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Mortality and Apolipoprotein E in African-American, and White Elders: An Attempted Replication

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We have tried, with only partial success, to confirm findings in a recently reported study in this journal on the relationship of APOE genotype to mortality in community representative Hispanics (n = 659), Whites (n = 272), and African-Americans (n = 450), aged 65 and over, living in Northern Manhattan, New York. That study found that using proportional hazards models adjusted for sex and lipid levels, Hispanics and Whites with the E2/E3 genotype, but not African-Americans, had the lowest mortality risk. Those under age 75 had risks comparable to those over age 75, suggesting minimal survivor bias. Nearly 50% of the mortality risk associated with the APOE genotype appeared to act through heart disease, diabetes, and stroke. The current study of African-Americans (n = 1,083) and Whites (n = 915) aged 71 and over living in the more rural Southeastern US, found no protective effect of the E2/E3 genotype for either African-Americans or Whites. Among younger Whites (age 71–75), point estimates suggested that the E2/E3 genotype might be protective, but at a nonsignificant level; self-reported African-American race, but not genotype, was a risk factor for mortality in this age group. Neither lipid level nor health condition attenuated the effect of APOE genotype. Differences in findings may reflect issues of sampling, age, the relative distribution of the APOE alleles, or some other factor. Until such time as studies use truly representative samples and include younger ages, findings in this area must be treated with caution. © 2003 Wiley-Liss, Inc.

KEY WORDS: mortality; apolipoprotein E; African-American; White

INTRODUCTION

Much of science is built on an accumulation of confirmatory findings. To determine whether recent report from the Manhattan study of racial/ethnic differences in the impact of apoliprotein E (APOE) genotype on mortality could be confirmed [Lee et al., 2001], we examined data from the Duke site of the Established Populations for Epidemiologic Studies of the Elderly (EPESE) [Cornoni-Huntley et al., 1990; Blazer et al., 1991]. Findings using this more rural community-based population of African-American and White elderly living in the southern part of the United States do not consistently confirm those in the study in Manhattan. In the Manhattan study, Lee and colleagues examined the relation between APOE genotype and mortality in a randomly ascertained population of Whites, Hispanics, and African-Americans. The Manhattan cohort showed that Whites and Hispanics with the E2/E3 genotype had the lowest mortality risk, while those with the E4/E3 genotype did not. The protective effect of the E2/E3 genotype, however, was not observed in African-Americans. We present a comparison of the findings here, together with information on the similarities and differences between the Manhattan and Duke EPESE studies and suggestions for further steps, so that other investigators may be aware of issues, which could cloud findings in this area.

METHODS

Subjects

Very briefly, subjects for the Manhattan study were randomly selected from Medicare rolls. They were

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65 years of age and older, and spoke English or Spanish. There was a 62% participation rate.

Subjects for the Duke Established Populations for Epidemiologic Studies of the Elderly (EPESE) were selected using a multi-stage, stratified, random household design in five adjacent counties (one urban, four rural). Of those contacted, 80% (N = 4,162, 65 years of age and over) participated [details are given in Cornoni-Huntley et al., 1990; Blazer et al., 1991]. Of this group, 54% were African-American (who were deliberately oversampled to increase statistical precision for this group), and all but 26 of the remainder were White. Participants were interviewed in person at home by trained interviewers using structured questionnaires at baseline (1986–1987) and 3, 6, and 10 years later. Telephone interviews were conducted annually between baseline and year 3, and between year 3 and year 6. At the third in-person visit (1992–1993) blood was drawn, and later genotyped. At this visit, 2,550 persons were present (absence was primarily due to death, dropout for other reasons was minor) and genotype was obtained on 1999 persons. Date of death could not be ascertained for one subject, who was dropped from the analyses. The resulting sample consists of 1998 persons. The study was approved by the Institutional Review Board of Duke University Medical Center. Informed consent was obtained from each participant, or legal representative if cognitively impaired.

Outcomes

Survival status was determined by search of the National Death Index through 12/31/1999.

