APOLIPOPROTEIN ∈4 ALLELE FREQUENCY IN YOUNG AFRICANS OF UGANDAN DESCENT VERSUS AFRICAN AMERICANS

Floyd Willis, MD, Neill Graff-Radford, MBBch, FRCP, Martin Pinto, MD, LaShaune Lawson, Jennifer Adamson, Dawn Epstein, Francine Parfitt, Mike Hutton, PhD, Peter C. O'Brien, PhD

Jacksonville, Florida, and Rochester, Minnesota

Through its role in lipid metabolism, Apolipoprotein ∈4 (ApoE4) may affect "brain repair" in stroke, brain hemorrhage, Alzheimer's disease, and other brain injury syndromes for which African Americans may have greater morbidity and mortality. Cross-cultural evaluations of these and other genetic factors may provide insight on possible ethnic differences in risk of morbidity to acute central nervous system (CNS) injury and chronic neurodegenerative processes.

As an initial step toward expanding knowledge of ApoE allele frequencies for persons of African descent, we compared ApoE genotype of a group of 70 young Ugandans to 59 (subset of a larger group of 342 African Americans of all ages) age-matched African Americans and to published frequencies for Caucasians and Asians. We found that the ApoE4 and ∈2 alleles are more frequent in Ugandans (U) than Caucasians (C) or Asians (A) with corresponding alleles showing significant elevations of ∈2 (U 15.71%, C 8.40%, A 4.20%) and Î4 (U 25%, C 13.70%, A 8.90%) ($p < .001$). Comparing the differences between Ugandans and age-appropriate African Americans (AA) was not statically significant, but this outcome may be due to small sample size.

These results provide the only published ApoE frequencies for Ugandans and the complete set of data provides the largest published community group of ApoE frequencies for African Americans. (*J Natl Med Assoc.* 2003;95:71–76.)

Key words: apolipoprotein ∈ ♦ Alzheimer's disease

Apolipoprotein ∈ plays a role in lipid metabolism as a component of very low-density lipoproteins (VLDL), high density lipoproteins (HDL), and chylomicron remnants.[1] In humans, there are three common alleles of ApoE (∈2, ∈3, ∈4), which are encoded on chromosome 19 and thus produce six different genotypes (2/2, 2/3, 2/4, 3/3, 3/4, and 4/4). Frequencies of ApoE vary in different age and racial groups, with ∈4 generally shown to be higher among African Americans and in many other groups of African dissent.[2,3] The ∈4 allele has been associated with several medical conditions, including cardiovascular disease and gallstone formation.[4,5]

Apolipoprotein ∈4 also has been associated with the development of Alzheimer's disease (AD), poor recovery from head injury and stroke, arteriosclerosis and amyloid angiopathy.[6-8]

 From the Mayo Clinic, Division of Family Medicine, Department of Neurology and Department of Neuroscience, Jacksonville, FL, and the Mayo Clinic, Department of Biostatistics, Rochester, MN. Direct correspondence to: Floyd Willis, MD, Mayo Clinic, 4500 San Pablo Rd., Jacksonville, FL 32224; phone (904) 953-2000; fax (904) 953-0625; or send e-mail to willis.floyd@mayo.edu.

Although we have recently reported that ApoE4 is a risk factor for Alzheimer's disease in African Americans (Graff-Radford et al: Association between ApoE genotype and AD in African Americans: in press—Archives of Neurology), minimal attention has been given to the influence that ApoE has on other health factors in blacks. Through its role in lipid metabolism, ApoE4 may affect "brain repair" in stroke, brain hemorrhage, and other brain injury syndromes for which African Americans may have greater morbidity and mortality. Cross-cultural evaluations of these and other genetic factors may provide insight into ethnic morbidity differences for CNS injury and chronic neurodegenerative processes.

In order to better understand the role of ApoE in neurovascular and neurodegenerative diseases in blacks, we need to know allele frequencies of each population. To our knowledge, there are no previous publications of ApoE allele frequencies in Ugandans. In this study, we compare frequencies of 70 Ugandan's with a sample of 59 age-matched African Americans (a subset of a larger group of 342 African Americans).

