ORIGINAL CONTRIBUTION

APOE and *APOC1* Promoter Polymorphisms and the Risk of Alzheimer Disease in African American and Caribbean Hispanic Individuals

Benjamin Tycko, MD, PhD; Joseph H. Lee, PhD; Alejandra Ciappa, MD; Anjana Saxena, PhD; Chi-Ming Li, PhD; Lin Feng, BA; Alex Arriaga, BA; Yaakov Stern, PhD; Rafael Lantigua, MD; Neil Shachter, MD; Richard Mayeux, MD, MSc

Background: The *APOE* ε4 allele is a genetic risk factor for Alzheimer disease (AD), though the strength of the association varies by ethnic group. Polymorphisms in regulatory sequences of *APOE* have also been related to AD, but the effects are inconsistent across studies.

Methods: We examined the association between AD and variants in 3 *APOE* promoters and in the promoter of the adjacent *APOC1* gene in African American and Caribbean Hispanic individuals. Polymorphisms tested were the –491A/T, –427T/C, and –219G/T (Th1/E47cs) in the *APOE* promoter and the *Hpa*I variant in the *APOC1* promoter. Using standard research criteria for AD, overall odds ratios were computed and repeated stratified by presence or absence of *APOE* ε4.

Results: The *APOC1 Hpa*I+ variant was associated with AD in Caribbean Hispanic individuals, but strong linkage disequilibrium with the *APOE* ε4 allele indicated that this was not an independent effect. No promoter variant in *APOE* or *APOC1* was associated with AD before or after adjusting for age, education, sex, and multiple comparisons. Estimated haplotypes including –219G/T, *APOE*, and *APOC1* differed significantly in Caribbean Hispanic patients and controls but not in African American participants. This effect was primarily owing to the –219G/T-*APOE* haplotype, but we did not detect significant allele-specific differences in promoter activity comparing reporter constructs containing the *APOE* –219G and –219 T alleles.

Conclusion: These findings exclude a strong or independent influence of *APOE* or *APOC1* promoter polymorphisms on the variation in *APOE*-related risk of AD in African American and Caribbean Hispanic individuals.

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Author Affiliations:
Department of Pathology (Drs Tycko, Saxena, and Li and Ms Feng), Division of Neuropathology (Dr Tycko), Taub Institute for Research on Alzheimer's Disease and the Aging Brain (Drs Tycko, Ciappa, Stern, Lantigua, and Mayeux), Gertrude H. Sergievsky Center (Drs Lee, Stern, and Mayeux and Mr Arriaga), and the Departments of Neurology (Drs Stern and Mayeux) and Medicine (Drs Lantigua and Shachter), Columbia University College of Physicians and Surgeons, New York, NY.

THE ε4 VARIANT OF THE *APOE* gene is a well-established risk factor for sporadic and familial Alzheimer disease (AD),[1,2] although risk appears less consistent among African American and Caribbean Hispanic individuals with sporadic AD.[2,3] An attractive hypothesis has been that genetic polymorphisms in regulatory sequences of the *APOE* locus, either in the immediate upstream promoter region or in the downstream enhancer regions, or a nearby gene might modify the *APOE*-associated risk. Thus, overexpression of the ε4 allele in the aging brain may be detrimental, possibly by promoting the extracellular aggregation of amyloid-β peptide, whereas overexpression of the ε3 or ε2 alleles may be neutral or protective. Data from transgenic mice that express human APOE protein have tended to support this hypothesis.[4,5] An association between 1 or more *APOE* promoter polymorphisms and AD has been reported.[6-15] Promoter polymorphisms associated with AD in 1 or more of these studies are, numbered relative to the transcriptional start site: –491A/T, –427T/C, and –219G/T (also known as Th1/E47cs). However, for each of these markers there are conflicting data.[13,16-22] The *Hpa*I+ variant in the promoter of the *APOC1* gene, located closely downstream of *APOE*, has also been associated with AD.[23-25] It is not clear whether this is an independent association or due to linkage disequilibrium with the *APOE* ε4 allele. Herein we report the allele frequencies of each of these polymorphisms in a large sample of African American and Caribbean Hispanic patients with AD and elderly controls, and we test for *APOE* promoter activity of 1 variant, –219G/T.

