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Genetics of Intracranial Aneurysms

Ynte M. Ruigrok, MD; Gabriel J.E. Rinkel, MD

Background and Purpose—Genetic determinants probably play a role in the development of intracranial aneurysms. We review the present knowledge on this issue.

Methods—This work entailed a comprehensive search of the literature, a critical appraisal of the identified papers, and a personal view of limitations and future directions.

Results—We identified 10 genome-wide linkage studies in families and sib pairs with intracranial aneurysms. These studies have identified several loci, but only 4 (1p34.3–p36.13, 7q11, 19q13.3, and Xp22) have been replicated in different populations. For the loci on 1p34.3–p36.13 and 7q11 association with positional candidate genes has also been demonstrated: for locus on 1p34.3–p36.13 association with the perlecan gene and for 7q11 association with the elastin and collagen type 1 A2 genes.

Conclusions—The progress in identifying genetic determinants for intracranial aneurysms is modest. Reasons for this modest progress include limitations of the present studies, limitations because of the nature of the disease, and limitations in our concept of aneurysms and aneurysm development. Future studies may benefit from strict definitions of familial aneurysms, reduced phenotypic heterogeneity (separating ruptured from unruptured aneurysms, and within these subsets probably also reduce morphological heterogeneity, eg, by grouping similar sites of aneurysms), taking into account age and other risk factors of the patients with aneurysms, and sufficiently large numbers of patients. In future studies we should not only look for genetic determinants of aneurysms, but also for genetic determinants of rupture of aneurysms. This would help in selecting patients for preventive treatment of aneurysms. (Stroke. 2008;39:1049-1055.)

Key Words: aneurysm ■ genetics ■ subarachnoid hemorrhage

Subarachnoid hemorrhage (SAH) from rupture of an intracranial aneurysm has a poor prognosis. In a systematic review on population based studies published before 1995, the case fatality of aneurysmal SAH was around 50%, whereas another 20% remain dependent in activities of daily living. More recent data suggest that case fatality rate has decreased in the last 2 decades. The incidence of SAH increases with age, but half the patients are younger than 55 years at the time of the SAH. Because of the young age at which SAH occurs and the poor prognosis, the loss of productive life years from SAH in the population is as large as that from ischemic stroke. Risk factors for aneurysmal SAH include smoking, hypertension, excessive use of alcohol, and familial occurrence of SAH. The modifiable risk factors are the most prevalent ones, but familial occurrence is the strongest risk factor. Undoubtedly the familial clustering of SAH can to some extent be explained by shared acquired risk factors, but these acquired risk factors explain only part of the familial aggregation. Thus, the familial occurrence suggests genetic factors to be involved in the development of intracranial aneurysms. This suggestion is further supported by the findings that some aneurysm characteristics differ between patients with and those without familial occurrence of aneurysms. In comparison to sporadic aneurysms, familial aneurysms are generally larger at time of rupture, more often located at the middle cerebral artery, and more likely to be multiple than sporadic. Moreover, in familial SAH aneurysms rupture at an earlier age. The concept of genetic factors being involved in the development of aneurysms has led to many studies on genetic determinants for intracranial aneurysms in the last decade. Here, we review results of genetic studies, discuss possible reasons for why progress in this field has been modest, and give some suggestions for future directions of research.

