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COMT, *BDNF*, and *DTNBP1* polymorphisms and cognitive functions in patients with brain tumors

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Background. Cognitive dysfunction is common among patients with brain tumors and can be associated with the disease and treatment with radiotherapy and chemotherapy. However, little is known about genetic risk factors that may moderate the vulnerability for developing cognitive dysfunction. In this study, we examined the association of single nucleotide polymorphisms (SNPs) in the catechol-O-methyl transferase (*COMT*), brain-derived neurotrophic factor (*BDNF*), and dystrobrevin-binding protein 1 (*DTNBP1*) genes with cognitive functions and neuroimaging outcomes in patients with brain tumors.

Methods. One hundred and fifty patients with brain tumors completed neuropsychological tests of attention, executive functions, and memory and were genotyped for polymorphisms in the *COMT*, *BDNF*, and *DTNBP1* genes. Ratings of white matter (WM) abnormalities on magnetic resonance imaging scans were performed.

Results. Multivariate regression shrinkage analyses, adjusted for age, education, treatment type, time since treatment completion, and tumor location, indicated a significant association between the *COMT* SNP rs4680 (Val158Met) and memory with lower scores in delayed recall ($P < .01$) among homozygotes (valine/valine). Additional *COMT*, *BDNF* and *DTNBP1* SNPs were significantly associated with attention, executive functions, and memory scores.

Conclusion. This is the first study to suggest that known and newly described polymorphisms in genes associated with executive and memory functions in healthy individuals and other clinical populations may modulate cognitive outcome in patients with brain tumors.

Keywords: BDNF, brain tumors, cognitive, COMT, DTNBP1.

Cognitive dysfunction is frequent among brain tumor survivors[1] and is associated with disease and radiotherapy (RT) or chemotherapy treatment.[2] The cognitive domains sensitive to the adverse effects of treatment include attention, executive functions, and memory.[3] However, little is known about individual factors that may influence the vulnerability for treatment-related neurotoxicity, or its severity, and that contribute to interpatient variability. We reported recently that brain tumor patients with at least one apolipoprotein E (*APOE*) ε4 allele had significantly lower scores in verbal learning and delayed recall in comparison with noncarriers of a ε-4 allele and that additional *APOE* SNPs were significantly associated with attention, executive, and memory abilities.[4] In addition, patients with at least one ε-4 allele and history of cigarette smoking had significantly higher scores in working memory and verbal learning than ε-4 carriers who never smoked. In order to further investigate the likelihood that variability in cognitive outcome in brain tumor patients may in part be due to genetic constitutive traits, we studied 3 genes with known associations with cognitive functions: the catechol-O-methyl transferase (*COMT*), brain-derived neurotrophic factor (*BDNF*), and dystrobrevin-binding protein 1 (*DTNBP1*). These 3 genes have been described in association with memory and executive functions in healthy individuals and in other clinical populations and could be of relevance in cancer patients, considering that these are the cognitive domains most often disrupted by treatment.

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The role of COMT in modulating frontostriatal networks and executive functions has been reported in healthy individuals and patients with psychiatric disorders.[5,6] The functional single nucleotide polymorphism (SNP) rs4680 (G>A) consists of a substitution of valine with methionine at codon 158 (Val158Met). The Val (G) allele is associated with higher enzymatic activity and faster dopamine degradation, leading to dopamine depletion in the prefrontal cortex.[7,8] There is evidence that dopamine availability in the frontal lobes plays an important role in cognitive function[5] and that carriers of the Val (G) allele perform more poorly on tests of executive functions compared with Met (A) carriers.[6,9,10]

BDNF participates in neural repair and plasticity and long-term potentiation in the hippocampus.[5,11,12] The SNP rs6265 (G>A) consists of a valine/methionine polymorphism at codon 66 (Val66Met). The Met allele alters BDNF trafficking and decreases its secretion, leading to less effective neuroplasticity.[13] Studies have reported that carriers of the Met allele have reduced hippocampal volume and activity and perform more poorly in episodic memory and working memory tests.[12,14,15] DTNBP1 influences glutaminergic neurotransmission and modulates GABAergic, nicotinic, and dopaminergic neurotransmitter systems,[16,17] and the gene is expressed in neurons in the prefrontal cortex, hippocampus and cerebellum.[18] Polymorphisms in *DTNBP1* have been linked to schizophrenia[19] and are associated with difficulties in attention, executive function, and memory in healthy adults and patients with schizophrenia.[18,20–23]