METHODS

SUBJECTS AND DIAGNOSIS

Data were derived from participants in the Washington Heights-Inwood Columbia Ag-

ing Project identified from a random sample of healthy Medicare beneficiaries aged 65 years or older residing in northern Manhattan, NY, between 1991 and 1996. The sampling procedures have been described elsewhere.[3] Participants underwent an in-person, structured interview of health and function in daily activities at the time of study entry followed by a standard medical history, physical and neurological examination, and neuropsychological battery. Participants were followed at approximately 18-month intervals, repeating the evaluations used at baseline at each follow-up.

Ethnic group was classified by self-report using the format of the 1990 US Census Bureau. First, each individual chose a particular racial or ethnic group. Then they were asked whether they were of Hispanic origin. Using this information, individuals were separated into 3 ethnic groups: African American, Hispanic, or white (non-Hispanic). For Hispanic individuals, we also asked their country of birth or origin. For those who agreed to participate, an in-person interview and a standardized assessment including a medical history, physical and neurological examination, and neuropsychological battery were completed.[26] For cases, the diagnosis of AD was established at a consensus conference of physicians and neuropsychologists. The clinical diagnosis of AD was based on criteria of the National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer's Disease and Related Disorders Associations.[27] When available, all medical records and imaging studies were used in the evaluation, as were data from the initial and follow-up examinations. *APOE* genotypes were not available to the physicians at any point during the diagnostic process. Patients classified as having AD included only individuals with probable AD[27] and those with a Clinical Dementia Rating Scale[28] score of 1.0 or higher. The comparison group consisted of individuals who had not received a diagnosis of dementia or either possible or probable AD at any point during follow-up across the 7 years. Patients with Parkinson disease, stroke, or non-AD dementia were excluded from the current study. The Columbia University institutional review board reviewed and approved this project.

STATISTICAL ANALYSIS

APOE allele frequencies were determined by counting each allele and by calculating sample proportions. For comparison of cases and controls within and across ethnic groups, *APOE* allele frequencies were calculated for all subjects and compared by χ^2 analysis. Logistic regression was used to compute the odds ratio for the association between AD and markers flanking *APOE*. Data were stratified by the presence or absence of an *APOE* ε4 allele and by adjusting for differences in age and education. Logistic regression analyses were conducted separately for each ethnic group. We tested for Hardy-Weinberg equilibrium using χ^2 analysis. In addition, we compared allele frequencies for *APOE* and adjacent markers in affected vs unaffected individuals and tested statistical significance by χ^2 analysis. Multivariate logistic regression was used to compute the odds ratio for the association between AD and *APOE* promoter polymorphisms, adjusting for age, sex, and education. To study whether the *APOE* ε4 allele interacts genetically with the upstream markers, we conducted multivariate logistic regression, stratified by the presence or absence of this allele.

A haplotype analysis was used to determine whether the haplotype frequencies including these genes in cases differed from those in unrelated controls. Because the genetic parameters (eg, mode of inheritance, disease allele frequencies, penetrance, etc) of late-onset sporadic AD remain unknown, we tested a heterogeneity model using the EHPlus program.[29,30]

GENOTYPING

APOE coding polymorphisms were scored by the method of Hixson and Vernier[31] using *Hha*I (*Cfo*I) restriction digestion of polymerase chain reaction (PCR) products and visualization of the fragment sizes on ethidium-stained 8% polyacrylamide gels. The –491A/T *APOE* promoter polymorphism was scored after nested PCR with an internal primer designed to incorporate a diagnostic *Dra*I site, as described elsewhere.[9] The –427T/C polymorphism was scored after the same nested PCR, using digestion with *Alu*I, followed by fragment analysis on 2.5% MetaPhor agarose gels (FMC BioProducts, Rockland, Me). All PCR reactions used standard PCR buffer (Roche, Basel, Switzerland), containing 5% dimethylsulfoxide, except for the *APOE* coding polymorphism PCR, which was optimized using 10% dimethylsulfoxide. The *Hpa*I variant in *APOC1* was genotyped as previously described.[32] The accuracy of genotyping was greater than 97% for each marker, as indicated by redetermining the genotypes in 150 to 250 subjects.

