Addressing the Heterogeneity of the Ischemic Stroke Phenotype in Human Genetics Research

James F. Meschia, MD

Background and Purpose—Many investigators have approached ischemic stroke as a complex phenotype by dividing the ischemic stroke population into distinct subtypes. The purpose of this study was to review systematically the methods used to subtype ischemic stroke in recent genetic studies.

Methods—The MEDLINE database was searched for articles pertaining to research on the genetics of human ischemic stroke published from January 2000 through January 2002. Abstracts and full-length reports were then sequentially screened to select articles pertaining to original case-control or cohort studies.

Results—The initial search yielded 153 publications. Of 41 relevant articles, ischemic stroke was subtyped in 25 (61%). The most common standard subtyping system was the Cerebrovascular Classification of Diseases III system (9 articles). Of the subtyping systems used, 3 had previously published interrater reliability. The subtyping system was reported to have been prespecified in 1 study. Four articles reported using central adjudication. Two articles reported that the person doing the subtyping was blinded to genotype, and 2 reported that the person doing the genotyping was blinded to the patient’s subtype status.

Conclusions—When investigators subtyped ischemic stroke, they typically used either nonstandard classification systems or systems of undetermined reliability. Important methodological issues, including blinding and prespecification of the classification system, were rarely reported. Advances in methodology and scientific reporting standards would foster identification of subtype-specific genetic risk factors. (Stroke. 2002;33:2770-2774.)

Key Words: cerebrovascular disorders ■ genetics, medical ■ phenotype ■ stroke, ischemic

Many successful clinical trials of procedures for secondary stroke prevention have regarded ischemic stroke as a heterogeneous entity, eg, targeting patients with stroke of presumed atherothrombotic origin1 or stroke of presumed cardioembolic origin.2 Strokes experienced by individuals with certain single-gene and mitochondrial disorders tend to fall within recognizable clinical syndromes. Cerebral autosomal dominant arteriopathy with subcortical infaracts and leukoencephalopathy causes small-vessel white-matter infarction,3 and mitochondrial encephalopathy with lactic acidosis and stroke-like episodes causes cortical-subcortical lesions with a predilection for occipitoparietal distribution.4 For so-called sporadic stroke, there may be value in narrowing the search for genetic risk factors to patients with well-defined stroke subtypes. Investigators of the genetics of sporadic stroke have used mechanistic and anatomical systems for subtyping ischemic stroke.5 The purpose of this study was to review systematically the systems used to subtype ischemic stroke in recent studies of genetic risk factors.

Methods
The MEDLINE database was electronically searched for studies on the genetics of ischemic stroke with the following search criteria.

The search was limited to human subjects, the English language, and a publication date from January 2000 through January 2002. The search terms included the medical subject heading term “cerebrovascular disorders, genetics of” combined with the text words “ischemic” or “ischemic” and “stroke.” The search excluded letters and reports on sickle cell disease. Abstracts of all citations found by means of this search were reviewed and included if they pertained to original case-control or cohort studies of human genetic risk factors for ischemic stroke. Citations were excluded if they pertained to formal or informal reviews, family history studies without a molecular genetic component, studies focused on carotid stenosis or dissection, studies limited to 1 single-gene or mitochondrial disorder, or studies of white-matter disease detected by MRI or so-called silent stroke. If it was unclear from the abstract whether a study was relevant or if no abstract was available, the citation was rejected or accepted on the basis of review of the full-length article.

Each report was evaluated by means of a standardized form on which were recorded year of publication, number of patients with ischemic stroke, and geographic location of patient recruitment. It was determined whether a system was used to subtype ischemic stroke into ≥2 categories on the basis of clinical characteristics, results of a diagnostic workup, or autopsy findings. In rare instances, articles cited earlier publications describing the systems used for subtyping. In such cases, these earlier publications were also reviewed. The nature of the subtyping system was assessed, and the system was classified as standard or nonstandard. Standard was defined as a subtyping system that had been developed in a formal consensus process or had been tested for interrater reliability.

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Conclusions—When investigators subtyped ischemic stroke, they typically used either nonstandard classification systems or systems of undetermined reliability. Important methodological issues, including blinding and prespecification of the classification system, were rarely reported. Advances in methodology and scientific reporting standards would foster identification of subtype-specific genetic risk factors. (Stroke. 2002;33:2770-2774.)