In this study, we explored the associations of *COMT*, *BDNF*, and *DTNBP1* SNPs and cognitive and neuroimaging outcomes along with possible interactions with *APOE* ε4 status in a subset of patients with brain tumors who participated in our prior study assessing *APOE* polymorphisms and cognitive outcome.[4]

Materials and Methods

Participants

One hundred and fifty 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 between 2009 and 2012. Study eligibility included no evidence of active disease on serial MRIs prior to accrual; completion of treatment with radiotherapy (RT) or chemotherapy at least 6 months prior to enrollment; no history of psychiatric or other neurological disorders, and fluency in English. The research protocol was approved by the Institutional Review Board, and written informed consent was obtained from all participants. This cohort represents a subset of the 211 patients who participated in our prior study assessing *APOE* polymorphisms and cognitive functions[4] and for whom more comprehensive genotyping is available.

Fifty-nine patients (39%) were diagnosed with a high grade tumor (ie, glioblastoma, anaplastic astrocytoma, or anaplastic oligodendroglioma), 35 (23%) with a low grade tumor (ie, oligodendroglioma, oligoastrocytoma), 40 (27%) with primary CNS lymphoma (PCNSL), and 16 (11%) had other brain tumors (ie, meningioma, ependymoma). One-hundred and five patients (70%) received treatment with RT±chemotherapy, and 45 (30%) had chemotherapy-only regimens. Eighty-five patients had focal RT (57%), and 20 had whole-brain RT (14%); RT dose ranged from 2340 to 6840 cGy. All patients completed a neuropsychological evaluation and provided a blood sample for genotyping. *APOE* genotyping was obtained previously for all participants,[4] and 35 (23%) were carriers of at least one ε4 allele.

Measures

Neuropsychological Assessment

Neuropsychological tests with reported sensitivity to the adverse effects of cancer therapy[3] were selected to evaluate the following cognitive domains.

- Auditory Attention and Working Memory: Digit Span subtest of the WMS-III (Digit Span Forward-DSF; Digit Span Backward-DSB); Brief Test of Attention (BTA).
- Graphomotor Speed and Executive Functions: Trail Making Test Parts A & B – TMT-A, TMT-B; Phonemic Verbal Fluency Test (VF).
- Verbal Memory: Hopkins Verbal Learning Test-Revised – Learning (HVLT-L), Delayed Recall (HVLT-D), Discrimination Index (HVLT-DI).

The test battery was administered either by a neuropsychologist (DDC) or by 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. Z scores have a mean of zero and a standard deviation of 1. A positive z score value indicates that a score is higher than the mean, which corresponds to better cognitive test performance.

Genotyping

SNPs were selected based on reports in the literature on association with cognitive functions in healthy and clinical populations and evidence from imaging and in vitro assays.[6,8–10,12–15,18,20,22,24] We also included SNPs predicted to overlap with seed miRNA regions or transcription factor binding sites, and tagging SNPs (obtained with HaploView software) as reported in Caucasians and with a correlation (r^2) > 0.80 with other SNPs. The inclusion criteria (chromosome location, genomic context, and expected allelic prevalence) for the tested SNPs are listed in Supplementary material, Table S1. Thirty-five SNPs were genotyped using the GoldenGate assay (Illumina Inc.), and 2 SNPs were genotyped using the Sequenom MassARRAY iPLEX genotyping platform (Sequenom Inc.) as previously described.[25,26] Assays were considered optimal according to degree of clustering, specificity, and reproducibility. One SNP was genotyped by restriction fragment length polymorphism (RFLP).[27] Hardy-Weinberg equilibrium (HWE) was calculated to identify major genotyping issues; however, the cohort consisted solely of cases with the disease, and absence of HWE can be due to the SNP conferring risk for the condition. SNPs that were monoallelic, had >5% missing data, failed during earlier stages of the assay design, or showed poor clustering were excluded from further analysis. A total of 38 SNPs passed the quality control and were included in subsequent statistical analyses.