PROMOTER-REPORTER ASSAYS

To create the promoter-reporter plasmids, an 806–base pair (bp) fragment of the *APOE* promoter, from positions –773 to +33 relative to the transcriptional start site, was amplified by PCR with high-fidelity Taq polymerase (Roche), using upstream and downstream primers with the sequences AGCAGGTACCTCATACTGTTCCCAC and TCCTCGAGAAGGACGTCCTTCACC, respectively, into which were incorporated *Kpn*I and *Xho*I restriction sites. This PCR amplification was done using genomic DNA from an individual heterozygous for the –219G/T polymorphism and carrying the common –491A and –427T alleles. The PCR products were digested with *Kpn*I and *Xho*I and ligated into these sites of the pGL3-Basic reporter plasmid (Promega, Madison, Wis), upstream of the luciferase gene. Multiple plasmid clones were sequenced to establish whether they contained the –219G or –219T allele. HepG2 hepatoma cells (American Type Culture Collection, Rockville, Md) were grown on gelatin-coated plates and transfected at 70% confluence, using FuGene 6 reagent (Roche) and plasmids at 1 µg per 35-mm^2 plate, according to the manufacturer's specifications. A β-galactosidase expression plasmid (pSV-β-galactosidase; Promega) was cotransfected to allow normalization for transfection efficiency. The cells were washed with phosphate-buffered saline and lysed in the plate using 250 µL of Reporter Lysis Buffer (β-Galactosidase Enzyme Assay System; Promega). The cell extract was centrifuged for 5 minutes at 10 000 g, and the supernatant was collected. An aliquot (20 µL) was used for determining luciferase activity with 100 µL of Luciferase Assay Buffer (Promega) in a Berthold luminometer. β-galactosidase assays (β-Galactosidase Assay System; Promega) were performed according to the manufacturer's protocol using 10 to 20 µL of the cell lysate. Luciferase values were then normalized to β-galactosidase activity.

RESULTS

POPULATION CHARACTERISTICS AND ALLELE FREQUENCIES

The mean ± SD age of the participants was 79.5 ± 6.6 years (range, 65-103 years) and did not differ by ethnic group. The number of years of formal education differed in the 2 groups, with the mean level of education being 9.6 years for African American participants and 5.9 years for Caribbean Hispanic participants. The individuals who de-

Table 1. Allele Frequencies of *APOE* Flanking and Coding Polymorphisms in African American and Caribbean Hispanic Participants

Allele	African American Participants, %	Caribbean Hispanic Participants, %
–491		
A	71.8	72.3
T	28.2	27.7
No. of alleles counted	606	956
–427		
T	95.1	95.0
C	4.9	5.0
No. of alleles counted	530	873
–219*		
G	73.7	59.3
T	26.3	40.7
No. of alleles counted	786	1178
*APOE**		
ε2	11.1	8.4
ε3	68.8	76.7
ε4	20.1	15.0
No. of alleles counted	910	1332
*APOC1**		
*Hpa*I–	75.4	83.3
*Hpa*I+	24.6	16.7
No. of alleles counted	720	1061

*There are significant differences in the frequencies of the *APOE* coding polymorphisms, the *Hpa*I variant in the *APOC1* promoter, and the –219G/T polymorphism in African American participants compared with Caribbean Hispanic participants.

Table 2. Genotype Frequencies and Genotypic Risks Related to *APOE* and *APOC1* in African American and Caribbean Hispanic Participants

