms in a white population: evidence of racial/ethnic differences. Stroke 32: 2689–2694
55. Krischek B, Inoue I, Kasuya H (2005) Response to letter: collagen morphology is not associated with the Ala549Pro polymorphism of the COL1A2 gene. Stroke 36: 2068–2955
56. Krischek B, Inoue I (2006a) The genetics of intracranial aneurysms. J Hum Genet 51: 587–594
57. Krischek B, Kasuya H, Akagawa H, Tajima A, Narita A, Onda H, Hori T, Inoue I (2006b) Using endothelial nitric oxide synthase gene polymorphisms to identify intracranial aneurysms more prone to rupture in Japanese patients. J Neurosurg 105: 717–722
58. Krischek B, Narita A, Akagawa H, Kasuya H, Tajima A, Onda H, Yoneyama T, Hori T, Inoue I (2006c) Is there any evidence for linkage on chromosome 17cen in affected Japanese sib-pairs with an intracranial aneurysm? J Hum Genet 51: 491–494
59. Kuivaniemi H, Prockop DJ, Wu Y, Madhatheri SL, Kleinert C, Earley JJ, Jokinen A, Stolle C, Majamaa K, Myllyla VV, Norrgard O, Schievink WI, Mokri B, Fukawa O, ter Berg JWM, De Paepe A, Lozano AM, Leblanc R, Ryynanen M, Baxter BT, Shikata H, Ferrell RE, Tromp G (1993) Exclusion of mutations in the gene for type III collagen (COL3A1) as a common cause of intracranial aneurysms or cervical artery dissections: results from sequence analysis of the coding sequences of type III collagen from 55 unrelated patients. Neurology 43: 2652–2658
60. Labauge P, Denier C, Bergametti F, Tournier-Lasserve E (2007) Genetics of cavernous angiomas. Lancet Neurol 6: 237–244
61. Mazighi M, Tchetche D, Goueffic Y, San Juan A, Louedec L, Henin D, Michel JB, Jacob MP, Feldman LJ (2006) Percutaneous transplantation of genetically-modified autologous fibroblasts in the rabbit femoral artery: a novel approach for cardiovascular gene therapy. J Vasc Surg 44: 1067–1075
62. Meschia JF, Brott TG, Brown RD Jr (2005) Genetics of cerebrovascular disorders. Mayo Clin Proc 80: 122–132

63. Morita A, Fujiwara S, Hashi K, Ohtsu H, Kirino T (2005) Risk of rupture associated with intact cerebral aneurysms in the Japanese population: a systematic review of the literature from Japan. J Neurosurg 102: 601–606
64. Nahed BV, Bydon M, Ozturk AK, Bilguvar K, Bayrakli F, Gunel M (2007) Genetics of intracranial aneurysms. Neurosurgery 60: 213–225
65. Nahed BV, Seker A, Guclu B, Ozturk AK, Finberg K, Hawkins AA, DiLuna ML, State M, Lifton RP, Gunel M (2005) Mapping a Mendelian form of intracranial aneurysm to 1p34.3-p36.13. Am J Hum Genet 76: 172–179
66. Nanba R, Kuroda S, Tada M, Ishikawa T, Houkin K, Iwasaki Y (2006) Clinical features of familial moyamoya disease. Childs Nerv Syst 22: 258–262
67. NY Cp (2006) The abdominal aortic aneurysm. Genetics, pathophysiology, and molecular biology. Proceedings of a conference. April 3–5, 2006. New York, USA. Ann NY Acad Sci 1085: 1–408
68. Olson JM, Vongpunsawad S, Kuivaniemi H, Ronkainen A, Hernesniemi J, Ryynanen M, Kim LL, Tromp G (2002) Search for intracranial aneurysm susceptibility gene(s) using Finnish families. BMC Med Genet 3: 7
69. Onda H, Kasuya H, Yoneyama T, Hori T, Nakajima T, Inoue I (2003) Endoglin is not a major susceptibility gene for intracranial aneurysm among Japanese. Stroke 34: 1640–1644
