APOE polymorphisms and cognitive functions in patients with brain tumors

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ABSTRACT

Objective: The goal of this study was to assess whether the *APOE* ε4 allele and other *APOE* single nucleotide polymorphisms (SNPs) influence neuropsychological and neuroimaging outcomes in patients with brain tumors.

Methods: Two hundred eleven patients with brain tumors participated in the study. All patients completed standardized neuropsychological tests and provided a blood sample for *APOE* genotyping. Ratings of white matter abnormalities were performed on MRI scans. Patients were classified into 2 groups based on the presence (n = 50) or absence (n = 161) of at least one *APOE* ε4 allele. Additional *APOE* SNPs were genotyped in a subset of 150 patients.

Results: Patients with at least one *APOE* ε4 allele had significantly lower scores in verbal learning and delayed recall, and marginally significant lower scores in executive function, in comparison to noncarriers of an ε4 allele. Patients with at least one ε4 allele and history of cigarette smoking had significantly higher scores in working memory and verbal learning than ε4 carriers who never smoked. Nine additional *APOE* SNPs were significantly associated with attention and executive and memory abilities. There were no significant differences between ε4 carriers and noncarriers on the extent of white matter abnormalities on MRI.

Conclusions: The findings suggest that patients with brain tumors who are carriers of the *APOE* ε4 allele may have increased vulnerability to developing memory and executive dysfunction, and that additional SNPs in the *APOE* gene may be associated with cognitive outcome. *Neurology*® 2014;83:320–327

GLOSSARY

HVLT-D = Hopkins Verbal Learning Test–Delayed Recall; **HVLT-DI** = Hopkins Verbal Learning Test–Discrimination Index; **HVLT-L** = Hopkins Verbal Learning Test–Learning; **MAF** = minor allele frequency; **MSKCC** = Memorial Sloan-Kettering Cancer Center; **RT** = radiotherapy; **SNP** = single nucleotide polymorphism; **TMT** = Trail Making Test; **WM** = white matter.

Cognitive dysfunction in patients with primary brain tumors is associated with the disease and treatment with radiotherapy (RT) and chemotherapy,[1] and is the most frequent complication among long-term survivors.[2] The cognitive domains sensitive to adverse treatment effects include attention, executive functions, and learning and retrieval of information.[1] Marked interpatient variability is recognized clinically, but little is known about individual factors that may increase the vulnerability for treatment-related neurotoxicity.

One potential genetic risk factor for cognitive decline is the presence of *APOE* ε4 alleles. *APOE* is polymorphic and has 3 common isoforms, ε2, ε3, and ε4, encoded by 2 single nucleotide polymorphisms (SNPs). Approximately 25% of the population carries at least one *APOE* ε4 allele.[3] The ε4 allele has been associated with increased risk of late-onset Alzheimer disease[4] and with poor outcomes after traumatic head injury.[5] Preliminary evidence suggests that the *APOE* ε4 allele may increase the vulnerability to cognitive dysfunction in patients with breast cancer and lymphoma treated with chemotherapy,[6] and in patients with low-grade gliomas.[7] These initial studies suggest that the *APOE* gene may moderate the development of treatment-related neurotoxicity in patients with cancer. In this study, we assessed neuropsychological and neuroimaging outcomes in patients with brain tumors with

Supplemental data at Neurology.org

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Presented in part at the Society for Neuro-Oncology Annual Meeting, November 2013, San Francisco, CA.

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and without at least one ε4 allele of the *APOE* gene. We investigated additional *APOE* SNPs in a subset of patients.

METHODS **Subjects.** Two hundred eleven patients diagnosed with a brain tumor were recruited from a cohort of survivors followed in the Department of Neurology at Memorial Sloan-Kettering Cancer Center (MSKCC); 61 patients were recruited between 2001 and 2009, and 150 between 2009 and 2012. Information on a subset of these patients was provided previously.[7] Study eligibility included the following: no evidence of active disease on serial MRIs before accrual; completion of treatment with RT or chemotherapy at least 6 months before enrollment, or surgical resection at least 2 months before accrual if no other treatment was administered; no history of psychiatric or other neurologic disorders; and fluency in English.