APOE AND NEURODEGENERATIVE DISEASES IN BLACKS

Increased incidence of Alzheimer's disease has been linked to genetic risk factors such as ApoE4 in Caucasians, but similar findings have yet to be confirmed in Africans.[9] Furthermore, the link between ApoE4 and AD in African Americans is shown to be weaker than in Caucasians in some studies[3,10,11] and similar to Caucasians in others (Graff-Radford et al: Association between ApoE genotype and AD in African Americans: in press). Tang et al. concluded that the ApoE4 allele is a determinate of Alzheimer's disease risk in Caucasians, but that blacks are at increased risk of Alzheimer's disease regardless of ApoE genotype. These findings suggest that other unknown factors may be involved in conferring risk of Alzheimer's disease in black populations. Cross-cultural studies may provide insight on racial and ethnic differences in risk of Alzheimer's and other neurodegenerative diseases in blacks.

African Americans and Caribbeans of African decent descent have higher morbidity and mortality from cerebrovascular and vascular diseases.[12,13] Although ApoE4 genotype (which is associated with elevated low-density lipoproteins) and elevated lipids may explain individual cases of stroke and heart disease, there is no documented evidence showing that lipids are generally higher in blacks as compared to whites, or that lipid profile explains the increased morbidity and mortality of vascular diseases in blacks.[14,15] Some have postulated that the African American population's risk factors for arteriosclerosis (hypertension, diabetes, smoking, etc.) leads to more vascular disease, and thereby the possibility of more vascular dementia and mixed vascular/Alzheimer's type dementia.[16] Although this association may eventually prove to be true in blacks, Slooter's study in a Caucasian population suggests that arteriosclerosis is not the link between ApoE and dementia.[17] This suggests that ApoE's role in neurovascular and cardiovascular diseases in blacks is associated with environment, other disease factors, or perhaps a yet undefined role that ApoE plays in neuronal repair in blacks.

Better understanding of ApoE's role as a factor in central nervous system repair is important in general, but may be particularly important for blacks in that death rate from stroke is 2.4 times more likely in African Americans than Caucasian Americans.[18,19] This strongly suggests the need for further study on ApoE's impact on neuronal repair in people of African ancestry.

PATIENTS AND METHODS

The native African participants were recruited from an international conference of Ugandans held in the United States in August of 1999. All volunteers identified themselves as natives of Uganda, currently living either in Uganda, the United States, or Canada. The African American participants are a part of an ongoing longitudinal study established to gather information on normal memory in African Americans. All participants provided written informed consent under an IRB-approved protocol.

ApoE Genotype Determination

Blood was collected into EDTA tubes from 70 adults from Uganda and 342 African Americans from the United States. The genotype was determined using basic methods described in the single-day apolipoprotein ∈ genotyping by Crook et al.,[20] with the following modifications noted. The DNA was amplified using a Hybaid Touchdown Thermal Cycler. Polymerase Chain Reaction (PCR) conditions were an initial denaturation at 94°C for five minutes, followed by 35 cycles overall of a 94°C denaturation step for 30 seconds, annealing step consisting of a 22 cycle 60°C to 50°C touchdown at 0.5°/cycle for 30 seconds, and a 72°C extension step for 45 seconds, then a final extension at 72°C for 10 minutes, completing amplification. Genomic DNA was amplified using the following primer sequences:

upstream=5'-TAAGCTTGGCACGGCTGTCCAAGGA-3'

downstream=5'-ACAGAATTCGCCCCGGCCTGGTACAC-3'

After amplification, 10 units of enzyme, Cfo I (Promega) and its buffer were added directly to 20 microliters of PCR product. The mixture was incubated at 37°C for five hours giving main fragment sizes of 91, 83, 72, and 48 base pairs. The digest was run on a 4.5% agarose gel with 1xTBE buffer.

Statistical Methods

Comparison of the allele frequencies between groups was carried out using the chi-square test with 2° of freedom.

RESULTS

As seen in Table 1, when compared to previously published frequencies for larger, older control populations of Caucasians (C) and Asians (A), Ugandans (U) show significant elevations of ∈2 (U 15.71%, C 8.40%, A 4.20%) and ∈4 (U 25%, C 13.70%, A 8.90%) (p values < .001). Though not statistically significant, ∈2 and ∈4

Table 1. SUMMARY ALLELE FREQUENCY BY GROUP

Group	E2	E3	E4
Old African American Normals (N=566)	54 (9.54%)	384 (67.84%)	128 (22.61%)
Old and Young AA Normals (N=684)	69 (10.09%)	465 (67.98%)	150 (21.93%)
Young African American Normals (n=118)	15 (12.71%)	81 (68.64%)	22 (18.64%)
Ugandans (N=140)	22 (15.71%)	83 (59.29%)	35 (25.00%)
Caucasians (N=12524)*	858 (8.40%)	7957 (77.9%)	1399 (13.70%)
Japanese (N=3954)*	166 (4.20%)	3436 (86.90%)	352 (8.90%)