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 (SK, JL) who were blind to the cognitive test results. WM abnormalities were rated on a fluid-attenuated inversion recovery (FLAIR) sequence for most patients; if these were not available, T2-weighted sequences were used. Radiographic endpoints were measured according to the modified Fazekas scale[28] and included no WM change (grade 0), minimal patchy WM foci (grade 1), start of confluence of WM disease (grade 2), large confluent areas (grade 3), confluence with cortical and subcortical involvement (grade 4), leukoencephalopathy (grade 5), and possible radiation necrosis (grade 6). The tumor and surrounding edema were visually excluded from these measurements. One total score was obtained for each patient.

Statistical Analyses

For each cognitive outcome measure, we used linear regression analysis to examine the association between the 38 SNPs using a multivariable model adjusted for age, education, tumor location, treatment with RT±chemotherapy, and time since treatment.[2,4] To address potential multiple comparison issues in the multivariable model, we used an empirical Bayes-type shrinkage analysis, which adjusts for the analysis of multiple SNPs within each outcome.[29] This shrinkage analysis specifies that all SNPs within the same gene confer the same effect on a cognitive test. This effect will be the null value of zero if none of the SNPs in that gene is associated with a cognitive test score or if only one or few SNPs having small effects are associated with a cognitive test score. In this manner, this approach shrinks the effects of the seemingly null SNPs towards zero. We have demonstrated previously[29] that this approach has superior sensitivity (for detecting true associations) and specificity (for not detecting null associations) than standard multivariable analysis approaches when evaluating multiple putative risk factors; at the 5% significance level, we would expect around 2 (= 38 * 0.05) significant results. In all analyses, a SNP was treated as a binary variable if any of its genotype category was smaller than 10%. Otherwise, it was treated as a categorical variable having 3 categories, and the effects of the heterozygous and homozygous (for minor allele) genotypes were estimated in the analyses. Additional Bonferroni corrections for evaluating 9 cognitive outcomes were not conducted given the exploratory nature of our study. SNPs having P values $< .05$ were deemed significant. We also considered P values in the range .05 to .10 to be noteworthy findings, although these are not statistically significant.

Logistic regression was used for WM ratings, which were classified into 2 categories: none/minimal (grade = 0-1) and moderate/severe (grade 2–6), consistent with our prior study assessing *APOE* polymorphisms and cognition.[4]

Interaction Analysis

We examined the interaction of each SNP in the *COMT*, *BDNF*, *DTNBP1*, and the *APOE* ε-4 allele and (i) smoking history, (ii) vascular risk factors, and (iii) treatment with RT± chemotherapy. We used analysis of ANOVA for the interaction analysis and corrected the interaction P values for multiple comparisons.[30] All regression analyses were performed using the STATS library in R version 3.0.1,[31] and the R function for shrinkage estimation program is available at the following webpage URL: https://www.mskcc.org/departments/epidemiology-biostatistics/biostatistics/shrinkage-estimation-analysis-cognitive-functions-and-other-cancer-related-outcomes.

Results

Patient demographics, disease, and treatment variables are included in Table 1. In addition to the widely reported *COMT* rs4680 (Val158Met), and *BDNF* rs6265 (Val66Met) SNPs, we genotyped other SNPs in the 3 genes of interest that were described in the literature,[14,15,32] which were likely functional as per *in silico* tools or were tagging SNPs (Supplementary material Table S1). Mean cognitive test z scores according to genotypes in the *COMT*, *BDNF*, and *DTNBP1* genes are reported in Supplementary material Table S2.

The results of multivariate regression shrinkage analyses (adjusting for age, education, tumor location, treatment with RT±chemotherapy, and time since treatment) showed that there were significant associations between the cognitive outcomes and 11 *COMT* SNPs, 4 *BDNF* SNPs, and 1 *DTNBP1* SNP. As there were no significant differences on the cognitive outcomes according to tumor type, this variable was not included in the regression model. A total of 146 participants had complete genotype data and were included in the multivariate regression shrinkage analyses. Four participants were excluded due to missing data for SNPs in the *COMT* (rs740603), *BDNF* (rs10767664, rs2030324), and *DTNBP1* (rs9476886) genes. Only 3 SNPs (*COMT* SNP rs737865, rs2020917, *BDNF* SNP rs7103873) were removed from the analysis to avoid multicollinearity issues as they were in strong (>0.90) linkage disequilibrium with SNP rs1800706 and with SNP rs2030324, respectively. The number of individuals in each genotype class, estimated effects of the SNPs, their standard errors, and statistical significance are included in Tables 2–4 for the *COMT*, *BDNF*, and *DTNBP1* SNPs, respectively. Only SNPs and cognitive tests showing significant or noteworthy, albeit nonsignificant, associations are included in each table.

