Clinical Cancer Research

Genetic Modulation of Neurocognitive Function in Glioma Patients

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Abstract

Purpose: Accumulating evidence supports the contention that genetic variation is associated with neurocognitive function in healthy individuals and increased risk for neurocognitive decline in a variety of patient populations, including cancer patients. However, this has rarely been studied in glioma patients.

Experimental Design: To identify the effect of genetic variants on neurocognitive function, we examined the relationship between the genotype frequencies of 10,967 single-nucleotide polymorphisms in 580 genes related to five pathways (inflammation, DNA repair, metabolism, cognitive, and telomerase) and neurocognitive function in 233 newly diagnosed glioma patients before surgical resection. Four neuropsychologic tests that measured memory (Hopkins Verbal Learning Test—Revised), processing speed (Trail Making Test A), and executive function (Trail Making Test B, Controlled Oral Word Association) were examined.

Results: Eighteen polymorphisms were associated with processing speed and 12 polymorphisms with executive function. For processing speed, the strongest signals were in *IRS1* rs6725330 in the inflammation pathway ($P = 2.5 \times 10^{-10}$), *ERCC4* rs1573638 in the DNA repair pathway ($P = 3.4 \times 10^{-7}$), and *ABCC1* rs8187858 in metabolism pathway ($P = 6.6 \times 10^{-7}$). For executive function, the strongest associations were in *NOS1* rs11611788 ($P = 1.8 \times 10^{-8}$) and *IL16* rs1912124 ($P = 6.0 \times 10^{-7}$) in the inflammation pathway, and *POLE* rs5744761 ($P = 6.0 \times 10^{-7}$) in the DNA repair pathway. Joint effect analysis found significant gene polymorphism-dosage effects for processing speed ($P_{trend} = 9.4 \times 10^{-16}$) and executive function ($P_{trend} = 6.6 \times 10^{-15}$).

Conclusions: Polymorphisms in inflammation, DNA repair, and metabolism pathways are associated with neurocognitive function in glioma patients and may affect clinical outcomes. *Clin Cancer Res; 21(14); 3340–6. ©2015 AACR.*

Introduction

Impaired neurocognitive function (NCF) is extremely common in patients with brain tumor, with up to 91% of patients having at least one area of deficit compared with the normal population, and 71% demonstrating at least three deficits (1). These functions include attention, ability to acquire new memories, recall of stored memories, executive functions, speed of information processing, expressive speech, language comprehension, visual-perception, reasoning, fine motor speed, emotional behavior, interpersonal behavior, and so forth. In patients with malignant glioma, NCF has been reported as a prognostic factor for overall survival (2–6), tumor progression (7, 8), and quality of life (QOL; refs. 3, 9). However, despite the recognition that many factors can potentially affect NCF (i.e., tumor malignancy, epilepsy, anticonvulsants, radio- and chemotherapy, and psychologic distress), there remains heterogeneity in outcome, suggesting that additional genetic risk factors may modulate NCF. It is believed that genetic factors account for over half of the variance in adult NCF and may account for a large majority of the variance in those over the age of 80 years (10).

Accumulating evidence supports the contention that genetic variation is associated with NCF in healthy individuals and increased risk for neurocognitive decline in a variety of patient populations, including cancer patients. Several single-nucleotide polymorphisms (SNP) in genes in metabolism and cognitive pathways have been reported to affect NCF in different conditions such as head trauma, temporal lobe epilepsy, dementia pugilistica, multiple sclerosis and gliosis. Even subjects with no known neurologic disease perform more poorly

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Translational Relevance

Impaired neurocognitive function is extremely common in brain tumor patients, whether primary or metastatic. These functions include speed of information processing, memory, word retrieval, fine motor speed, and executive functions. Genetic variation may be associated with cognitive function, and patients with at-risk variant alleles may be at greater risk for impaired neurocognitive function. Our findings of genetic variants in inflammation, DNA repair, and metabolism pathways genes associated with glioma patients' neurocognitive performance (memory, processing speed, and executive function) before surgical resection have implications for clinical practice and could allow for the development of new neuroprotective therapies to reduce neurocognitive dysfunction, and improve quality of life.

on tests of memory and executive function if they are carriers of an "at-risk" allele (11, 12). For example, human carriers of the *COMT* Val allele (Val158Met, rs4680) have been found to exhibit significantly lower executive function and inefficiency in working memory function (11). The *BDNF* Met allele (Val66Met, rs6265) is associated with poorer verbal episodic memory function (12, 13). The epsilon 4 allele of APOE is associated with increased vulnerability to cognitive decline in breast cancer, brain tumor patients, and aging (14–18). Polymorphisms in these cognitive-related genes may be mediators or moderators of cognitive and brain reserve (16), and individuals with the variant alleles may be at greater risk for impaired NCF.