Results of Genetic Studies

For the identification of genetic factors responsible for a complex disease (ie, many genetic and environmental factors
involved) such as intracranial aneurysms, different approaches can be used. One approach is a hypothesis-based approach involving characterization of genes that because of their function are candidates to be involved in the development of aneurysms (so called functional candidate genes, such as elastin). For this approach, association studies are widely used. In this type of study association between aneurysms and a specific allele within the functional candidate gene is analyzed between aneurysm patients and controls. A disadvantage of this hypothesis-based approach is that genes involved in the pathogenesis of aneurysms through unknown pathways are overlooked. To overcome this drawback, genes involved in the development of aneurysms may be detected through a hypothesis-free approach by whole genome screening with linkage studies or by genome-wide association studies. Genome-wide association studies investigate differences in the frequencies of specific alleles between patients and controls. Major advantages of genome-wide association studies are the ease of the design and the ability to detect genetic factors with only small effects. A disadvantage is that disease genes are not detected if aneurysms are caused by several rare variants in a gene.11 Thus far, no genome-wide association studies have been performed for intracranial aneurysms. Linkage studies analyze whether cosegregation of aneurysms (or another disease phenotype) with DNA markers of known location, located throughout the genome, occurs in diseased families. Linkage of the disease phenotype (and thus the disease-causing gene) with a specific DNA marker means that the marker and the disease-related gene are located in close proximity to each other on the DNA. Linkage studies will identify so-called loci where the disease-related gene is located. The genes thus identified are so called positional candidate genes. Linkage studies are often followed by association studies analyzing positional candidate genes located in the identified loci.

We focus on the results of the hypothesis-free approach of whole genome linkage studies. In addition, we discuss the results of the association studies of the candidate genes situated in the loci identified by these linkage studies. The results of association studies in genes that were selected only because of their function and presumed role in the pathogenesis of aneurysms have been conflicting or have not been replicated. Most of these studies had small sample sizes precluding robust results. These studies have been reviewed elsewhere.12-14 The literature search for the present review was based on a Pubmed search (April 2007) with key words “aneurysm,” “intracranial,” “cerebral,” “subarachnoid hemorrhage,” “genes,” “genetics” in different combinations and on a personal database (GJER) that has been prospectively built by daily search of PubMed in the past 15 years with terms related to subarachnoid hemorrhage and intracranial aneurysms.

We identified 10 linkage studies (Table).15-24 Some of these studies used a “model-based “ (presumed pattern of inheritance) design of linkage analysis, which generates a so-called logarithm of odds (LOD) score, whereas others used a “model free” analysis, which generates a nonparametric

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*loci with LOD scores >2.2 and nonparametric LOD (NPL) score >3.2.
#loci with LOD and NPL scores above 2 but under the levels for “suggestive linkage.”
LOD (NPL) score. As these 2 scores are calculated in different ways, the threshold levels for “suggestive linkage” are different for the 2 scores. We included only loci with LOD scores or NPL scores > 2. Loci with LOD scores > 2.2 or NPL scores > 3.2 (probability value of 7.4 × 10^-4) were considered “suggestive loci,” those with LOD and NPL scores above 2 but under the levels for suggestive linkage were considered “possible loci.”

Suggestive Loci for Intracranial Aneurysms

Chromosome 1p

Locus
In a Northern American family with aneurysms segregating as a dominant trait, linkage was found to a locus on chromosome 1p34.3–p36.13 (LOD score 4.2). This locus overlaps with a locus on chromosome 1 at 1p36.11–p36.13 (NPL of 3.18) that we recently identified in a large Dutch family.

Candidate Genes
A plausible candidate gene in the 1p locus is the perlecan gene, which codes for a heparan sulfate proteoglycan involved in the maintenance of the extracellular matrix (ECM) of the arterial wall. In a case-control study with 2 independent populations, we recently demonstrated single nucleotide polymorphisms (SNPs) in the perlecan gene to be associated with intracranial aneurysms (probability value 0.0005), which further underlines the possible involvement of this gene in the pathogenesis of intracranial aneurysms. In the Northern American family other possible candidate genes of the 1p locus, including polycystic kidney disease-like 1, brain-specific angiogenesis inhibitor 2, fibronectin type III domain–containing gene, and collagen type XVI A1 were screened but no mutations in these genes segregating on the affected chromosomes could be demonstrated.

Chromosome 2p13

In a large Dutch consanguineous family, initially linkage was found to 2p13 using under the assumption of a recessive mode of inheritance (LOD score 3.6). This family was reanalyzed after follow-up screening had detected newly developed aneurysms in previously unaffected relatives. These newly detected relatives did not carry the disease haplotype, and therefore the previously found linkage was lost.