Catechol-O-methyl Transferase

As shown in Table 2, the *COMT* SNP rs4680 (Val158Met) was significantly associated with delayed recall (HVLT-D) performance, with GG (Val/Val) carriers having significantly *lower* scores relative to AA (Met/Met) carriers. Significant associations were also seen for SNP rs16815 and learning (HVLT-T) and SNP rs4646316 and recognition memory (HVLT-DI), with carriers of the variant alleles obtaining *lower* scores than carriers of the reference alleles. For SNPs rs9332377, rs165815, rs4646312, rs5993883, and rs4646312, carriers of the variant alleles had significantly *lower* scores than carriers of the reference allele on tests of attention (DSF), working memory, and executive functions (BTA, TMTB, and VF). For SNPs rs4818, rs5746847, and rs6269, carriers of the variant alleles had significantly *higher* scores than

Table 1. Demographic characteristics and disease/treatment history (N = 150)

Characteristics	
Male	68 (45%)
Right-handed	131 (87%)
Caucasian[a]	133 (89%)
Age at study entry (y)	
Mean (SD)	51 (13.4)
Median (range)	52 (21–83)
Mean education (y)	16 (2.8)
Mean estimated VIQ	112 (8.6)
Tumor type	
Low-grade glioma	34 (23%)
High-grade glioma	57 (38%)
Primary CNS lymphoma	42 (28%)
Other	17 (11%)
Tumor location	
Frontal/frontal-temporal/frontal-parietal	81 (54%)
Temporal/parietal/occipital	36 (24%)
Cortical/subcortical	33 (22%)
Predominant tumor side	
Left	53 (36%)
Right	68 (45%)
Bilateral	29 (19%)
Treatment type[b]	
RT ± chemotherapy	150 (70%)
Chemotherapy	45 (30%)
Time since treatment completion, mo	
Mean (SD)	45 (50.7)
Median (range)	27 (6–370)[c]
Relapse history[d]	37 (25%)
Smoking history	
Yes	66 (44%)
Vascular risk	
Yes	58 (39%)
Antiepileptics[e]	
Yes	76 (51%)

Abbreviations: mo, months; RT, Radiotherapy; SD, Standard Deviation; VIQ, Verbal IQ; y, years.
[a]Additional ethnicity: Asian = 5%, Black = 4%, Other = 2%.
[b]Treatment history = all therapy received including at relapse, if applicable.
[c]Two patients had longer time since treatment completion compared with others (ie, highest values = 370 & 314 months; third highest value = 155 months).
[d]History of disease relapse prior to study participation.
[e]Medication at the time of the cognitive evaluation.

carriers of the reference allele on a test of executive function (TMTB).

Brain-derived Neurotrophic Factor

As shown in Table 3, for *BDNF* SNP rs11030104, AA (most common allele) carriers had significantly *lower* scores in delayed recall (HVLT-D). There was also a noteworthy, albeit nonsignificant, association with AA carriers of SNPs rs11030104 and rs7127507 having *lower* scores in learning (HVLT-L; $P = .07$) and/or recognition memory (HVLT-DI; $P = .07$) relative to homozygote and heterozygote carriers of the variant alleles (AG.GG). Significant associations with delayed recall (HVLT-D) or recognition memory (HVLT-DI) were evident for 2 additional SNPs (rs10767664, rs10835210), with variant allele carriers showing *worse* performance compared with carriers of the common alleles. SNP rs2030324 was significantly associated with executive functions (TMT-B, BTA), with AG carriers showing *worse* performance compared with AA carriers. There were no significant associations between rs6265 (Val66Met) and any of the cognitive outcomes.

Dystrobrevin-binding Protein 1

As shown in Table 4, for *DTNBP1* SNP rs742106, GG carriers had significantly *lower* scores in recognition memory (HVLT-DI) relative to carriers of the variant alleles (AA.AG). There was a noteworthy, albeit nonsignificant, association for SNP rs742106 with GG carriers having *lower* scores in delayed recall (HVLT-D; $P = .09$) relative to carriers of the variant alleles.