Genotype	No. (%) of Cases	No. (%) of Controls	Odds Ratio (95% Confidence Interval)
Caribbean Hispanic Participants			
APOE			
ε4/ε4	11 (4.2)	7 (1.8)	2.9 (1.1-7.7)
ε4/–	84 (32.1)	76 (19.2)	2.1 (1.4-2.9)
–/–	167 (63.7)	313 (79)	1.0 (Referent)
APOC1			
*Hpa*I +/+	14 (7.3)	12 (3.6)	2.4 (1.1-5.4)
*Hpa*I +/–	56 (29)	70 (20.7)	1.7 (1.1-2.5)
*Hpa*I –/–	123 (63.7)	256 (75.7)	1.0 (Referent)
ε4+			
*Hpa*I +/+	13 (22.8)	10 (15.9)	1.8 (0.6-5.2)
*Hpa*I +/–	30 (52.6)	34 (54)	1.2 (0.5-2.8)
*Hpa*I –/–	14 (24.6)	19 (30.2)	1.0 (Referent)
ε4–			
*Hpa*I +/+	1 (0.7)	2 (0.7)	1.1 (0.1-12.1)
*Hpa*I +/–	26 (19.1)	36 (13.1)	1.6 (0.9-2.7)
*Hpa*I –/–	109 (80.1)	237 (86.2)	1.0 (Referent)
African American Participants			
APOE			
ε4/ε4	8 (5.1)	4 (1.5)	3.6 (1.1-12.2)
ε4/–	52 (33.1)	97 (35.3)	1.0 (0.6-1.5)
–/–	97 (61.8)	174 (63.3)	1.0 (Referent)
APOC1			
*Hpa*I +/+	14 (10.8)	15 (6.5)	1.7 (0.8-3.8)
*Hpa*I +/–	42 (32.3)	77 (33.5)	1.1 (0.6-1.6)
*Hpa*I –/–	74 (56.9)	138 (60.0)	1.0 (Referent)
ε4+			
*Hpa*I +/+	9 (18.0)	11 (13.5)	1.0 (0.4-2.9)
*Hpa*I +/–	17 (34.0)	39 (48.8)	0.5 (0.25-1.20)
*Hpa*I –/–	24 (48.0)	30 (37.5)	1.0 (Referent)
ε4–			
*Hpa*I +/+	5 (6.3)	4 (2.7)	2.7 (0.7-10.5)
*Hpa*I +/–	25 (31.3)	38 (25.3)	1.4 (0.8-2.6)
*Hpa*I –/–	50 (62.5)	108 (72.0)	1.0 (Referent)

veloped AD were significantly older (African American participants with AD, 82.3 years vs African American participants without dementia, 78.2 years; Caribbean Hispanic participants with AD, 81.9 years vs Caribbean Hispanic participants without dementia, 77.5 years; $P<.001$ for both comparisons) and less well educated (African American participants with AD, 8.5 years vs African American participants without dementia, 10.2 years; Caribbean Hispanic participants with AD, 4.8 years vs Caribbean Hispanic participants without dementia, 6.6 years; $P<.001$ for both comparisons). In both ethnic groups, a greater proportion of participants were women (328 [72%] of 455 African American participants and 480 [72%] of 666 Caribbean Hispanic participants).

The allele frequencies of the *APOE* coding and flanking polymorphisms are shown in **Table 1**. After correcting for multiple testing, no significant Hardy-Weinberg disequilibrium for any of the allelic systems was detected. There were significant differences between Caribbean Hispanic and African American participants in the frequencies of the *APOE* coding polymorphisms, the *Hpa*I variant in the *APOC1* promoter, and the –219G/T promoter (Table 1), but there were no differences in the allele frequencies of the –491A/T and –427T/C promoter/polymorphisms.

ASSOCIATIONS BETWEEN *APOE* AND *APOC1* PROMOTER POLYMORPHISMS AND AD

We compared the allele frequencies of variants for *APOE* and *APOC1* independently and, because of the differences in the allele frequencies, stratified by ethnic group. In the Caribbean Hispanic participants, the *APOE* ε4 allele was more frequent among patients with AD than controls (22.2% vs 11.5%; $\chi^2=19.1$; $P<.001$). (The numbers of participants with each genotype are listed in **Table 2**.) On the other hand, in African American participants, there were no overall differences in the frequency of the *APOE* coding alleles between patients and controls. Nonetheless, compared with those without an ε4 genotype, the *APOE* ε4 homozygous genotype was associated with AD both in African American and Caribbean Hispanic participants, while the *APOE* ε4 heterozygous genotype was associated with AD only in Caribbean Hispanic participants (Table 2).

The *APOC1 Hpa*I+ variant was also more frequent among patients with AD than controls but only in the Caribbean Hispanic participants (21.6% vs 13.9%; $\chi^2=10.33$; $P=.001$). (The numbers of participants with each genotype are listed in Table 2.) We repeated this analysis stratifying by the presence or absence of *APOE* ε4. Considering both heterozygotes and homozygotes, the *Hpa*I+

Table 3. Genotype Frequencies of *APOE* Flanking Polymorphisms in Alzheimer Disease Cases and Controls*

