Heritability of Ischemic Stroke in Relation to Age, Vascular Risk Factors, and Subtypes of Incident Stroke in Population-Based Studies

U.G.R. Schulz, MRCP, MD; E. Flossmann, MRCP; P.M. Rothwell, FRCP, PhD

Background—Appropriate design of molecular genetic studies of ischemic stroke requires an understanding of the genetic epidemiology of stroke. However, there are no published population-based data on heritability of aetiological subtypes of ischemic stroke, confounding by heritability of other vascular risk factors, or the relationship between heritability and age of onset.

Methods—We studied family history of stroke (FHxStroke) and of myocardial infarction (FHxMI) in first-degree relatives in 2 population-based studies (Oxford Vascular Study [OxVASC]; Oxfordshire Community Stroke Project [OCSP]). We related FHxStroke and FHxMI to subtype of ischemic stroke, age, and the presence of vascular risk factors and performed a systematic review of all studies of FHxStroke by stroke subtype.

Results—In our population-based studies and in 3 hospital-based studies, FHxStroke was least frequent in cardioembolic stroke (OR=0.74, 95%CI=0.58 to 0.95, P=0.02) but was equally frequent in the other subtypes. In OxVASC and OCSP, FHxStroke (P=0.02), FHxMI (P=0.04), and FHs of either (P=0.006) were associated with stroke at a younger age. Only FHxStroke was associated with previous hypertension (OR=1.59, 95%CI=1.08 to 2.35, P=0.02). FHxMI was more frequent in large-artery stroke (OR=1.63, 95%CI=0.99 to 2.69, P=0.05).

Conclusion—Consistent results in our population-based studies and previous hospital-based studies suggest that inclusion bias is not a major problem for studies of the genetic epidemiology of stroke. Molecular genetic studies might be best targeted at non-cardioembolic stroke and younger patients. However, genetic susceptibility to hypertension may account for a significant proportion of the heritability of ischemic stroke. (Stroke. 2004;35:819-825.)

Key Words: ischemia ■ stroke ■ history ■ risk factors

Susceptibility to ischemic stroke may be influenced by genetic factors. Some Mendelian disorders have been identified, and animal models also suggest that susceptibility to stroke may be genetically determined. However, human candidate gene studies in apparently sporadic stroke have so far been either inconsistent or negative. To target molecular genetic studies appropriately, it is first necessary to understand the basic genetic aetiology of ischemic stroke. Although there have been several epidemiological studies of the heritability of ischemic stroke, results have also been inconsistent. A recent systematic review of such studies found that although there probably is a genetic contribution to stroke, publication bias and heterogeneity between the studies did not allow reliable interpretation of results. Most studies failed to differentiate between ischemic stroke subtypes, and many studies combined ischemic and hemorrhagic stroke.

It is likely that genetic susceptibility to the pathological mechanisms underlying ischemic stroke differs between the subtypes. However, the few family history (FHx) studies that have differentiated between subtypes were insufficiently powered and were all hospital-based. There are no published population-based studies of the heritability of aetiological subtypes of ischemic stroke. Population-based studies are worthwhile because hospital-based studies may be subject to inclusion bias. Bias might occur in genetic epidemiological studies if, for example, hospital admission or extent of investigation were dependent on age or severity of stroke, both of which might be related to heritability. We therefore studied FHx of stroke (FHxStroke) and FHx of myocardial infarction (FHxMI) in pooled data from 2 population-based studies of incident ischemic stroke (Oxfordshire Community Stroke Project [OCSP]; Oxford Vascular Study [OxVASC]). To avoid recall bias associated with case-control comparisons, we confined our study to case–case comparisons. Given that some of the risk factors for ischemic stroke, such as hypertension or hyperlipidemia, are partly genetically determined, we also compared the frequency of vascular risk factors in patients with and without FHxStroke. We performed

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the same analyses for FHxMI to determine whether stroke subtype or risk factor associations were specific to FHxStroke. Finally, to identify any possible bias in previous hospital-based studies and to summarize all currently available data, we conducted a systematic review of all studies of FHxStroke in stroke subtypes.