Sixty-four patients (30%) had a high-grade tumor (i.e., glioblastoma, anaplastic astrocytoma, or anaplastic oligodendroglioma), 66 (31%) had a low-grade tumor (i.e., oligodendroglioma, oligoastrocytoma), 65 (31%) had primary CNS lymphoma, and 16 (8%) had other brain tumors (i.e., meningioma, ependymoma). One hundred thirty-one patients (62%) received treatment with conventional fractionated RT ± chemotherapy, 64 (30%) received chemotherapy-only regimens, and 16 (8%) had no treatment (except for surgical resection). One hundred seven patients (51%) had focal RT and 24 (11%) had whole-brain RT; RT dose ranged from 2,340 to 6,840 cGy. All patients completed a neuropsychological evaluation and provided a blood sample for *APOE* genotyping.

Measures. ***Neuropsychological assessment.*** Neuropsychological tests sensitive to the adverse effects of cancer therapy[8] were selected to evaluate the following cognitive domains:

- Auditory attention: Digit Span subtests of the Wechsler Memory Scale, third edition (Digit Span Forward, Digit Span Backward); Brief Test of Attention (BTA).
- Executive function: Trail Making Test (TMT) Parts A and B; Phonemic Verbal Fluency Test.
- Memory: Hopkins Verbal Learning Test-Revised–Learning (HVLT-L), –Delayed Recall (HVLT-D), and –Discrimination Index (HVLT-DI).

The test battery was administered by a neuropsychologist (D.D.C.) or a trained research assistant. Raw cognitive test scores were compared with published normative values according to age, and when available, to education, and converted into *z* scores.

Table 1 Patient demographic characteristics

Demographics	*APOE* ε4 positive (n = 50)	*APOE* ε4 negative (n = 161)
Male, %	56	50
Right handed, %	90	85
Age at study entry, y		
Mean (SD)	51 (14.2)	52 (13.3)
Median (range)	54 (25–84)	50 (21–85)
Mean education, y (SD)	16.1 (3.1)	15.8 (2.9)
Mean estimated VIQ (SD)	111 (9.2)	112 (8.7)
Occupation at study entry, %		
Not working	52	50
Working	48	50

Abbreviation: VIQ = verbal IQ (North American Adult Reading Test or Barona Index).

DNA extraction, selection of SNPs, and genotyping. *APOE* ε allelic data were available for 61 patients who were previously genotyped by restriction fragment length polymorphism analysis[7] or by Serial Invasive Signal Amplification Reaction (Athena Diagnostics, Worcester, MA).

In 150 patients, blood DNA was extracted with the Qiagen FlexiGene DNA kit (Qiagen, Valencia, CA) according to the manufacturer's protocols, and standard quality control procedures were followed throughout. Genotyping was performed with 2 complementary approaches: (1) DNA sequencing, and (2) the GoldenGate genotyping assay, to include the *APOE* SNPs rs429358 and rs7412, which encode for the polymorphisms ε2, ε3, and ε4.[9] We also included the promoter polymorphisms, rs449647 and rs405509, which have also been associated with Alzheimer disease, cognitive dysfunction, and altered cortical expression.[10–12] We also targeted additional *APOE* SNPs that can affect the gene product, transcription, or messenger RNA stability through missense, nonsense, or frameshift mutations, overlap with seed microRNA regions and/or transcription factors binding sites, with >15% minor allele frequency (MAF) in Caucasians as per HapMap_CEU: rs6857, rs405697, rs439401, rs5112, and rs442706; intronic synonymous SNPs conserved across species, or map near 5′ region with >25% MAF among Caucasians: rs446037, rs405509, rs584007, rs769446, and rs3207187. Haplotype tagging SNPs were searched with the HaploView software and none were identified with a correlation (r^2) >0.80 with other SNPs.

Genotyping. In the first approach, the *APOE* gene was partially sequenced using 4 overlapping amplicons spanning positions hg19: 45408564–45430336. Bidirectional sequencing was performed in the Beene Core at MSKCC following standard procedures. Nucleotide changes were detected using an automated detection pipeline at the MSKCC Bioinformatics Core. To avoid false-positives, only point mutations supported by at least one bidirectional read pair and one sample mutation called by PolyPhred were considered, and all traces were reviewed and compared with a reference visually and with the Mutation Surveyor software (SoftGenetics, State College, PA). Epsilon isoforms ε2, ε3, and ε4 encoded by rs429358 and rs7412 were annotated according to Mahley and Rall.[13] In the second approach, additional *APOE* SNPs were genotyped using previously described procedures.[14] SNPs that were monoallelic, had >5% missing data, failed during earlier stages of the assay design, and/or showed poor clustering were excluded from further analysis: rs442706, rs3207187, rs446037. The genomic context and inclusion criteria for the *APOE* SNPs are described in table e-1 on the *Neurology*® Web site at Neurology.org.