We have previously published an overview of candidate genes association studies mainly focused on the DNA repair, metabolism, and inflammation pathways and the results are encouraging (19). Also, our group (20, 21) and others (22) found, using genome-wide association study (GWAS) methods, seven susceptibility loci for glioma risk: 5p15.33 *TERT*, 7p11.2 *EGFR*, 7q36.1 *XRCC2*, 8q24.21 *CCDC26*, 9p21.3 *CDKN2A-CDKN2B*, 11q23.3 *PHLDB1*, and 20q13.33 *RTEL1*. It is interesting to note that of the seven glioma susceptibility genes identified by GWAS, five genes (*XRCC2*, *RTEL1*, *TERT*, *CCDC26*, and *CDKN2B*) are crucial for both the repairing of DNA double-strand breaks and telomere maintenance (23). Taken together, these data provide strong evidence that common variation in DNA repair, metabolism, inflammation, and telomerase pathway genes contributes to glioma predisposition. However, none of these genes polymorphisms have been explored in relation to NCF in patients with brain tumor.

The aim of our study was to examine the association between genetic polymorphisms and NCF in glioma patients before surgical resection. We hypothesize that polymorphisms in cognitive, metabolism, inflammation, DNA repair, and telomerase pathway genes are associated with NCF, and could potentially modulate treatment response, disease progression, and neurocognitive sequelae. This exploratory approach will permit us to assess the individual contribution of SNPs in each gene to NCF, and also potentially allow us to assess the joint effect of multiple SNPs and pathways on NCF.

Materials and Methods

Study subjects

The population for this study was a subset of patients from a prospective epidemiologic study of malignant glioma patients consecutively diagnosed and treated at The University of Texas MD Anderson Cancer Center (Houston, TX) between 1992 and 2009 (20, 24). The patients included in this analysis were newly diagnosed and previously untreated (no tumor resection, chemotherapy, or radiotherapy) malignant gliomas. Histology was subsequently confirmed after surgical resection. Of these 1,247 patients, 233 had been clinically referred for and completed comprehensive neuropsychologic evaluation prior to surgical resection and had genotype data available for analysis. Clinical data, including date of diagnosis, histology, tumor location, and medication information, were extracted from patients' medical records. The study was approved by The University of Texas MD Anderson Institutional Review Board.

Neuropsychologic tests

All patients participated in a comprehensive neuropsychologic assessment before surgical resection with tests administered by licensed and board certified neuropsychologists or neuropsychology trainees and psychometrists who were trained in standardized assessment and scoring procedures. The whole neuropsychologic assessment (including patient and family interview as well as testing) typically required 2 to 3 hours to complete. As patients were referred for clinical purposes not all patients received the same set of cognitive tests. The most common cognitive domains assessed and their respective tests included verbal memory (Hopkins Verbal Learning Test—Revised, HVLT-R, Total Recall; ref. 25), processing speed (Trail Making Test A, TMTA; ref. 26), and executive function [Trail Making Test B, TMTB (26) and Controlled Oral Word Association Test, COWA (27)]. All test scores for each cognitive test were converted to demographically adjusted *z*-scores using published normative data from healthy controls adjusting for age, education, and gender when necessary. NCF test performance was considered impaired if the *z*-score was at or below −1.5.

Selection of the pathway genes SNPs and genotyping assays

Genomic DNA extracted from venous blood samples was genotyped as part of the parent epidemiologic study as previously described (20) using the Human610-Quad Bead Chips according to the manufacturer's protocols (Illumina). We selected all the genes listed in the Human DNA repair genes reviewed by Wood and colleagues (ref. 28; http://www.cgal.icnet.uk/DNA_Repair_Genes.html) for the DNA repair pathway and genes listed in the PANTHER database (http://www.pantherdb.org/pathway/), KEGG (http://www.genome.jp/kegg/pathway.html), and BioCarta (http://www.biocarta.com/) for the inflammation, metabolism, cognitive, and telomerase-related pathways. We identified a set of 580 candidate genes involved in the five pathways, including DNA repair ($n = 176$), inflammation ($n = 267$), metabolism ($n = 66$), cognitive ($n = 13$), and telomerase-related ($n = 58$). A total of 12,661 SNPs belonging to the above 580 genes in these five pathways were identified from the Human 610-Quad Bead Chip. After excluding the monomorphic SNPs and SNPs with minor allele frequency (MAF) lower than 0.05, the vast majority of our final 10,967 SNPs were located in flanking and intronic regions (Table 1).