Materials and Methods
We studied FHxStroke and FHxMI in first-degree relatives in 2 population-based stroke incidence studies that conformed to the standard quality criteria for such studies. The methods and results of the OCSP have been published previously. The OCSP study started in April 2002 and used identical ascertainment methods to the OCSP. Briefly, by close collaboration with family practitioners (FPs) (50 FPs in OCSP, 63 FPs in OXVASC), an urban and rural population (105 000 people in OCSP, 91 000 in OXVASC) was studied. Patients with a mild stroke were seen at a daily study clinic, allowing more rapid assessment and treatment than currently the norm in the British health care system. This provided an incentive for FPs to report all patients who might have had a transient ischemic attack or stroke during the study periods. However, we double-checked FP databases and records to identify any strokes that might have been missed. Stroke patients requiring admission to hospital were identified by daily assessment of admission registers and visits to the relevant wards of hospitals within the study catchment. We also reviewed hospital discharge coding, referrals for brain and vascular imaging, and all death certificates and coroner reports relating to our study population. Both studies were approved by the local ethics committee.

In both OXVASC and OCSP, a study neurologist assessed all cases as soon as possible after notification, and CT brain imaging was obtained. Details of the presenting event, clinical characteristics, medical history, and FHx were recorded from the patient, FP records, and/or hospital records. FHx data were obtained separately for stroke and for MI, and FHx was regarded as positive if at least 1 first-degree relative was affected.

In OXVASC, patients routinely undergo Doppler scanning of the cerebral arteries and echocardiography. Stroke aetiology is classified prospectively according to the Trial of Org 10172 in Acute Stroke Treatment (TOAST) criteria. In the OCSP, the subtype of ischemic stroke had been categorized according to the Bamford classification, but the investigators had also originally prospectively categorized stroke according to cause, and detailed clinical and imaging data had been collected. It has been shown that the TOAST classification can be applied retrospectively, and that this is accurate and reproducible. As reported previously, the details available in the OCSP allowed us to reclassify all ischemic strokes according to the same causative categories as used in the TOAST study.

Statistical Analysis
Because the OCSP and OXVASC were conducted in the same population in collaboration with the same family practices using very similar methods, we pooled the data to increase statistical power. However, we allowed for possible differences between the studies by adjusting analyses by “study” when appropriate. We studied FHxStroke and FHxMI in relation to stroke subtype and in relation to age, sex, cholesterol level, and history of previous transient ischemic attack. A history of hypertension, hypercholesterolemia, and diabetes mellitus was also recorded and regarded as positive if confirmed by the patient or medical notes, or if the patient was using treatment.

We expressed the prevalence of FHxStroke or of FHxMI as simple proportions and compared these between stroke subtypes by \( \chi^2 \) test. To test for independent associations between FHx and stroke subtypes, we performed a logistic regression analysis, adjusting for age, sex, study, and other vascular risk factors. To study differences in baseline characteristics between patients with and without a FHx, we used the \( \chi^2 \) test and analysis of variance as appropriate. For any factor that was associated with FHx overall or with a particular stroke subtype, we also performed a logistic regression analysis, adjusting for age, sex, and study.

We identified previous published reports of FHxStroke as reported elsewhere. Briefly, 2 independent observers searched Medline, Embase, and the reference lists of all articles that met the inclusion criteria. Studies were included in the current review if they reported FHx by subtype of ischemic stroke according to TOAST criteria or a similar classification. We calculated the odds for a positive FHx in specific stroke subtypes compared with the remainder within individual studies and, when appropriate, performed fixed-effects meta-analysis according to the Mantel-Haenszel method. SPSS for Windows version 10.0 (SPSS 1999) was used for all statistical analyses.

Results
The OCSP registered 675 patients with a first-ever stroke and 545 patients with a first-ever ischemic stroke; 56 patients were excluded from the analysis because no details were available on FHx, stroke subtype, or risk factors. In the first year of OXVASC (April 2002 to March 31, 2003), 116 patients were registered with a first-ever ischemic stroke, of whom FHx data were available in 107. Of the 596 patients in the combined “Oxfordshire” cohort, 137 (23.0%) had FHxStroke, 137 (23.0%) had FHxMI, 40 (6.7%) had both, and 234 (39.3%) had FHx of either stroke or myocardial infarction. The frequency of FHxStroke, or of FHxMI did not differ between hospitalized and non-hospitalized patients (Table 1). Brain imaging or postmortem data was available in 89% of patients (88% in OCSP and 96% in OXVASC).