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Methods
The MEDLINE database was electronically searched for studies on the genetics of ischemic stroke with the following search criteria.
Standard and nonstandard systems were classified as either mechanistic or anatomic. Mechanistic systems classified strokes on the basis of inferred origin of cerebrovascular occlusion (eg, atherothrombotic versus cardioembolic). Anatomic systems classified strokes on the basis of location or size of the lesion within the brain (eg, cortical versus subcortical) using findings on neurological examination, head imaging studies, or postmortem examination.

It was noted whether decisions regarding ischemic stroke subtype were reported to have been adjudicated centrally. Adjudication was considered centralized if a single person or a committee of ≤3 people adjudicated every subtype diagnosis. It was also noted whether the articles explicitly indicated that the subtype adjudicator was blinded to genotype data or that the personnel responsible for generating the genotype data were blinded to stroke subtype. Furthermore, it was noted whether the subtyping system was prespecified. A subtyping system was considered prespecified if it was defined before patient recruitment was initiated.

Results

The electronic search yielded 153 citations. By the end of the screening process, 112 citations (73%) were rejected for the following reasons: 40 citations (36%) were meta-analyses, review articles, or editorials; 4 citations (4%) were family history studies that did not include a molecular genetic component; 5 citations (4%) were studies of carotid stenosis or carotid dissection rather than stroke; 22 citations (20%) were studies of a single mendelian or mitochondrial disorder; and 6 citations (5%) were studies of white-matter signal abnormalities detected on MRI or studies of silent stroke. Thirty-five citations (31%) did not meet eligibility criteria for other reasons.

The final search yielded 41 relevant citations (27% of the total 153). There were no duplicate publications. Twenty articles (49%) were published in 2000, 20 (49%) were published in 2001, and 1 (2%) was published in 2002. Nineteen of the studies (46%) were carried out in Europe, 16 (39%) in Australian or Asian populations, 5 (12%) in North America, and 1 (2%) in South America. Eight studies (20%) enrolled ≤100 patients with stroke. Fourteen studies (34%) enrolled 101 to 200 patients with stroke, and 19 studies (46%) enrolled >200 patients with stroke.

Of the 41 studies, 5 did not report the sex distribution of study subjects. In the remaining 36 studies, 57.2% of study participants were male and 42.8% were female. The age distribution for cases was reported as a mean or median in 36 studies, was given only as a range in 4 studies, and was not reported in 1 study. Of the studies reporting mean or median age, the mean or median was <40 years in 4 studies, 40 to 59 years in 9 studies, 60 to 69 years in 20 studies, and ≥70 years in 3 studies. Of the studies reporting only age ranges for cases, the ranges were 10 to 18 months in 2 studies, 18 to 45 years in 1 study, and 18 to 84 years in 1 study. Studies recruiting subjects outside of North America typically did not describe a racial or ethnic distribution. Of the 5 North American studies, 400 of 521 study subjects (77%) were white, 110 of 521 (21%) were black, and 11 of 521 (2%) were reported as “other.”

The Table indicates the findings of the review of subtyping in the 41 eligible articles. Ischemic stroke was subtyped in less than two thirds of the studies, and considerable variability existed among studies in the subtyping systems used. The most frequently used standard system for subtyping stroke was the National Institute for Neurologic Disorders and Stroke (NINDS) Classification of Cerebrovascular Diseases III system. Two standard ischemic stroke subtyping systems, the Stroke Data Bank system and the Oxfordshire Community Stroke Project system, have established reliability and were used in a total of 3 studies (7%). International Classification of Diseases (ICD) codes were used in 2 studies. One of these studies used the 8th revision (ICD-8),
and the other study did not specify which version of ICD was used. The subtyping system was reported to have been prespecified in 1 study. Results of subgroup analyses based on ischemic stroke subtype were given in the abstracts of 13 reports (32%).

Of the 25 studies in which ischemic stroke was subtyped, 4 used central adjudication and identified the adjudicator in the full-length report. Two of these articles indicated whether the person adjudicating stroke subtype had knowledge of the study subjects’ genotypes, and 2 articles indicated whether the person assigning genotype had knowledge of the study subjects’ ischemic stroke subtype diagnoses.

