Clinically Translated Ischemic Stroke Genomics

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Abstract—Cohort studies show that having a positive family history of stroke increases the odds of having a stroke by \( \approx 30\% \). The heritability of stroke appears to be heterogeneous across ischemic stroke subtypes, with cardioembolic stroke being least heritable. The relative influence of stroke risk attenuates with age, but genetics does not cease to be relevant in later adulthood. Recent family history and twin studies suggest that genetic factors remain relevant even beyond the seventh decade of life. One of the challenges of gene discovery in stroke relates to the complexities of phenotype. The complexities of phenotype can be addressed by focusing on individual ischemic stroke subtypes or by studying intermediate phenotypes like leukaraiosis, which has a heritability of \( \approx 70\% \). Although most stroke genetics research has focused on the identification of risk factor genes, an independent set of genes likely influences poststroke outcomes (for example, apolipoprotein E) and response to drug therapies (example, \( \alpha \)-adducin and diuretic therapy).

(Stroke. 2004;35[suppl I]:2735-2739.)

Key Words: genetics ■ outcome ■ stroke, ischemic

There have been recent successes in stroke genomics, including the discovery of phosphodiesterase 4D\(^1\) and 5-lipoxygenase activating protein\(^2\) as potential risk factor genes. This review does not attempt to catalog the scores of stroke genetics studies that have been reported over the years, but instead attempts to put stroke genetics research into a clinical context.

Evidence for Inherited Stroke Risk

Even without sequencing a single gene, there is substantial evidence for inherited risk of stroke. Cohort, case-control, and twin studies generally support an inherited risk. Flossmann et al\(^3\) at the Radcliffe Infirmary recently generated quantitative estimates of this risk in a systematic review of 3 twin, 33 case-control, and 17 cohort studies identified in a search from 1966 through May 2003. Monozygotic twins had 65% greater odds than dizygotic twins of being concordant for stroke (ie, both twins having stroke or neither having stroke). Among case-control studies, having a positive family history of stroke increased the odds of stroke by 76%. Among cohort studies, having a positive family history of stroke increased the odds of having a stroke by 30%. The most statistically powerful studies found significantly smaller odds ratios than studies of intermediate and low power, suggesting publication bias.

Marenberg et al\(^4\) reported 35-year follow-up results of the Swedish Twin Registry, which included 21 004 twins born between 1886 and 1925. The concordance rates for stroke mortality were 20.6% for monozygotic female twins and 16.4% for dizygotic female twins.

Few studies have assessed the heritability of individual subtypes of ischemic stroke. Two case-control studies of ischemic stroke classified proband stroke type using TOAST criteria.\(^5\) Jerrard-Dunne et al\(^6\) found that a family history of stroke before the age of 65 years increased the odds of having stroke by 38% after adjusting for age, sex, hypertension, diabetes mellitus, serum cholesterol, and smoking status. Family history of stroke before age 65 years was a risk factor for ischemic stroke overall and for the large-vessel subtype. A family history of stroke before age 65 years was not as significant a risk factor for small-vessel stroke, cardioembolic stroke, or stroke of undetermined cause. Polychronopoulos et al\(^7\) found that a family history of stroke was a significant risk factor for ischemic stroke overall and for large-vessel and small-vessel subtypes after adjusting for age, sex, hypertension, smoking, and diabetes. However, family history of stroke was not a significant risk factor for patients with cardioembolic stroke or stroke of undetermined cause. Both studies showed that family history of stroke had the least effect on odds of stroke if the stroke was cardioembolic in cause.\(^6,7\)

Age at Phenotype Expression and Inherited Risk

The relative influence of genetics on stroke risk attenuates with age of phenotypic expression. This phenomenon is well illustrated by the case-control study of Jerrard-Dunne et al\(^6\) of 1000 consecutive cases with ischemic stroke and 800 controls matched for age and sex. Subjects were recruited from 2 south London hospitals. Family history of stroke in first-
degree relatives was obtained by structured interview. A family history of stroke occurring at any age was not a statistically significant risk factor (odds ratio [OR], 1.22; 95% confidence interval [CI], 0.90 to 1.39). However, a family history of stroke occurring in a relative before the age of 65 years was a significant risk factor (OR 1.38; 95% CI, 1.01 to 1.90).

Jerrard-Dunne et al also studied the relationship between age of stroke in the proband and a positive family history of early (occurring by age 65 years) stroke for patients with small-vessel and large-vessel stroke. For probands with small-vessel stroke, a positive family history of early stroke carried an odds ratio for stroke by age 55 years of 3.99. The OR decreased to 2.69 for having a stroke by age 65 years. The OR decreased still further to 1.55 and was no longer significant for having a stroke by age 75 years. For probands with large-vessel stroke, a positive family history of early stroke carried an OR of for stroke at age 55 years of 4.46. The odds ratio decreased to 2.34 for having a stroke by age 65 years. The odds ratio remained significant, but decreased still further to 1.88 for having a stroke by age 75 years.