The distribution of stroke subtypes did not differ between patients with and without FHxStroke or FHxMI (Table 1, Figure 1), and there were no significant associations between stroke subtypes and FHx after adjusting for potential confounders (Table 2). However, FHxStroke tended to be least frequent in patients with cardioembolic stroke (Figure 2). FHxStroke was associated with a young age of onset (Figure 3), with significant heterogeneity in the frequency of FHx across 10-year age bands (P = 0.01) and highest rates in patients aged 60 years or younger (OR = 1.73, 95% CI = 1.02 to 2.91). The trend toward a higher frequency of FHxStroke in patients younger than age 60 was present for each stroke subtype: large vessel, OR = 2.57 (95% CI = 0.84 to 7.88), P = 0.09; small vessel, 1.43 (0.50 to 4.09), P = 0.34; cardioembolic, 2.17 (0.38 to 12.6), P = 0.33; and undetermined, 2.51 (1.00 to 6.26), P = 0.04. A similar trend toward increasing FHx in younger patients was also present for FHxMI (P = 0.04 for trend across 10-year age bands) and for FHxStroke or FHxMI (P = 0.001, Figure 3).

The prevalence of vascular risk factors and their association with FHxStroke or FHxMI is shown in Table 1. FHxStroke was associated with a history of hypertension before (OR = 1.59, 95% CI = 1.08 to 2.35, P = 0.02) and after adjusting for age, sex, study, and stroke subtype (OR = 1.52, 95% CI = 1.02 to 2.26, P = 0.04). There was a borderline-significant (P = 0.05) trend for FHxMI to be associated with large-vessel stroke (OR = 1.63, 95% CI = 0.99 to 2.69), particularly in patients with FHxMI in 2 first-degree relatives (OR = 1.79, 95% CI = 0.85 to 3.77, P = 0.09). This trend was present in comparison to all other subtypes: large-vessel stroke versus small-vessel stroke (OR = 1.52, 95% CI = 0.82 to 2.79, P = 0.18), large-vessel stroke versus cardioembolic stroke.
We identified 4 previous studies that provided data on the prevalence of FHx Stroke in subtypes of ischemic stroke according to the TOAST classification, all of which were hospital-based. Only 1 study also collected details on FHx MI. Three studies defined FHx as at least 1 first-degree relative affected, and 1 study did not provide a clear definition of FHx. Two studies were case-control studies, one of which reported sufficient data to allow re-analysis as a case–case comparison. We contacted the authors to obtain the required details for the other study. The 2 other studies were cross-sectional studies, in which FHx Stroke was one of several factors that were related to stroke subtype. We excluded 1 study because of its very selected population (young Taiwanese stroke patients in a tertiary referral center). In this study, large-vessel stroke was associated with FHx Stroke (OR = 3.10, 95% CI = 1.18 to 8.14), but no other associations were found. Both case-control studies found that in comparison to healthy controls, FHx Stroke was associated with FHx MI (OR = 3.01, 95% CI = 1.03 to 8.44) and FHx Stroke was associated with FHx MI (OR = 3.01, 95% CI = 1.03 to 8.44).

### Table 1: Association Between Vascular Risk Factors, Stroke Subtypes, and FHx Stroke or FHx MI in the Oxfordshire Cohort

