Genetic Aspects of Cerebrovascular Disease

Mark J. Alberts, MD

In the past conventional wisdom held that stroke and other forms of cardiovascular disease were due primarily to environmental factors and that genetic background played a relatively minor role in disease pathogenesis. However, recent discoveries in the fields of molecular and population genetics have suggested the need to reexamine the role of genetic factors in the pathogenesis of vascular disease.

Research into the genetics of stroke presents some unique challenges since stroke is a heterogeneous disease with multiple risk factors. However, such studies provide an opportunity to elucidate the etiology of stroke at a molecular level and to attempt true primary prevention. This article reviews some of the principles of inherited disorders and genetic research techniques and applies the concepts to the study of the genetics of specific stroke types.

Over 3,000 genetic disorders have been described in man, but the gene defect responsible for a specific disease has been identified for only a handful of diseases. Many genetic diseases are termed “simple” diseases: they follow a clear Mendelian pattern of inheritance (e.g., autosomal dominant, X-linked recessive) and are due to a defect in only a single gene, and every individual with the gene defect will display the mutant phenotype. Examples of simple neurogenetic diseases include Huntington’s chorea, Duchenne’s muscular dystrophy, and myotonic muscular dystrophy.

The “complex” genetic disorders are thought to be due to multiple gene interactions (perhaps influenced by environmental factors), with variable clinical expression of the disease phenotype. Examples include diabetes, cancer, and atherosclerosis. The study of complex genetic disorders is complicated by numerous factors:

1) Environmental factors: Extrinsic influences such as diet, smoking, and stress may be responsible for the modified expression of certain genetic traits. For example, an individual with the genetic propensity for hyperlipidemia may reduce serum cholesterol by eating a modified diet. Likewise, genetic factors may have a role in determining how much an individual eats, how one handles stress, and who chooses to smoke.

2) Risk factors: It is apparent that the development of certain major risk factors such as hypertension and diabetes are under some genetic influence. It is likely that these risk factors are themselves synthetic traits (see below) and are also partially influenced by environmental factors.

3) Synthetic traits: Multiple genes can interact to produce a trait. Atherosclerosis or hypertension may be examples of synthetic traits. Since complex genetic diseases may be due to several synthetic traits acting together, analysis of such interactions becomes exceedingly complex.

4) Polygenic trait: In this case abnormalities of multiple genes are necessary to produce a specific trait.

5) Genetic heterogeneity: Defects at more than one genetic allele can produce similar phenotypic abnormalities.

6) Phenocopy: An environmental factor can produce a mutant phenotype identical to that caused by a defective gene. For example, a dissection of the carotid artery due to neck trauma can produce a stroke with clinical findings similar to atherosclerotic occlusion of the carotid artery.

Two genetic terms which are often misunderstood are penetrance and expressivity. Penetrance is an all-or-nothing phenomenon: a genetic disease is completely penetrant if every person carrying the mutant genotype expresses the mutant phenotype and incompletely penetrant if some individuals carrying the mutant genotype do not have the mutant phenotype. Variable expressivity refers to the propensity of some genetic disorders to produce quite varied phenotypic findings. Neurofibromatosis is an example of a genetic disorder with variable expressivity.

The issues described above are important for understanding genetic diseases in populations. The techniques described below are used for studying the molecular genetic abnormalities responsible for these complex diseases.

Molecular Genetic Techniques

The goal of molecular genetic research is to identify and characterize the genetic defect responsible for a specific genetic disease. The magnitude of this task can be better understood when it is realized that the human genome has over 3 billion bases of DNA organized into about 100,000 genes. Since only a small number of specific genes have been isolated...
and characterized, the genes responsible for most genetic diseases have not been identified.

