ABSTRACT

Objective: We sought to determine whether APOE genotype influences a previously observed decline in serum total cholesterol (TC) and low-density lipoprotein (LDL) levels preceding primary intracerebral hemorrhage (ICH), as a potential demonstration of nonamyloid mechanisms of APOE in ICH risk.

Methods: We performed a single-center retrospective longitudinal analysis using patients with known APOE genotype drawn from an ongoing cohort study of ICH. Serum lipid measurements for TC, triglycerides (TGs), LDL, and high-density lipoprotein (HDL) collected within 2 years before and after index ICH were extracted from electronic medical records. Piecewise linear mixed-effects models were used to compare APOE allele–specific effects on temporal serum lipid trends in ICH. Demographics, medical history, medications, and health maintenance data were included as fixed effects. Inter- and intraindividual variations in lipid levels were modeled as random effects.

Results: A total of 124 ICH cases were analyzed. APOE e4 carriers had greater rates of decline in serum TC and LDL within 6 months preceding ICH (TC: 27.30 mg/dL/mo, p = 0.0035; LDL: 28.44 mg/dL/mo, p = 0.0001). Conversely, serum TC and LDL levels in APOE e2 carriers were unchanged within the same time period. APOE genotype had no associations with serum HDL or TG trends.

Conclusions: APOE allele status predicts serum TC and LDL changes preceding acute ICH. Our results have implications for ongoing efforts in dissecting the role of dyslipidemia in cerebrovascular disease risk. APOE genotype–specific influence on lipid trends provides a clue for one mechanism by which APOE may influence risk of ICH. Further characterization of the metabolic roles of APOE is needed to improve the understanding of APOE biology in cerebrovascular disease risk. Neurology Genet 2016;2:e81; doi: 10.1212/NXG.0000000000000081

GLOSSARY

HDL = high-density lipoprotein; ICH = intracerebral hemorrhage; LDL = low-density lipoprotein; PLME = piecewise linear mixed-effects; TC = total cholesterol; TG = triglyceride.

Primary intracerebral hemorrhage (ICH) accounts for 10%–15% of all strokes but is the most severe form of acute cerebrovascular disease, with 90-day mortality rates of 40%–50% and with fewer than a third of survivors regaining functional independence by 12 months. Previous studies have established ε2/ε4 alleles of the APOE gene as potent determinants of ICH risk, severity, and outcome. APOE ε2 and ε4 are associated with increased risk of ICH occurring in the lobar regions of the brain, whereas APOE ε4, but not ε2, is associated with risk of nonlobar ICH. Separately, several epidemiologic studies have also observed an association between serum lipid levels and ICH risk and outcome.
with reduced risk of ICH,8–13 fewer cerebral microbleeds, and improved outcome after ICH.15,16 However, despite known functions of APOE gene products in lipid transport and regulating circulating lipid levels,17 the biological mechanisms mediating the roles of APOE and serum lipids on ICH risk remain unclear. A previous finding that serum low-density lipoprotein (LDL) mediates APOE ε4–associated nonlobar ICH risk7 suggests that the effect of APOE on ICH may be at least in part because of its effect on lipids.

We have recently demonstrated that ICH is preceded by declines in serum total cholesterol (TC) and LDL levels.18 We hypothesized that APOE genotype may influence these temporal lipid trends in ICH and tested this hypothesis by investigating APOE allele–specific effects on changes in serum lipid trends over time in a cohort of ICH patients with longitudinal lipid data.

**METHODS**

**Study design.** Patients were drawn from an ongoing prospective longitudinal cohort study of primary ICH at Massachusetts General Hospital (MGH)19 (figure 1). All aspects of this study were approved by the MGH Institutional Review Board (IRB), and written informed consent was obtained from all patients or their legal guardians before study participation.

