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Stroke Genetics Update: 2011

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Abstract

Although stroke remains a leading cause of disability and mortality worldwide, recently there have been significant advances related to our understanding of the genetic basis of stroke. Ongoing research efforts put us on the cusp for major breakthroughs in the field. In this review, we present the substantial evidence for the contribution of genetic variation to the development of stroke, and the difficulties posed in the study of stroke given the numerous genetically driven risk factors and stroke subtypes. We emphasize recent findings implementing candidate gene and genome-wide association approaches. We then discuss how emerging knowledge is informing and reshaping our understanding of stroke biology and how, in the near term, genetics may be used clinically to identify individuals who are at risk of disease or who may derive benefit from specific treatment modalities. Lastly, we address ongoing and future approaches that will continue to improve our understanding of stroke genetics.

Keywords

Stroke; Genetics; Genome-wide association studies

Introduction

Stroke is a prototypical complex disease occurring secondary to the interaction of multiple genes in combination with both lifestyle and environmental factors. Complicating our ability to understand these relationships is that many of the major stroke risk factors, such as hypertension, diabetes, and hyperlipidemia, among others, are considered complex diseases unto themselves. Further, both forms of common stroke, including ischemic and hemorrhagic, each have several distinct subtypes that can also be thought of as unique diseases, each with their own genetic and environmental risk factor profiles. Numerous studies demonstrate unique risk loci for the various ischemic subtypes including large vessel atherosclerotic, cardioembolic, and small vessel or lacunar stroke, as well as intracerebral hemorrhage subtypes including lobar and deep. Although there is strong evidence for stroke clustering in families, stroke lacks a clear-cut pattern of inheritance. As such, it is difficult to determine a person's risk of inheriting or passing on either form of common stroke, and even less clearly the risk of developing any specific subtype. In this review, we describe

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recent advances in our understanding of stroke genetics relevant to the pathophysiology of stroke and to advancing diagnosis, prognosis, and care.

Stroke Subtypes

Broadly, stroke can be thought of as the clinical manifestations of an occluded or ruptured cerebral blood vessel, yet it is also a complex end phenotype with many differing genetic predispositions and etiologies. From both a clinical and research standpoint, strokes are typically classified into one of the two major forms, either ischemic or hemorrhagic. In the acute clinical setting, a stat head CT in the emergency room is typically implemented to define which form is present. Although such a distinction is critical for therapeutic decision making, such as the administration of thrombolytic agents or clot evacuation as examples, it is likely that these two forms of stroke have both similar and differing genetic predispositions and etiologic overlaps. Further, the patients' global risk factor profile at the time of the stroke is believed to significantly influence the form of stroke that occurs. For example, hypertension is a strong risk factor for both forms of stroke; however, extremely poor control is known to markedly escalate non-lobar hemorrhage risk [1]. Continuing with hypertension as an example, genetic predisposition not only plays a role in the development of hypertension, but genetics also play a role in each patient's ability to tolerate the ill-effects of hypertension. Some patients' vessels may develop wall thickening whereas others may develop aneurysms and rupture. Such physiologic responses are under genetic control but may be further influenced by the presence or absence of other risk factors. As described, the pathophysiology of stroke is complex, thereby making the study of the genetic basis of stroke challenging. Given these difficulties, the two forms of common stroke, ischemic and hemorrhagic, are typically studied in isolation; however, it is likely that as our understanding of the genetic basis of stroke improves, both shared and unique genetic predispositions across both forms of stroke will be identified.

It is not only important to identify the major form of stroke, but it is also important to classify the stroke accurately into a specific subtype. Such classifications have both clinical and research implications given that both ischemic and hemorrhagic stroke subtypes differ in cause, pathophysiology, treatment, and outcome. The various subtypes and their relative incidences are listed in Table 1 [2–5]. The Trial of Org 10172 in Acute Stroke Treatment (TOAST) subtypes are commonly used to classify ischemic stroke [6]. Common subtypes of hemorrhagic stroke are subarachnoid hemorrhage and intracerebral hemorrhage, typically further classified as related to amyloid angiopathy or hypertension. A minority of strokes result from vascular occlusion in the cerebral venous system, termed cerebral venous thrombosis [2–5].