Classic genetic research has attempted first to find the defective protein and then to isolate the abnormal gene. However, since the abnormal protein responsible for most genetic diseases has not been found, a new approach is warranted. This approach, termed reverse genetics, uses genetic linkage analysis to identify chromosomal regions that are close to disease loci. One advantage of reverse genetics is that no a priori information about the location of the mutant gene is necessary. Linkage can be tested with probes (fragments of DNA) that are scattered throughout the human genome. The specific techniques and terminology of reverse genetics have been reviewed recently, and the interested reader is referred to these excellent references for more detailed information.1,2

One of the key steps in studying genetic linkage is to identify sufficiently large families with the disease of interest. If the familial form of the disease is rare or the available families do not have multiple generations or many living members available for testing, the usefulness of classic genetic linkage studies becomes somewhat limited. Because of the likely multifactorial pathogenesis of stroke, classic genetic linkage analysis may be insufficient to find linkage.

Some of these problems may be overcome by using newer methods of linkage analysis. The affected pedigree member (APM) method, developed by Weeks and Lange,3 uses marker phenotypes for APMs only. This technique is more sensitive for distantly related relatives that are affected and share the same rare allele. Another advantage of the APM method is that no assumptions about the mode of inheritance are required.

Another potentially powerful technique is the affected relative pairs (ARP) technique.4 Various combinations of pairs of affected relatives (siblings, first cousins, half sibs, etc.) can be used to identify major or minor disease susceptibility loci. This approach is attractive in cases where large immediate families may not be available but members of extended pedigrees are still alive. Both the APM and ARP methods may be most useful for identifying major gene effects in the pathogenesis of complex diseases rather than identifying every gene involved in an inherited disease.

Stroke Genetics

Risk Factors

The well-documented risk factors described for stroke include hypertension, diabetes, lipid abnormalities, heart disease, advanced age, fibrinogen levels, smoking, and coagulation defects. Many of these risk factors are probably synthetic traits themselves (e.g., hypertension, lipid levels) while others have a stronger environmental component (i.e., smoking). A recent study evaluated the relative importance of genetic and environmental factors in determining blood pressure, lipid levels, and body mass index in over 300 twins and 1102 healthy adults. This study found that genetic background had a significantly greater influence on these risk factors than environmental components.5

Another risk factor that is receiving increasing attention is fibrinogen. Several studies have shown a correlation between plasma levels of fibrinogen, stroke risk, and the rate of progression of carotid atherosclerosis.6,7 The control of plasma fibrinogen levels may be under both environmental and genetic control. Since fibrinogen is an acute phase reactant, it increases as a result of various injuries (including stroke or myocardial infarction), inflammation, and cigarette smoking. Genetic studies have shown that plasma fibrinogen levels are significantly elevated in individuals who are homozygous for the rare B2 allele within the fibrinogen gene. Therefore, fibrinogen may represent an important risk factor under both genetic and environmental control. Screening at-risk individuals for the potentially harmful B2 allele might help identify people who should be closely monitored and who should avoid environmental factors that could accelerate atherosclerosis.

Various lipid and lipoprotein abnormalities at the protein and gene level have been correlated with the development of cardiovascular disease.6,9 These disorders are also under environmental and genetic control. Familial hyperalphalipoproteinemia is an autosomal dominant trait that is a common cause of high density lipoprotein (HDL) deficiency. It has been associated with premature cardiovascular disease and strokes.10 Other disorders such as familial hypercholesterolemia (homozygous form), type III and type IV hyperlipoproteinemia, and Tangier disease have been associated with premature atherosclerosis and stroke.11,12 As characterization of the complex genetic pathways responsible for lipid metabolism improves, more high-risk alleles and mutations will be identified.

Epidemiologic Studies

If genetic factors play a role in the etiology of stroke, one might expect epidemiologic studies to identify a family history of stroke as a significant risk factor. Although almost a dozen large-scale studies reported since 1966 have attempted to analyze this issue,13-23 they are limited by some intrinsic weaknesses. Most of the studies did not separate strokes into various subtypes and pathogenic mechanisms, thereby severely limiting any genetic analysis. For example, a large hemispheric stroke could be caused by a cardiac embolism (from atrial fibrillation or valvular heart disease) or by atherosclerotic occlusion of the internal carotid artery. The resulting phenotype is the same, but the underlying etiology is quite different. Likewise, it is unclear if lacunar strokes should be considered etiologically similar to carotid occlusion although they share some of the same risk factors. Some misclassification of stroke type could have occurred since many of the studies...
were done before computed tomographic (CT) scans became widely available.