APOE Genotyping and Covariates

APOE genotyping was done according to standard procedures [Blazer et al., 2001]. In addition, cholesterol level was determined (but not high density lipoprotein (HDL), low density lipoprotein (LDL), and triglycerides separately). On each interviewing occasion, information was obtained on selected health conditions, including heart attack, diabetes, and stroke.

Statistical Methods

Analytic approaches were the same as in the Manhattan study, that is, chi square and Cox proportional hazards modeling. When estimating mortality risk as a function of ethnicity and genotype (E2/E3, E3/E3, E4/E3) we used Whites with the E2/E3 genotype as the reference group. In these analyses, we adjusted for sex and cholesterol level, which was used as a substitute for HDL, LDL, and triglycerides, which were not available. When adjusting also for chronic health conditions we excluded cholesterol level (which was not statistically significant) in order to retain in the analysis the 263 participants for whom cholesterol data was missing.

RESULTS

Table I shows the demographic characteristics and APOE genotypes of those in the Duke EPESE study.

TABLE I. Demographic and Apolipoprotein Characteristics of Duke EPESE Participants by Race/Ethnicity

	Total (n = 1,998)		White (n = 915)		African-American (n = 1,083)	
Variable	N	%	N	%	N	%
Gender						
Male	655	32.8	306	33.4	349	32.2
Female	1,343	67.2	609	66.6	734	67.8
Vital status						
Deceased	481	24.1	218	23.8	263	24.3
Surviving	1,517	75.9	697	76.2	820	75.7
Education						
≤6 years	563	28.2	150	16.4	413	38.1
7–11 years	917	45.9	412	45.0	505	46.6
≥12 years	518	25.9	353	38.6	165	15.2
Age						
≤75 years	806	40.3	371	40.6	435	40.2
>75 years	1,192	59.7	544	59.5	648	59.8
APOE genotype						
E4/E4	63	3.2	22	2.4	41	3.8
E3/E4	489	24.5	171	18.7	318	29.4
E3/E3	1,064	53.3	570	62.3	494	45.6
E2/E4	87	4.4	23	2.5	64	5.9
E2/E3	282	12.4	126	13.8	156	14.4
E2/E2	13	0.7	3	0.3	10	0.9

African-Americans (n = 1,083) slightly outnumbered Whites (915, 54%:46%, respectively). Nearly two-thirds of each group were women. Just under a quarter of the participants (481, 24.1%) were deceased by the end of the study, including similar proportions of African-Americans and Whites. Education was higher in Whites than in African-Americans.

Participants ranged in age from 71 to 102 years, with an average age for both African-Americans and Whites of 78.0 years; 60% of each group was over 75 years of age. For both African-Americans and Whites the E3/E3 genotype was the most common; however, its prevalence among Whites was greater than among African-Americans (62.3% vs. 45.6%). The E3/E4 genotype was the next most prevalent, with African-Americans having a higher percentage with this genotype than Whites (29.4% vs. 18.7%). The E2/E3 was the third most prevalent genotype, with African-Americans having a slightly higher percentage with this genotype also (14.4–13.8%).

Table II presents the allele frequencies by race and survival status. Among both African-Americans and Whites the E2 allele was slightly more prevalent among survivors, but it was far from the fourfold difference found for the White Manhattan participants. Further, we found the E4 allele to be only marginally more prevalent in deceased African-Americans. Overall, we found no statistically significant relationships between allele and survival status.

Table III presents the mortality risks associated with the APOE genotype, adjusting for sex and total cholesterol level. Whites with the E2/E3 genotype were the reference group. Women had a reduced risk of mortality (RR = 0.467, 95% CI = 0.381–0.571), while the level of cholesterol bore no relationship to mortality. Each of the race/genotype groups demonstrated a higher

TABLE II. Allele Frequency by Vital Status for Duke EPESE Whites and African-Americans

Allele	Allele frequency		
	Total %	Survivors %	Deceased %
Whites	(n = 1,830)	(n = 1,394)	(n = 436)
E2	8.5	8.8	7.3
E3	78.5	78.6	78.4
E4	13.0	12.6	14.2
African-American	(n = 2,166)	(n = 1,640)	(n = 526)
E2	11.1	11.7	9.1
E3	67.5	67.1	68.6
E4	21.4	21.2	22.2

risk of mortality than Whites with the E2/E3 genotype; however, in no case was the difference statistically significant.

