Update on the Genetics of Stroke and Cerebrovascular Disease 2008

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2008 has brought us the first fruits from genomewide association studies (GWASs), an unbiased and comprehensive approach to identify common risk alleles for complex diseases of adulthood. By genotyping >310 000 single nucleotide polymorphisms (SNPs) in over 1700 intracranial aneurysm (IA) cases and 7400 controls from Finland and the Netherlands, a multinational team of investigators recently identified several common SNPs that showed significant association with IA.1 SNPs on chromosomes 2q, 8q and 9p were found to replicate in an independent sample from Japan with similar odds ratios (OR) in all 3 samples. Pooled OR were between 1.24 and 1.36, and analyses of the combined effects suggested a significant linear relationship with risk score in each cohort, with a more than 3-fold increase from the lowest to highest strata. Collectively, the 3 loci were calculated to account for 38% to 46% of the population-attributable fraction of IA, which is substantial. Although additional work in other ethnic groups is required, the consistency emphasizes the robustness of the findings.

The critical genetic intervals on chromosomes 8q and 9p both harbor genes that are implicated in the regulation of stem (progenitor) cell populations and expressed in the adult vasculature. SOX17, the main candidate gene on 8q, is required for both endothelial formation and maintenance—an interesting aspect when considering the predominant location of IA at arterial branch points and sites of endothelial shear stress.

The association between common variants at 9p and IA had already been reported in another study earlier last year.2 The 9p21.3 region was originally identified as a major risk locus for coronary artery disease and myocardial infarction.3 Subsequent analyses revealed that the same chromosomal region is also implicated in the risk of IA and abdominal aortic aneurysms. The estimated risk for IA conferred by the main risk allele (rs10757278-G) was very similar across sample sets from Iceland, Finland, and the Netherlands and comparable to that for abdominal aortic aneurysms and coronary artery disease. Moreover, the risk for abdominal aortic aneurysms and IA was found to be independent of the risk for coronary artery disease, thus indicating a broader role of the 9p21.3 region in arterial disease.2

Extending these findings, investigators of the international stroke genetics consortium recently demonstrated associations between several SNPs in the 9p21.3 region and large artery stroke.4 The study included 4376 ischemic stroke patients, 970 of whom were classified as having large artery stroke. ORs for the lead SNP (rs1537378-C) were remarkably consistent across populations from different geographical regions and ethnic backgrounds (pooled OR = 1.21). In contrast, no associations were found with other etiologic stroke subtypes, thus stressing the specific impact of the 9p21.3 region on large artery disease. The estimate for the population-attributable fraction was 20%, which renders the 9p21.3 region a major focus for large artery stroke.

The influence of the 9p21.3 region on risk of vascular disease seems to be independent of conventional vascular risk factors. This would suggest that we are dealing with an entirely new risk factor for various arterial phenotypes.2,4 There is some indication that the effects of 9p21.3 are mediated through vascular remodeling. However, additional studies are needed to disentangle the mechanisms leading to such diverse arterial phenotypes. The next challenge now is to identify the responsible genes and associated mechanisms. Positional candidates include CDKN2B (encoding p15INK4b), CDKN2A (encoding p16INK4a, and ARF) and ANRIL (a nonprotein-coding transcript), all of which are expressed in the vasculature. Of interest, p16INK4a like SOX17 has been shown to be implicated in the regulation of stem (progenitor) cells.5

Another important finding has been the identification of risk variants for ischemic stroke on chromosome 4q25. Investigators from Iceland, Germany, Sweden and the United Kingdom recently reported a multistage GWAS including 6222 ischemic stroke patients and 29 474 controls. A single SNP (rs2200733-T) on chromosome 4q25 was significantly associated with cardioembolic stroke in various European populations. Again, ORs were remarkably similar between samples, the combined OR being 1.52 (P=5.8×10^{-7}).6 The same variant has previously been associated with atrial fibrillation,7 which suggests that the risk of stroke is mediated through atrial fibrillation, although this was not specifically addressed in the study by Gretarsdottir et al. Of interest, rs2200733-T was also associated with ischemic stroke not classified as cardioembolic stroke (combined

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OR = 1.18), suggesting that atrial fibrillation may be underdiagnosed in this group of patients. In theory, genotyping of rs2200733 might help identify subjects at risk for intermittent or future permanent atrial fibrillation. However, it is currently unclear whether such an approach has diagnostic use. Ideally, guidance on the use of gene testing should come from randomized prospective trials (see below).