**Patient selection.** Individuals enrolled in the MGH longitudinal ICH study presenting to the MGH Emergency Department between June 1993 and June 2014 were screened for eligibility for the present study based on the following: (1) availability of APOE genotype, (2) survival up to 2 years after ICH, and (3) possession of at least 3 serum lipid values for each lipid fraction of interest including TC, LDL, triglycerides (TGs), and high-density lipoprotein (HDL) drawn ≥6 months apart within 24 months before and after the date of acute ICH. Patients with recurrent ICH or other non-ICH hospitalization events during the time period of interest were excluded to minimize confounding by variations in serum lipid levels during periods of acute illness.20,21

![Figure 1 Study cohort and analysis plan](image-url)
Abbreviations: DM - diabetes mellitus; HTN - hypertension; ICH - intracerebral hemorrhage.

**Table 1** Baseline characteristics of the study cohort

| Variable          | APOE ε2 (n = 19) | APOE ε3/3 (n = 66) | APOE ε4 (n = 39) |
|-------------------|------------------|-------------------|------------------|
| Age, y, mean ± SD | 75.2 ± 9.7       | 73.6 ± 10.7       | 70.7 ± 11.5      |
| Females, n (%)    | 8 (42.1)         | 25 (37.9)         | 22 (56.4)        |
| White, n (%)      | 17 (89.5)        | 59 (89.4)         | 33 (84.6)        |
| HTN, n (%)        | 12 (63.2)*       | 57 (86.4)         | 38 (97.4)        |
| DM, n (%)         | 3 (25.0)         | 22 (44.0)         | 9 (47.4)         |
| Alcohol use, n (%)| 10 (62.5)        | 33 (56.9)         | 20 (51.2)        |
| Smokers, n (%)    | 1 (5.9)          | 3 (5.0)           | 7 (18.0)*        |
| Statin use, n (%) | 8 (42.1)         | 27 (41.5)         | 17 (43.6)        |
| Lobar ICH, n (%)  | 8 (42.1)         | 25 (37.9)         | 24 (61.5)*       |

*p < 0.05 in comparison with the reference group (APOE ε3/3).

Statistical methods. Study individuals were grouped by APOE ε2 and ε4 carrier status (having either 1 or 2 allelic copies of ε2 and ε4, respectively) with APOE ε3/3 individuals serving as a reference cohort as the ε3 allele is not associated with ICH risk. Patients with ε2ε4 APOE genotype (n = 5) were excluded because of an inability to assign a single carrier status. Comparisons of differences in cohort characteristics between ε2 or ε4 carriers and the reference ε3ε3 group were made by univariate analyses using unpaired t test, Mann-Whitney rank sum test, or Fisher exact test, as appropriate. Continuous numeric variables were expressed as mean ± SD.

Piecewise linear mixed-effects (PLME) random-coefficient models were used to evaluate APOE allele-specific effects on temporal variation in serum lipid trends in ICH patients within prespecified time periods, which are fixed in relation to acute ICH occurrence. This allowed for modeling of change in serum lipid trends in predetermined time intervals of interest to differ within and between ε2 or ε4 carriers and noncarriers. In a previous case-control analysis comparing ICH patients and non-ICH controls, we demonstrated significant decline in serum lipid trends in the 6-month interval immediately preceding the occurrence of ICH, which was not observed in non-ICH controls.

Accordingly, fixed knots were placed at the date of acute ICH and the date 6 months before acute ICH to mark transitions in time periods of interest corresponding to the time period 6–24 months pre-ICH (P1), the time period 0–6 months immediately pre-ICH (P2), and time period 0–24 months post-ICH (P3).

Separate multivariate linear mixed models were constructed for ε2 and ε4 carriers, with carrier status and covariates whose p values were <0.20 on univariate analyses or with known potential to influence serum lipid levels included as fixed effects. The final multivariate model was adjusted for variables: age, sex, race, pre-ICH history of hypertension, statin use (yes/no), smoking history (ever smoked), and ICH location. Interindividual and intra-individual variation in serum lipid levels were modeled as random effects. Model validity was examined using a likelihood ratio test. Unstructured covariance was used as the covariance model. Comparisons of the significance in change in serum lipid trends (slope) at the time period of interest, P1 (0–6 months pre-ICH), by APOE allele carrier status were made using the Wald test. Subgroup analyses stratified by ICH location (lobar and nonlobar) for ε2 and ε4 carrier status were separately performed but not shown because of insufficient statistical power. Significance threshold was set at p < 0.05 (2-tailed) for univariate analyses and at p < 0.0125 (Bonferroni correction for 4 tests) for individual serum lipid fraction mixed-model analyses. All statistical analyses were performed using STATA 10.0 (StataCorp LP, College Station, TX).