Genotype	Caribbean Hispanic Participants			African American Participants		
	No. (%) of Cases	No. (%) of Controls	Odds Ratio (95% Confidence Interval)	No. (%) of Cases	No. (%) of Controls	Odds Ratio (95% Confidence Interval)
–491AT						
T/T	14 (8.4)	33 (10.6)	1.20 (0.60-2.50)	11 (10.1)	22 (11.3)	0.95 (0.43-2.10)
A/T	59 (35.3)	112 (36.0)	1.30 (0.68-2.60)	41 (37.6)	64 (33.0)	1.20 (0.70-2.00)
A/A	94 (56.3)	166 (53.4)	1.00 (Referent)	57 (52.3)	108 (55.7)	1.00 (Referent)
ε4+						
T/T	6 (12.2)	11 (20.0)	1.70 (0.51-5.40)	6 (14.3)	10 (14.1)	1.10 (0.34-3.30)
A/T	18 (36.7)	20 (36.4)	1.90 (0.61-5.90)	19 (45.2)	30 (42.3)	1.20 (0.51-2.60)
A/A	25 (5.0)	24 (43.6)	1.00 (Referent)	17 (40.5)	31 (43.7)	1.00 (Referent)
ε4–						
T/T	8 (6.8)	22 (8.6)	1.20 (0.50-2.90)	5 (7.5)	12 (9.8)	0.80 (0.26-2.40)
A/T	41 (34.7)	92 (35.9)	1.30 (0.55-3.10)	22 (32.8)	34 (27.6)	1.20 (0.65-2.40)
A/A	69 (58.5)	142 (55.5)	1.00 (Referent)	40 (59.7)	77 (62.6)	1.00 (Referent)
–427TC						
C/C	1 (0.7)	1 (0.3)	1.90 (0.10-29.9)	2 (2.1)	1 (0.6)	3.50 (0.30-39.00)
T/C	11 (7.3)	30 (10.5)	0.70 (0.30-1.30)	4 (4.3)	16 (9.4)	0.44 (0.14-1.40)
T/T	138 (92.0)	256 (89.2)	1.00 (Referent)	88 (93.6)	154 (90.1)	1.00 (Referent)
ε4+						
C/C	0	1 (2.3)	NA	0	0	NA
T/C	31 (7.0)	8 (15.4)	0.78 (0.10-1.70)	0	9 (13.0)	NA
T/T	3911 (90.7)	44 (84.6)	1.00 (Referent)	35 (100.0)	56 (86.2)	1.00 (Referent)
ε4–						
C/C	0	1 (0.4)	NA	2 (3.4)	1 (0.9)	3.70 (0.30-41.70)
T/C	8 (7.5)	22 (9.4)	0.42 (0.33-1.80)	4 (6.8)	7 (6.6)	1.10 (0.30-3.80)
T/T	99 (92.5)	212 (90.2)	1.00 (Referent)	53 (89.9)	98 (92.5)	1.00 (Referent)
–219GT						
T/T	38 (16.5)	61 (17.0)	1.10 (0.68-1.70)	9 (6.3)	20 (8.0)	0.72 (0.30-1.65)
G/T	114 (49.6)	168 (46.8)	0.89 (0.59-1.60)	50 (35.2)	99 (39.4)	0.80 (0.52-1.20)
G/G	78 (33.9)	130 (36.2)	1.00 (Referent)	83 (58.5)	132 (52.6)	1.00 (Referent)
ε4+						
T/T	14 (17.5)	19 (25.7)	1.10 (0.40-2.90)	5 (9.4)	16 (17.6)	0.50 (0.16-1.50)
G/T	53 (66.3)	36 (48.6)	2.10 (0.90-4.90)	27 (50.9)	42 (46.2)	1.00 (0.50-2.10)
G/G	13 (16.3)	19 (25.7)	1.00 (Referent)	21 (39.6)	33 (36.3)	1.00 (Referent)
ε4–						
T/T	24 (16.0)	42 (14.7)	1.00 (0.54-1.70)	4 (4.5)	4 (2.5)	1.60 (0.40-6.60)
G/T	61 (40.7)	132 (46.3)	0.80 (0.50-1.20)	23 (25.8)	57 (35.6)	0.60 (0.40-1.20)
G/G	65 (43.3)	111 (38.9)	1.00 (Referent)	62 (69.7)	99 (61.9)	1.00 (Referent)

Abbreviation: NA, not able to be calculated.
*Confidence intervals are from the univariate analysis; values corrected for age, sex, and education are given in the text.

variant was more frequent in Caribbean Hispanic patients with AD compared with controls but only in those individuals with an *APOE* ε4 allele (16.2% vs 10.6%; $\chi^2=6.09$; $P=.01$). (The numbers of participants with each genotype are listed in Table 2.) There were no differences in *APOC1 Hpa*I allele frequencies between African American patients and controls, regardless of the presence or absence of *APOE* ε4.