Neuroimaging. White matter (WM) abnormalities were rated on clinical brain MRI scans performed within 3 months of the cognitive evaluation. The ratings were performed by 2 radiologists who were blinded to the cognitive test results. WM abnormalities were rated on a fluid-attenuated inversion recovery sequence for most patients, and if not available, T2-weighted sequences were used. Radiographic endpoints were measured according to the modified Fazekas scale,[15] and the tumor and surrounding edema were excluded from these measurements.

Standard protocol approvals, registrations, and patient consents. The MSKCC institutional review board approved the research protocols. All participants provided written informed consent.

Statistical analyses. The relation between demographic and clinical variables and the outcome measures was assessed using Wilcoxon rank sums tests for neuropsychological test scores, and Fisher exact tests for WM ratings. Of particular interest were

Table 2 Disease and treatment history

Disease/treatment history[a]	*APOE* ε4 positive (n = 50)	*APOE* ε4 negative (n = 161)
Tumor type, %		
Low-grade glioma	32	31
High-grade glioma	32	30
Primary CNS lymphoma	28	32
Other	8	7
Tumor location, %		
Frontal	42	35
Frontal-temporal/parietal	16	17
Temporal/parietal/occipital	22	26
Cortical/subcortical	20	22
Predominant tumor side, %		
Left	42	34
Right	42	50
Bilateral	16	16
Treatment type, %		
RT ± chemotherapy	60	63
Chemotherapy	30	30
None (except surgery)	10	7
Time since diagnosis, mo		
Mean (SD)	70 (66.9)	66 (58.9)
Median (range)	48 (24–84)	48 (24–85)
Time since treatment completion, mo		
Mean (SD)	49 (48.9)	40 (47.6)
Median (range)	31 (7–199)	24 (2–370)[b]
Smoking history		
Yes, %	40	43
Vascular risk		
Yes, %	40	42
Antiepileptics[c]		
Yes, %	58	52

Abbreviation: RT = radiotherapy.
[a] Treatment history reflects all therapy received including treatment at relapse, if applicable.
[b] Two ε4-negative patients had considerably longer time since treatment completion compared with others (i.e., highest values = 370 and 314 months; third highest value = 155 months).
[c] Medication at the time of the cognitive evaluation.

associations between the outcome measures and *APOE* ε4 carrier status, i.e., patients who were carriers of at least one ε4 allele (ε4-positive) vs noncarriers of the ε4 allele (ε4-negative).

Linear regression was used to evaluate the associations between cognitive test scores and ε4 carrier status controlling for age, education, treatment with RT ± chemotherapy, and cigarette smoking history (i.e., any past or current use). These control variables were selected based on evidence for the association between cognitive performance and age, education, and the *APOE* ε4 allele,[16,17] between cognitive outcome and treatment with RT and chemotherapy in patients with brain tumors,[1] and between smoking and *APOE* status.[18] Logistic regression was used similarly for WM ratings, which were classified into 2 categories: none/minimal (grade = 0–1) and moderate/severe (grade ≥2).

The joint effects of *APOE* SNPs on the outcome measures were assessed by backward selection regression analysis. For each outcome, a full model including all *APOE* SNPs was fit, controlling for age, education, treatment with RT ± chemotherapy, and cigarette smoking history. Starting with this full model, the SNP providing the poorest fit was removed from the model, where "poorest fit" meant that, compared with the other SNPs, removal of the SNP resulted in the largest decrease in Akaike Information Criterion. This process was repeated until removal of any remaining SNPs resulted in an increase in Akaike Information Criterion. Given the exploratory nature of the study, adjustments for multiple testing were not used on any analyses. Statistical analyses were performed using R version 3.0.1.[19]

RESULTS ***APOE* ε status.** *APOE* ε allele type results were available for all 211 patients. Fifty patients (24%) carried at least one *APOE* ε4 allele (ε2/ε4 = 3%, ε3/ε4 = 20%, and ε4/ε4 = 1%), a percentage consistent with the general population distribution (i.e., ε2/ε4 = 3%, ε3/ε4 = 21%, and ε4/ε4 = 2%).[20] There were no significant differences between *APOE* ε4 carriers and noncarriers on any of the demographic, disease, or treatment variables (tables 1 and 2).