The findings were similar when cholesterol was excluded and the three health conditions—self-report of heart attack, diabetes, and stroke—were included in the model. Female gender was protective (RR = 0.409, 95% CI = 0.338–0.496), but each of the health conditions demonstrated a significant risk for mortality (heart attack: RR = 1.695, 95% CI = 1.386–2.073; stroke: RR = 1.714, 95% CI = 1.331–2.207; diabetes: RR = 1.556; 95% CI = 1.261–1.919). While each of the race/genotype groups had a higher risk of mortality compared to Whites with the E2/E3 genotype, the differences, again, were not statistically significant.

Table IV shows the results of the analyses run separately for those 75 and younger and those 76 and older to determine whether the above findings represented a survivor effect. In the younger group, female gender and cholesterol have similar effects to those in the total model—significantly reduced risks for women, no effect for cholesterol, but there are significant differences between the African-American and the White genotype groups. For both African-Americans and Whites, relative risk for mortality is elevated compared to Whites with the E2/E3 genotype. Each of the three genotypes for African-Americans has a relative risk for mortality that is approximately four times that of Whites with the E2/E3 genotype, and is statistically significant (African-Americans E2/E3, $P = 0.038$; E3/E3, $P = 0.042$; E4/E3, $P = 0.032$). Whites with the E3/E3 and E3/E4 genotypes had increased risks of mortality, but compared to the Whites with the E2/E3 genotype these differences were not statistically significant. Among the older sample members, there were no significant differences among the race/genotype groups.

Findings were similar when the three health conditions were substituted for cholesterol, although now the risk for younger African-Americans with the E3/E3 genotype did not reach statistical significance, although a significant difference still held for African-Americans with the E2/E3 and the E4/E3 genotype. In the older group, Whites with the E3/E3 or the E3/E4 genotype had nonsignificantly higher risks of mortality than those with the comparison E2/E3 genotype, while the African-Americans had nonsignificantly lower risks, but overall the risks associated with race/genotype group disappeared.

DISCUSSION

Science is built on confirmation of findings. Using a sample of older African-Americans and Whites drawn from both urban and rural regions of the Piedmont area of North Carolina, we have tried to replicate a study on African-American and White participants living in the heavily urban area of North Manhattan. There is only partial agreement in the findings of these two studies. We have not been able to confirm the protective effects for mortality of the E2 allele in Whites, although our findings agree on a lack of effect in African-Americans. We found no statistically significant differences with respect to mortality among the genotypes for either group, neither did we find a marked decline in genotype-related risk when chronic health conditions were substituted for cholesterol level. Unlike the comparison study, we did find differential effects with respect to age at entry into the study (71–75 vs. >75), although only for African-Americans. African-Americans (71–75 years of age) were at increased risk of mortality for each of the three genotypes examined (E2/E3, E3/E3, E4/E3) compared to Whites with the lowest risk, those with the E2/E3 genotype. This suggests that among those

TABLE III. Effect of Genotype on Mortality in Models Controlling for Sex and Cholesterol Level, and Sex and Health Conditions

Variable	Adjusted for cholesterol		Adjusted for health condition	
	RR	95% CI	RR	95% CI
Female	0.467	0.381–0.571	0.409	0.338–0.496
Cholesterol	1.000	0.998–1.003		
Heart attack			1.695	1.386–2.073
Stroke			1.714	1.331–2.207
Diabetes			1.556	1.261–1.919
White E2/E3	Reference		Reference	
White E3/E3	1.313	0.860–2.004	1.258	0.832–1.903
White E4/E3	1.551	0.939–2.563	1.497	0.925–2.424
African-American E2/E3	1.173	0.703–1.956	1.136	0.689–1.874
African-American E3/E3	1.258	0.818–1.935	1.104	0.726–1.678
African-American E4/E3	1.382	0.880–2.170	1.232	0.797–1.902

RR, risk ratio; CI, confidence interval.