70. Onda H, Kasuya H, Yoneyama T, Takakura K, Hori T, Takeda J, Nakajima T, Inoue I (2001) Genomewide-linkage and haplotype-association studies map intracranial aneurysm to chromosome 7q11. Am J Hum Genet 69: 804–819
71. Ooboshi H, Welsh MJ, Rios CD, Davidson BL, Heistad DD (1995) Adenovirus-mediated gene transfer in vivo to cerebral blood vessels and perivascular tissue. Circ Res 77: 7–13
72. Ozturk AK, Nahed BV, Bydon M, Bilguvar K, Goksu E, Bademci G, Guclu B, Johnson MH, Amar A, Lifton RP, Gunel M (2006) Molecular genetic analysis of two large kindreds with intracranial aneurysms demonstrates linkage to 11q24-25 and 14q23-31. Stroke 37: 1021–1027
73. Pradat PF, Kennel P, Naimi-Sadaoui S, Finiels F, Orsini C, Revah F, Delaere P, Mallet J (2001) Continuous delivery of neurotrophin 3 by gene therapy has a neuroprotective effect in experimental models of diabetic and acrylamide neuropathies. Hum Gene Ther 12: 2237–2249
74. Ribourtout E, Raymond J (2004) Gene therapy and endovascular treatment of intracranial aneurysms. Stroke 35: 786–793
75. Richling B (2006) History of endovascular surgery: personal accounts of the evolution. Neurosurgery 59: S30–S38
76. Rinkel GJ, Djibuti M, Algra A, van Gijn J (1998) Prevalence and risk of rupture of intracranial aneurysms: a systematic review. Stroke 29: 251–256
77. Ronkainen A, Hernesniemi J, Puranen M, Niemitukia L, Vanninen R, Ryynanen M, Kuivaniemi H, Tromp G (1997) Familial intracranial aneurysms. Lancet 349: 380–384
78. Ross M, Pawlina W (2006) Histology: text & atlas. Lippincott Williams & Wilkins, Baltimore
79. Ruigrok YM, Rinkel GJ, Wijmenga C (2005) Genetics of intracranial aneurysms. Lancet Neurol 4: 179–189
80. Schievink WI, Michels VV, Piepgras DG (1994) Neurovascular manifestations of heritable connective tissue disorders. A review. Stroke 25: 889–903

81. Schievink WI, Schaid DJ, Michels VV, Piepgras DG (1995) Familial aneurysmal subarachnoid hemorrhage: a community-based study. J Neurosurg 83: 426–429
82. Simon M, Franke D, Ludwig M, Aliashkevich AF, Koster G, Oldenburg J, Bostrom A, Ziegler A, Schramm J (2006) Association of a polymorphism of the ACVRL1 gene with sporadic arteriovenous malformations of the central nervous system. J Neurosurg 104: 945–949
83. Stapf C, Mohr J (2004) Aneurysms and subarachnoid hemorrhage – epidemiology. In: Le Roux P, Winn H, Newell D (eds) Management of cerebral aneurysms. Saunders, Philadelphia
84. Stefansson H, Sigurdsson E, Steinthorsdottir V, Bjornsdottir S, Sigmundsson T, Ghosh S, Brynjolfsson J, Gunnarsdottir S, Ivarsson O, Chou TT, Hjaltason O, Birgisdottir B, Jonsson H, Gudnadottir VG, Gudmundsdottir E, Bjornsson A, Ingvarsson B, Ingason A, Sigfusson S, Hardardottir H, Harvey RP, Lai D, Zhou M, Brunner D, Mutel V, Gonzalo A, Lemke G, Sainz J, Johannesson G, Andresson T, Gudbjartsson D, Manolescu A, Frigge ML, Gurney ME, Kong A, Gulcher JR, Petursson H, Stefansson K (2002) Neuregulin 1 and susceptibility to schizophrenia. Am J Hum Genet 71: 877–892
85. Stehbens WE (1989) Etiology of intracranial berry aneurysms. J Neurosurg 70: 823–831
86. Stehbens WE (1999) Relationship of cerebral aneurysms and medial raphes. Surg Neurol 52: 536–538
