Apolipoprotein E, Statins, and Risk of Intracerebral Hemorrhage

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Background and Purpose—Apolipoprotein E (ApoE) genotypes have been associated with lobar intracerebral hemorrhage (ICH). Although statins have been associated with an increased risk of ICH, meta-analyses have not consistently shown a statin-induced risk of ICH. Here, we test whether hypercholesterolemia (HC) and ApoE polymorphisms affect the risk of ICH by statin use.

Methods—The Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS) study is a prospective, demographically matched case–control study of ICH. A similar study of ICH, Genetic Risks for Medication-Related Hemorrhagic Stroke (GOCHA), was used as a replication cohort. Subjects were classified as normocholesterolemia, HC without statin use, and HC with statin use. Statistical comparisons were performed using Fisher exact test, $\chi^2$ tests, and the Breslow–Day test.

Results—The discovery cohort consisted of 558 ICH cases and 1444 controls, and the replication cohort consisted of 1020 ICH cases and 382 controls. The association of lower risk for HC was not attenuated by statin use. Statin use was observed to confer a higher risk for lobar ICH in those carrying ApoE4/E4 and ApoE2/E4 genotypes in both discovery and replication cohorts, and a test for interaction showed a trend towards significance ($P=0.11$ for statin and ApoE4/E4).

Conclusions—Statin use does not seem to attenuate the association of HC with decreased risk for nonlobar ICH. Our data support a gene-by-drug effect for lobar ICH, but larger sample sizes are needed to confirm the association before any clinical change is warranted.


Key Words: apolipoprotein E ■ epidemiology ■ genetics ■ hydroxymethylglutaryl-CoA reductase inhibitors ■ intracerebral hemorrhage ■ pharmacogenomics

A randomized placebo-controlled trial, the Stroke Prevention by Aggressive Reduction in Cholesterol Levels (SPARCL) study, found a higher risk of hemorrhagic stroke among subjects who had previously had ischemic stroke and were treated with a high-dose statin medication, atorvastatin, compared with placebo (odds ratio [OR], 1.68; 95% confidence interval [CI], 1.09–2.59), although the absolute number of hemorrhagic events was small and did not correlate with low-density lipoprotein levels. However, in a recent large meta-analysis of statin use and risk of ICH, McKinney and Kostis did not find this risk. The authors evaluated 31 randomized controlled trials in which active treatment with statins was compared with treatment with placebo or low-dose statin. This meta-analysis,
which included 91,588 subjects in the active treatment group and 91,215 subjects in the comparison group, found no significant difference in the rate of ICH (OR, 1.08; 95% CI, 0.88–1.32; P=0.47).

Although the result of meta-analysis by McKinney and Kostis11 suggests that statin use is not associated with ICH in a general population, the SPARCL data suggest that the use of statins may pose a risk of ICH among patients with stroke. In a decision analysis that used SPARCL’s estimates of ICH risk with statin use, Westover et al12 reported that statin use should be avoided in ICH patients, given the increased risk of ICH recurrence, high morbidity, and mortality associated with recurrent ICH. The major pathophysiological mechanism of lobar ICH is cerebral amyloid angiopathy, and apolipoprotein E alleles with the more common E3 allele.13

We hypothesize that 1 explanation for the disparate reports in the literature could be an ApoE/statin or ApoE/HC interaction. If statin use is associated with the increased risk of ICH specifically in the presence of ApoE2 or ApoE4 alleles, then the relative proportion of these genotypes in a cohort could influence whether the risk was identified. We sought to determine whether a history of HC alone influenced the association of ApoE alleles with the risk of lobar ICH. In addition, we sought to determine whether statin users who carry ApoE2 or ApoE4 alleles had a higher risk of ICH compared with statin users with ApoE/E3 (wild-type) genotype among lobar and nonlobar ICH patients and also whether statin use attenuated the association with decreased risk observed in nonlobar ICH for HC.