As the study by Jerrard-Dunne illustrates, the risk imparted by family history attenuates with age. It is also well-known that the risk of stroke increases exponentially with age. The question arises whether the genetic component to stroke risk overwhelms the nonspecific increasing risk with age. This was assessed in a prospective family history registry of 310 probands with recent ischemic stroke. Regression analyses adjusted for sibship size demonstrated that increasing proband age significantly increased the probability of having a concordant sibling, but not the probability of having a living concordant sibling or the probability of having a concordant parent. Attempts at modeling the relationship using a quadratic function or a 2-stage linear function did not explain the probability of having an affected sibling better than a simple linear model. One interpretation of these findings is that there was no point of inflection where the influence of genetics overwhelmed the influence of aging. Our results argued against limiting affected sibling pair studies to any single group of probands defined by age.

Approaches to Phenotyping

So-called common or sporadic stroke is a complex phenotype in terms of the diversity of its clinical presentation and the diversity of its pathophysiology. Genomic research has approached the complexity of the phenotype by focusing on the study of intermediate phenotypes or by focusing on the study of relatively homogenous individual subtypes of stroke (Figure). Although intermediate phenotypes are associated with stroke, they are not necessarily obligate transitional phases to stroke. Intermediate phenotypes are analogous to surrogate end points in clinical trials. Any polymorphism or haplotype found to be associated with an intermediate phenotype should be validated as a risk factor for stroke itself.

Carotid intima-media thickness and leukoaraiosis can be measured among individuals who are otherwise asymptomatic for cerebrovascular disease. Therefore, the number of potential study participants is relatively large, extending to asymptomatic and symptomatic individuals.

The heritability of an intermediate phenotype can be substantially greater than the heritability for the ultimate phenotype. The NHLBI twins study estimated heritability as high as 73%. Magnetic resonance imaging (MRI) determination of leukoaraiosis volume was assessed as part of the Genetic Epidemiology Network of Arteriopathy study in 483 non-Hispanic white subjects. The estimated heritability was 80±10%. Adjustments for sex, age, and systolic blood pressure and brain volume reduced the heritability estimate to 67±11%.

Leukoaraiosis is a risk factor for stroke, but not all polymorphisms that are risk factors for leukoaraiosis are necessarily risk factors for stroke. Cerebral autosomal-dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) is caused by genetic mutations in the notch 3 gene. The MRI abnormalities seen with CADASIL are strikingly similar to the abnormalities seen with severe sporadic leukoaraiosis. Although CADASIL is associated with small-vessel stroke and can even have small-vessel stroke as its presenting feature, CADASIL does not appear to be associated with other types of ischemic stroke.

Numerous genetics researchers have broken ischemic into more clinically homogenous subtypes. Some genetic association studies have supported the notion that phenotypic heterogeneity can parallel genetic heterogeneity. Martiskainen et al have
found that A+ genotype for the fibrinogen gene promoter –455A was a powerful predictor of having 3 or more lacunar infarcts (OR, 2.57).19 Myllykangas et al found the Ser447Ter polymorphism of lipoprotein lipase to be negatively associated with infarcts <1.5 centimeters.20 Unfortunately, much of the research into the genetic determinants of stroke subtype has been hampered by the lack of coherent use of classification systems.21

**Genetic Determinants of Outcome**

There is preclinical evidence for genetic determinants of outcome after cerebral infarction. Jeffs et al22 performed an experiment to identify the genetic component responsible for large infarct volumes in the stroke-prone spontaneous hypertensive rat (SHRSP) in response to a focal ischemic insult. They performed a genome scan in an F2 cross derived from the SHRSP and the normotensive reference WKY strain. The F2 hybrids were subjected to permanent MCA occlusion, and infarct volume was assessed histologically. A genome scan revealed a quantitative trait locus on rat chromosome 5 that accounted for 67% of the total variance in infarct volume. The microsatellite marker known as anf sits within the gene encoding for atrial natriuretic factor. F2 hybrids that were homozygous for the SHRSP allele (ss) had the largest infarct volume (208 mm³). F2 hybrids heterozygous for the SHRSP allele (ws) had an intermediate infarct volume (155.5 mm³). F2 hybrids homozygous for the WKY allele (ww) had the smallest infarct volume (79.2 mm³). The effects of the anf marker on infarct volume were independent of systolic or mean arterial blood pressure.

In a similar experiment, Gratton et al23 studied determinants of infarct volume assessed by MRI after permanent MCA occlusion in the SHRSP, WKY, and F1 hybrids. Adult SHRSP rats and SHRSP x WKY F1 hybrids had comparable infarct volumes, but both SHRSP strains had significantly larger infarct volumes than the WKY rats. The differences could not be explained by differences in blood pressure.

There have been few studies of determinants of stroke outcomes in humans (Table). Most have focused on apolipoprotein E (apoE) as a possible influence on outcomes in ischemic and hemorrhagic stroke, with mixed results. One might expect that several genes harbor common functional variants that can influence outcomes after stroke. Gene products play a role in mechanisms of cell death, proteolysis of the neurovascular matrix, and the inflammatory cascade triggered by ischemia.24 Powerful nongenetic effects on outcome like duration, location, and severity of the ischemic insult will make identifying genes of even moderate effect challenging.