TABLE IV. Effect of Genotype on Mortality by Age Group in Models Controlling for Sex and Cholesterol Level, and Sex and Health Conditions

	Adjusted for cholesterol		Adjusted for health condition	
Variable	RR	95% CI	RR	95% CI
Age ≤75 years				
Female	0.487	0.329–0.719	0.387	0.266–0.564
Cholesterol	0.996	0.991–1.001		
Heart attack			2.063	1.408–3.021
Stroke			1.876	1.170–3.007
Diabetes			1.518	1.035–2.225
White E2/E3	Reference		Reference	
White E3/E3	3.577	0.863–14.831	3.479	0.839–14.431
White E4/E3	3.113	0.682–14.220	3.184	0.704–14.389
African-American E2/E3	4.869	1.090–21.762*	4.876	1.089–21.828*
African-American E3/E3	4.403	1.052–18.418*	3.738	0.893–15.639
African-American E4/E3	4.859	1.147–20.592*	4.449	1.055–18.754*
Age >75 years				
Female	0.510	0.399–0.652	0.462	0.366–0.582
Cholesterol	1.001	0.999–1.004		
Heart attack			1.540	1.211–1.958
Stroke			1.695	1.252–2.295
Diabetes			1.501	1.167–1.930
White E2/E3	Reference		Reference	
White E3/E3	1.063	0.676–1.671	1.007	0.647–1.566
White E4/E3	1.385	0.799–2.399	1.324	0.784–2.236
African-American E2/E3	0.816	0.457–1.456	0.790	0.450–1.387
African-American E3/E3	0.973	0.613–1.543	0.879	0.563–1.372
African-American E4/E3	1.025	0.627–1.676	0.926	0.578–1.483

*$P = 0.03–0.04$; RR, risk ratio; CI, confidence interval.

71–75 years of age, being African-American is an important risk factor for death; genotype is not relevant. It also indicates the possibility of survivor bias alluded to in Lee et al. [2001].

Why such discrepancy in findings? These two studies differ in certain respects. They have used different sampling designs. Sample selection for the Manhattan study was based on Medicare rolls, that for Duke EPESE on a counting and listing of households. Both are accepted methods of obtaining representative samples. The time interval between assessment and determination of survival status appears to be similar, with 21.4% dying in the Manhattan study and 24.1% in Duke EPESE. Both studies suffer from absent genotype information for a notable proportion of participants, 34.6% for the Manhattan study, 22% for Duke EPESE. In Duke EPESE, material for genotyping was not obtained on those who were geographically out of range, who had medical conditions making blood draw inappropriate, who refused, who died between time of interview and appointment for blood drawing, and, most notable and the largest group, those who were unable personally to sign a consent form (i.e., were cognitively impaired). Examination of those not genotyped indicated that they tended to be older, were more likely to be cognitively impaired, and had a higher mortality rate. Insofar as there is a relationship between allele status and cognitive functioning, the E4 allele and those in whom it is present, may be under-represented in Duke EPESE. Had this group of individuals been included, the distribution of the allele frequencies, and the risk associated with each ethnicity-genotype group might have changed.

Results in the two studies could disagree if the absolute risk of mortality for the comparison group (Whites with the E2/E3 genotype) is different in the two studies. Since the proportional hazards model computes hazards experienced by one group compared with another, differences in baseline hazards will lead to different relative risks. Thus if the baseline risk for Duke EPESE differs from that for the Manhattan group, the magnitude of relative risks associated with racial/ethnic-genotype categories can differ, even though an increase in absolute risk may be comparable for the two studies. Examination indicates that the proportion of Whites with the E2/E3 allele who die differs in the two studies. It is 6% in the Manhattan group, but 21% in the Duke sample. This difference may well explain the findings. In the Duke sample the E2/E3 death rate is comparable to the proportion dying with the E3/E3 genotype (24% of Whites, 24% of African-Americans) and the E4/E3 genotype (24% Whites, 26% African-Americans). In the Manhattan group, the proportion with the E3/E3 genotype who die (20% of Whites, 22% of African-Americans), is similar to that in the Duke sample, but differs for those with the E4/E3 genotype who die (12% of Whites, 11% of African-Americans).