RESULTS Cohort characteristics. A total of 212 ICH patients enrolled between June 1993 and June 2014 with longitudinal serum lipid levels measured that met our inclusion criteria; 129 of these patients were genotyped for APOE. The 83 ICH patients removed because of the absence of APOE genotype (figure 1) did not differ in clinical characteristics from the group of patients who ultimately were included in our analyses (table e-1 at Neurology.org/ng). After removing the 5 patients with APOE ε2ε2, we analyzed 124 individuals including 19 ε2 carriers, 39 ε4 carriers, and 66 ε3ε3 patients (table 1). Compared with the reference group (APOE ε3ε3), ε2 carriers were less likely to have a pre-ICH history of hypertension, and
ε4 carriers were more likely to be smokers and have ICH located in the lobar region (all \( p < 0.05 \)). There were no differences in the rates of statin use between the 3 groups. \( APOE \) allelic frequencies in our analysis cohort were consistent with previously observed population estimates for North American Caucasians.\(^{23}\)

\( APOE \) alleles and serum lipid levels in ICH patients. We first sought to confirm previously observed effects of \( APOE \) on serum lipid levels, as seen in previous population-level genome-wide association studies of lipids.\(^{17}\) Comparisons of mean serum levels of TC, TG, LDL, and HDL before ICH by \( APOE \) allele status revealed an expected allelic dose-dependent increase in mean serum TC and LDL levels in ε4 carriers compared with noncarriers, whereas levels of both lipid fractions were decreased in ε2 carriers compared with noncarriers (TC: \( 217.21 \pm 58.37 \) mg/dL in ε4 carriers, \( 157.67 \pm 41.12 \) mg/dL in ε2 carriers; LDL: \( 130.43 \pm 48.67 \) mg/dL in ε4 carriers, \( 69.90 \pm 19.86 \) mg/dL in ε2 carriers). No associations were observed for serum TG and HDL levels, consistent with known absence of \( APOE \) effects on these lipid fractions.

\( APOE \) alleles influence 24-month pre-ICH serum lipid trends. Temporal lipid patterns in our analysis cohort revealed a decline in both serum TC and LDL levels beginning several months preceding acute ICH occurrence consistent with observed trends seen previously in a larger cohort\(^{18}\) (figure 2). Subgroup analysis by \( APOE \) carrier status revealed distinct differences in temporal serum lipid trends, visualized using Loess smoothed curves, during this time period. \( APOE \) ε4 carriers experienced an overall decline in serum TC and LDL levels in the 24 months pre-ICH. In contrast, both serum TC and LDL trends remained relatively flat in non-ε4 carriers during the same time period preceding ICH (figure 3).

*Figure 2* Temporal trends in individual serum lipid fractions in ICH patients

(A–D) Loess smoothed curves of serum lipid levels (mg/dL) against time (in months) before and after ICH. Gray areas indicate standard error (SE). Time period of interest is indicated by shaded boxes (P2 0-6 months pre-ICH). \(^* p < 0.0125\), rate of change of serum lipids by Wald test for the time period P2. ICH = intracerebral hemorrhage.
Comparisons of serum lipid trends by APOE allele demonstrated an overall decline in serum TC and LDL levels during the pre-ICH time period in ε4 carriers compared with noncarriers (p = 0.049 and p = 0.014, respectively), whereas no changes were observed in ε2 carriers.