Chi-Square *p*-values N=alleles

- Old AA Normals vs. Young AA Normals—0.431
- Old AA Normals vs. Ugandans—0.064
- Young AA Normals vs. Ugandans—0.293
- Old an Young African-American Normals vs. Ugandans—0.076
- Japanese vs. Ugandans—0.001
- Caucasians vs. Ugandans—0.001

*Farrer meta-analyses

Table 2. SUMMARY OF AGE DATA

Group	Mean	Median	Range
Old African American Normals (N=283)	72.0	72	(53, 97)
Young African American Normals (N=59)	35.6	34	(20, 55)
Ugandans (N=70)	35.7	36	(17, 80)

was higher, as well, in Ugandans than in age appropriate African Americans (AA) (p=0.293). The observed matching allele frequencies for an older group of African Americans was also lower for ∈2 and ∈4 frequencies than in Ugandans (N=283; all > or = 65 years/mean age 72.0/∈2-10.09%, ∈3-67.98%, ∈4-21.93%) and trended toward statistical significance (p value = 0.064). Allele frequencies in our total group of African Americans (all ages, N=342) were ∈2-10.09%, ∈3-67.98%, and ∈4- 21.93%. Frequencies for ApoE are in Hardy Weinberg equilibrium.

The mean ages of the groups was 35.6 years for the younger AAs, 34.9 years for the Ugandans, and 72 years for the older AA group (65 years or older) (Table 2).

When looking specifically at persons of African descent, there is a predominance of the ∈4 allele.[21] When reviewing seven African-based population studies of 50 or more participants from specific ethnic groups, all have increased ∈4 alleles when compared to most non-African groups (Table 3).

DISCUSSION

These results provide the only published ApoE frequencies for Ugandans and the largest published community group of ApoE4 genotype frequencies for African Americans, and they are concordant with previous reports that show that ApoE4 frequencies are higher among Africans compared to African Americans, and higher among African Americans compared to Caucasian and Japanese populations.

The specific reasons for the population heterogeneity of ApoE allele frequency is not yet well explained, but debate in literature suggests sampling variation or population subculture as possible causes.[2] Population differences are likely the explanation of the frequencies seen in Ugandan data presented here, as the higher ∈4 and ∈2 frequencies are also well documented in most African populations studied. One exception to this generalization is for the Tanzanian frequencies as reported by Kalaria. This group of Tanzanians is shown to have higher ∈2 than either African Americans or Caucasians, but ∈4 frequencies lower than most African groups and similar to African American groups (Table 3). It is unclear whether this apparent similarity between Tanzanians and African Americans is due to racial admixture or sampling bias. However, racial admixture could explain the frequencies of African American and Tanzanian populations, both appearing to have ∈4 frequencies that are intermediary between those of Caucasians and other African groups.

It is plausible that our younger group of Africans (all but one under age 65) could have an artificially high ∈4 frequency due to decreased age-related mortality ApoE4 is thought to be associated with cardiovascular disease[4,22,23] and one might postulate that an African group of advanced age might have a lower ∈4 frequency secondary to ∈4 associated cardiovascular mortality. Similar postulations could be made for young vs. older groups of African Americans. However, the ∈4 allele frequency in the younger African Americans was not observed to be higher than the frequency observed in our large group of African American elderly; and review of the literature shows the only group that reported age-differentiated allele frequencies for Africans[24] showed no ∈4 allele frequency difference between two age groups of 65-years and older vs. under 65 years. In these published reports, as well as in our group, there is no suggested inverse association of ApoE4 allele frequency and advanced age. Further study is needed on ApoE associated risk of cardiovascular disease and death as it relates to cross cultural populations.

Table 3. COMPARISON OF APOE ALLELE FREQUENCIES OF MODERATELY-SIZED (N>50) GROUPS OF AFRICANS, AFRICAN AMERICANS, AND OTHERS