Few of the studies corrected for patients' ages although it is well known that the risk of stroke increases significantly with advancing age. When a similar error was corrected for in the study of families with Alzheimer's disease, a marked familial aggregation was detected, consistent with an autosomal dominant trait.

Only four studies used modern statistical multivariate analysis to evaluate the role which standard risk factors played in determining stroke risk. Despite these limitations it is interesting to note that three of the four studies using multivariate statistical analysis and correction for age did find a significant contribution of genetic factors to the development of stroke.

These findings must be weighed against the results of the Honolulu Heart Study, which evaluated the occurrence of stroke and heart disease in a cohort of men who migrated from Japan to Hawaii. This study found that environmental factors were of primary importance in determining the occurrence of cardiovascular disease. However, the results could be interpreted as showing that environmental factors modified the expression of the underlying genetic predisposition for vascular disease by accelerating or retarding the rate of atherosclerosis.

Since stroke shares many common epidemiologic and pathogenic features with coronary artery disease (CAD), studies of the genetics of CAD may provide clues about common genetic influences. The epidemiologic study of CAD has been underway for over 40 years with many well-described familial associations. The presence of CAD in a first degree relative prior to age 55 increases by tenfold the risk to other family members. In several twin studies of CAD, most showed a strong genetic component. Familial associations of high-risk lipid alleles have also been demonstrated. Although genetic factors appear to play a major role in determining the risk and development of CAD, direct comparisons between CAD and stroke are difficult since CAD is a fairly homogeneous entity and stroke is clinically and etiologically heterogeneous.

**Familial Stroke**

An alternative approach to large population-based studies is to identify specific families with particular types of stroke. While such familial aggregations are uncommon, an analysis of such families could provide significant information about the pathogenesis of stroke in sporadic cases. Also, the study of such families could lead to the development of larger pedigrees that could be sufficient for genetic linkage analysis.

There have been several reports of well-documented pedigrees with familial cerebral infarction. A French group has described a large family with an apparent autosomal dominant disease that presents with strokes or transient ischemic attacks (TIAs) in the 30s and 40s. These patients appear to have mostly white matter infarcts. There is no significant aggregation of risk factors and no evidence of an inherited coagulopathy. Angiographic studies have been negative. Head CT or magnetic resonance imaging (MRI) showed white matter lucency that in some cases was dramatic. This may be a form of inheritedBinswanger's disease or perhaps a defect in white matter metabolism.

In 1987 Sonninen and Savontaus reported a 51-member family (16 affected) with an inherited form of multi-infarct dementia. Examination of 14 patients showed a mean age of onset of 46 years and mean disease duration of 10.6 years. Head CT showed white matter infarcts that were confirmed on postmortem examination of one patient.

The familial occurrence of vascular malformations, particularly arteriovenous malformations (AVM) and cavernous angiomas, has been reported. In 1985 Boyd et al reported four males in two generations who had AVMs documented by the head CT scan. Several other cases of apparent familial AVMs have been noted. The pattern of inheritance in this report and others suggests an autosomal dominant trait, perhaps with variable penetrance. Since few studies have evaluated asymptomatic relatives of apparently sporadic cases, the true incidence and hereditary pattern are unclear.

In a study of cavernous malformations by Rigamonti et al found a familial aggregation in 13 of 24 probands. In several asymptomatic at-risk individuals brain MRI showed lesions consistent with cavernous malformations. This familial form may be particularly common among Mexican-American families and appears to be inherited as an autosomal dominant trait, perhaps with incomplete penetrance. Pettigrew et al reported another large kindred of 75 individuals with hereditary cavernous hemangiomas.