APOE alleles influence differential change in serum lipid trends immediately preceding ICH occurrence. Visual inspection of the Loess smoothed curves also revealed distinct differences in temporal serum lipid trends in the 6-month time period immediately preceding ICH occurrence by APOE allele status. Subacute declines in mean serum TC and LDL levels beginning around 6 months pre-ICH were observed only in ε4 carriers (figure 3). This accelerated decline in the 6-month period before ICH is consistent with our previous report in a larger cohort. Covariate-adjusted PLME was used to compare differences in serum lipid trends by APOE genotype in the immediate 0- to 6-month interval pre-ICH and in the antecedent interval (6–24 months pre-ICH) (table 2). APOE ε4 carriers experienced acceleration in the rates of decline in serum TC and LDL levels in the 6 months before an acute ICH event compared with trends in the antecedent 18-month time interval. The observed association remained significant after the inclusion of potential confounders in multivariate analysis, including hypertension (table 2). Comparatively, serum TC trends were unchanged in the same 6-month time period immediately pre-ICH in both APOE ε2 carriers and APOE ε3/ε3 individuals.

Figure 3: Temporal trends in individual serum lipid fractions in ICH patients by APOE allele carrier status

(A–D) Loess smoothed curves of serum lipid levels (mg/dL) against time (in months) before and after ICH. Gray areas indicate standard error (SE). Time period of interest is indicated by shaded boxes (P2 0–6 months pre-ICH). *p < 0.0125, rate of change of serum lipids by Wald test for time period P2 in APOE ε2 or ε4 carrier status compared with reference (APOE ε3/ε3). ICH = intracerebral hemorrhage.
Our results demonstrate that temporal variation in serum lipids in ICH patients are influenced by APOE allele status and differ from APOE associations with steady-state serum lipid levels.\textsuperscript{17} APOE ε4 carriers experienced drops in TC and LDL levels in the 24-month period before their ICH, in comparison with non-APOE ε4 carriers. Furthermore, in the 6-month period immediately preceding ICH, APOE ε4 carriers displayed increased rates of decline in serum TC and LDL levels. This observation of genotype-specific differences in temporal serum lipid trends builds on our previous observation of subacute decline in serum TC and LDL levels before acute ICH and suggests that APOE gene products may exert at least some of their effect on risk of ICH through modulation of serum lipids.

Demonstration of APOE epsilon allele–specific effects on serum lipid trends in ICH patients raises several hypotheses regarding the role of APOE in ICH risk. Both ε2 and ε4 are risk factors for lobar ICH, in part, through their effects on amyloid processing.\textsuperscript{4} APOE ε4 is also independently associated with increased risk of nonlobar ICH,\textsuperscript{4} presumably through nonamyloid-related mechanisms given that cerebral amyloid angiopathy is almost universally absent from the deeper small vessels.\textsuperscript{24} A growing body of evidence supports the association of hypocholesterolemia with elevated risk of ICH\textsuperscript{17-14} and with progression of ICH-related phenotypes such as cerebral microbleeds.\textsuperscript{14,25} This association has been hypothesized to be the result of loss in vascular integrity in low circulating cholesterol states, which predisposes toward vessel rupture in ICH, although the complex role of lipids in cellular biology, inflammation, and signalling,\textsuperscript{26-29} in addition to cell membrane integrity, makes it difficult to attribute the observed associations to any one mechanism.\textsuperscript{30-33}

Known APOE effects in lipid metabolism and vascular amyloid deposition raise the possibility that APOE may influence ICH risk through amyloid and nonamyloid effects. Our results seem to support the hypothesis of a nonamyloid role of APOE in ICH risk through the observed APOE genotype–specific associations with subacute serum lipid changes before primary ICH. Given that APOE ε4 associates with higher average TC and LDL levels, our results also raise the hypothesis that the rate of change in serum levels, rather than the baseline average, is an important determinant of ICH risk imparted by the APOE ε4 genotype. Furthermore, our demonstration of APOE allele–specific effects on temporal serum lipid trends in ICH corroborates the notion of divergent mechanistic pathways between ε2 and ε4 alleles in the pathophysiology of ICH.\textsuperscript{34,35} Our observations of similar APOE allele–specific serum TC and LDL trends pre-ICH in subgroup analyses stratified by ICH location (lobar and nonlobar) likewise support such a notion, but our study was insufficiently
powered to detect statistically significant differences because of the small sample size.