Methods

Study Design

The discovery cohort is from the Genetic and Environmental Risk Factors for Hemorrhagic Stroke (GERFHS) study, a case–control study of hemorrhagic stroke that uses prospective, population-based case ascertainment with recruitment within a 50-mile radius from the University of Cincinnati.13 Cases and controls from the multicenter Genetic Risks for Medication-Related Hemorrhagic Stroke study of ICH (GOCHA)14 were used as a replication cohort.

Setting

The methods of the 2 studies have been previously described.13-15 ICH is defined as the nontraumatic abrupt onset of severe headache, altered level of consciousness, and/or focal neurologic deficit associated with a focal collection of blood within the brain parenchyma as observed on neuroimaging or at autopsy (adapted from Classification of Cerebrovascular Disease III, 1989).13 Data were abstracted from medical charts of all individuals with apparent hemorrhagic stroke. Case status was verified by study physicians.

Standard Protocol Approvals and Patient Consents

The Institutional Review Board of each hospital system approved the studies. A Certificate of Confidentiality was obtained from the Department of Health and Human Services. Informed consent was obtained from all subjects who underwent interview and genetic analysis.

Participants

GERFHS cases were eligible for the study if they were ≥18 years of age and resided within a 50-mile radius from the University of Cincinnati. Hemorrhages associated with trauma, brain tumor, encephalitis, endarterectomy, hemorrhagic cerebral infarction, or thrombolytic treatment of ischemic stroke did not meet study criteria. Patients with ICH associated with anticoagulation, primary intraventricular hemorrhage, or previous history of ischemic stroke were included. Study neurologists reviewed clinical and neuroimaging information for each patient and made the final decision about case eligibility. Controls for the GERFHS study were identified by random digit dialing to match cases by age (±5 years), race, and sex. For the current analysis, we disregarded the original matching to conduct stratified analysis by cholesterol and genotype status. All analyses included adjustments for age, race, sex, and significantly associated risk factors. The GOCHA study is a case–control study of both non–warfarin- and warfarin-related ICH. Enrolled cases included subjects with acute ICH aged ≥55 years presenting to the Massachusetts General Hospital. Exclusion criteria included trauma, brain tumor, hemorrhagic transformation of an ischemic stroke, vascular malformation, or any other perceived cause of secondary ICH. Controls were enrolled from the same population that gave rise to the cases and included individuals aged ≥55 years attending ambulatory clinics.

Variables

Medical records were abstracted on standardized forms. Each hemorrhage was classified as lobar (involving predominantly the cortex and underlying white matter of the cerebral hemisphere), deep (involving predominately the basal ganglia, periventricular white matter, thalamus, or internal capsule), cerebellar, or brain stem by a study neurologist. When categorization was unclear, the film was adjudicated by a group of study neurologists for consensus.

Each consented case (or proxy) and control was interviewed face-to-face in a highly structured, identical manner.13 Race/ethnicity and risk factor variables were determined by self-report. We used self-reported history of hypertension and HC rather than measurements from the acute setting because blood pressure may increase in the acute setting and fasting status was not uniformly available. We also recorded medication use by the cases (before ICH onset) and controls. Statin use was determined by self-report of medications that included any of the following: atorvastatin, cerivastatin (discontinued in 2001), fluvastatin, lovastatin, pravastatin, rosuvastatin, or simvastatin, or a combination medications that included a statin (eg, amloidipine/atorvastatin, ezetimibe/simvastatin). Subjects were classified as normcholesterolemia (NC), HC treated with statins (HC-S), or HC not treated with statins (HC-NS). HC-NS subjects included those treated with nonstatin lipid-lowering medications (ie, clofibrate, colestipol, cholestyramine, colseveleam, colestipol, ezetimibe, fenofibrate, gemfibrozil, niacin), as well as those not treated with any medications for HC. For ApoE genotyping, 4 buccal brush samples or whole blood were obtained from each case and control at the time of interview in GERFHS and from blood samples in GOCHA. ApoE genotype was determined by a polymerase chain reaction–based method to determine genotype at single nucleotide polymorphisms rs429358 and rs7412.13,16 The allelic reads from the 2 assays were then translated to ApoE genotypes (e3e3, e3e4, e4e4, e3e2, e2e2, and e2e4). All genotyping personnel were blinded to clinical and neuroimaging data.