Mean cognitive test scores were within 1 SD below the normative mean on most measures for both groups, but ε4-positive patients had mean z scores more than 1 SD below normative values on the HVLT-L and HVLT-D, and the TMT-B. *APOE* ε4 carriers had significantly lower mean z scores than noncarriers on the TMT-B and HVLT-D ($p < 0.05$), and marginally significantly lower HVLT-L scores ($p = 0.065$) (table 3). These differences remained notable on linear regression analyses adjusting for age, education, treatment with RT ± chemotherapy, and cigarette smoking history; specifically, ε4-positive patients had significantly lower scores in verbal learning (HVLT-L; $t_{203} = -2.11$, $p = 0.036$) and delayed recall (HVLT-D; $t_{203} = -2.12$, $p = 0.035$), and marginally significant lower scores in executive function (TMT-B; $t_{202} = -1.92$, $p = 0.056$), relative to ε4-negative patients.

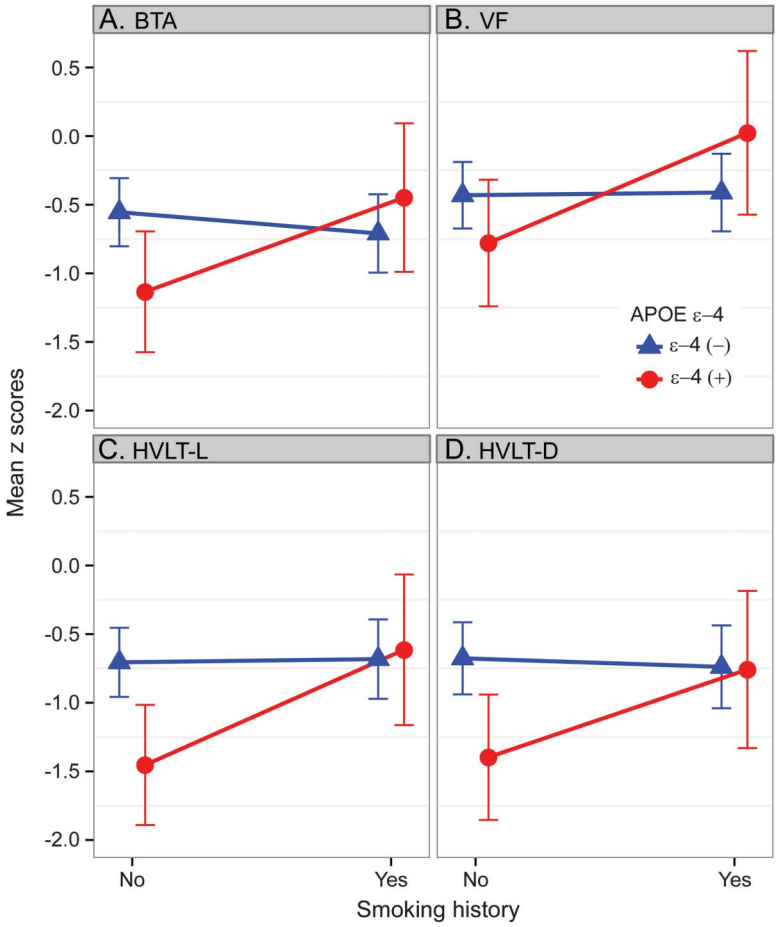
We examined the modifying effect of cigarette smoking history on the association between *APOE* ε4 status and cognitive test performance, adjusting for age, education, and treatment with RT ± chemotherapy. The results showed that ε4-positive patients with a history of cigarette smoking obtained significantly higher scores in attention (Brief Test of Attention, interaction $t_{201} = 2.09$, $p = 0.038$) and verbal learning (HVLT-L, interaction $t_{202} = 2.01$, $p = 0.046$) than ε4-positive patients who never smoked. Marginally significant interactions were seen for delayed recall (HVLT-D, interaction $t_{202} = 1.65$, $p = 0.099$) and phonemic verbal fluency (interaction $t_{191} = 1.84$, $p = 0.067$), with ε4-positive patients with a history of cigarette smoking obtaining higher scores than ε4-positive patients who never smoked (figure). Among ε4-negative patients,