**Discussion**

There is a need for greater use of standardized and reliable systems of subtyping ischemic stroke in studies of genetic risk factors. Investigators in just over half of the studies regarded ischemic stroke as a heterogeneous phenotype, but more than a third of the studies made no attempt to address the heterogeneity of ischemic stroke. Nonstandard subtyping systems were used almost as often as standard systems. Isolated studies have suggested that subtype-specific risk factors exist. For example, Myllykangas and colleagues reported that the lipoprotein lipase Ser447Ter polymorphism showed a particularly strong negative association with pathologically confirmed small (<1.5 cm) infarcts. Greater standardization in the subtyping of ischemic stroke would provide for greater validity of meta-analyses attempting to confirm the presence of subtype-specific risk factors. More widespread use of standardized systems would also increase the validity of comparisons of genetic risk factors among racially divergent populations and facilitate confirmatory studies in similar populations.

It is possible that the research protocols of some of the studies had salient methodological features that were not specifically described in the published reports. However, this review shows that the standards for reporting methods of subtyping ischemic stroke are suboptimal in genetic association studies. It was rare for a full-length report to identify the subtype adjudicator, to state whether the subtyping system was selected before genotyping, to state whether the genotype was blinded to subtype diagnosis, or to state whether the subtype adjudicator was blinded to genotype. The standards of reporting genetic association studies of ischemic stroke are in stark contrast to the standards of reporting randomized clinical trials, in which investigators and journal editors have developed and use the Consolidated Standards of Reporting Trials (CONSORT). After the introduction of CONSORT into the peer-reviewed publication process, there was measurable improvement in the quality of randomized clinical trials reporting.

Although efforts were made to be comprehensive in the retrieval of studies published in English, it is possible that relevant studies were inadvertently missed. Furthermore, limiting the study to articles published in English may have introduced a bias. For example, Moher and colleagues have shown that overviews of randomized clinical trials can be biased if the overview pertains only to studies reported in English. Thus, the study findings may not be generalizable to the non-English literature.

It is not yet certain whether ischemic stroke should be viewed as 1 phenotype or several phenotypes when attempting to discover genetic risk factors. However, when the aim of a study is to identify subtype-specific risk factors, investigators should consider a double-blinded study design in which genotyping is done independently of phenotyping (in this case, subtyping ischemic stroke) and phenotyping is done independently of genotyping. Subtyping ischemic stroke requires clinical judgment and is therefore subject to interrater variability and bias. Blinding, prospectively defining the subtyping system, and central adjudication can minimize these potential methodological problems. Blinding is routinely done in randomized clinical trials to reduce differential assessment of outcomes (information bias). It is likely that information bias can occur during adjudication of ischemic stroke subtype if the adjudicator is aware of the results of genetic testing, although information bias has never been empirically assessed in this context.

There may be a tendency to classify patients with ischemic stroke on the basis of subtyping systems chosen after a DNA bank has been established. Investigators may appreciate patterns of clustering of polymorphisms along unanticipated lines after data analysis has begun. Although these types of exploratory analyses are valid, it should be recognized that subgroup analyses can lead to spurious results. Any subgroups chosen after data collection should be clearly identified.

Centralized adjudication of stroke subtype would minimize the methodological problem of variability among physicians in assigning subtype diagnoses. Even well-described systems like the Trial of ORG 10172 in Acute Stroke Treatment (TOAST) and the Oxfordshire systems show only moderate to good interobserver agreement. Central adjudication is commonly used to assess end points in clinical trials, although the optimal number of adjudicators is not yet clearly established. To date, central adjudication of subtypes has been rare in genetic studies of stroke.

When ICD codes are used as the system for subtyping, it is essential to specify the ICD version. Switching from ICD-9 to ICD-10 has been shown to alter the ranking of leading causes of death. The possibility exists that the use of different versions of ICD might substantively alter the conclusions of genetic studies. The validity of ICD codes as a subtyping system in the context of genetic research is uncertain. In a review of hospital charts from the Durham Veterans Affairs Medical Center, Goldstein found that 15% to 20% of patients with the indicated primary ICD-9-CM discharge codes had conditions other than acute ischemic stroke. However, such findings cannot be generalized to a study in which ICD codes are applied prospectively by a panel of neurologists.

In conclusion, many genetic studies have attempted to address phenotypic heterogeneity of ischemic stroke through subtyping, but more methodological rigor and higher reporting standards are needed. Organizations such as the NINDS might consider convening a working group to develop a policy analogous to CONSORT. A widely accepted policy
would elevate the standards of reporting of molecular genetic studies of stroke.

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 References


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