From a clinical standpoint, it is important to note that whereas ischemic strokes arise secondary to an occluded artery, the occlusion may occur due to a thrombus that develops at the site of occlusion or may be due to an embolus that forms more proximally in the circulation. Regardless of the source, in the absence of blood flow the brain parenchyma and vasculature distal to the clot quickly become ischemic. As such, if the vessel wall is weakened secondary to ischemia and if the clot is subsequently removed, either by the natural fibrinolytic system or via the use of tissue-plasminogen activator (tPA) or mechanical thrombectomy, the weakened vessel wall is prone to rupture. In such situations an ischemic stroke becomes hemorrhagic and is termed a "reperfusion hemorrhage." Although the frequency of such events is not firmly established across all subtypes, genetics are thought to play a role, with predisposition to such events defined in part by the patient's genetically derived ability to withstand variable amounts of ischemia.

From a research standpoint, subtyping the common forms of stroke and studying such subtype-specific patients as compared with matched controls is the typical methodology implemented in most stroke genetics studies. However, other techniques are also implemented, including analyses focusing on early-onset disease, using affected family members or affected sibling pairs, and by evaluating groups of patients with a paucity of standard vascular risk factors. Prospective studies are also employed, but such studies can be limited by the number of stroke events that occur in any time period, particularly when considering the various stroke subtypes.

Family History as a Risk Factor

Twin studies and familial aggregation studies suggest a stronger genetic contribution to early-onset than later-onset stroke. Reports from the National Academy of Sciences–National Research Council Twin Registry showed a greater concordance in monozygotic twins relative to dizygotic twins when the twin cohort was younger [7, 8]. Evidence from prior research, including meta-analyses [9], has consistently supported a greater degree of familial aggregation and a greater inherited component of stroke among early-onset compared to late-onset ischemic stroke cases. These findings were replicated in a young stroke dataset demonstrating that siblings of stroke cases had more than four times the risk of stroke compared to siblings of controls (odds ratio [OR] 4.17; 95% CI, 1.9–8.8), and mothers of stroke cases had twice the risk of stroke compared to mothers of controls (OR 2.02; 95% CI, 1.4–3.0). Furthermore, the association between stroke in probands and family history of stroke was strongest among women ages 15 to 24 years (OR 2.5; 95% CI, 0.4–15.1) and diminished with increasing proband age (OR 1.6; 95% CI, 0.8–3.3 among women 25–34 years of age and OR 1.5; 95% CI, 1.1–1.9 among women 35–49 years) [10]. Taken in aggregate, the evidence supports the point of view that the extreme disease phenotype, young age-of-onset stroke, has more familial aggregation and may be more genetically driven than later-onset forms of the disease.

A recent Framingham study greatly added to understanding the relationship between parental history of stroke and risk of stroke in offspring. Parental occurrence of stroke by age 65 years increased the risk of stroke in offspring by threefold [11•]. This elevated risk persisted after adjusting for conventional risk factors. Individuals with a parental history of stroke had higher risk than people without a parental history of stroke across all five quintiles of baseline risk estimated using the Framingham Stroke Risk Profile, a validated tool that incorporates clinical and demographic information such as the presence of left ventricular hypertrophy on electrocardiography. The greatest effect of parental history of stroke occurred at the highest quintile of risk. In some settings, targeted screening appears to be more cost-effective for cardiovascular primary prevention [12]. Screening for a parental history of stroke may be one way to screen for high-risk populations for targeted stroke prevention.

Studies evaluating the familial risk of intracerebral hemorrhage (ICH) demonstrate that ICH in a first-degree relative increases a subject's odds by as much as two- to sixfold [13]. Subarachnoid hemorrhage (SAH) has a distinct clinical profile, and a family history of SAH has been associated with an increased risk for SAH of about fourfold [14]; because of this association, screening of the SAH patient's first-degree relatives for asymptomatic aneurysms is recommended [15]. Further, evidence of a gene–environment interaction with smoking, another risk factor for SAH, has also been demonstrated. In one such study [16•], 339 cases of aneurysmal SAH (aSAH) were matched to 1016 controls. Compared to current nonsmokers with no first-degree relatives with aSAH (–FH), the OR for aSAH for current nonsmokers with +FH was 2.5 (95% CI, 0.9–6.9); for current smokers with –FH, the OR was 3.1 (95% CI, 2.2–4.4); and for current smokers with +FH, the OR was 6.4 (95% CI,

3.1–13. 2) [16•]. These results emphasize the importance of smoking cessation in patients with a positive family history of SAH.