Table 3 Cognitive test *z* scores

Cognitive tests	*APOE* ε4 positive (n = 50)	*APOE* ε4 negative (n = 161)
DSF	−0.17 (1.0)	0.05 (1.1)
DSB	0.02 (0.9)	0.08 (1.0)
TMT-A	−0.59 (1.3)	−0.55 (1.2)
TMT-B	−1.04 (1.4)	−0.65 (1.3)[a]
BTA	−0.82 (1.3)	−0.63 (1.2)
VF	−0.42 (1.4)	−0.42 (1.2)
HVLT-L	−1.11 (1.2)	−0.7 (1.4)[b]
HVLT-D	−1.12 (1.4)	−0.71 (1.4)[a]
HVLT-DI	−0.62 (1.4)	−0.37 (1.3)

Abbreviations: BTA = Brief Test of Attention; DSB = Digit Span Backward; DSF = Digit Span Forward; HVLT-D = Hopkins Verbal Learning Test–Delayed Recall; HVLT-DI = Hopkins Verbal Learning Test–Discrimination Index; HVLT-L = Hopkins Verbal Learning Test–Learning; TMT-A = Trail Making Test, Part A; TMT-B = Trail Making Test, Part B; VF = Verbal Fluency Test.
Data represent mean (SD).
Wilcoxon rank sums: [a] $p < 0.05$, [b] $p = 0.065$.

Figure *APOE* status, smoking history, and cognitive functions



The effects of smoking on the association between *APOE* status and performance on the (A) BTA ($p = 0.038$), (B) VF ($p = 0.067$), (C) HVLT-L ($p = 0.046$), and (D) HVLT-D ($p = 0.099$). BTA = Brief Test of Attention; HVLT-D = Hopkins Verbal Learning Test–Delayed Recall; HVLT-L = Hopkins Verbal Learning Test–Learning; VF = Verbal Fluency Test.

cognitive test performance was similar between those with and without a history of smoking. Although there was considerable overlap between history of smoking and other vascular risk factors (e.g., hypertension, hypercholesterolemia, diabetes) among patients, we also examined their possible moderating effects by testing the interaction between ε4 status and a 3-category variable (i.e., no vascular risk factors, nonsmoking vascular risk factors, or smoking history). These results showed no evidence that nonsmoking vascular risk factors moderated the associations between the *APOE* ε4 allele and cognitive test performance.

Twenty-two ε4-positive patients (44%) were rated as having moderate/severe WM abnormalities, compared with 62 ε4-negative patients (39%) (Fisher exact $p = 0.51$). The results of logistic regression analyses adjusting for age, education, treatment with RT ± chemotherapy, and smoking history showed no significant differences between ε4-positive and ε4-negative patients on the WM abnormality ratings.

Additional *APOE* SNPs. More in-depth genotyping was performed in patients for whom germline DNA was available (n = 150). In addition to the widely reported SNPs rs429358 and rs7412, we genotyped other SNPs described in the literature,[10–12] or that were likely functional as per in silico tools: rs449647, rs405509, rs6857, rs405697, rs439401, rs5112, rs584007, and rs769446. Seven additional SNPs were incidentally found during sequencing: rs72654473, rs72654472, hg19:45430118 (C>T), hg19:45430250 (G>A), hg19:45430336 (T>C), hg19:45408815 (C>T), and hg19:45412080 (G>T). Table e-1 provides details on MAF, genomic position, as well as known/predicted SNP features. All incidentally found SNPs were excluded from further analysis because of low MAF (<2%), except for rs72654473. Therefore, a total of 11 *APOE* SNPs qualified for statistical analyses.

The results of multiple regression analyses using backward selection of SNPs and adjusting for age, education, treatment with RT ± chemotherapy, and cigarette smoking history showed that a total of 9 *APOE* SNPs were retained in the final models for the cognitive outcomes. SNP rs405509 was retained in the final model for tests of memory including HVLT-L, HVLT-D, and HVLT-DI, and rs6857 for HVLT-L. Two to 6 SNPs were retained for tests of attention and executive functions. The estimated effects of these 9 SNPs, their standard errors, and statistical significance are included in table 4. The results of backward selection adjusting for age, education, treatment with RT ± chemotherapy, and cigarette smoking history showed that none of the *APOE* SNPs provided a good fit for the WM abnormality ratings.