<table>
<thead>
<tr>
<th>Stroke subtype</th>
<th>Total (%)</th>
<th>FHxStroke +</th>
<th>FHxStroke -</th>
<th>P Value (het)</th>
<th>FHxMI +</th>
<th>FHxMI -</th>
<th>P Value (het)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Large vessel</td>
<td>14.6 (11.8–17.4)</td>
<td>16.1 (9.9–22.2)</td>
<td>14.2 (11.0–17.4)</td>
<td>0.581</td>
<td>19.7 (13.0–26.4)</td>
<td>13.1 (10.0–16.2)</td>
<td>0.054</td>
</tr>
<tr>
<td>Small vessel</td>
<td>22.0 (18.7–25.3)</td>
<td>21.9 (15.0–28.8)</td>
<td>22.0 (18.2–25.8)</td>
<td>0.979</td>
<td>21.9 (15.0–28.8)</td>
<td>22.0 (18.2–25.8)</td>
<td>0.979</td>
</tr>
<tr>
<td>Cardioembolic</td>
<td>21.6 (18.3–25.0)</td>
<td>18.2 (11.8–24.7)</td>
<td>22.7 (18.8–26.5)</td>
<td>0.271</td>
<td>19.7 (13.0–26.4)</td>
<td>22.2 (18.4–26.0)</td>
<td>0.531</td>
</tr>
<tr>
<td>Other defined</td>
<td>5.7 (4.0–7.9)</td>
<td>6.6 (3.1–12.1)</td>
<td>5.4 (3.6–7.9)</td>
<td>0.619</td>
<td>4.4 (1.6–9.3)</td>
<td>6.1 (4.1–8.7)</td>
<td>0.446</td>
</tr>
<tr>
<td>Undetermined</td>
<td>36.1 (32.2–39.9)</td>
<td>37.2 (29.1–45.3)</td>
<td>35.7 (31.3–40.1)</td>
<td>0.785</td>
<td>34.3 (26.4–42.3)</td>
<td>36.6 (32.2–41.0)</td>
<td>0.591</td>
</tr>
<tr>
<td>Hypertension</td>
<td>51.4 (47.4–55.5)</td>
<td>60.3 (52.1–68.5)</td>
<td>48.8 (44.2–53.4)</td>
<td>0.019</td>
<td>55.9 (47.5–64.2)</td>
<td>50.1 (45.5–54.7)</td>
<td>0.237</td>
</tr>
<tr>
<td>Diabetes</td>
<td>10.1 (7.7–12.5)</td>
<td>12.5 (6.9–18.1)</td>
<td>9.4 (6.9–12.5)</td>
<td>0.294</td>
<td>14.7 (8.8–20.7)</td>
<td>8.8 (6.3–11.7)</td>
<td>0.043</td>
</tr>
<tr>
<td>Previous transient ischemic attack</td>
<td>19.1 (15.9–22.3)</td>
<td>21.5 (14.6–28.4)</td>
<td>18.4 (14.9–22.0)</td>
<td>0.427</td>
<td>19.1 (12.5–25.7)</td>
<td>19.1 (15.5–22.7)</td>
<td>0.999</td>
</tr>
<tr>
<td>Current smoker</td>
<td>25.9 (22.4–29.5)</td>
<td>24.8 (18.0–32.7)</td>
<td>26.3 (22.2–30.4)</td>
<td>0.731</td>
<td>27.4 (19.9–34.9)</td>
<td>25.5 (21.5–29.5)</td>
<td>0.658</td>
</tr>
<tr>
<td>Hereditary hypercholesterolemia</td>
<td>50.4 (46.2–54.5)</td>
<td>48.4 (39.7–57.1)</td>
<td>50.9 (46.2–55.7)</td>
<td>0.619</td>
<td>54.7 (46.1–63.3)</td>
<td>49.1 (44.3–53.8)</td>
<td>0.264</td>
</tr>
<tr>
<td>Mean cholesterol</td>
<td>6.23 (6.0–6.36)</td>
<td>6.35 (6.04–6.66)</td>
<td>6.20 (6.06–6.34)</td>
<td>0.354</td>
<td>6.10 (5.78–6.41)</td>
<td>6.27 (6.13–6.41)</td>
<td>0.266</td>
</tr>
</tbody>
</table>

The columns indicate proportions or mean values within the entire study and in patients with or without FHx Stroke or FHx MI. The P value for heterogeneity shows whether the risk factor prevalence differed significantly between patients with and without a FHx.
controls, FHx Stroke was more common in large-vessel and small-vessel strokes. In addition, one study reported that a FHx MI was associated with large vessel strokes. In the meta-analysis of the Oxfordshire cohort and the 3 published hospital-based studies, FHx Stroke was consistently less frequent in cardioembolic stroke overall (OR = 0.74, 95% CI = 0.58 to 0.95, P = 0.02; Figure 1) and in comparison with each of the other subtypes individually (Figure 2). The prevalence of FHx Stroke did not differ between the other stroke subtypes: large-vessel stroke versus lacunar stroke (OR = 1.11, 95% CI = 0.86 to 1.42, P = 0.42), large-vessel stroke versus stroke of undetermined cause (OR = 1.17, 95% CI = 0.93 to 1.49, P = 0.19), small-vessel stroke versus stroke of undetermined cause (OR = 1.06, 95% CI = 0.83 to 1.35, P = 0.64). We did not include strokes of “other determined aetiology” in the meta-analysis because of their heterogeneous aetiology and, in some cases, proven genetic background.