Statistical Analysis

All analyses were conducted separately for lobar and nonlobar groups using SAS version 9.3 (SAS Institute, Cary, NC). Demographic characteristics are reported as mean±SD or n (%), and comparisons between cases and controls are based on unpaired t tests, χ² tests, or Fisher exact test, as appropriate. To examine the role of the rare E2 and E4 alleles, genotypic groups were classified into 2 different ways for analysis: ApoE2 carriers (E2/E2, E2/E4) versus E3/E3 and E4 carriers (E2/E4, E3/E4, E4/E4) versus E3/E3. Associations between case status and ApoE2 or ApoE4 alleles were tested by χ² and Fisher exact analysis of 2×2 contingency tables (case/control status versus presence/absence of allele). Homogeneity of ORs across clinical strata (eg, HC versus NC, HC-NS versus HC-S) was tested
Results

After removing cases and controls with missing ApoE results, the discovery cohort included 558 cases of spontaneous ICH (354 nonlobar, 204 lobar) who were compared with 1444 controls (946 matched to nonlobar ICH and 508 to lobar ICH). In the replication cohort, 1020 cases (539 nonlobar, 481 lobar) were compared with 382 controls. Table 1 presents the demographic characteristics of the case–control cohorts. ApoE2- and E4-containing genotypes were associated with the risk of ICH in lobar regions but not in nonlobar regions for both the discovery and replication cohorts.

HC had been previously reported to be associated with a decreased risk of nonlobar ICH. We evaluated whether statin use attenuated the decreased risk of nonlobar ICH with HC (Table 2). Compared with NC in both the discovery and replication cohorts, history of HC was associated with decreased risk of lobar ICH, and this protective association was not attenuated by statin use in either the discovery or replication cohorts.

We next evaluated whether HC status itself (with or without statin use) modified the increased risk of lobar ICH associated with ApoE alleles (Table 3). In the discovery sample, ApoE2 carriers exhibited an increased risk of lobar ICH among those with NC (OR, 2.06; 95% CI, 1.21–3.51), which increased with HC (OR, 3.93, 95% CI, 2.04–7.55). However, a formal test for interaction did not reach statistical significance (P=0.13), and the finding of differential risk by cholesterol status was not observed in the replication cohort. When evaluating ApoE4 alleles, carriers had a similar risk of lobar ICH with and without a history of HC. Thus, a history of HC did not seem to modify the association of either ApoE2 or ApoE4 with lobar ICH.

Finally, we separately evaluated the risk of lobar ICH for specific ApoE genotypes among statin users (HC-S) and the HC-NS and NC groups using ApoE3/E3 as the referent genotype. Statin users with ApoE4/E4 had a significant risk of lobar ICH (OR, 4.5; 95% CI, 1.3–16.0; P=0.02), whereas the risk was nonsignificant for the ApoE4/E4 genotype among the NC (OR, 1.9; 95% CI, 0.53–6.7; P=0.30) and HC-NS (OR, 1.6; 95% CI, 0.27–9.4; P=0.63) groups. In the replication cohort, ApoE4/E4 statin users also had a much higher OR (OR, 12; 95% CI, 2.5–54; P<0.0001) than the NC group (OR, 4.9; 95% CI, 1.1–22; P=0.04) and HC-NS (OR, 3.8; 95% CI, 0.2–90; P=0.82) groups. Similarly, the ApoE2/E4 HC-S group had a higher risk for lobar ICH (OR, 11.3; 95% CI, 2.0–64; P=0.005) than the NC group (OR, 2.0; 95% CI, 0.8–5.2; P=0.18). This was also found to be true in the replication sample: HC-S (OR, 7.4; 95% CI, 1.5–3.7; P=0.008) compared with NC (OR, 3.7; 95% CI, 1.3–11; P=0.01). ApoE2/E4 was not significantly associated with lobar ICH for the HC-NS group in either the discovery or the replication cohort. When performing a formal test for interaction between statin use and genotype combining the discovery and replication cohorts, a trend was observed for ApoE2/E4 (P=0.09 across all groups) and for ApoE4/E4 (P=0.11). As expected, given that ApoE alleles have not been associated with nonlobar ICH, similar analysis of nonlobar ICH revealed no evidence of statin use/ ApoE interaction modifying the risk (data not shown).

