PC Reviewed: 05/06/04   **Status: A**   **Priority: 2**
SC Reviewed: 05/07/04   **Status: A**   **Priority: 2**

1.a. Full Title: Apolipoprotein E Genotype and Risk of Ischemic Stroke

  b. Abbreviated Title (Length 26 characters): **APOE Genotype and Stroke**

2.   Writing Group (list individual with lead responsibility first):

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3.   Timeline: April 2004-October 2004

4.   Rationale:
Stroke is a major cause of death and disability in the United States and other industrialized countries. In the United States stroke is the 3rd leading cause of death and a major cause of disability. There are 500,000 first strokes each year, and of those 88 percent are ischemic [1]. While recent medical advances have improved acute ischemic stroke survival, prevention remains the best hope for reduction in stroke mortality [2].

The main risk factors for ischemic stroke have been reported to be age, hypertension, smoking, heart disease, atrial fibrillation, congestive heart failure, diabetes, hyperglycemia, and prior TIA or stroke [13]. Some potential risk factors have been contested within the literature. The relationship between apolipoprotein E genotype and ischemic stroke has remained unclear despite many published papers.

Apolipoprotein E genotype was first recognized as a potential determinant of coronary heart disease after it was shown that ε2 alleles lowered blood cholesterol levels, and the ε4 alleles consistently raised them, across several populations and studies [3]. The Framingham study demonstrated an increased risk of coronary heart disease for ε4 vs. ε3 after adjustment for hypertension, smoking, obesity, diabetes, HDL, and LDL [4].   A more recent summary in the American Journal of Epidemiology reported that APOE maintains a modest relation with coronary heart disease outcomes [5].

Efforts to link stroke and APOE genotype have been more inconsistent. McCarron et al performed a meta-analysis of APOE genotype in 1999. In their analysis of 9 case control studies they found an overall odds ratio of 1.68 (95% CI 1.36, 2.06) for APOE frequency among stroke

cases versus controls [6]. In their analysis six of the studies had reported null results. Since this meta-analysis there have been at least five null case control studies published, as well as one modestly positive case control study [7-11], and one null result from ARIC discussed below [12]. A lack of association between APOE genotype and ischemic stroke may be consistent with little relation between serum cholesterol and ischemic stroke [14], except at younger ages [16]. Yet, cholesterol lowering with statins does reduce stroke risk [15].

ARIC has some advantages for addressing the role of APOE genotype in incident ischemic stroke. ARIC has a well-established cohort with very high quality data and measures. ARIC has a more diverse population than previous studies, and allows the opportunity to look at potential interactions with race. ARIC has an estimated 500 incident ischemic stroke cases (40% in blacks), and will be one of the largest studies looking at APOE genotype and stroke to date. This will allow us to examine potential interactions of APOE genotype and other risk factors.

As mentioned above, stroke and APOE genotype have been looked at previously in ARIC by Morrison et al (ARIC manuscript 732). Morrison examined 218 strokes in a nested case-control study with an average follow-time of 7.5 years. Our intention is to extend the work done by Morrison, as we now have APOE genotyping of the entire cohort and approximately 500 strokes. Morrison reported the possibility of their null results being due to a lack of power, and the current proposal would have a greater ability to detect an association as well as look at potential interactions. Morrison conducted their analysis as a nested case-control study and we will be analyzing the cohort. Finally, while Morrison adjusted by race, we anticipate having sufficient cases to report race-specific findings, which is important given the widely different APOE genotype for whites and blacks [5].

5. Main Hypothesis /Study Questions: The incidence of ischemic stoke is not significantly different between those with an APOE ε4, ε3, or ε2 alleles (ε4=ε3=ε2). A secondary, but important, question is whether APOE genotype interacts with race, hypertension, and other risk factors for stroke. As a supplemental aim we will explore whether lipid levels might explain any association observed with APOE genotype; however, we do not expect this to be the case since lipids were largely unrelated to stroke in an earlier ARIC paper (14).

6. Data (variables, time window, source, inclusions/exclusions):
Inclusions: ARIC cohort.
Exclusions: Prior stroke at baseline.
Dependent variables: Ischemic stroke incidence through most recent closure.
Independent variables: APOE genotype.
Other Covariates: Age, sex, race, blood pressure, anti-hypertensive medications, anthropometrics, diabetes, blood glucose, insulin, physical activity, smoking status, alcohol use, medications, hormone replacement therapy status, prior CHD, ECG Left-ventricular hypertrophy, fibrinogen, and blood lipids.
Analysis Plan: We plan on conducting a cohort analysis examining ischemic stroke and APOE genotype controlled for other known risk factors. The independent and dependent variables are listed above. Categorical variables will be analyzed using their natural categories (ex: male and female for gender) and continuous variables will be analyzed as both continuous (blood pressure measure) and discrete (blood pressure divided into quartiles, etc). We plan to report incidence rates and relative risks for APOE as three categories: E2 (ε2/ε2, ε2/ε3, ε2 /ε4), E3 (ε3/ε3) and E4 (ε4/ε3, ε4/ε4). We will then compare incidence between these categories, using the E3 group as

the referent. We may also consider a dichotomous categorization of E4 vs. non-E4, with the non-E4 being the reference group. We will also report relative risks created using Cox proportional hazard regression models. We will produce our model by examining the contribution of possible confounders and effect modifiers, and removing those that do not significantly contribute to our model. We anticipate including confounders such as age and sex in our model. We will be able to report separate risk ratios by race, as a possible effect modifier. Our race categories will be Black and White. ARIC has some measures that were taken at baseline and throughout the time of the cohort, while some were only taken at baseline. We may use time-dependant covariates to account for this data, but are waiting until analysis to determine the best approach. Adjustment for lipids (potential intermediate variables) will be considered carefully. We will be using SAS for our analysis.