Discussion

In this study, FHx Stroke was consistently less common in cardioembolic stroke than in other subtypes in population-based and in hospital-based cohorts. FHx Stroke was equally frequent in the other subtypes of ischemic stroke, but FHx MI

<table>
<thead>
<tr>
<th>Subgroup</th>
<th>Odds Ratio 95% CI</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Large vessel vs cardioembolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxford studies</td>
<td>1.14 (0.67-1.93)</td>
<td>0.640</td>
</tr>
<tr>
<td>Jerrard-Danne</td>
<td>1.11 (0.63-1.60)</td>
<td>0.984</td>
</tr>
<tr>
<td>Polychnepochova</td>
<td>0.81 (0.49-1.32)</td>
<td>0.326</td>
</tr>
<tr>
<td>Meschia</td>
<td>1.40 (0.62-3.18)</td>
<td>0.422</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1.27 (0.72-2.25)</td>
<td>0.531</td>
</tr>
<tr>
<td><strong>Small vessel vs cardioembolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxford studies</td>
<td>1.09 (0.63-1.63)</td>
<td>0.950</td>
</tr>
<tr>
<td>Jerrard-Danne</td>
<td>0.89 (0.36-2.21)</td>
<td>0.797</td>
</tr>
<tr>
<td>Polychnepochova</td>
<td>0.78 (0.30-2.00)</td>
<td>0.604</td>
</tr>
<tr>
<td>Meschia</td>
<td>1.52 (0.62-3.71)</td>
<td>0.421</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1.01 (0.68-1.50)</td>
<td>0.792</td>
</tr>
<tr>
<td><strong>Undetermined vs cardioembolic</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxford studies</td>
<td>1.38 (0.63-3.06)</td>
<td>0.422</td>
</tr>
<tr>
<td>Jerrard-Danne</td>
<td>1.40 (0.62-3.18)</td>
<td>0.421</td>
</tr>
<tr>
<td>Polychnepochova</td>
<td>1.52 (0.62-3.71)</td>
<td>0.358</td>
</tr>
<tr>
<td>Meschia</td>
<td>1.01 (0.68-1.50)</td>
<td>0.792</td>
</tr>
<tr>
<td>TOTAL</td>
<td>1.06 (0.71-1.58)</td>
<td>0.792</td>
</tr>
</tbody>
</table>

A indicates adjusted for study (OCSP or OXVASC); B, adjusted for study, age, and sex; C, adjusted for study, age, sex, hypertension, smoking, diabetes mellitus, hypercholesterolemia, and history of previous transient ischemic attack; LV, large vessel; SV, small vessel; CE, cardioembolic; OTH, other; ND, not determined.

Figure 2. Meta-analysis of the prevalence of FHx Stroke in each of the stroke subtypes compared with cardioembolic stroke. For each study, the odds of the prevalence of FHx Stroke in a subtype of stroke versus cardioembolic stroke are shown. “Total” shows the pooled odds ratio. P values for heterogeneity between the studies (P-het) and for overall significance are shown on the right side of this figure.
was associated with large-artery stroke. In the Oxfordshire cohorts, there were positive associations between FHxStroke and previous hypertension and between young age at stroke onset and both FHxStroke and FHxMI.

One advantage of our study methodology was that by performing case–case comparisons of patients with different stroke subtypes, we are likely to have avoided the recall bias that can undermine case–control studies, with stroke cases being more aware of any FHx than controls. A further advantage of the Oxfordshire studies was that they were population-based, ie, all ages and all degrees of severity of stroke were included. However, our study also had several potential shortcomings. First, we classified stroke subtypes according to the TOAST criteria, because it is currently the most widely used aetiological classification of stroke. However, it is still relatively crude, with some categories probably comprising several underlying disorders with differing genetic influences. Second, the OCSP was conducted in the early 1980s. The standard of investigations has improved since then, and techniques such as MRI scanning or transesophageal echocardiography are now much more readily available. More detailed investigations might have allowed more accurate subtyping. However, the associations between stroke subtype and FHxStroke were consistent with more recent studies. Finally, we used FHx as a measure of heritability. However, familial clustering of a disease may be due not only to genetic factors, but also to a shared environment.