DISCUSSION The study findings provide new evidence that the *APOE* ε4 allele and additional SNPs

Table 4 Multivariate associations of *APOE* SNPs with cognitive test *z* scores

	DSF	DSB	TMT-A	TMT-B	BTA	VF	HVLT-L	HVLT-D	HVLT-DI
rs769446 (ref = TT)									
CC/TC		0.36 (0.22)		0.52 (0.28)[a]	0.71 (0.24)[b]				
rs405509 (ref = TG)									
TT		−0.11 (0.23)					−0.18 (0.25)	−0.41 (0.26)	−0.44 (0.25)[a]
GG		−0.44 (0.23)[a]					−0.59 (0.23)[b]	−0.40 (0.24)[a]	−0.69 (0.22)[b]
rs429358 (ref = TT)									
CC/TC	−0.46 (0.26)[a]	−0.42 (0.22)[a]		−0.54 (0.25)[c]		−0.63 (0.24)[c]			
rs7412 (ref = CC)									
CT/TT	−0.83 (0.42)[a]	−0.48 (0.23)[c]	0.73 (0.4)[a]						
rs72654473 (ref = CC)									
CA/AA	0.55 (0.38)		−0.78 (0.36)[c]		−0.52 (0.23)[c]				
rs439401 (ref = CC)									
CT	−0.34 (0.23)	−0.4 (0.24)[a]			0.66 (0.23)[b]	−0.28 (0.22)			
TT	−0.89 (0.31)[b]	−0.82 (0.35)[c]			0.17 (0.3)	−0.59 (0.3)[a]			
rs5112 (ref = CG)									
CC	0.02 (0.25)	−0.27 (0.23)		−0.10 (0.31)					
GG	−0.46 (0.22)[c]	−0.46 (0.20)[c]		−0.51 (0.24)[c]					
rs405697 (ref = AA/AG)									
GG					0.36 (0.21)[a]				
rs6857 (ref = AA/AG)									
GG							0.31 (0.22)		
Full model AIC[d]	28.43	6.64	60.19	97.94	48.61	71.21	67.97	88.9	64.99
Final model AIC	17.83	−2.89	46.16	82.04	35.51	54.53	51.85	67.8	48.66

Abbreviations: AIC = Akaike Information Criterion; BTA = Brief Test of Attention; DSB = Digit Span Backward; DSF = Digit Span Forward; HVLT-D = Hopkins Verbal Learning Test–Delayed Recall; HVLT-DI = Hopkins Verbal Learning Test–Discrimination Index; HVLT-L = Hopkins Verbal Learning Test–Learning; ref = reference; SNP = single nucleotide polymorphism; TMT-A = Trail Making Test, Part A; TMT-B = Trail Making Test, Part B; VF = Verbal Fluency Test.

Beta and standard error values for the 9 SNPs retained in the backward selection regression models, controlling for age, education, treatment with radiotherapy ± chemotherapy, and cigarette smoking history (i.e., any past or current use). Blank cells indicate that the SNP was not in the final model for the given cognitive test.

[a] $p < 0.10$.

[b] $p < 0.01$.

[c] $p < 0.05$.

[d] AIC for the models that contained all 10 SNPs, in addition to the control variables.

in the *APOE* gene may increase the vulnerability of patients with brain tumors to cognitive dysfunction. Carriers of at least one *APOE* ε4 allele had significantly lower scores in verbal learning and delayed recall, and a trend toward worse performance in executive functions, relative to noncarriers of the ε4 allele. The presence of the ε4 allele is associated with increased risk of late-onset Alzheimer disease.[4] The ε4 allele has been shown to increase the risk and severity of cognitive dysfunction and poor outcomes in traumatic brain injury[5] and cardiovascular disease and stroke.[21] Several studies described an association between the ε4 allele and diminished memory performance, particularly in encoding and retrieval, and executive functions in middle-aged[17] and healthy, older adults,[22] and a faster rate of cognitive decline in healthy, older *APOE* ε4 carriers.[16,23]