Chromosome 9p21.3 Locus

This locus was first discovered by genomewide association to be a risk factor for coronary artery disease [17–19] before studies showed a relationship with ischemic stroke [20, 21•]. The locus also associates with intracranial and aortic aneurysms and peripheral vascular disease [22]. The causative variant remains unknown. Recently, chromosome 9p21.3 has also been associated with platelet reactivity [23]. This increased platelet reactivity may explain the association with myocardial infarction and stroke; it is less clear how increased platelet reactivity might relate to aneurysm formation. The 9p21.3 locus includes a non-coding RNA called ANRIL, which in turn alters expression of several genes related to cellular proliferation [24]. Recent data also indicates that 9p21 polymorphisms influence inflammatory signaling [25] and vascular cell proliferation [26]. An improved explanation for the pleiotropic effects of the 9p21.3 locus on multiple vascular beds should emerge in the near future.

Cerebral Autosomal Dominant Arteriopathy with Subcortical Infarcts and Leukoencephalopathy (CADASIL) and Conventional Risk Factors

Mutations in *Notch3*, a gene encoding a large transmembrane receptor, cause cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) [27]. The function of the human Notch3 receptor remains poorly understood. Although CADASIL remains incurable, recent clinical observations suggest that the risk of stroke in patients with this disease may be modifiable. A review of 200 consecutive subjects enrolled in the UK CADASIL National Referral Service showed that the risk of stroke increased in the presence of conventional risk factors [28]. The odds of stroke were 2.5 times greater for patients with hypertension. The number of pack-years of smoking was associated with risk of stroke, and current smoking was associated with earlier age at onset of stroke. These findings support aggressive vascular risk factor modification in this patient population.

Hereditary Angiopathy with Nephropathy, Aneurysms, and Muscle Cramps and Carotid Aneurysms

A clearer picture is emerging about the cerebrovascular manifestations of hereditary angiopathy with nephropathy, aneurysms, and muscle cramps [29]. This condition, which is caused by mutations in *COL4A1* involving glycine residues in the $\alpha 1$ chain of Type IV collagen, can cause hematuria, renal cysts, elevations in creatine phosphokinase, and retinal arterial tortuosity. Patients can also have lacunar infarcts, cerebrovascular micro-hemorrhages, white matter changes, and dilated perivascular spaces. Aneurysms confined to the carotid siphons should heighten suspicion for this condition. Risk of rupture from these carotid aneurysms appears to be low. It is not known whether the risk is lower than the natural history of unruptured aneurysms in unselected patients [30].

Mitochondrial Genetic Risk of Ischemic Stroke

Interest in mitochondrial genetics resurged after the report of an association between a common haplogroup and stroke [31]. A multicenter mitochondrial genome-wide association study found an association with ischemic stroke and a genetic risk score that included summation of the contributions of individual mitochondrial variants [32]. No individual variant was significantly associated with ischemic stroke. This can be explained by the low

power resulting from low minor allele frequencies and low effect sizes. It may not be possible to use association methods to detect effects for individual mitochondrial variants. Post hoc statistical power calculations suggest that to do this would require > 80,000 cases.

Established mitochondrial diseases, such as Mitochondrial Encephalopathy, Lactic Acidosis, and Stroke-like episodes (MELAS), remain an active focus of research, particularly as related to therapeutic interventions. This maternally inherited syndrome caused by mutations in mitochondrial DNA typically presents with stroke before age 40 years, encephalopathy characterized by seizures or dementia, and blood lactic acidosis or ragged red fibers in skeletal muscle biopsy specimens [33]. The causal hypotheses for Stroke-like episodes (SE) in MELAS presented to date are angiopathy, cytopathy, and neuronal hyperexcitability [34]. Administration of L-Arginine (L-Arg) to MELAS patients has been successful in reducing neurologic symptoms due to acute strokes and preventing recurrences of SEs in the chronic phase. L-Arg is believed to have dual pharmaceutical effects on both angiopathy and cytopathy in MELAS [34].