The above results illustrate the need for cautious phenotyping. Most likely, the majority of risk alleles contribute to etiologic stroke subtypes rather than stroke per se. Thus, “lumping” stroke subtypes together will attenuate most of the signals in association studies. Analyses according to subtypes pose a challenge to both sample size and phenotyping quality. Sample size issues can be solved by collaborative efforts such as those within the Ischemic Stroke Genetics Consortium (www.strokegenetics.org). In contrast, harmonization of phenotyping protocols and stroke subtype classification between sites remains a major task. Standardized data on conventional risk factors, intermediate phenotypes such as intima-media thickness, infarct volumes and outcomes are desirable but not always possible to obtain. Such data will eventually be needed to disentangle the mechanisms by which genetic variants contribute to stroke risk.

The initial results from GWASs might represent the “low hanging fruit” for genetic susceptibility to stroke although some alleles with moderate relative risks may have gone undetected. Future studies to identify risk alleles with smaller relative risk ratios (say ≈ 1.10 or less) will probably require tens of thousands of patients. While biologically important, the clinical relevance of such markers is currently not clear.

**Pharmacogenomics**

Recent progress in pharmacogenomics might soon be translatable into the clinic. Two pertinent examples include characterization of genetic differences in sensitivity to anticoagulation by warfarin and the identification of risk alleles for serious myopathy with statins.

Observational studies and randomized trials have shown that genotypes of the cytochrome P450 2C9 gene, CYP2C9, and the vitamin K epoxide reductase complex 1 gene, VKORC1, influence warfarin response across diverse patient groups. Furthermore, CYP2C9 variant genotypes are associated with increased risk of serious bleeding events. But despite the excitement about this research, routine genotyping of CYP2C9 and VKORC1 in the general patient population to guide warfarin dosing is not yet supported by current evidence. This might change with accumulation of prospective studies, which are currently underway (www.clinicaltrials.gov).

A common adverse effect encountered in patients taking statins is dose-related myopathy. A recent GWAS showed a strong association of simvastatin-related myopathy with a common SNP located within the drug transporter gene SLC01B1. This SNP had a prevalence of ≈ 15% in the study population, and 1 or 2 copies of the risk allele increased the odds of severe myopathy by ≈ 4 and ≈ 17-fold, respectively. Although these findings might not be generalizable to patients with milder myalgias or to those taking statins other than simvastatin, the study provided proof-of-concept of genetic susceptibility to this adverse effect. In light of the obvious benefit of statin drugs, it is not yet clear whether this SNP should be routinely genotyped, because ≈ 85% of homozygotes for the risk allele tolerated simvastatin without incident. Perhaps the initiation dose of statin could be reduced in genetically predisposed individuals, but even this idea would require further clinical evaluation.

**More Candidate Gene Studies in Stroke**

As illustrated above, complex genetics is moving toward unbiased genomewide approaches. Nevertheless, the past year has continued to see numerous candidate gene-association studies. A broader theme in many of these studies has been the inflammatory system. Thus, for example, investigators from Austria found an association between a deletion mutation in the chemokine receptor CCR5 and a lower risk of incident cardiovascular disease including stroke. The same mutation was associated with lower levels of C-reactive protein and a lower carotid intima-media thickness, suggesting an intriguing link between the genetic variant, inflammation, an intermediate phenotype, and clinical end points. However, as always replication in independent cohorts remains the litmus test in genetic association studies.

**Outlook**

Genetics remains a rapidly developing field. The coming months and years will see additional GWASs on ischemic stroke, hemorrhagic stroke, and related phenotypes such as white matter hyperintensities and intima-media thickness. Advances in genotyping methodology and analysis tools now also allow for the identification of rare (micro)deletions, duplications and insertions on a genome-wide level. Such variants—collectively termed copy numbers variations (CNV)—have recently been recognized to contribute to complex multifactorial disease. Rare variants may account for a larger fraction of the overall genetic risk than previously assumed. One example is the recent identification of rare independent mutations in renal salt-handling genes contributing to blood pressure variation. The most complete profile of genomic differences between patients and controls will eventually come from next-generation whole genome sequencing, which is already within our reach if not our grasp. Expectations are high that genetic discoveries will eventually be translated into improved diagnostics and treatment.

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None.

**References**


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