ASSOCIATIONS BETWEEN *APOE* PROMOTER POLYMORPHISMS AND AD RISK

We compared the allele frequencies of each *APOE* promoter polymorphism between patients and controls, conducting the analysis separately in the African American and Caribbean Hispanic participants. Because the influence of such flanking polymorphisms may depend on the *APOE* coding polymorphism to which they are linked in *cis*, we also tabulated the allele frequencies in patients and controls stratified by the *APOE* ε4 allele. As shown in **Table 3**, none of the *APOE* upstream markers showed a significant association with AD.

Setting the β at 0.20 (80% power) and the α at .05, we calculated the lowest detectable odds ratios for the sample sizes for each ethnic group separately. For African American and Caribbean Hispanic participants, we had large enough samples to detect odds ratios of 2.0 with –491A/T, –219G/T, and *APOC*1. Because the frequency of a genotype containing the variant allele of –427TC was 10%, the lowest detectable odds ratio was 2.8 for African American participants and 2.5 for Caribbean Hispanic participants.

ESTIMATED HAPLOTYPES AND AD RISK

We calculated haplotypes using the 3 loci (–219G/T-*APOE*-*APOC1*) that are in linkage disequilibrium to determine whether the distribution of haplotypes differed

in patients and controls. Among Caribbean Hispanic participants, –219G/T and *APOE* together showed a significant difference in patients and controls in the estimated haplotype distribution (χ^2_5=35.40; P<.001), while *APOE-APOC1* did not (χ^2_5=7.00; P=.22). The 3 loci examined together also indicated significant differences between patients and controls (χ^2_{11}=33.44; P<.001), driven primarily by the –219GT-*APOE* haplotype. We then recomputed the *P* values using Monte Carlo methods based on 5000 replicates to minimize the influence of less-frequent haplotypes. The *P* values for the haplotypes using the 2 loci (–219G/T-*APOE*) and the 3 loci (–219G/T-*APOE-APOC1*) remained unchanged at .00001 and .0004, respectively. Among African American participants, on the other hand, none of the estimate haplotypes differed significantly between cases and controls for the same 3 sets of analyses.

PROMOTER-REPORTER ASSAYS FOR FUNCTIONAL EFFECTS OF THE –219G/T POLYMORPHISM

The –219G/T promoter polymorphism has been reported to produce modest but significant effects on allele-specific *APOE* messenger RNA expression,[9,12] and our haplotype analysis suggested a possible influence of –219G/T promoter polymorphism in conjunction with the adjacent loci. We tested whether the –219G vs –219T sequence variants could cause differences in *APOE* transcription when they were present in the context of a minimal *APOE* promoter sequence. Accordingly, we cloned a series of matched 806-bp promoter fragments, extending from –773 to +33 relative to the transcriptional start site and containing either the –219G or –219T alleles, upstream of the luciferase reporter gene. These were transfected into HepG2 cells in multiple repeated experiments. As expected, this minimal promoter region strongly stimulated expression of the luciferase reporter gene. However, although the T allele of the –219G/T polymorphism gave slightly reduced *APOE* promoter activity, this reduction was within the range of error of the assay. As a positive control, a plasmid containing an artificial point mutation, introduced at another site in the promoter sequence, showed significantly reduced activity.

The PrD1 plasmid contained the *APOE* promoter with G at position –219. The PrD3 and PrD10 plasmids were independent but identical clones, both with a T at position –219. The PrD2 plasmid was an artificial mutant allele with –219T and an additional T-to-C substitution at position –97. Each experiment consisted of triplicate determinations of luciferase activity divided by β-galactosidase activity. The mean values of luciferase/β-galactosidase obtained with the PrD2, PrD3, and PrD10 plasmids in each experiment were then normalized to the mean value obtained in that experiment with the PrD1 plasmid. For luciferase/β-galactosidase, the mean ± SD value for G (PrD1) was 1; Tmut (PrD2), 0.40 ± 0.12 (16 experiments); T (PrD3), 0.78 ± 0.35 (17 experiments); and T (PrD10), 0.80 ± 0.34 (9 experiments). In all experiments, the pGL3-Basic vector lacking any promoter cassette gave luciferase/β-galactosidase values at least 10-fold less than the PrD1 plasmid.