**Stroke Pharmacogenomics**

Numerous host genetic factors likely influence the response to acute stroke pharmacotherapy and pharmacological approaches to primary and secondary stroke prevention. An example of the interaction of host genetic factors with response to acute pharmacotherapy comes from a subgroup analysis of the National Institute of Neurological Disorders and Stroke tPA Stroke Study.25 In an analysis that included 409 patients with an available plasma sample, investigators studied the interaction of apoE2 and apoE4 status on stroke outcome. A treatment versus apoE4 status interaction was not detected, even after adjusting for various baseline covariates known to influence outcome. ApoE2 status itself was not associated with 3-month favorable outcomes, but there was a significant interaction between treatment with tissue plasminogen activator (tPA) and apoE2. The odds ratio for the global test for favorable outcome in patients who were apoE2-negative and received tPA versus patients who were apoE2-...
negative and received placebo was 5.4 ($P=0.03$). The odds ratio for the global test for favorable outcome when adjusting for baseline covariates that predicted outcome was 6.4 for apoE2-positive patients who received tPA versus apoE2-negative patients who received placebo ($P=0.01$). This study shows the potential for using genetic testing to identify a high-responder population for thrombolytic therapy, a goal that has mainly been pursued to this point based on identification of certain MRI signal characteristics.

Stroke pharmacogenomics is likely to be most widely applicable in the outpatient clinic where it could be used to tailor primary and secondary stroke prevention. In a population-based case-control study of patients enrolled in a health maintenance organization, Psaty et al$^{26}$ looked for an interaction between antihypertensive therapy and a common alpha-adducin gene variant (Gly460Trp) that is associated with renal sodium retention in a salt-sensitive form of hypertension in some patient populations. Among patients with the adducin wild-type genotype, diuretic therapy was not associated with risk of myocardial infarction or stroke. However, among the 385 carriers of the adducin gene variant, diuretic therapy was associated with a lower risk of myocardial infarction and stroke than other antihypertensive therapies (OR, 0.49). The current practice of selecting among the various antihypertensive agents, including diuretics, beta blockers, calcium channel blockers, angiotensin-converting enzyme inhibitors, and angiotensin receptor blockers, has been based on practical considerations of cost and side effects as well as nongenetic characteristics of individual patients.$^{27}$ However, the study by Psaty et al$^{26}$ suggests that more effective stroke prophylaxis may eventually be based on specific genetic information.

Current secondary stroke prevention is tailored without the use of genetic information based on the presence or absence of comorbidities like atrial fibrillation or symptomatic moderate-grade to high-grade carotid stenosis. In the Warfarin-Aspirin Recurrent Stroke Study (WARSS),$^{28}$ investigators looked for but did not find a difference between aspirin and warfarin in the prevention of recurrent ischemic stroke or death or in rates of intracranial hemorrhage over a 2-year period of follow-up. In a cohort study within WARSS, Levine et al$^{29}$ found that the presence of antiphospholipid antibodies (either lupus anticoagulant or anticardiolipin antibodies) among patients with ischemic stroke did not predict either increased risk of subsequent vascular occlusive events or a differential response to aspirin or warfarin. Nor was a differential response to aspirin versus warfarin detected based on patent foramen ovale status in a transesophageal echocardiography substudy.$^{30}$ It is possible that within the WARSS noncardioembolic nonatherosclerotic stroke population, there may have been patients who responded differentially to aspirin or warfarin. However, at this time there is no practical, reliable means of separating out the high-responders versus low-responders for either therapy. Pharmacogenomics may allow for selection of high-responder versus low-responder populations to antithrombotic drug regimens in the future.

The African-American Antiplaletate Stroke Prevention Study (AAASPS)$^{31}$ calls into question the value of racial/ethnic status as a means of identifying high-responder versus low-responder populations in secondary stroke prevention. AAASPS was a randomized double-blind multicenter trial of aspirin versus ticlopidine for secondary stroke prevention among 1809 black men and women who had recently had a noncardioembolic ischemic stroke. During a 2-year follow-up, investigators found no significant difference between ticlopidine and aspirin in the prevention of recurrent stroke, myocardial infarction, or vascular death. The rationale for the study relied mainly on a subgroup analysis of the Ticlopidine Aspirin Stroke Study (TASS), which suggested a more favorable risk benefit profile for nonwhites than whites.$^{32}$ Associated demographic variables are unlikely to be adequate substitutes for molecular genetic information.

## Conclusion

Taking model-free genome-wide approaches to gene discovery can seem like a fishing expedition. The human genome has so many single nucleotide polymorphisms and even more potential genotype combinations that the nets that have been cast are weighed down by the so-called curse of dimensionality.$^{33}$ Fortunately, methods of data mining continue to advance. Recent successes using the Iceland Healthcare Database demonstrate that genomic approaches to stroke can yield specific risk factor genes. Genetic testing will likely have a routine role in optimizing prevention and treatment of stroke.

## References


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Stroke. 2004;35:2735-2739; originally published online September 9, 2004;
doi: 10.1161/01.STR.0000143156.32467.fd
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2004 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/35/11_suppl_1/2735

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