Ahles et al.[6] documented worse performance on visual memory, and spatial and psychomotor functions, in *APOE* ε4 carriers than in noncarriers treated with chemotherapy for breast cancer or lymphoma. We described in a small cohort of patients with low-grade gliomas that carriers of the *APOE* ε4 allele had relatively lower scores than noncarriers in verbal memory.[7] The current study extends these initial findings and provides support for the role of the *APOE* ε4 allele in moderating memory and executive functions in patients with brain tumors. The *APOE* ε4 allele may increase amyloid deposition[20] and the susceptibility to oxidative stress and mitochondrial damage,[24] reduce cholinergic integrity and function,[25] disrupt neuronal repair and utilization of glucose,[26] and influence the regulation of phospholipid and cholesterol[25] after brain injury. It may also accelerate age-related changes in the breakdown of myelin[27] and loss of hippocampal volume.[28] Preliminary evidence also suggests that RT may be associated with an increase in amyloid deposition.[29] It is possible, therefore, that the *APOE* ε4 allele has an important role in influencing the response to CNS injury from RT or chemotherapy, which may involve vascular damage, depletion of glial progenitor cells, oxidative stress, inflammation, demyelination, and disruption of hippocampal neurogenesis.[30,31]

The study findings also suggest that the *APOE* ε4 allele may interact with cigarette smoking and affect cognition. Patients with at least one *APOE* ε4 allele and a history of smoking had higher scores in attention, learning and delayed recall, and verbal fluency, relative to ε4 carriers who never smoked. A higher risk of dementia and cognitive decline has been described among smokers but only for noncarriers of the *APOE* ε4 allele.[32,33] In a large cohort of healthy, middle-aged adults,[18] *APOE* ε4 carriers with a history of smoking had higher scores in verbal fluency than nonsmokers. However, other studies found no association among *APOE* ε4 genotype, smoking, and cognitive functions.[34] Patients with Alzheimer disease who are carriers of the *APOE* ε4 allele were reported to have fewer CNS nicotinic binding sites and lower choline acetyltransferase activity than noncarriers.[25] Although the mechanisms are poorly understood, cigarette smoking may counterbalance the deficiency in nicotinic receptors in carriers of the ε4 allele by facilitating the release of acetylcholine or increasing the density of nicotinic receptors.[35,36] Vascular risk factors have been variably associated with cognitive decline among ε4 carriers,[16,37] but this was not observed in our study. This may have been related in part to the overlap between smoking and other vascular risks among patients, but the absence of detailed vascular risk factor information may also have limited a more comprehensive assessment.

We did not identify a significant relationship between *APOE* ε4 and extent of WM abnormalities. In healthy, older adults, the presence of the ε4 allele has been associated with increased hippocampal atrophy and WM hyperintensity volumes[28] and changes in WM integrity.[27] It is possible that the sensitivity of the rating scale, which measures global WM abnormalities, was inadequate to detect significant associations between extent and distribution of WM lesions and *APOE* ε4 status. Advanced neuroimaging methods for measuring regional brain volume and WM integrity may provide greater sensitivity to detect the possible moderating effects of *APOE* in the development of treatment-related changes in brain structure.

Our findings also suggested that other *APOE* genetic variants moderate cognitive outcome in patients with brain tumors. The results showed that several SNPs were associated with attention and executive functions, and 2 SNPs, including rs405509 and rs6857, were associated with memory. Previous studies described that 3 *APOE* SNPs, including rs449647, rs769446, and rs405509, were associated with increased susceptibility to Alzheimer disease.[10–12] SNP rs405509 was associated with a poorer recovery after traumatic brain injury.[5] Recently, a genome-wide association longitudinal study[38] reported that the *APOE* SNP rs429358 was associated with cognitive decline in older, healthy adults. In our study, rs429358 (TT allele) and rs769446 (TT allele) were associated with lower scores in attention and executive functions. These results suggest that examining *APOE* genetic variants in addition to identification of the ε4 allele provides relevant information regarding cognitive outcome, and should be considered in future studies.

Given the cross-sectional design of our study, we cannot exclude the possibility that the lower scores in memory and executive functions in ε4-positive patients and in association with several *APOE* SNPs were related to an interaction with other factors, such as the disease itself, age-related increased vulnerability to cognitive decline observed in ε4 carriers,[23,38] or to

preexisting differences in hippocampal morphology.[39] We also acknowledge that the heterogeneity of the patient population and the variable posttreatment intervals of the neuropsychological evaluations are potential confounding factors. The sample size may have limited the power to detect small to moderate size effects, and to study possible interactions with disease-related factors, such as brain tumor type and location, and the possible contribution of focal vs whole-brain RT. We could not assess a dose effect because there were too few homozygous for the ε4 allele. Nevertheless, this is the largest study of its kind, and our findings provide new evidence that the *APOE* ε4 allele and other *APOE* SNPs are important in moderating cognitive outcome in patients with brain tumors. A multisite, prospective, longitudinal study would be warranted to further examine the role of *APOE* and other relevant genes in this population.