87. Styrkarsdottir U, Cazier JB, Kong A, Rolfsson O, Larsen H, Bjarnadottir E, Johannsdottir VD, Sigurdardottir MS, Bagger Y, Christiansen C, Reynisdottir I, Grant SF, Jonasson K, Frigge ML, Gulcher JR, Sigurdsson G, Stefansson K (2003) Linkage of osteoporosis to chromosome 20p12 and association to BMP2. PLoS Biol 1: E69
88. Tabor HK, Risch NJ, Myers RM (2002) Candidate-gene approaches for studying complex genetic traits: practical considerations. Nat Rev 3: 391–397
89. Takenaka K, Sakai H, Yamakawa H, Yoshimura S, Kumagai M, Nakashima S, Nozawa Y, Sakai N (1999) Polymorphism of the endoglin gene in patients with intracranial saccular aneurysms. J Neurosurg 90: 935–938
90. Tanaka H, Ueda Y, Date C, Baba T, Yamashita H, Hayashi M, Shoji H, Owada K, Baba KI, Shibuya M, Kon T, Detels R (1981) Incidence of stroke in Shibata, Japan: 1976–1978. Stroke 12: 460–466
91. Tournier-Lasserve E, Joutel A, Melki J, Weissenbach J, Lathrop GM, Chabriat H, Mas JL, Cabanis EA, Baudrimont M, Maciazek J, Bach MA, Bousser MG (1993) Cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy maps to chromosome 19q12. Nat Genet 3: 256–259
92. Tulamo R, Frosen J, Junnikkala S, Paetau A, Pitkaniemi J, Kangasniemi M, Niemela M, Jaaskelainen J, Jokitalo E, Karatas A, Hernesniemi J, Meri S (2006) Complement activation associates with saccular cerebral artery aneurysm wall degeneration and rupture. Neurosurgery 59: 1069–1076
93. van den Berg JS, Pals G, Arwert F, Hennekam RC, Albrecht KW, Westerveld A, Limburg M (1999) Type III collagen deficiency in saccular intracranial aneurysms. Defect in gene regulation? Stroke 30: 1628–1631
94. von der Leyen HE, Gibbons GH, Morishita R, Lewis NP, Zhang L, Nakajima M, Kaneda Y, Cooke JP, Dzau VJ (1995) Gene therapy inhibiting neointimal vascular lesion: in vivo transfer of endothelial cell nitric oxide synthase gene. Proc Natl Acad Sci USA 92: 1137–1141

95. Wermer MJ, van der Schaaf IC, Algra A, Rinkel GJ (2007) Risk of rupture of unruptured intracranial aneurysms in relation to patient and aneurysm characteristics. An updated meta-analysis. Stroke 38: 1404–1410
96. Winn HR, Jane JA Sr, Taylor J, Kaiser D, Britz GW (2002) Prevalence of asymptomatic incidental aneurysms: review of 4568 arteriograms. J Neurosurg 96: 43–49
97. Wolfberg AJ (2006) Genes on the Web – direct-to-consumer marketing of genetic testing. N Engl J Med 355: 543–545
98. Yamada S, Utsunomiya M, Inoue K, Nozaki K, Inoue S, Takenaka K, Hashimoto N, Koizumi A (2004) Genome-wide scan for Japanese familial intracranial aneurysms: linkage to several chromosomal regions. Circulation 110: 3727–3733
99. Yamada S, Utsunomiya M, Inoue K, Nozaki K, Miyamoto S, Hashimoto N, Takenaka K, Yoshinaga T, Koizumi A (2003) Absence of linkage of familial intracranial aneurysms to 7q11 in highly aggregated Japanese families. Stroke 34: 892–900
100. Yoneyama T, Kasuya H, Onda H, Akagawa H, Hashiguchi K, Nakajima T, Hori T, Inoue I (2004) Collagen type I alpha2 (COL1A2) is the susceptible gene for intracranial aneurysms. Stroke 35: 443–448
101. Yoshimoto T, Mizoi K (1997) Importance of management of unruptured cerebral aneurysms. Surg Neurol 47: 522–525
102. Yoshimura K, Aoki H, Ikeda Y, Fujii K, Akiyama N, Furutani A, Hoshii Y, Tanaka N, Ricci R, Ishihara T, Esato K, Hamano K, Matsuzaki M (2005) Regression of abdominal aortic aneurysm by inhibition of c-Jun N-terminal kinase. Nat Med 11: 1330–1338