Group	Author	N	ϵ2	ϵ3	ϵ4
Africans					
Ugandans	***Present Study***	***70***	***15.71%***	***59.29%***	***25.0%***
Sudanese	Hallman (2)	103	8%	62%	30%
African Pygmies	Zekraoui (25)	70	5.7%	53.6%	40.7%
Khoi San (Bushmen) of South Africa	Sandholzer (26)	247	7.7%	55.3%	37.0%
Black South Africans	Loktionov (27)	100	14.5%	57.0%	28.5%
Nigerian Blacks	Sepehrnia (28)	365	2.7	67.7%	29.6%
Tanzanians	Kalaria (24)	143	14.3%	64.7%	21.0%
Kenyans	Kalaria (24)	61	9.0%	59.0%	32.0%
US Blacks					
US Blacks (all ages)	***Present Study***	***342***	***10.09%***	***67.98%***	***21.93%***
US Blacks	Tang (10)	256	10.2%	69.1%	20.7%
US Blacks	Sahota (11)	216	10.5%	67.5%	21.76%
US Blacks	Maestre (29)	57	2.0%	74%	24%
Others					
African Americans	Farrer meta-analysis(3)	240	8.3%	72.2%	19.0%
Caucasians	Farrer meta-analysis(3)	6262	8.4%	77.9%	13.7%
Japanese	Farrer meta-analysis(3)	1977	4.2%	86.9%	8.9%
Hispanics	Farrer meta-analysis(3)	267	6.7%	82.3%	11.0%

This study has several limitations. First, like most previously published community-based studies on ApoE allele frequencies, the samples of both the African Americans and Ugandans are composed of volunteers and are not representative of either general population. Secondly, the Ugandans were younger and predominately upper middle class. Thirdly, the sample size for the Young African American normals (N=59) was smaller than the sample size for the other comparison groups, and this may account for the lack of statistical significance for the comparison of this group to the Ugandans. Pursuit of larger, population based, age balanced samples may provide clarification of these issues.

ACKNOWLEDGEMENTS

The technical assistance of Rita Fletcher and Nadine Reese is gratefully acknowledged.

Financial and material support was provided by the following grants: Genetic Markers in Africans vs. African Americans (#0-399-98), Mayo funded; NIH Supplemental Grant (AG16574); and Dementia Screen Test and Genetic Markers in African Americans (#B-68-96), NIH funded. None of the authors have affiliation or financial involvement with any organization or entity with a financial interest except for the NIH ADRC grant.

REFERENCES

1. Mahley RW. Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science.* 1988;240:622-630.

2. Hallman DM, Boerwinkle E, Saha N, Sandholzer C, Menzel HJ, Csazar A, et al. The apolipoprotein E polymorphism: a comparison of allele frequencies and effects in nine populations. *Am J Hum Genet.* 1991;49:338-349.

3. Farrer LA, Cupples LA, Haines JL, Hyman B, Kukull WA, Mayeux R, et al. Effects of age, sex, and ethnicity on the association between apolipoprotein E genotype and Alzheimer disease. A meta-analysis. APOE and Alzheimer Disease Meta Analysis Consortium. *JAMA.* 1997;278:1349-1356.

4. Davignon J, Gregg RE, Sing CF. Apolipoprotein E polymorphism and atherosclerosis. *Arteriosclerosis.* 1988;8:1-21.

5. Juvonen T, Kervinen K, Kairaluoma MI, Lajunen LH, Kesaniemi YA. Gallstone cholesterol content is related to apolipoprotein E polymorphism. *Gastroenterology.* 1993; 104:1806-1813.

6. Laskowitz DT, Horsburgh K, Roses AD. Apolipoprotein E and the CNS response to injury. *J Cereb Blood Flow Metab.* 1998;18:465-471.

7. Alberts MJ, Graffagnino C, McClenny C, DeLong D, Strittmatter W, Saunders AM, et al. ApoE genotype and survival from intracerebral haemorrhage. *Lancet.* 1995;346:575.

8. Slooter AJ, Tang MX, van Duijn CM, Stern Y, Ott A, Bell K, et al. Apolipoprotein E epsilon4 and the risk of dementia with stroke. A population-based investigation. *JAMA.* 1997; 277:818-821.

9. Osuntokun BO, Sahota A, Ogunniyi AO, Gureje O, Baiyewu O, Adeyinka A, et al. Lack of an association between apolipoprotein E epsilon 4 and Alzheimer's disease in elderly Nigerians. *Ann Neurol.* 1995;38:463-465.

10. Tang MX, Stern Y, Marder K, Bell K, Gurland B, Lantigua R, et al. The APOE-E4 allele and the risk of Alzheimer disease among African Americans, whites, and Hispanics. *JAMA.* 1998;279:751-755.

11. Sahota A, Yang M, Gao S, Hui SL, Baiyewu O, Gureje O, et al. Apolipoprotein E-associated risk for Alzheimer's disease in the African- American population is genotype dependent. *Ann Neurol.* 1997;42:659-661.

12. Geronimus AT, Bound J, Waidmann TA, Hillemeier MM, Burns PB. Excess mortality among blacks and whites in the United States. *N Engl J Med.* 1996;335:1552-1558.