White Matter Ratings

Sixty-five (43%) participants were rated as having moderate/severe (grade $\geq$ 2) WM abnormalities, and 85 (57%) had none/minimal (grade 0-1) WM abnormalities. The results of logistic regression analyses (adjusting for age, education, tumor location, treatment with RT±chemotherapy, and time since treatment) showed that none of the *COMT*, *BDNF*, and *DTNBP1* SNPs were significantly associated with WM abnormalities. There was a noteworthy, albeit nonsignificant, association between WM ratings and *COMT* SNPs rs174696 (AG.GG) ($P = .07$) and rs165774 (GG) ($P = .06$), and *BDNF* SNP rs10767664 (AT.TT) ($P = .09$), with carriers of 2 or any minor allele showing more extensive WM abnormalities.

Multivariate regression shrinkage analyses of cognitive scores (adjusting for WM ratings in addition to age, education, tumor location, treatment with RT±chemotherapy, and time since treatment) showed comparable significant associations between the cognitive outcomes and *COMT*, *BDNF*, and *DTNBP1* SNPs, as in the shrinkage analysis described above (without adjusting for WM).

Interactions and Associations with Variables of Interest

For cognitive outcomes, there was no significant interaction between the *COMT*, *BDNF*, and *DTNBP1* SNPs and (i) the *APOE* ε-4 allele, (ii) cigarette smoking, (iii) other vascular risk factors (eg, hypertension, hypercholesterolemia, diabetes), (iv) treatment with RT ± chemotherapy, and (v) WM ratings. There were no significant associations between the cognitive outcomes and *APOE* ε-4 allele, cigarette smoking, or other vascular risk factors in this cohort of 150 patients.

Discussion

This study provides new evidence that polymorphisms in the *COMT*, *BDNF*, and *DTNBP1* genes may be functionally relevant and influence memory, attention, and executive functions in

Table 2. Multivariate associations of COMT single nucleotide polymorphisms with cognitive test z scores

SNP	Allele	N	DSF	DSB	TMT-B	BTA	VF	HVLT-L	HVLT-D	HVLT-DI
rs4818	CC (ref)	31								
	CG	74			**1.97 (0.86)**[b]					
	GG	45		−0.59(0.31)[a]						
rs9332377	AA.AG (ref)	54								
	GG	96	**−0.60 (0.31)**[b]		**−0.79 (0.39)**[b]	**−0.87 (0.33)**[c]	−0.53 (0.31)[aΔ]			
rs165656	CC	29								
	CG	72			−1.34 (0.81)[a]					
	GG	49								
rs5746847	AA (ref)	22								
	AG	66			**1.17 (0.49)**[b]					
	GG	62			**1.51 (0.66)**[b]		0.87 (0.46)[a]			
rs165815	AA (ref)	101								
	AG.GG	49					**−0.84 (0.32)**[c]	**−0.68 (0.31)**[b]		
rs4680	AA (ref)	27								
	AG	73								
	GG	50							**−2.05 (0.90)**[b]	
rs5993883	AA (ref)	26								
	AC	70	−0.77 (0.46)[a]						−0.96 (0.54)[a]	−0.84 (0.46)[a]
	CC	54	**−1.22 (0.59)**[b]	−0.71 (0.39)[a]						−1.05 (0.59)[a]
rs1800706	AA (ref)	18								
	AG	66								
	GG	66					0.77 (0.46)[a]			
rs4646312	AA (ref)	46								
	AG	74			**−3.03 (1.44)**[b]					
	GG	30								
rs740603	AA (ref)	28								
	AG	68	**0.84 (0.41)**[b]			0.76 (0.40)[a]				
	GG	53			**−1.19 (0.61)**[b]					
rs165774	AA.AG (ref)	68								
	GG	82	**−0.77 (0.26)**[c]						0.55 (0.32)[a]	0.54 (0.31)
rs6269	AA (ref)	42								
	AG.GG	108			**2.16 (0.81)**[c]				1.06 (0.63)[a]	
rs4646316	C (ref)	82								
	CT.T	68							−0.61 (0.32)[a]	**−0.69 (0.31)**[b]

Beta and standard error values for 13 COMT single nucleotide polymorphisms (SNPs) retained in the multivariate regression models, controlling for age, education, treatment with RT±chemotherapy, time since treatment, and tumor location.

Blank cells indicate that the SNP was not associated with the given cognitive test. Only SNPs and cognitive tests showing significant or noteworthy albeit non-significant associations are included.