Genetics of Intracerebral Hemorrhage

Convincing evidence for associations between apolipoprotein alleles $\epsilon 2$ and $\epsilon 4$ and lobar ICH was recently generated by the International Stroke Genetics Consortium [35]. The collaboration involved 2189 cases of ICH and 4041 control subjects from seven cohorts. This study included the first genetic association study of lobar ICH to reach genomewide significance.

Genome-wide Association Studies

The first genome-wide association study (GWAS) of ischemic stroke was reported from the Ischemic Stroke Genetics Study (ISGS) in 2007 [36]. No genetic locus was specifically and robustly associated with stroke, but the sample size was quite modest, including only about 250 patients and a comparable number of stroke-free controls. Since that time, few stroke GWAS have been reported in literature [37•], although several are ongoing and soon to be reported. In one recently reported study, a prospective GWAS was performed by the Cohorts for Heart and Aging Research in Genomic Epidemiology (CHARGE) Consortium demonstrating a novel association between ischemic stroke and two single nucleotide polymorphisms (SNPs) on chromosome 12p13. The primary study population, consisting of nearly 19,600 subjects with 1544 incident strokes from four prospective white cohorts, identified two SNPs, rs12425791 and rs11833579, in the region of 12p13. Results of the initial GWAS suggested that each copy of a minor allele at these two loci increased the hazard ratio for total stroke by about 1.3 (95% CI, 1.19–1.44) and for ischemic stroke by about 1.4 (95% CI, 1.27–1.56). The corresponding population-attributable risks were 11% to 13% for total stroke and 14% to 17% for ischemic stroke. They further tested for an association between the two implicated SNPs and ischemic stroke in two independent replication samples, an African-American community-based cohort and a Dutch case-control sample, and observed that rs12425791 was significantly associated with the incidence of ischemic stroke, especially atherothrombotic stroke in both samples. This SNP was located close to the *ninjurin 2* gene (*NINJ2*: <http://www.ncbi.nlm.nih.gov/gene/4815>), which encodes an adhesion molecule expressed in glia that shows increased expression after nerve injury. The International Stroke Genetics Consortium (ISGC) and the Wellcome Trust Case-Control Consortium 2 (WTCCC2) performed a meta-analysis combining case-control data among persons of European ancestry and a population-based genome-wide cohort study but did not replicate this association [38•]. In addition, an Italian and a Swedish case-control study also failed to show any association between the two genetic variants on chromosome 12p13 and ischemic stroke [39, 40]. Conversely, a large population-based,

case-control study found a significant association between atherothrombotic stroke and rs12425791 in Japan [41].

One ongoing effort with respect to GWAS includes collaboration between the WTCCC and members of the ISGC. These efforts, which will soon be published, implemented a multistage GWAS of ischemic stroke among whites of European ancestry. Recently, the National Institute of Neurological Disorders and Stroke (NINDS) funded a large international GWAS in ischemic stroke, formally called the Stroke Genetics Network (SiGN). In this exciting study, a large multiethnic population consisting of an estimated 12,000 ischemic stroke cases and 17,000 controls will undergo GWAS and subsequent subtype analyses. Interestingly, a new subtyping algorithm, termed the Causative Classification System (CCS), is being implemented among all the cases (accessible at: https://ccs.mgh.harvard.edu/ccs_title.php). The CCS is an automated version of the SSS-TOAST system [42, 43, 44•], and implements an evidence-based classification system that harmonizes multiple aspects of the diagnostic stroke evaluation in a standardized manner to determine the most likely etiology of stroke. The algorithm has been developed to maximize inter-examiner reliability in stroke classification while utilizing the full diagnostic data set from a typical “stroke work-up”; such data would include brain and vessel imaging, cardiac tests, and laboratory data, among other available information. The automated CCS system limits inter-examiner variability in interpretation of stroke-related characteristics, ensures consistency in data entry, and thereby, further adds in the evidence-based classification algorithm’s (SSS-TOAST) reliability in etiologic stroke classification. Use of the CCS in the National Institutes of Health’s NINDS SiGN study is expected to allow many additional analyses based upon the robust data set created.

