Update on the Genetics of Stroke and Cerebrovascular Disease 2006

Martin Dichgans, MD; Robert A. Hegele, MD, FRCP, FACP

The last year has again seen several advances in the genetics of cerebrovascular disease covering a spectrum of disorders ranging from small vessel disease to intracranial aneurysms to ischemic stroke.

Collagen Type IVα1 and Small Vessel Disease
A key discovery was the identification of collagen type IV α1 (COL4A1) mutations in families with cerebral small vessel disease.1,2 Col4a1 was initially identified as the causative gene in a mouse mutant with perinatal cerebral hemorrhage and porencephalopathy.3 Heterozygous (Col4a1<sup>-1/4α1</sup>) mice develop recurrent hemorrhages in the basal ganglia—the typical site of intracerebral hemorrhage in hypertensive patients. Subsequent analysis of affected family members with porencephalopathy and cerebral small vessel disease revealed several mutations in the human COL4A1 gene.1,3-5

Type IV collagens are an integral component of the vascular basement membrane. COL4A1 and COL4A2, the most abundant type IV collagens, form hetero-trimers. The triple helix domain contains repeated glycine-proline-X motifs, which are critical for helix formation during collagen assembly. Most identified mutations involve glycine residues within such motifs. It was therefore hypothesized that COL4A1 mutations interfere with triple helix formation or heterotrimer secretion; in fact, there is evidence from Col4a1<sup>−1/4α1</sup> embryonic tissue that mutations inhibit collagen secretion into the basement membrane. Ultrastructural abnormalities in capillaries from COL4A1 mutation carriers indicate disordered basement membrane assembly.

The phenotypic spectrum associated with COL4A1 mutations is broad and strongly connected to small vessel disease. Key features include leukoencephalopathy, microhemorrhages, and clinically overt hemorrhage. The structural compromise of small blood vessels is illustrated by the fact that birth trauma, brain trauma, and oral anticoagulants may trigger intracerebral hemorrhage in COL4A1 mutation carriers.1,2 Furthermore, the precipitating role of these factors exemplifies gene-environment interactions. Genes encoding vascular basement membrane-associated proteins remain attractive candidates for intracerebral hemorrhage and leukoencephalopathy.

Genes Implicated in Intracranial Aneurysms
Epidemiological studies demonstrate a strong genetic influence on the development of intracranial aneurysms (IA). The risk of subarachnoid hemorrhage is increased by a factor of 6 and 4, respectively, in siblings and other first-degree relatives of patients with subarachnoid hemorrhage. Within recent years, genetic loci for IA have been mapped to regions on chromosomes 1p34, 2p13, 5q22–31, 7q11, 11q24–25, 17cen, 19q, and Xp22. However, few genetic defects have been characterized at the nucleotide level.

A recent study showed that a functional at-risk haplotype spanning the 3′-untranslated region (3′-UTR) of the elastin gene (ELN) and the entire LIM domain kinase 1 (LIMK1) gene confers susceptibility to IA.4 The ELN gene is located within the chromosome 7q11 linkage region and was recognized earlier to be a positional and functional candidate gene. However, allelic association studies yielded variable results.5-7 The novel findings from Akagawa et al are based on a systematic analysis of 166 single nucleotide polymorphisms (SNPs) and haplotypes that reside within the chromosome 7 linkage peak. These investigators identified a highly significant association (P<0.000002) between IA and a distinct linkage disequilibrium block containing the 3′-UTR of ELN and the promoter region of LIMK1.4 The strongest association was found with the ELN G(+695)C tag-SNP for an at-risk haplotype comprised of the functional ELN [+502]A insertion and the LIMK1 C[−187]T SNP. Both the genotype and haplotype associations were replicated in an independent cohort. Functional studies revealed that the ELN [+502]A insertion decreases ELN transcription, whereas the LIMK1 C[−187] SNP reduces promoter activity. Synergism between genetic variants in ELN and LIMK1 on vascular stability and distensibility seems plausible because: (1) elastin is a major structural component of the internal elastic lamina in cerebral arteries; (2) ELN plays a key role in vascular development and remodelling; (3) secreted elastin activates a G-protein-coupled signaling pathway that stimulates actin stress fiber organization; and (4) LIMK1 is a regulator of the major actin cytoskeleton.

Another exciting new finding is that sequence variation in the tumor necrosis factor receptor superfamily, member 13B

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Stroke is available at http://www.strokeaha.org DOI: 10.1161/01.STR.0000254710.32761.44

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(TNFRSF13B) gene may contribute to IA risk. Using sequence analysis of genes under the linkage peak on chromosome 17, Inoue et al identified several potentially deleterious changes in TNFRSF13B that segregated with IAs in pedigrees. Sequence analysis of a portion of TNFRSF13B in a large case-control sample showed that several potentially functional rare variants were significantly more frequent in cases (14/304) than in controls (5/332). Finally, association analyses suggested that one of the TNFRSF13B haplotypes was protective. These findings require replication in other cohorts, but they illustrate the need to consider both rare and common genetic variants in the pathogenesis of IA. Interactions of genetic factors with known risk factors for aneurysm formation such as smoking and hypertension remain another important area of research.