In the Oxfordshire studies, the frequency of FHxStroke did not differ between subtypes of stroke, although there was a trend toward lower rates in cardioembolic stroke. This was consistent with similar trends in the 3 previous hospital-based studies, was statistically significant when the results of all studies were combined (Figure 1), and was present in comparison with each of the individual subtypes (Figure 2). These results are consistent with the findings of previous case–control studies, which found that patients with large-vascular disease and patients with small-vascular disease, but not patients with cardioembolic stroke, were more likely to have a FHxStroke than healthy controls. The relatively low heritability of cardioembolic stroke may be explained by the fact that the underlying cardiac disorders, eg, atrial fibrillation or valvular disease, are not highly hereditable and do not invariably cause stroke.

We found a borderline-significant positive association between FHxMI and large-vessel stroke. This was also found by the only other study of FHxMI. Large-vessel disease and ischemic heart disease reflect similar pathological processes, and the association with FHxMI may indicate an inherited tendency for atherosclerotic disease to develop. We also found a positive association between a history of hypertension and FHxStroke, which was not present for FHxMI. This is consistent with previous general population studies that have shown that FHxStroke is more common in hypertensive than normotensive subjects and a previous case–control study in which stroke patients were more likely to have a FHx of hypertension than controls. Given that hypertension has a major genetic component, heritability of stroke may partly be conferred by an inherited tendency for hypertension.

There are few published data on the relationship between age of stroke onset and FHx of vascular disease, and no study has differentiated between the subtypes of ischemic stroke. Studies used different age cut-offs or were restricted to relatively young patients, and results have been conflicting. We found significantly higher rates of FHxStroke, FHxMI, and FHx of either stroke or myocardial infarction in younger patients overall and similar trends within each cause subtype. However, given the conflicting trends in the published data, more studies are required to determine the relationship between age and heritability of stroke and to analyze the degree to which these results are influenced by potential better recall in younger patients versus older patients and the consequent increase in likelihood of stroke of parents and siblings of older patients.

Consistent results in our population-based studies and previous hospital-based studies suggest that inclusion bias is not a major problem for studies of the genetic cause of stroke. Molecular genetic studies might be best targeted at non-cardioembolic stroke and younger patients. However, genetic susceptibility to hypertension may account for a significant proportion of the heritability of ischemic stroke.

Acknowledgments

Dr Rothwell and Dr Flossmann are funded by the UK Medical Research Council (MRC). Dr Schulz is funded by the Wellcome Trust. We thank Professor Charles Warlow and the OCSP Collaborators for allowing us access to their data, the OXVASC Collaborators for their help with this study, and Hugh S. Markus for providing unpublished data.
References

Editorial Comment
Unraveling the Pagodian Architecture of Stroke as a Complex Disorder

The last decade has witnessed an explosion of hospital-based association studies aimed at deciphering the genetic architecture of complex disorders. Most of these studies attempt such major undertakings without taking into consideration the phenotypic heterogeneity of these disorders. The population-based meta-analyses presented by Schulz and colleagues in this issue of Stroke brings home a very significant point: a complex disorder is not a uniform entity, but a composite of several elemental pathologies affecting particular organ system(s).

Most genetic association studies on stroke, for example, pool its various subtypes, amalgamate several ethnicities for the sake of increasing sample size, and genotype only one or a few polymorphisms within candidate genes. In their endeavor to associate a single biallelic polymorphism with a complex disorder, such studies overlook the Factor(s) X which mediate the pathology. In essence, such an association is a jump across the Atlantic. The result is that the literature is flooded with a large number of published papers with extensive heterogeneity and publication bias.