Chromosome 5p

Locus
In a large French Canadian family a locus on chromosome 5p15.2 to 14.3 (LOD score 3.6) was demonstrated. This locus includes about 25 known genes. Two suggested candidate genes in this locus are catenin δ-2 (CTNND2), which is involved in neuronal cell adhesion, tissue morphogenesis and integrity by regulating adhesion molecules, and triple functional domain protein. No association studies of these candidate genes have been performed thus far.

Chromosome 7q

Locus
A Japanese study in 104 affected sib pairs identified 1 suggestive locus (7q11; NPL 3.2) and 2 possible loci (5q22–q31 with NPL 2.2, and 14q22 with NPL 2.3). The last 2 loci will be discussed in the next paragraph. The marker with the best evidence of linkage in this study on region 7q11 was in the vicinity of the elastin gene. In a study on 13 families with intracranial aneurysms from Utah, linkage to 7q11 was confirmed (LOD score 2.3). In another study on linkage to the elastin gene, 14 Japanese families were tested. In this study inclusion criteria for familial aneurysms were more stringent (at least 3 affected members per family) than in the Japanese genome-wide linkage study (at least 2 affected members per family). Linkage to the elastin gene could be excluded in 11 of these 14 families and was inconclusive in the other 3. In a recent genome-wide scan on 41 affected relatives from 9 Japanese families, linkage to the 7q11 region could not be confirmed under the assumption of a autosomal dominant mode of inheritance. Possible explanations for the discrepant findings are the different definitions for familial aneurysms and the different approaches, ie, model free versus an assumed mode of inheritance, between the studies.

Candidate Genes
The elastin gene codes for elastin, which is an important element of the arterial wall and responsible for its dilatation and recoil. In a further analysis of the elastin gene a haplotype between the SNPs at intron 20 and intron 23 was strongly associated with aneurysms (P = 3.81 × 10^-6). A follow-up study in Japan on the 7q11 locus showed a susceptibility locus covering part of the elastin gene and the entire region of the closely to elastin positioned LIM domain kinase 1 gene. An association analysis in 404 patients and 458 controls showed an at-risk haplotype including 2 functional SNPs, the elastin 3′-UTR (+502) A insertion and the LIM domain kinase 1 promoter C(-187)T SNP. Further analyses demonstrated that both these SNPs are associated with a decrease of transcript levels. In contrast to these findings in Japanese populations, in the Utah population (where linkage to 7q11 had been confirmed), no mutation in the elastin gene was found segregating in the pedigrees in which linkage was found.

In 2 German studies comparing 120 aneurysm patients with 172 controls or 205 patients with 235 controls, no allelic association was found with the haplotype associated with intracranial aneurysms in Japanese patients. The differences between the Japanese and European studies may be explained by allelic heterogeneity between the Japanese and Europe’s population of aneurysm patients.

The chromosome region 7q11 also includes the candidate gene collagen type 1 A2. The collagen type 1 fibers represent together with the collagen type 3 fibers up to 90% of the total arterial collagen and are important for the strength of arterial wall. The collagen type 1 A2 gene was analyzed in a Japanese population. Three different SNPs, of which one results in an amino acid substitution, showed significant differences in allelic frequencies between cases and controls, especially the SNP resulting in an amino acid substitution in familial cases.
markers evidence for linkage was reduced in this region and it was not further explored. A susceptibility locus for intracranial aneurysms on the X-chromosome may explain the preponderance of affected women with intracranial aneurysms, as many genes on chromosome Xp escape X inactivation.

Candidate Genes

The identified locus contains the angiotensin I converting enzyme 2 gene, but association with this gene could not be demonstrated in 2 Japanese studies.

Possible Loci for Intracranial Aneurysms

Chromosome 5q

Locus

Possible linkage to chromosome 5q22–31 was suggested in 1 study in Japan (NPL score of 2.2). Thus far no studies attempting to replicate this locus have been published.