However, caution must be exercised in making mechanistic links because of the biological complexity of the underlying disease and APOE pleiotropy. Based on our observation, we can only speculate as to whether serum lipid declines directly drive the process of increased vessel wall vulnerability, leading ultimately to vessel rupture and ICH or serve as a surrogate marker of a separate process affecting cerebral small vessels. Active inflammation is associated with lower serum TC and LDL levels, whereas an APOE genotype–specific elevation in proinflammatory response has been observed in APOE ε4 carriers compared with APOE ε2 and APOE ε3 carriers in transgenic murine models. Thus, it is possible that the influence of APOE polymorphisms on temporal lipid trends in ICH may instead reflect APOE genotype–specific differences in innate inflammatory processes in the cerebral small vessels.

A strength of this study is the use of a unique data set combining both APOE genotype and longitudinal lipid data in a rigorously phenotyped ICH cohort. The additional information conferred by serum lipid variations over time both before and after primary ICH revealed APOE genotype–specific associations distinct from known steady-state relationships. This in turn allowed for the dissection of lipid-dependent associations of APOE in primary ICH risk.

There are limitations to our study. First, we had to exclude almost 40% of eligible cases identified because of the absence of APOE genotype data. It should be noted, however, that the analysis cohort remained representative of the larger ICH cohort, with no differences in any covariates of interest to suggest a sampling bias. Furthermore, distribution of the APOE alleles in our small study was also consistent with frequency estimates in the population at large. We were also unable to account completely for selection bias arising from subject-specific indications for serial serum lipid measurements, although, in our particular study cohort, previous analysis showed no differences in clinical characteristics between ICH patients with and without serum lipid data. A third limitation was our relatively small sample size, particularly with regard to the total number of APOE ε2 individuals, which may influence our ability to more accurately assess association with serum lipid trends in those individuals. We attempted to address this by using mixed-effect modeling to increase statistical power through additional use of interindividual change with time. Nevertheless, future studies incorporating a longitudinal design with available APOE genotypes and relatively frequently recorded lipid levels will be necessary to validate and confirm these results. Fourth, we were unable to exhaustively address the broad range of all the possible external environmental factors that can influence biological variation in serum lipid levels. We did attempt to minimize potential confounding by including these measures, where available, by including covariates of age, statin, and alcohol use in our models and using a longitudinal trial design of sufficiently long duration (4 years), which limits the effect of seasonal variations in serum lipids. In addition, we were also unable to account for either statin dose or type and potential intermittent use. However, because the decline in both serum TC and LDL levels immediately preceding ICH occurrence were previously noted to be independent of statin use, the differential degree of lipid lowering conferred by nuances in statin use is unlikely to contribute substantial confounding. Fifth, although there is a high likelihood that LDL may be a major mediator of TC effects seen in APOE ε4 carriers, our study design prohibits formal mediation analysis because of violation of several assumptions needed for establishing a correctly specified mediation model. Finally, although the demonstration of temporal changes in serum lipids preceding ICH strongly suggests a correlation between serum lipid changes and ICH development, we are unable to confirm a causal relationship because of the retrospective study design.

APOE ε4 strongly predicts pre-ICH trends in serum TC and LDL levels and the acute decline in serum TC and LDL in the 6-month period before acute ICH. Our results have implications for ongoing efforts in dissecting the role of dyslipidemia in cerebrovascular disease risk and provide novel insight regarding nonamyloid APOE mechanisms in ICH risk.

AUTHOR CONTRIBUTIONS
Dr. C.-L. Phuah participated in study design, data acquisition, statistical analyses, drafting, and revision of the manuscript. M.R. Raffeld and A.M. Ayres were responsible for data collection. Drs. A. Viswanathan, M. Edip Gurol, S.M. Greenberg, and J. Rosand participated in the final editing of the manuscript. Dr. C.D. Anderson participated in the study design and funding and in the revision of the manuscript.

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APOE polymorphisms influence longitudinal lipid trends preceding intracerebral hemorrhage
Chia-Ling Phuah, Miriam R. Raffeld, Alison M. Ayres, et al.

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