Sporadic stroke, as are most complex disorders, is heterogeneous. It can be divided into two major categories, hemorrhagic and ischemic stroke, each having several subcategories which can be identified as distinct phenotypes with their own etiologies. In spite of the recognition of these concepts for a long time, Schulz and colleagues are among the very first to provide a proof. They present clear evidence that confirms prior notions about the genetic architecture of stroke: (1) there is greater genetic influence in pathogenesis if the disease develops at a younger age; (2) large vessel disease is predominantly due to atherosclerosis and shares common genetic denominators with hypertension and myocardial infarction; (3) cardioembolism is essentially a mechanical phenomenon and would unlikely be genetically determined. Investigators, in just over half of the studies reviewed recently, regarded ischemic stroke as a heterogeneous phenotype and more than a third of the studies made no attempt to address the clinical heterogeneity of the disease. Various systems have been used for subtyping ischemic stroke, including nonstandard ones, and there is little consistency among studies. Thus, there is a need for greater use of standardized and reliable systems for subtyping complex disorders in studies of genetic risk factors.

Complex disorders do not exhibit a flat architecture, but in fact possess a high degree of plasticity mediated by several nonlinear dynamic processes. A large number of these processes may be genetically determined and all would be partially influenced. The overall design is comparable to that of a pagoda with several independent units stacked on top of one another constituting the holistic phenotype. Yet, the units...
are partially interdependent as well. Rarely, we may stumble on polymorphisms which constitute the pillars of the pagoda and their influence transcends to the very top of the structure, such as the *APOE e4* in Alzheimer’s. Chance would certainly smile on us then, but only if we have been prepared enough to eliminate the confounding effect of ethnic and phenotypic heterogeneity.

In light of the same thought, it is not ostentatious to presume that some polymorphisms only influence the structure of a single pagodian unit; others shape several, yet the effect of some only manifests in the presence of polymorphisms which alter the structure of the preceding unit. This kind of dynamic interaction of multiple gene effects is responsible for shaping the overall architecture of a complex disorder. Thus, it would be difficult, if not impossible, to hit absolute associations of a polymorphism within a candidate gene in a complex disorder. The polymorphism may certainly be involved, via several nonlinear dynamic processes and its influence may be significant, yet limited.

To tease out such effects, which indeed constitute the large majority, it is beneficial to look for associations on a limited scale, taking advantage of the fractal behavior of complex disorders. Genetic polymorphisms may alter the structure or expression of a protein, leading to a dysfunctional biologic pathway. Over time, this disturbance may manifest as an altered physiological characteristic of the organism. An example is *ACE* gene polymorphisms leading to renin-angiotensin system dysfunction which causes atherosclerosis, manifesting as increased intima media thickness (IMT) or systolic blood pressure variability due to rigid arteries. Indeed, these physiological characteristics can be easily measured and their development can be followed over time, since they are continuous in nature. Keeping in mind the fractal characteristics of complex disorders it is not presumptuous to infer that polymorphisms influencing measurable intermediate phenotypes certainly have a role to play in structuring the overall architecture of the complex disorder, even if no association is observed with the holistic phenotype. Additionally, polymorphisms influencing one complex disorder might be involved in the causation of another such disorder as well, as they might operate through the same intermediate phenotype(s). This is in line with the suggestion by Schulz and colleagues that the heritability of ischemic stroke may be partly conferred by an inherited tendency for hypertension.

In view of the above, I would suggest a structured approach to genetic association studies of stroke. Polymorphisms in the exonic or promoter regions of candidate genes should be searched for associations with intermediate phenotypes, such as carotid IMT. The power of such studies can be enhanced by haplotyping and by using improvised study designs, such as sib-pair or TDT-trios, which strengthen the studies by tracking genetic transmission of haplotypic markers. The candidate genes yielding positive associations should be explored further for associations with stroke after accurate phenotyping. As suggested by Schulz et al, molecular genetic studies should target noncardioembolic stroke. Additionally, stroke association studies should focus separately on large vessel and small vessel disease, as well as independently evaluate hemorrhagic stroke. It is advantageous to look for and quantitate the combined effect of the polymorphisms in the various candidate genes on the stroke subtype and to verify the results in at least two ethnically distinct, homogenous populations of adequate sample sizes. Schulz et al demonstrate that hospital-based association studies do not suffer from the problem of inclusion-bias, which eases molecular genetic investigations for stroke. These might further benefit from recruiting younger diseased individuals (age < 60 y) since the heritability is high, as suggested by the present study and the genetic effects of candidate polymorphisms is enhanced. Furthermore, unraveling complex disorders is a colossal task which will benefit greatly by the establishment of international multilateral collaborations and their organization into large consortiums.

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