Comment: Can *APOE* genotyping predict cognitive damage from brain tumor therapy?

Brain tumor therapy conveys risk of neurocognitive deficits as well as neuroimaging effects including white matter changes and brain atrophy. While these are best studied for radiation therapy, increasing evidence supports a potential contribution of chemotherapy. Some risk factors for developing these adverse effects are recognized, e.g., increasing dose and volume of radiation treatment, and synergistic toxicity of radiation with methotrexate. The individual patient risk factor of age is well-known, while other individual risk factors are speculative (e.g., preexisting small-vessel disease). Knowledge regarding individual risk factors would be clinically useful. As a risk factor for dementia, *APOE* ε4 is a logical target to study, and some preliminary data already suggest it may be a risk factor for cancer treatment–related neurotoxicity.

The current study[1] analyzed 211 brain tumor patients off treatment without active tumor who had undergone neuropsychological testing and *APOE* genotyping over a 12-year period. The patient population was extremely heterogeneous, including gliomas, primary CNS lymphoma, meningiomas, and other tumors; treatments were equally heterogeneous, with 62% of patients receiving radiation therapy at varying doses and field sizes and 30% chemotherapy alone.

The most interesting finding was that ε4 carriers had worse neurocognitive performance; the authors concluded that ε4 and additional *APOE* single nucleotide polymorphisms may increase vulnerability of patients with brain tumor to treatment-related cognitive dysfunction. The underlying pathogeneses of radiation and chemotherapy-related cognitive dysfunction, and how *APOE* allelic status may moderate this, remain speculative.

Caution is advised in interpreting these thought-provoking results. The cross-sectional design is less than ideal; moreover, heterogeneity of the study population and their treatments, and the variable time points from treatment at which neuropsychometric testing was acquired, are potential confounding variables. Additionally, the relatively short follow-up of most patients is an unavoidable limitation. Further studies to explore the role of ε4 in this population as well as some of the other hypothesis-generating findings the authors reported will be worthwhile.

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Study funding: No targeted funding reported.

Disclosure: D. Schiff serves on data safety and monitoring boards for Celldex and as a consultant to Tau Pharmaceuticals without personal compensation. He has served on advisory boards for Genentech and Sigma Tau and on a data safety and monitoring board for Vascular Biogenics. Go to Neurology.org for full disclosures.

AUTHOR CONTRIBUTIONS

Dr. Correa was responsible for the study concept and design, oversight of the data acquisition and analysis, and for the interpretation of the results and manuscript writing. Dr. Satagopan and Mr. Baser were involved in the overall data analysis and interpretation, and manuscript writing. Mr. Cheung participated in the genetic analyses and interpretation of the results, and critical revision of the manuscript. Dr. Richards participated in data acquisition, organization and interpretation, and critical revision of the manuscript. Dr. Lin was involved in the genetic data analysis and interpretation, and critical revision of the manuscript. Dr. Karimi and Dr. Lyo were involved in the neuroimaging data analysis and interpretation, and critical revision of the manuscript. Dr. DeAngelis was involved in the study concept and design, interpretation of the results, and critical revision of the manuscript. Dr. Orlow was involved in the study concept and design, genetic data analysis and interpretation of the results, and manuscript writing.

STUDY FUNDING

Supported by the Society of MSKCC and the Charles Dana Foundation (Dr. Denise Correa). NCI grant R01CA137420 provided partial support for analytic contributions (Dr. Satagopan).

DISCLOSURE

D. Correa serves on the editorial board of *Neuro-Oncology Practice*. J. Satagopan serves on the editorial board of *Genetic Epidemiology* and *Sankhya*. R. Baser, K. Cheung, E. Richards, M. Lin, S. Karimi, and J. Lyo report no disclosures relevant to the manuscript. L. DeAngelis serves on the editorial board of *Neurology*, *Journal of Neuro-Oncology*, *Neuro-Oncology*, *Neuro-Oncology Practice*, and *The BMJ*. She serves as a mentor for CTSC KL2 Scholar Award, KL2TR000458; A Pilot Trial of Enoxaparin vs Aspirin in Patients with Cancer and Stroke. I. Orlow reports no disclosures relevant to the manuscript. Go to Neurology.org for full disclosures.

Received October 24, 2013. Accepted in final form March 26, 2014.

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