The data above suggest that the observed difference in genotype risk associated with mortality in the Manhattan sample, but not in the Duke EPESE sample, is due to low risk associated with the E2/E3 genotype in the Manhattan sample. However, the reason for the observed protective effect of the E2/E3 genotype in the Manhattan sample is unclear. There is a difference in the lower age range of the participants in the two studies—age 65 in the Manhattan study and age 71 in

Duke EPESE. Nevertheless, racial/ethnic (but not genotype) differences were found in Duke EPESE, although not in the Manhattan study, for those 75 years of age and under. It is possible that the effect of E2/E3 genotype is strongest in the younger cohort (<71 years of age). This can be discussed in terms of timing of gene expression [Jarvik et al., 1997; De Benedictis et al., 1998; Toupance et al., 1998]. Farrer and colleagues [Farrer et al., 1997], in a meta-analysis, showed that the risk for Alzheimer's disease associated with the E4 allele diminishes after age 70. Thus, an unequal age distribution between the two study populations may contribute to differing allelic associations, which may lead to differing estimates of risk. To better compare the two study populations, the Manhattan sample was restricted to those 71 years of age and older, and re-analyzed. The relative risks did not change substantially, suggesting that the differential genotypic effect is not purely due to having a higher proportion of individuals in the younger cohort in the Manhattan sample.

There are also some differences in the control variables used. Duke EPESE did not have separate information on HDL, LDL, and triglycerides; consequently total cholesterol level was substituted. The impact of total cholesterol may be different from that of the selected components. Because information on cholesterol level was lacking on 263 people, and the impact of cholesterol was minimal, cholesterol was not retained in the Duke EPESE model when including health conditions in the analysis. Further, in Duke EPESE, health conditions were self-reported [although such reports have been found to be reliable; Horner et al., 2001] as opposed to clinically determined, and heart attack represented the broader designation of heart disease. As expected, however, heart attack carried a significant risk for death.

Further studies are needed using representative samples of community residents, where genotype status is known for a larger proportion of participants, and who are younger than 65 years of age, and so less likely to be affected by survivor bias. Data from one such—the Rotterdam study—has been reported recently [Slooter et al., 2001]. The Rotterdam study had a response rate of 78% (slightly less than Duke EPESE), but APOE genotype was available for 86% (n = 6,852). Participants were 55 years of age and older. They were followed for a mean of 5.4 years, during which 15.8% died. We may assume that participants were primarily White. No association was found between APOE genotype and mortality; only age, however, appears to have been controlled. The authors suggest that studies to determine the relationship of APOE genotype to mortality should use yet younger ages.

Aside from the details on differences between the studies, these two studies clearly illustrate inherent difficulties in studying longevity. Previous studies showed that 25–33% of the heritability for longevity is explained by genetic variance, while 67–75% of the variance is due to environment. Since longevity is not a rare autosomal trait, each genetic locus will confer weak to modest influence, with much of the phenotypic variance being explained by interaction between gene and environment [Weiss and Terwilliger, 2000; Terwilliger et al., 2002]. Thus, it is not at all surprising that the findings regarding the relation between APOE and mortality are equivocal. In addition, it is well known that allelic association based on a case-control or cross-sectional design can be influenced by population stratification; that is, the observed allelic association can arise from differences in allele frequency among underlying sub-populations, rather than from biological reasons. Further, the allele frequencies at the gene itself as well as flanking regions, which may regulate the functional expression of the gene, can differ in different sub-populations. To assess the degree of population stratification, it may be beneficial to compare allele frequencies for randomly selected markers across the genome in the two-study populations [Reich and Goldstein, 2001].

In comparing the Manhattan and Duke EPESE-based studies, we cannot say which is "right." We also have little reason to believe that a meta-analysis, combining information from a number of studies, would necessarily provide a better answer, for meta-analysis does not control for problems in a study. Rather, looking at an issue such as mortality we need to be sure to take into account other characteristics known to affect survival status, such as social resources, education, income, health insurance, and use of medical care. We also need to be aware of limitations imposed by missing data. Perhaps, then, we can more assuredly identify the influence of selected genotypes on mortality.

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