References:
1. American Heart Association. Heart Disease and Stroke Statistics – 2004 Update. Dallas, Texas: AHA; 2004
2. Broderick JP. Willima M. Feinberg Lecture: Stroke Therapy in the Year 2025. Stoke. 2004; 35: 205-211.
3. Davignon J, Gregg RE, et al. Apolipoprotein E Polymorhism and Atherosclerosis. Atheriosclerosis 8:1-21, Jan/Feb 1988.
4. Wilson PWF, Myers RH, et al. Apolipoprotein E Alleles, Dyslipidemia, and Coronary Heart Disease: The Framingham Offspring Study. JAMA. 1994; 272:1666-1671.
5. Eichner JE, Dunn ST, et al. Apolipoprotein E Polymorphism and Cardiovascular Disease: A HuGE Review. Am J Epidemiol 2002; 155:487-95.
6. McCarron MO, Delong D, et al. APOE genotype as a risk factor for ischemic cerebrovascular disease: A meta-analysis. Neurology 1999; 43: 1308-1311.
7. Catto AJ, McCormack LJ, et al. Apolipoprotein E polymorphism in cerebrovascular disease. Acta Neurol Scand 2000; 101: 399-404
8. MacLeod MJ, De Lange RP, et al. Lack of association between apolipoprotein E genotype and ischemic stroke in a Scottish population. Eur J Clin Invest 2001; 31(7): 570-573.
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10. Luthra K, Prasad K, et al. Apolipoprotein E polymorphism in cerebrovascular disease: a case-control study. Clin Genet 2002: 62: 39-44.
11. Souza DRS, Campos BF, et al. Influence of the polymorphism of Apolipoprotein E in cerebrovascular disease. Arq Neuropsiquiatr 2003; 61(1): 7-13.
12. Morrison AC, Ballantyne CM, et al. LPL polymorphism predicts Stroke Risk in Men. Genet Epidemiol 22:233-242, 2002.
13. Sacco RL, Wolf PA, et al. Risk factors and their management for stroke prevention: Outlook for 1999 and beyond. Neurology 1999; 53(Supp 4): S15-S24.
14. Shahar E, Chambless LE, et al. Plasma Lipid Profile and Incident Ischemic Stroke: ARIC. Stroke. 2003; 34:623-631.
15. Bucher HC, Griffith LE, et al. Effects of HMGcoA reductase inhibitors on stroke: a meta-analysis of randomized, controlled trials. Ann Intern Med. 1998; 128:89-95.
16. Prospective Studies Collaboration. Cholesterol, diastolic blood pressure, and stroke: 13 000 strokes in 450 000 people in 45 prospective cohorts. Lancet 1995; 346: 1647-1653.

7.a. Will the data be used for non-CVD analysis in this manuscript? _____ Yes __X_ No

b. **If Yes, is the author aware that the file ICTDER02 must be used to exclude persons with a value RES_OTH = "CVD Research" for non-DNA analysis, and for DNA analysis RES_DNA = "CVD Research" would be used?** _____ **Yes** _____ **No**
(This file ICTDER02 has been distributed to ARIC PIs, and contains the responses to consent updates related to stored sample use for research.)

8.a. **Will the DNA data be used in this manuscript?** __**X**_ **Yes** _____ **No**

8.b. **If yes, is the author aware that either DNA data distributed by the Coordinating Center must be used, or the file ICTDER02 must be used to exclude those with value RES_DNA = "No use/storage DNA"?** __**X**_ **Yes** _____ **No**

9.The lead author of this manuscript proposal has reviewed the list of existing ARIC Study manuscript proposals and has found no overlap between this proposal and previously approved manuscript proposals either published or still in active status. ARIC Investigators have access to the publications lists under the Study Members Area of the web site at:
http://www.cscc.unc.edu/ARIC/search.php

__**X**__ Yes _____ No

Previous work by Morrison (manuscript 421) is discussed above. Several of the previous authors are contributing their expertise to this work.

10. **What are the most related manuscript proposals in ARIC (authors are encouraged to contact lead authors of these proposals for comments on the new proposal or collaboration)?**
ARIC manuscript 421
    Shahar E, Chambless LE, et al. Plasma Lipid Profile and Incident Ischemic Stroke: ARIC. Stroke. 2003; 34:623-631.
ARIC manuscript 732
    Morrison AC, Ballantyne CM, et al. LPL polymorphism predicts Stroke Risk in Men.
Genet Epidemiol 22:233-242, 2002.

11. **Manuscript preparation is expected to be completed in one to three years. If a manuscript is not submitted for ARIC review at the end of the 3-years from the date of the approval, the manuscript proposal will expire.**