Underlined SNPs have NOT been reported previously in association with cognition.

[a]$P < 0.10$; [b]$P \leq 0.05$; [c]$P < 0.01$.

Abbreviations: A, Adenine; C, Cytosine; G, Guanine; T, Thymine; BTA, Brief Test of Attention; DSB, Digit Span Backward; DSF, Digit Span Forward; HVLT-DI, Hopkins Verbal Learning Test-Discrimination Index; HVLT- L, Hopkins Verbal Learning Test-Learning; TMT-B, Trail Making Test, Part B; VF, Verbal Fluency Test.

Table 3. Multivariate associations of brain-derived neurotrophic factor single nucleotide polymorphisms with cognitive test z scores

SNP	Allele	N	DSB	TMT-B	BTA	HVLT-L	HVLT-D	HVLT-DI
rs10767664	AA (ref)	91						
	AT.TT	57		−1.24 (0.73)[a]			**−1.46 (0.68)**[b]	
rs10835210	AA (ref)	37						
	AC	57						
	CC	56			−1.03 (0.54)[a]	−0.74 (0.43)[a]	**−1.36 (0.69)**[b]	**−1.52 (0.74)**[b]
rs11030101	AA (ref)	52						
	AT	59						
	TT	39						−1.59 (0.87)[a]
rs11030104	AA (ref)	93						
	AG.GG	57				0.96 (0.54)[a]	**1.98 (0.78)**[b]	1.28 (0.68)[a]
rs11030107	AA (ref)	98						
	AG.GG	52	−0.50 (0.31)[a]					
rs2030324	AA (ref)	46						
	AG	62		**−1.74 (0.64)**[c]	**−1.15 (0.49)**[b]		−1.01 (0.52)[a]	
	GG	41						
rs7127507	AA (ref)	81						
	AG.GG	69						1.24 (0.72)[a]

Beta and Standard Error values for 7 BDNF the single nucleotide polymorphisms (SNPs) retained in the multivariate regression models, controlling for age, education, treatment with RT ± chemotherapy, time since treatment, and tumor location.
Blank cells indicate that the SNP was not associated with the given cognitive test. Only SNPs and cognitive tests showing significant or noteworthy, albeit nonsignificant, associations are included.
Underlined SNPs have not been reported previously in association with cognition.
Abbreviations: A, Adenine; C, Cytosine; G, Guanine; T, Thymine; BTA, Brief Test of Attention; DSB, Digit Span Backward; HVLT-D, Hopkins Verbal Learning Test- Delay; HVLT-DI, Hopkins Verbal Learning Test- Discrimination Index; HVLT-L, Hopkins Verbal Learning Test-Learning; TMT-B, Trail Making Test, Part B.
[a], $P < .10$; [b], $P \leq .05$; [c], $P < .01$

Table 4. Multivariate associations of DTNBP1 single nucleotide polymorphisms with cognitive test z scores

SNP	Allele	N	DSF	BTA	HVLT-D	HVLT-DI
rs1047631	AA (ref)	115				
	AG.GG	35		0.50 (0.28)[a]		
rs3829893	AA.AG (ref)	56				
	GG	94	−0.51 (0.29)[a]			
rs742106	AA.AG (ref)	80				
	GG	70			−0.55 (0.32)[a]	**−0.64 (0.31)**[b]
rs9476886	AA.AG (ref)	96				
	GG	52	0.48(0.27)[a]	0.53(0.29)[a]		

Beta and Standard Error values for 4 DTNBP1 single nucleotide polymorphisms (SNPs) retained in the multivariate regression models, controlling for age, education, treatment with RT ± chemotherapy, time since treatment, and tumor location.
Blank cells indicate that the SNP was not associated with the given cognitive test. Only cognitive tests and SNPs showing significant or noteworthy albeit non-significant associations are included.
Underlined SNPs have not been reported previously in association with cognition.
Abbreviations: A, Adenine; C, Cytosine; G, Guanine; T, Thymine; BTA, Brief Test of Attention; DSF, Digit Span Forward; HVLT- D, Hopkins Verbal Learning Test- Delay; HVLT-DI, Hopkins Verbal Learning Test- Discrimination Index.
[a], $P < .10$; [b], $P \leq .05$.

patients with brain tumors (several of which were not previously reported in the literature), further supporting the potential effect of *COMT*, *BDNF*, and *DTNBP1*genetic variants on various aspects of cognition.