Intracranial aneurysms have been associated with several other inherited disorders such as polycystic kidney disease, Ehlers-Danlos syndrome type IV, and Marfan's syndrome. There have been a total of 177 patients from 74 kindreds with familial aneurysms reported in the literature. A multivariate analysis showed that intracranial aneurysms in siblings tended to occur at identical or mirror sites and that familial aneurysms tended to rupture at smaller sizes. The exact percentage of cases of aneurysm that are familial is unclear since asymptomatic relatives would not usually be studied if the proband had a negative family history. An autosomal dominant mode of inheritance has been suggested. Several candidate genes for familial and sporadic intracranial aneurysms are being studied, including type I and type III collagen. However, in a recent study LeBlanc et al failed to identify a collagen defect in some cases of familial intracranial aneurysm.

A prime example of the successful interaction of genetic and population research in stroke is the study of Icelandic amyloid angiopathy. This is an autosomal dominant disease with the onset of severe intracerebral hemorrhages before age 40. The cerebral vasculature shows depositions of cystatin C, an...
amyloid-like protein. The cystatin C protein and gene have been isolated and sequenced. Family studies have shown a specific DNA mutation of the cystatin C gene in every clinically affected family member. While the exact pathophysiology of the defect has not been clarified, the finding of a disease-specific mutation will accelerate the understanding of the disease process and offer an accurate tool for genetic counseling. Another recent study by Levy et al identified a pathogenic mutation of the amyloid gene presumed responsible for hereditary cerebral hemorrhage, Dutch type.

There have been other scattered reports of genetic factors underlying the pathogenesis of moyamoya disease, amyloid angiopathy, and transient global amnesia. A recent review described many other inherited conditions (coagulopathies, malformations, metabolic disorders) associated with stroke.

Although the collection of isolated families with specific stroke types may introduce ascertainment and population biases into genetic studies, this approach has the advantage of focusing research on a specific disease while minimizing the influence of a myriad of risk factors. Because stroke is such a complex disorder, population-based genetic studies may not be practical at this time. Directed efforts using families with a specific stroke type or studying various candidate genes in a well-defined population may avoid some of the limitations of past research.

Animal studies of stroke genetics have been limited by a paucity of models with inherited stroke. The spontaneously hypertensive stroke-prone rat has been used in most studies, but the overwhelming genetic influence of the hypertension trait is a limitation. Also, anatomic variations may account for some of the genetic influences. Two reports have found evidence for either a recessive trait or a multifactorial gene effect.

**Future Directions**

Large-scale epidemiologic studies of stroke have been attempted in the past with variable results. Perhaps a more efficient approach would be the identification and study of kindreds with a familial aggregation of a particular stroke type. If several families could be developed, genetic linkage studies could begin. More sophisticated methods of linkage analysis such as APM and ARP could be used to isolate major gene effects, even if extended families could not be ascertained. If a major gene effect is identified, apparent sporadic cases could be screened to determine the frequency of these high-risk alleles. Once major deleterious genes or alleles are identified, steps to prevent or alter gene expression could be instituted.

If large population-based studies are to be attempted again, several modifications may improve patient ascertainment and classification: 1) Identify patients with precursor lesions prior to becoming symptomatic. For example, the relatives of probands with carotid atherosclerosis could be screened by carotid ultrasound. 2) Reclassify stroke types so that similar phenotypes and pathologic lesions are grouped together. Patients with CAD may be considered similar to stroke patients with carotid disease. 3) Ascertain families with early onset disease since they are least likely to be significantly influenced by the standard risk factors.

In conclusion, the study of genetically complex diseases such as CAD, cancer, hypertension, and stroke is now beginning. Rapid advances in molecular genetic techniques and genetic epidemiology have provided the tools to identify and isolate major gene effects responsible for these disorders. Understanding these major genes will provide new avenues for the prevention and treatment of stroke.

**Acknowledgments**

The author wishes to thank Dr. Lawrence Brass for assistance in obtaining some reference material and Dr. Margaret Pericak-Vance for advice in population genetic studies.

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Key Words • cerebrovascular disorders • genetics
Genetic aspects of cerebrovascular disease.
M J Alberts

Stroke. 1991;22:276-280
doi: 10.1161/01.STR.22.2.276

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