13. Cappuccio FP, Cook DG, Atkinson RW, Strazzullo P. Prevalence, detection, and management of cardiovascular risk factors in different ethnic groups in south London. *Heart.* 1997;78:555-563.

14. Gaillard TR, Schuster DP, Bossetti BM, Green PA, Osei K. The impact of socioeconomic status on cardiovascular risk factors in African-Americans at high risk for type II diabetes. Implications for syndrome X. *Diabetes Care.* 1997;20:745-752.

15. Keil JE, Sutherland SE, Knapp RG, Gazes PC. Serum cholesterol—risk factor for coronary disease mortality in younger and older blacks and whites. The Charleston Heart Study, 1960-1988. *Ann Epidemiol.* 1992;2:93-99.

16. Gorelick PB, Brody J, Cohen D, Freels S, Levy P, Dollear W, et al. Risk factors for dementia associated with multiple cerebral infarcts. A case-control analysis in predominantly African-American hospital-based patients. *Arch Neurol.* 1993;50:714-720.

17. Slooter AJ, Cruts M, Ott A, Bots ML, Witteman JC, Hofman A, et al. The effect of ApoE on dementia is not through atherosclerosis: the Rotterdam Study. *Neurology.* 1999;53: 1593-1595.

18. Ness J, Aronow WS. Prevalence of coronary artery disease, ischemic stroke, peripheral arterial disease, and coronary revascularization in older African-Americans, Asians, Hispanics, whites, men, and women. *Am J Cardiol.* 1999;84:932-933, A937.

19. Sacco RL, Boden-Albala B, Gan R, Chen X, Kargman DE, Shea S, et al. Stroke incidence among white, black, and Hispanic residents of an urban community: the Northern Manhattan Stroke Study. *Am J Epidemiol.* 1998;147:259-268.

20. Crook R, Hardy J, Duff K. Single-day apolipoprotein E genotyping. *J Neurosci Methods.* 1994;53:125-127.

21. Gerdes LU, Klausen IC, Sihm I, Faergeman O. Apolipoprotein E polymorphism in a Danish population compared to findings in 45 other study populations around the world. *Genet Epidemiol.* 1992;9:155-167.

22. Wilson PW, Schaefer EJ, Larson MG, Ordovas JM. Apolipoprotein E alleles and risk of coronary disease. A meta-analysis. *Arterioscler Thromb Vasc Biol.* 1996;16:1250-1255.

23. Scuteri A, Bos AJ, Zonderman AB, Brant LJ, Lakatta EG, Fleg JL. Is the apoE4 allele an independent predictor of coronary events? *Am J Med.* 2001;110:28-32.

24. Kalaria RN, Ogeng'o JA, Patel NB, Sayi JG, Kitinya JN, Chande HM, et al. Evaluation of risk factors for Alzheimer's disease in elderly east Africans. *Brain Res Bull.* 1997;44:573-577.

25. Zekraoui L, Lagarde JP, Raisonnier A, Gerard N, Aouizerate A, Lucotte G. High frequency of the apolipoprotein E*4 allele in African pygmies and most of the African populations in sub-Saharan Africa. *Hum Biol.* 1997;69:575-581.

26. Sandholzer C, Delport R, Vermaak H, Utermann G. High frequency of the apo epsilon 4 allele in Khoi San from South Africa. *Hum Genet.* 1995;95:46-48.

27. Loktionov A, Vorster H, O'Neill IK, Nell T, Bingham SA, Runswick SA, et al. Apolipoprotein E and methylenetetrahydrofolate reductase genetic polymorphisms in relation to other risk factors for cardiovascular disease in UK Caucasians and Black South Africans. *Atherosclerosis.* 1999;145:125-135.

28. Sepehrnia B, Kamboh MI, Adams-Campbell LL, Nwankwo M, Ferrell RE. Genetic studies of human apolipoproteins. VII. Population distribution of polymorphisms of apolipoproteins A-I, A-II, A-IV, C-II, E, and H in Nigeria. *Am J Hum Genet.* 1988;43:847-853.

29. Maestre G, Ottman R, Stern Y, Gurland B, Chun M, Tang MX, et al. Apolipoprotein E and Alzheimer's disease: ethnic variation in genotypic risks. *Ann Neurol.* 1995;37:254-259.

We Welcome Your Comments

The *Journal of the National Medical Association* welcomes your Letters to the Editor about articles that appear in the *JNMA* or issues relevant to minority health care.

Address correspondence to Editor-in-Chief, JNMA, 1012 Tenth St., NW, Washington, DC, 20001; fax (202) 371-1162; or e-mail ktaylor@nmanet.org .