Discussion

We report several novel observations with respect to our understanding of the association of HC and ICH. For nonlobar ICH, statin use does not seem to diminish the protective

Table 1. Demographics

<table>
<thead>
<tr>
<th></th>
<th>Discovery Sample</th>
<th>Replication Sample</th>
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<tbody>
<tr>
<td></td>
<td>Lobar ICH</td>
<td>Nonlobar ICH</td>
</tr>
<tr>
<td></td>
<td>Cases</td>
<td>Controls</td>
</tr>
<tr>
<td>N</td>
<td>204</td>
<td>508</td>
</tr>
<tr>
<td>Age, y, mean±SD</td>
<td>66.4±16.2</td>
<td>63.1±15.6</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>91 (45)</td>
<td>216 (43)</td>
</tr>
<tr>
<td>Black race, n (%)</td>
<td>30 (15)</td>
<td>73 (14)</td>
</tr>
<tr>
<td>Hypertension, n (%)</td>
<td>111 (55)</td>
<td>267 (53)</td>
</tr>
<tr>
<td>Hypercholesterolemia, n (%)</td>
<td>85 (42)</td>
<td>223 (44)</td>
</tr>
<tr>
<td>Frequent alcohol use, n (%)</td>
<td>14 (7)</td>
<td>31 (6)</td>
</tr>
<tr>
<td>Previous ischemic stroke, n (%)</td>
<td>16 (8)</td>
<td>6 (1)</td>
</tr>
</tbody>
</table>

ApoE genotype, n (%)

<table>
<thead>
<tr>
<th></th>
<th>Discovery Sample</th>
<th>Replication Sample</th>
</tr>
</thead>
<tbody>
<tr>
<td>E2/E2</td>
<td>2 (1)</td>
<td>9 (2)</td>
</tr>
<tr>
<td>E2/E3</td>
<td>42 (21)</td>
<td>69 (14)</td>
</tr>
<tr>
<td>E2/E4</td>
<td>17 (8)</td>
<td>32 (7)</td>
</tr>
<tr>
<td>E3/E3</td>
<td>85 (42)</td>
<td>237 (49)</td>
</tr>
<tr>
<td>E3/E4</td>
<td>46 (23)</td>
<td>106 (22)</td>
</tr>
<tr>
<td>E4/E4</td>
<td>12 (6)</td>
<td>28 (6)</td>
</tr>
</tbody>
</table>

ApoE indicates apolipoprotein E; and ICH, intracerebral hemorrhage.
association of HC. If statin use can be presumed to lower cholesterol level, this would suggest that the actual cholesterol level may not be relevant to the protective association of a history of HC for nonlobar ICH, which is the predominant form of ICH. This would be consistent with the previous large meta-analyses that did not show an increased risk of ICH with statin use or cholesterol level.

However, for the less common lobar ICH, we found evidence in both the discovery and replication samples of a signal toward a higher risk of lobar ICH with statin use for specific genotypes. Despite the large sample size that our study began with, once limited by location and specific ApoE genotypes and by stratification by cholesterol status and treatment with statins, the ability to confirm the finding by direct interaction was limited. This preliminary finding should not warrant a change in management but may warrant more evaluation in future studies.

For >3 decades, data from around the world have consistently demonstrated an association with lower risk of ICH among those with HC. ApoE2 and E4 are both associated with lobar ICH but apparently by different mechanisms. ApoE2 is associated with lobar vessel rupture, whereas ApoE4 seems to be associated with cerebral amyloid angiopathy. ApoE4 is a well-established risk factor for cardiovascular disease, as well as Alzheimer disease, whereas ApoE2 has been associated with a decreased risk of these conditions. The association of statin use with an increased risk of lobar ICH in subjects with ApoE2 and E4 alleles suggests that lowering of serum lipid levels may be related to the development or progression of amyloid angiopathy and lobar ICH. Interestingly, the drug bexarotene has been associated with amyloid removal in mouse models of Alzheimer disease. Bexarotene is associated with increasing hyperlipidemia, and it is thought that bexarotene stimulates expression of ApoE, which leads to intracellular clearance of β-amyloid. By contrast, in predominantly hypertensive nonlobar ICH in which amyloid angiopathy plays little if any role, the protective association between HC and nonlobar ICH seems to be unaffected by statin agents that lower serum cholesterol. The mechanism by which hyperlipidemia reduces the risk of rupture of the small penetrating arteries and arterioles in the deep brain and brain stem remains unknown.