Candidate Genes

The versican gene is an interesting candidate gene located in the vicinity of the IA locus on 5q22–31, because versican plays an important role in the extracellular matrix. Recently we showed that SNPs in strong linkage disequilibrium in the versican gene are associated with aneurysms (OR 1.3; 95% CI 1.1 to 1.6). The linkage region on 5q22–31 also includes several other potential candidate genes, for example fibroblast growth factor 1, lysyl oxidase, and fibrillin-2. SNPs in these genes were analyzed in 172 Japanese patients with aneurysms and 192 controls, but no associations between these SNPs and aneurysms were observed. Also in 2 other studies, 1 with 25 German familial intracranial aneurysm patients and another with 2 independent populations totaling Dutch 692 patients with aneurysms and 718 controls, no association of SNPs in the lysyl oxidase with aneurysms could be demonstrated.

Chromosome 17cen

Locus

In the study with 29 Japanese families linkage to a locus on chromosome 17cen (NPL score of 3.0) was found. In the study with 104 affected Japanese sib pairs linkage to one marker (D17S925) with a probability value of 0.03 was found. Linkage to this region was also confirmed in 2 studies from Japan. The first study analyzed 29 Japanese families with 3 or more individuals with aneurysms. Using a model-free linkage analysis, an NPL score of 2.2 was found. In the second study, linkage was confirmed in a model based (dominant model) affected-only analysis in 41 affected members from nine unrelated families with aneurysms (maximum LOD score of 4.10).

Candidate Genes

This region identified in the Finnish population includes 135 genes of which 102 have been characterized. One of the genes is APOE, but no association of this gene with aneurysms could be demonstrated in 2 Japanese populations.

Chromosome Xp

Locus

In the large Dutch family in which a locus on chromosome 1p was found, also significant linkage to a locus on chromosome X (NPL of 4.5) at Xp22.2–p22.32 was found. Evidence for this locus was also found in the 29 Japanese families (NPL of 2.2). In addition, the Finnish linkage study in affected sib pairs found evidence for linkage to this Xp22 locus with a maximum LOD score of 2.1, but after genotyping additional
associations were found with the microfibril-associated protein 4 and the inducible nitric oxide synthase genes located in the 17cen locus.\textsuperscript{18,26,34}

Discussion

Genome-wide linkage studies in families and sib pairs with intracranial aneurysms have identified several loci on chromosomes. Of these loci, the ones on chromosomes 1p34.3–p36.13, 7q11, 19q13.3, and Xp22 are the most promising because these loci have been replicated in different populations, although 7q11 could not be confirmed in all populations studied. For the loci on 1p34.3–p36.13 and 7q11 association with positional candidate genes has also been demonstrated: for locus on 1p34.3–p36.13 association with the perlecan gene and for 7q11 association with the elastin gene and the collagen type 1A2 gene.

Our review shows that despite many years of research, progress in finding genetic determinants for aneurysms has been modest. There are many possible explanations for this modest progress and for the discrepant linkage findings between studies.

A first limitation regarding the present linkage studies is that varying definitions of familial aneurysms have been used. Some studies included only affected siblings, others included only families with 3 or more relatives affected, and again other studies included families with 2 relatives affected irrespective of the type of relation between the affected relatives. These different definitions may imply a different genetic load in the families studied, and thus may lead to different findings. A second limitation is that in some of these linkage studies an assumed mode of inheritance was included in the analysis, whereas the mode of inheritance is unknown and most likely heterogeneous.\textsuperscript{41,42} A third limitation is related to the disease itself, which has some inherent drawbacks for linkage studies. Within families it is difficult to determine whether a nonaffected sibling (no aneurysm on screening) really is nonaffected, because aneurysms develop during life (see paragraph on locus 2p13). In relatives of affected families with a negative screen, the chance of developing an aneurysm within 5 years of the screening is 7%.\textsuperscript{43} This problem can be avoided by an affected-only approach in families, or by affected sibling pairs approach. However, for this latter approach large numbers of pairs (at least 100) are needed for a proper analysis. It is very difficult to collect such large numbers of affected siblings because intracranial aneurysms have a late onset,\textsuperscript{44} a relatively low prevalence even in affected families,\textsuperscript{45} and a high case fatality after rupture.\textsuperscript{1} This high case fatality hampers collecting DNA in affected relatives, because many affected members may already have died before the family comes under attention. Large cooperative international studies probably are not a panacea because different populations and different ethnic groups have different risk for SAH and thus probably different genetic determinants for intracranial aneurysms.\textsuperscript{3,5} This genetic heterogeneity may not only play a role between different populations and different ethnic groups, but also within populations and ethnic groups. The genetic determinants in one family may be different from those in another family. Some of these genetic variants within families may be rare,\textsuperscript{46} which would make it much more difficult to detect such variants. A last issue regarding linkage studies is that we have thus far considered all intracranial saccular aneurysms alike, but it might very well be that the genetic determinants differ per site and per morphology of the aneurysm. The simple fact that characteristics of aneurysms differ between patients with and without familial occurrence of aneurysms already supports this notion.\textsuperscript{9,10} Thus, in intracranial aneurysms there may be not only genetic but also clinical heterogeneity.