As anticipated, we found a significant association of *COMT* rs4680 (Val158Met) with cognitive functions, with G (Val) carriers having significantly worse scores on delayed recall. Furthermore, we identified 5 additional *COMT* SNPs associated with worse scores on tests of attention, executive functions, and memory in our cohort. The *COMT* gene is important for regulating prefrontal dopamine levels,[5,9] with most studies reporting that rs4680 G (Val) carriers perform worse than A (Met) carriers

on tests of executive functions[6,9,10]; however, equivocal evidence for this association has also been reported in the literature.[33,34] Similar to our results, worse performance in episodic and semantic memory in G (Val) carriers has been described in some studies with healthy adults,[35–37] suggesting that the rs4680 G (Val) variant may also influence memory, possibly related to the role of executive function in some aspects of memory (eg, organization, retrieval, and semantic clustering). In breast cancer patients, Small et al.[38] reported worse attention performance in G (Val) carriers treated with chemotherapy compared with healthy untreated carriers, indicating that this genetic variant may increase the risk for cancer treatment-related cognitive dysfunction. The current study extends some of these initial findings and provides evidence for the role of additional, newly described *COMT* SNPs in influencing memory and executive functions in brain tumor survivors. The underlying mechanisms are unknown, but it is possible that in carriers of the variant alleles of rs4680 and other *COMT* SNPs, the disease and treatment further disrupt dopamine availability and the efficiency of cognitive functions mediated in part by the frontal lobes. Unlike other studies in healthy adults,[8] we found no association among haplotypes of *COMT* SNPs rs737865, rs4680, and rs165599 and cognitive outcomes, and this may be in part due to the relatively small sample size.

We found that 3 *BDNF* SNPs showed significant associations with tests of memory (learning, delayed recall, recognition), with variant allele carriers of SNPs rs10767664 and rs10835210 having *lower* scores, and variant allele carriers of SNP rs11030104 having *higher* scores. These results are consistent with the described involvement of *BDNF* in episodic memory and long-term potentiation in the hippocampus.[5,11,12] In addition, SNP rs2030324 was significantly associated with executive functions, with variant allele carriers having *lower* scores. There were no significant associations between rs6265 (Val66Met) and any of the cognitive outcomes in our cohort, unlike other published studies.[5,12,13] Decreased processing speed was reported in healthy older adults who were carriers of the rs6265 Met allele and the rs2030324T allele,[15] and worse delayed recall was seen in patients with traumatic brain injury and healthy controls who were Met carriers of rs6265 and in association with other SNPs (rs11030102, rs11030107, rs12273363, rs712507).[14] In the context of these findings, our results also suggest that other loci within the *BDNF* gene may influence cognition. This was observed in a rodent model, where a single-dose of 30 Gy of whole-brain RT induced persistent inhibition of *BDNF* gene transcription and cognitive dysfunction.[39] The noteworthy, albeit nonsignificant, associations between *BDNF* SNP rs10767664 and COMT SNPs rs174696 and rs165771 and more extensive WM abnormalities may suggest a role for these genes in WM integrity in our patient cohort, but further research would be required to clarify these associations. It is possible that variants in the *BDNF* and *COMT* genes may influence response to CNS injury from RT and chemotherapy, which often involves disruption of hippocampal neurogenesis, vascular damage, depletion of glial progenitor cells, inflammation, and demyelination.[40,41] Our results provide preliminary evidence that *COMT* and *BDNF* polymorphisms may be functionally important and may modulate aspects of memory and executive functions, and WM integrity in patients with brain tumors.

DTNBP1 SNP rs742106 was associated with memory (recognition), with carriers of the variant alleles having worse performance. Carriers of variant alleles of 2 other SNPs had better attention and executive scores, but the association was not significant. These findings are consistent with studies reporting that variants in *DTNBP1* influence cognitive functions mediated by dopaminergic and glutamatergic neurons in the prefrontal cortex[16,22] and are involved in regulation of neuroplasticity.[42] *DTNBP1* SNPs were associated with memory and executive functions in a large sample of older healthy adults[18] and with attention and memory performance in patients with schizophrenia and healthy controls.[20,43] Variants in the *DTNBP1* gene may influence cognition in patients with brain tumors through the modulation of neurotransmitter systems and regulation of neuroplasticity in response to CNS injury related to disease and treatment.