**PDE4D and Ischemic Stroke**

The initial enthusiasm that followed the identification of a highly significant association between an at-risk haplotype in the phosphodiesterase 4D (PDE4D) gene and ischemic stroke in Icelandic subjects a few years ago has been somewhat deflated because of difficulty in replicating the positive findings in other populations.

The original DeCode study identified several SNPs that were associated with the combined phenotype of large-vessel stroke and cardiogenic stroke. An at-risk haplotype (termed “GO”) built by a microsatellite marker (AC008818-1) and SNP (“SNP45”) conferred a relative risk of 1.5 for the combined phenotype compared with the wild-type haplotype (GX). Since then, numerous studies have examined the PDE4D gene in other populations. Some were negative, whereas others found weaker associations between other PDE4D SNPs or haplotypes and stroke subtypes. Importantly, the original association between the GO haplotype and the combined phenotype has yet to be replicated. Moreover, Rosand et al used the International HapMap Project dataset to show that no significantly associated SNP from any follow-up study was correlated with the original PDE4D GO haplotype. Despite these difficulties, the association signal from the DeCode study remains sufficiently impressive for some investigators to provide rationale for ongoing studies in other populations. These studies, together with analysis of the functional impact of specific PDE4D haplotypes and SNPs, should help clarify the role of PDE4D in ischemic stroke.

**Ischemic White Matter Disease**

Recent data suggest there is a strong genetic component to ischemic white matter (WM) lesions. Estimates of the heritability of incidental white matter T2-hyperintensity (WMH) volume range between 55% and 71% although the genes determining such lesions are still unknown. A recent genome-wide linkage analysis performed in 747 individuals (237 families) from the Framingham Heart Study provides evidence for a locus influencing WMH volume on chromosome 4. The signal on chromosome 4 was just above threshold for statistical significance (maximum multipoint logarithm of the odds score = 3.7) and a second nonsignificant linkage peak was found on chromosome 17. Although such evidence might seem weak, it is probably what would be expected in a multifactorial trait whose expression requires the interactions of multiple genes and environmental risk factors. Replication in other samples would strengthen the finding, but the experience with linkage studies in other complex disorders is that few loci are consistently detected across cohorts.

Additional support for multiple genes influencing ischemic WM lesions comes from CADASIL, which is a monogenic small vessel disease. Virtually all NOTCH3 mutation carriers will develop WM lesions and subcortical infarcts. Yet, lesion severity varies markedly between individuals matched for demographic factors. Recent data from 151 subjects from 95 families suggest the volume of ischemic brain lesions in CADASIL is strongly influenced by modifying genetic factors distinct from the causative NOTCH3 mutations. The heritability estimate (74%) for WMH volume in CADASIL is remarkably close to previous estimates for incidental WMH volume, and it is tempting to speculate that some genes that influence WM lesion volume in CADASIL might also be relevant to sporadic disease. The search for these genes remains an important area of research.

**More Candidate Gene Association Studies**

The past year has again seen various candidate gene association studies in the stroke field. An intriguing finding is the association between a haplotype in the vitamin K epoxide reductase complex subunit 1 (VKORC1) gene and multiple cardiovascular end points including stroke, coronary heart disease and aortic dissection. The stroke association was independently significant for ischemic and hemorrhagic stroke and for large artery and small vessel stroke. Potential mechanisms include effects on hemostasis, vessel calcification, and angiogenesis. Of note, VKORC1 sequence variants have recently been implicated in interindividual differences in warfarin sensitivity. Thus, VKORC1 variation and drug response represents a good example of the emerging area of pharmacogenomics.

Another interesting finding was the reported association between a promoter SNP of the glutamate transporter EAAT2 gene and early neurological worsening after stroke. The at-risk genotype was associated with elevated plasma glutamate concentration, possibly reflecting enhanced excitotoxicity in patients. In addition, there was a functional effect of the at-risk allele in cultured astrocytes. Of course, these EAAT2 associations need to be replicated in other cohorts. Nonetheless, this study by Mallolas has drawn attention to stroke outcome as a meaningful phenotype for genetic studies.

**Summary**

In summary, 2006 saw continued progress in identifying genes for monogenic forms of stroke and related phenotypes in families. Studies of more common and complex phenotypes continue to provide some promising leads but also some inconsistencies between populations. This mirrors the developments observed in the genetics of other disorders and medical fields. The coming year promises to be equally exciting, with the imminent characterization of new forms of
genomic variation, and further progress in rare disorders, pharmacogenomics and complex disease analysis.

**Sources of Funding**
Supported by grants from the Deutsche Forschungsgemeinschaft to M.D. (SFB596/TPA4) and from the Heart and Stroke Foundation of Ontario and Genome Canada, through the Ontario Genomics Institute to R.A.H.

**Disclosures**
None.

**References**


**Key Words:** association, COL4A1, Genetics, PDE4D, stroke, white matter
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Stroke. 2007;38:216-218; originally published online January 4, 2007;
doi: 10.1161/01.STR.0000254710.32761.44
Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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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/38/2/216

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