COMMENT

Since the first report of an association between an *APOE* promoter polymorphism and AD,[9] there has been considerable interest in testing whether regulatory polymorphisms near *APOE* might also affect AD susceptibility. Polymorphisms in regulatory sequences would presumably exert their effects by altering *APOE* messenger RNA expression in *cis*. Several studies[6-15,17,19-22,25] published to date have yielded inconsistent results, but it may be important to continue to test this hypothesis in additional populations. We examined multiple polymorphisms upstream of *APOE* and the promoter of the adjacent *APOC1* gene for associations with AD in a large cohort of Caribbean Hispanic and African American participants with extensive clinical follow-up.

Our findings have both positive and negative aspects. We found no convincing evidence of an independent association with AD for any variant in the promoter of *APOE*. In contrast, for the *Hpa*I variant of the *APOC1* polymorphism, we find evidence for a weak association with AD that was modified significantly by the presence or absence of an *APOE* ε4 allele. A haplotype analysis using the loci spanning –219G/T to *APOC* 1 yielded a significant difference in the haplotype distribution between cases and controls but only in Caribbean Hispanic participants. However, there was no significant association with any of the loci individually or as haplotypes among African American participants. Among the markers we have tested, the –219G/T polymorphism lies closest to the *APOE* transcriptional start site. Previously, slight differences in allele-specific expression of *APOE* messenger RNA in the brains of individuals with different –219G/T genotypes were reported.[12] Although we could not detect significant allele-specific differences in promoter activity in transfection assays using a minimal *APOE* promoter sequence, this may be owing to insufficient precision of this type of assay. Given the continuing interest in functional polymorphisms within human regulatory sequences, better assays for small effects of single-nucleotide polymorphisms in human promoter sequences are needed. Indeed, Stengard et al[33] have observed subtle phenotypic variation in lipid metabolism using information from additional sequence variation surrounding the *APOE* locus. A similar approach to investigate phenotypic variation in AD may be warranted.

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Correspondence: Richard Mayeux, MD, MSc, Sergievsky Center, Columbia University College of Physicians and Surgeons, 630 W 168th St, New York, NY 10032 (rpm2@columbia.edu).
Author Contributions: *Study concept and design:* Tycko, Lantigua, Shachter, and Mayeux. *Acquisition of data*: Tycko, Ciappa, Saxena, Feng, Stern, and Mayeux. *Analysis and interpretation of data*: Tycko, Lee, Ciappa, Li, Arriaga, and Mayeux. *Drafting of the manuscript*: Tycko, Lee, Saxena, and Mayeux. *Critical revision of the manuscript for important intellectual content*: Tycko, Lee, Ciappa, Li, Feng, Arriaga, Stern, Lantigua, Shachter, and Mayeux.

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tence of [this cluster] is to be expected" and that it is merely "an outlier"?

Ajit Kumar, DM
Susan Calne, CM
Michael Schulzer, MD, PhD
Edwin Mak, BSc
Zbigniew Wszolek, MD
Chris Van Netten, MD
Joseph K. C. Tsui, MD, FRCPC
A. Jon Stoessl, MD, FRCPC
Donald B. Calne, DM, FRSC

Correspondence: Dr Calne, Pacific Parkinson's Research Centre, Vancouver Hospital and Health Sciences Centre, Purdy Pavilion, 2221 Wesbrook Mall, Room M37, Vancouver, British Columbia, Canada V6T 2B5 (dbcalne@interchange.ubc.ca).

Correction

Error in Byline. In the Original Contribution by Tycko et al titled "*APOE* and *APOC1* Promoter Polymorphisms and the Risk of Alzheimer Disease in African American and Caribbean Hispanic Individuals," published in the September issue of the ARCHIVES (2004;61:1434-1439), an author name was omitted from the byline at article submission. The byline should have read Benjamin Tycko, MD, PhD; Joseph H. Lee, PhD; Alejandra Ciappa, MD; Anjana Saxena, PhD; Chi-Ming Li, PhD; Lin Feng, BA; Lin Yang, MD; Alex Arriaga, BA; Yaakov Stern, PhD; Rafael Lantigua, MD; Neil Shachter, MD; Richard Mayeux, MD, MSc.