Pharmacogenomics of Stroke Prevention

Through the use of personalized medicine, physicians will have a much improved ability to predict how each individual patient will respond to specific drug therapies. Because many adverse drug reactions are mediated via enzymes with established polymorphisms known to mediate such effects, the potential impact will be enormous, with such information leading to improved efficacy, fewer adverse drug reactions, and improved cost-to-benefit ratios [45]. Although the era of truly individualized pharmacogenomic medicine is not yet upon us, several important findings have been identified that directly relate to stroke prevention and treatment.

Warfarin

The promise of preventing cardioembolism and the perils of causing intracerebral hemorrhage are well known to physicians who treat patients with warfarin [46]. There is considerable inter-individual variation in response to warfarin dosing, posing challenges to initiation of therapy. A portion of the variable response is due to variations in genotypes in the cytochrome p450 isoform *CYP2C9* and the vitamin K epoxide reductase complex subunit 1 *VKORC1*. Genotype-guided initiation of warfarin has yet to prove better than 5-mg or 10-mg fixed initial doses in terms of achieving target international normalized ratios [47]. So far, no study has been powered to show differences in major bleeding.

As attempts are made to individualize treatment with warfarin using pharmacogenomics, novel medications are emerging for indications similar to warfarin. An example would be dabigatran etexilate, a direct thrombin inhibitor, which when given at a fixed dose showed comparable safety and improved efficacy to warfarin with regard to stroke prevention in the setting of atrial fibrillation [48•]. Dabigatran etexilate is an inactive pro-drug that gets converted to dabigatran, the active form, by esterase-catalyzed hydrolysis in the plasma and liver. Although specific medications such as quinidine, ketoconazole, amiodarone, and

verapamil can increase plasma concentrations of dabigatran [49], no genetic variants are known to alter metabolism or efficacy, although research on this topic is ongoing.

Clopidogrel

As an anti-platelet medication, clopidogrel is among those most commonly used to prevent ischemic stroke. Recent advances have led to an improved understanding of the genetic causes of clopidogrel resistance. Clopidogrel, a thienopyridine, is a prodrug that requires metabolism by the cytochrome P450 complex to act on its target, the platelet P2Y₁₂ receptor. Genetic studies nested within phase 3 cardiology trials have shown that a reduced-function allele of the CYP2C19 gene reduces the clinical effectiveness of clopidogrel in preventing recurrent myocardial infarction and in-stent thrombosis [50]. A current challenge for clinicians is that there now is a warning by the US Food and Drug Administration that some patients may be poor responders to clopidogrel and that gene tests exist for screening for poor responsiveness. It is not known how cost-effective screening would be in all patients in whom clopidogrel would otherwise be considered. However, it may be reasonable to test patients who have an ischemic stroke or transient ischemic attack on clopidogrel for the CYP2C19 reduced-function allele. Some have argued that patients might benefit more from platelet function assays rather than gene testing, but direct comparisons of various methods for trying to individualize stroke prevention with clopidogrel have yet to be done.

Safety concerns relating to a reduced pharmacodynamic effect of clopidogrel in combination with the use of proton pump inhibitors have recently emerged. Whether such drug interaction is a class effect, a drug effect, genetically influenced, or related to dosage timing remains a matter of debate. One recent study demonstrated that pantoprazole therapy used at high doses was not associated with modulation of the pharmacodynamic effects of clopidogrel, irrespective of timing of drug administration [51]. Additional studies specifically evaluating genetic effects are required.

Statins

Statin use is among the mainstays of current primary and secondary stroke prevention therapy. However, their adverse effects limit their appeal, with statin-induced myopathy occurring in about 1 in 1000 to 1 in 10,000 patients [52, 53]. One convincing recent finding relating to statin-induced myopathy relates to *SLC01B1*, which codes for an anion-transporting polypeptide OATP1B1 shown to regulate hepatic statin uptake. A relatively recent GWAS comparing subjects with and without myopathy, all of whom were taking 80 mg/d of simvastatin, found a SNP in the *SLC01B1* to be associated with a higher risk of statin-induced myopathy [54]. The identified noncoding SNP, rs4363657, was in strong linkage disequilibrium with a non-synonymous rs4149056 SNP, which has been linked to statin metabolism [55]. The prevalence of the rs4149056 C allele in the population was 15%. The odds ratio for myopathy was 4.5 per copy of the C allele, and 16.9 in CC as compared with TT homozygotes. More than 60% of these myopathy cases could be attributed to the C variant. The association of rs4149056 with myopathy was replicated in a related trial of 40 mg/d of simvastatin, which also showed an association between rs4149056 and the cholesterol-lowering effects of simvastatin.