There were no significant interactions among any of the *COMT*, *BDNF*, or *DTNBP1* SNPs, and the *APOE* ε-4 allele or among the SNPs and history of cigarette smoking or other vascular risk factors, suggesting that these variables do not jointly influence the associations of the SNPs and cognitive outcome. Recent studies have described significant interactions between *APOE* ε-4 and the *BDNF* Val66Met polymorphism in healthy adults, with carriers of the ε-4/Met alleles showing worse episodic memory performance,[44] and between *APOE* ε-4 and the *COMT* Val158Met polymorphism, with ε-4/Val allele carriers having worse semantic memory.[45] We have previously reported in this patient cohort that carriers of the *APOE* ε-4 allele had significantly lower scores in learning and delayed recall in comparison to non-ε-4 carriers.[4] The current findings suggest that, in addition to the *APOE* ε-4 allele, several SNPs in *COMT*, *BDNF* and *DTNBP1* were also associated with worse memory performance in this patient group. However, additional studies with larger sample sizes would be warranted to assess the possible additive effects of the *COMT*, *BDNF*, and *DTNBP1* SNPs, and the *APOE* ε-4 allele in modulating cognitive functions in patients with brain tumors.

There are several limitations to the present study. Considering the cross-sectional design, we cannot exclude the possibility that the lower scores in attention, executive functions, and memory seen in association with several SNPs and genotypes were related to pre-existing cognitive dysfunction in carriers of risk alleles or to an interaction with other factors such as the disease and its specific treatments. The relatively small sample size limited the power to detect small to moderate size effects and interactions among SNPs, and the assessment of associations with disease-related factors such as tumor grade and type, location, and treatment with RT or chemotherapy, as well as time elapsed since treatment. The findings that some SNPs had both adverse and beneficial effects across different cognitive tests may be due to other linked SNPs or other unknown factors. In addition, it is possible that the sensitivity of the WM rating scale was inadequate to detect associations between the severity and distribution of WM lesions and the genetic variants. Measurements of brain volume and WM integrity using advanced techniques such as diffusion tensor imaging may provide greater sensitivity to detect the potential involvement of these genes in the development of treatment-related changes in brain structure. These limitations notwithstanding, our study is the first of its kind, and our findings demonstrate

that SNPs in genes associated with cognition in healthy adults and other clinical populations with neurological, neurodegenerative, and psychiatric disorders[6,14,46,47] may also be important in modulating cognitive outcome in patients with brain tumors and may contribute to individual patient vulnerability to treatment-related neurotoxicity. Although these associations may not be specific to patients with brain tumors, it suggests that these patients may be at greater risk for developing cognitive dysfunction, possibly through impaired regulation of dopaminergic and other neurotransmitter systems and less efficient neuroplasticity in response to brain injury related to their disease and its treatment. A large prospective longitudinal study would be warranted to validate the role of the SNPs described in this study and to investigate additional relevant genes and underlying mechanisms. This line of research would contribute to the identification of patients at increased risk for treatment-related neurotoxicity and may guide the development of neuroprotective agents to reduce or remediate cognitive dysfunction in cancer patients and assist in individualized treatment planning through an assessment of neurotoxicity risk.

Supplementary material

Supplementary material is available online at *Neuro-Oncology* (http://neuro-oncology.oxfordjournals.org/).

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Conflicts of interest statement. Dr. Correa serves on the Editorial Board of *Neuro-Oncology Practice* and on the Neurotoxicity Advisory Board of Juno Therapeutics. Dr. DeAngelis serves on the Editorial Board of *Neurology, Journal of Neuro-Oncology, The Open Clinical Cancer Journal, CNS Oncology, Neuro-Oncology, Neuro-Oncology Practice,* and *The BMJ,* and on the Neurotoxicity Advisory Board of Juno Therapeutics Dr. DeAngelis serves as a mentor for CTSC KL2 Scholar Award, KL2TR000458; A Pilot Trial of Enoxaprin vs Aspirin in Patients with Cancer and Stroke. The following co-authors have no conflicts of interest: Dr. Satagopan, Mr. Cheung, Ms. Arora, Mr. Xu, Ms. Kryza-Lacombe, Dr. Karimi, Dr. Lyo, and Dr. Orlow.

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