In most association studies performed thus far age and other risk factors for aneurysms of the affected patients are not taken into account. It is very likely that the genetic impact of the development of an aneurysm differs between a 32-year-old woman with a positive family history and no history of smoking and hypertension and a 72-year-old man with hypertension and a 30 years history of smoking. A second issue regarding association studies is that in all these studies ruptured and unruptured aneurysms are lumped together. There are some obvious differences between ruptured and unruptured aneurysms (apart from the presence or absence of subarachnoid hemorrhage). Unruptured aneurysms are not so rare with 2% in the general population,\textsuperscript{44} and many of these will never rupture.\textsuperscript{47} Ruptured aneurysms are much rarer, with an incidence of only 9 per 100 000 (0.009%) per year.\textsuperscript{3} Moreover, distribution of sites differs between unruptured and ruptured aneurysms. In series of patients with ruptured aneurysms the most prevalent site of aneurysms is the anterior communicating artery, whereas in series of unruptured aneurysms anterior communicating artery aneurysms are rare, and aneurysms of middle cerebral artery and internal carotid artery are the most prevalent.\textsuperscript{44} Also, unruptured and ruptured aneurysms differ in histomorphological features.\textsuperscript{48} Keeping all these differences between unruptured and ruptured aneurysms in mind, it seems likely that there are differences in genetic determinants between unruptured and ruptured aneurysms. The occurrence of an SAH implies that an aneurysm has developed and has ruptured. If for rupture of an aneurysm additional genetic determinants need to be present on top of the determinants for development of aneurysms, this may provide a powerful tool for selecting patients with unruptured aneurysms who are at high risk of aneurysm rupture. Because only a minority of all unruptured aneurysms will eventually rupture,\textsuperscript{47} preventive treatment of aneurysms can than be restricted to this subset of patients.

Animal models of intracranial aneurysms can help to identify disease causing genes, but thus far no transgenic animal model with intracranial aneurysms has been developed.

Despite all these definite and potential difficulties for detecting the genetic determinants for intracranial aneurysms, more information on these determinants is needed. Knowledge on the genetic determinants may provide insight in the development of aneurysms, and thereby may give clues on how to stop aneurysm formation. It may also provide diagnostic tools for identifying individuals at increased risk for aneurysm formation; the identified persons can then be screened by imaging studies. Relatives of affected families who do not carry the disease trait can be reassured. Also, in future studies we should not only look for genetic determi-
nants of aneurysms, but also for genetic determinants of rupture of aneurysms. This would help in selecting patients for preventive treatment of aneurysms. In our view future genetic studies may benefit from strict definitions of familial aneurysms and sufficiently large numbers of affected relatives. It may also be helpful to reduce phenotypic heterogeneity. This should include at least separating ruptured from unruptured aneurysms. Within these subsets we should study if there are different endophenotypes regarding site and morphology of the aneurysm. If different endophenotypes exist, this should be taken into account in future studies. In association studies age and other risk factors for development of aneurysms should be taken into account.

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