Currently, routine pharmacogenomic testing for statin therapy is not recommended. Additional studies evaluating the described *SLC01B* variants in combination with other genetic variants will be required to determine the clinical utility and cost-effectiveness of pharmacogenomic testing [45]. Studies evaluating the specific statin, the dose, and concomitant use of other drugs are needed across ethnically diverse populations.

Criticisms and Future Directions

Although the field of stroke genetics is full of much promise, there are also some criticisms that should be mentioned.

One criticism, shared with other complex diseases, is that the variants discovered individually have only small effects on disease risk. For example, the increased risk of developing stroke as measured by odds ratios for most identified loci is on the order of 1.2 to 1.4, as consistent with the 9p21.3 locus previously described. However, it is important to note that effect sizes often do not necessarily reflect clinical or biological relevance [56]. For example, mutations in 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase explain only a small proportion of the variance in low-density lipoprotein (LDL) cholesterol [57], yet this enzyme is a target for many powerful LDL-lowering medications. Therefore, it is clear that small population effects should not be viewed as clinically or biologically unimportant.

A second concern is that the current thrust of the field is geared toward implementing GWAS-based approaches that are appropriate to identify common variants, but are not suited for situations where multiple rare disease-causing variants contribute to disease risk. This is because GWAS chips typically implement common variants as identified through the HapMap project. The ability to simultaneously examine hundreds of thousands of variants throughout the genome is both the strength and the weakness of the GWAS approach. The power of GWAS is that they provide a relatively unbiased examination of the entire genome for common risk variants; their weakness is that unless they have sufficient sample size, true risk variants may go undetected secondary to statistical noise from the vast numbers of markers that are not associated with disease [58]. To separate true signals from noise, researchers are forced to set an exceptionally high threshold that a marker needs to exceed before it is accepted as a likely disease-causing candidate. The most straightforward method to address this shortcoming is to increase the numbers of study participants in both the disease and control groups. Such a scheme steadily decreases the statistical noise from non-associated markers until even disease genes with small effects stand out. However, the cost and logistical efforts to attain such large sample sizes can be prohibitive.

Recognizing the inherent limitations of GWAS and the common disease-common variant hypothesis, further coupled with the fact that many GWAS results across a variety of complex diseases have failed to identify variants accounting for the majority of disease-specific cases, most agree that some non-trivial fraction of the genetic risk of common diseases will be the result of rare variants [59]. Current GWAS technologies are limited in their ability to unravel the contribution of rare variants. Although increasing sample size may help in this regard, the fundamental problem remains that current GWAS chips are unable to tag rare variation. To address rare variants, the answer lies in large-scale sequencing, which will provide a complete catalog of every variant in the genomes of both case patients and controls. Although such sequencing methods will identify rare variants, it is important to note that these techniques will also be subject to the problems as associated with multiple testing, requirements for large sample sizes, and may potentially require more complex analytical methodologies.

Conclusions

The field of stroke genetics has changed dramatically over the past decade and, with it, our understanding of stroke biology has been refined and expanded, yet much work is still to be done. Improvements in technology will lead to identification of more novel loci associated with stroke and help to define causal variants. Functional studies will determine how loci

contribute to disease risk and illuminate biological pathways that affect the understanding of stroke and other seemingly unrelated disorders. Ultimately, joint efforts by scientists and clinicians will help move the genetic information into practice and lead to the improvement in prevention, diagnosis, and treatment of individuals with stroke.

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Table 1

Major stroke types and subtypes

Type	Subtype or presumed major mechanism	Approximate frequency, %
Ischemic stroke		83
	Large artery atherosclerosis	14
	Cardioembolic	23
	Small-vessel disease	13
	Other determined	3
	Undetermined	30
Hemorrhagic stroke		17
Intracerebral hemorrhage	Hypertension (deep brain sites)	7
	Amyloid angiopathy (lobar)	3
Subarachnoid hemorrhage	Intracranial aneurysm	7
Cerebral venous thrombosis		< 1