Our study has several limitations. Fasting lipid levels were not available on controls, and cases did not have uniform collection of lipid levels; thus, fasting state could not be determined. Exposure to HC may lead to the effects observed, irrespective of cholesterol level at the time of entry into the study. Future studies that include fasting lipid profiles may help to clarify the relationship. A small number of cases were dropped for lack of ApoE genotype available (5 lobar ICH, 8 nonlobar ICH, and a total of 96 controls). Although we have no reason to suspect biased loss of sample by a specific genotype, it is possible that a missing data bias could have occurred in the discovery sample, but it should not have affected the replication sample in a similarly biased fashion. Another limitation is that although the current study represents one of the largest studies of ApoE, lobar ICH, and HC/statin use, the number of individuals for several of the specific ApoE genotypes is small. Larger studies are required to confirm this preliminary finding in which all ApoE-containing genotypes have sufficient sample to determine an allele-specific effect.

If confirmed, this finding may represent a rationale for genetic testing in patients with lobar ICH for those currently on or considered for statin therapy. The finding may have further interest for Alzheimer disease and cardiovascular disease in which statin use and HC and ApoE alleles may also affect risk.

### Table 2. Risk of Nonlobar ICH Among Subjects With Hypercholesterolemia, Stratified by Statin Use

<table>
<thead>
<tr>
<th></th>
<th>Discovery Sample</th>
<th>Replication Sample</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cases</td>
<td>Control</td>
<td>Unadjusted OR</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>114 (32%)</td>
<td>400 (43%)</td>
<td>0.64 (0.49–0.82)</td>
</tr>
<tr>
<td>without statin use</td>
<td>52 (15%)</td>
<td>201 (21%)</td>
<td>0.63 (0.45–0.88)</td>
</tr>
<tr>
<td>with statin use</td>
<td>62 (18%)</td>
<td>199 (21%)</td>
<td>0.79 (0.57–1.08)</td>
</tr>
</tbody>
</table>

ICH indicates intracerebral hemorrhage; and OR, odds ratio.

*Nonlobar ICH OR adjusted for age, sex, race, first-degree relative with ICH, and hypertension status.

### Table 3. Risk of Lobar ICH by ApoE Carrier Status and Hypercholesterolemia vs No Hypercholesterolemia

<table>
<thead>
<tr>
<th></th>
<th>Discovery Sample</th>
<th>Replication Sample</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Case</td>
<td>Control</td>
<td>OR (CI)</td>
</tr>
<tr>
<td>ApoE2 carriers vs E3/E3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normocholesterolemia</td>
<td>33 (39%)</td>
<td>53 (24%)</td>
<td>2.06 (1.21–3.51)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>28 (46%)</td>
<td>27 (18%)</td>
<td>3.93 (2.04–7.55)</td>
</tr>
<tr>
<td>ApoE4 carriers vs E3/E3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normocholesterolemia</td>
<td>42 (45%)</td>
<td>73 (30%)</td>
<td>1.90 (1.17–3.11)</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>33 (50%)</td>
<td>73 (37%)</td>
<td>1.71 (0.98–3.00)</td>
</tr>
</tbody>
</table>

ApoE indicates apolipoprotein E; CI, confidence interval; ICH, intracerebral hemorrhage; and OR, odds ratio.
In summary, risk of lobar ICH with statin use may be affected by ApoE genotype and may be greater in those with the uncommon to rare E2/E4 or E4/E4 genotypes. The association of HC with a decreased risk of nonlobar ICH does not seem to be attenuated by the use of statins.

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Disclosures
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References
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