Table 1. Pathway and gene selection

Pathways	Genes, *n*	SNPs in the chip, *n*	SNPs in the analysis, *n*
DNA repair	176	2,519	2,083
Inflammation	267	6,980	6,185
Metabolism	66	1,468	1,196
Cognitive	13	991	886
Telomerase	58	703	617
Total	580	12,661	10,967

Statistical methods

Descriptive statistics were generated for patient and treatment characteristics as well as for baseline NCF measures. The χ^2 tests were performed to confirm the presence or absence of allelic or genotypic associations. The effect of the genotypes on patients' NCF performances (HVLT-R, TMTA, TMTB, and COWA) was estimated using ANOVAs. Akaike's information criterion (AIC) was used to determine the best genetic model (codominant, dominant, recessive, over-dominant, and log-additive) for each SNP (29). To reduce the redundant information, loci in strong linkage disequilibrium (LD) with another marker ($D' \geq 0.9$) were dropped from further analysis. To account for multiple comparisons in our statistical testing procedures, we calculated and report false discovery rate (FDR; ref. 30)–adjusted *P* values.

We conducted a joint effect analysis to test the hypothesized dose–response relationship between SNP genotype on NCF, by adding up the number of at-risk alleles of the significant SNPs identified from the main effects analysis. At-risk alleles were defined as the minor allele of the risk SNPs and the common allele as the protective SNPs. Unless otherwise specified, SNPs significantly associated with NCF at the FDR-adjusted $P \leq 0.05$ in the main effects analyses were included in the multivariable regression models, along with clinical risk factors. Furthermore, we conducted multivariable regression models that included the significant clinical risk factors ($\alpha \leq 0.05$) and the SNPs identified from the individual SNP analysis. Finally, using stepwise minimization of the AIC, we built the most parsimonious models. All analyses were adjusted for age at the time of neurocognitive testing, education, tumor location, gender, and histology. All analyses were performed using SAS 9.2 software (SAS Institute).

Results

Patient characteristics and NCF performance

Demographic and clinical characteristics of the 233 participants are listed in Table 2. Most patients were diagnosed with a grade 4 glioma (53.65%), mean age at diagnosis was 45.7 years (median, 47 years; SD, 12.9 years), and 154 were men (66.1%). The sociodemographic and clinical characteristics of the patients included in this analysis were not different from those of the patient population included in the parent epidemiologic study (Supplementary Table S1).

NCF performance and rates of impairment on NCF tests are summarized in Table 3. Patients demonstrated significantly elevated rates of impairment in memory (HLVT-R Total Recall = 51%), executive function (TMTB = 34%; COWA = 20%), and processing speed (TMTA = 27%), compared with healthy controls from published normative data.

Individual SNP main effects on NCF

Of the 10,967 SNPs analyzed, 18 were significantly associated with processing speed as measured by TMTA and 12 SNPs were significantly associated with executive function as measured by TMTB (FDR-adjusted $P \leq 0.05$). No significant differences at the FDR-adjusted $P \leq 0.05$ level was found for verbal memory as measured by HLVT-R or executive function as measured by COWA. Only one SNP (DNA repair pathway gene *RAD51L1*) was identified as a potential mediator of HVLT-R ($0.05 < \text{FDR} < 0.1$), and two SNPs (telomerase pathway genes, *MCPH1* and *TANK*) showed marginal associations with COWA (Table 3).

The genotype distributions of these 33 significant SNPs are summarized in Table 4. At the very low FDR level of 0.001, six SNPs remained associated with processing speed (TMTA), and four SNPs remained associated with executive function (TMTB). The strongest association for TMTA was *IRS1* rs6725330 ($P = 2.5 \times 10^{-10}$; $P_{\text{adjusted}} = 1.2 \times 10^{-6}$), for TMTB was *NOS1* rs11611788 ($P = 1.8 \times 10^{-8}$; $P_{\text{adjusted}} = 8.6 \times 10^{-5}$); both *IRS1* and *NOS1* were from the inflammation pathway. Several other SNPs demonstrated strong associations with TMTA,

Table 2. Sociodemographic and clinical characteristics of patients with NCF test ($N = 233$)

Characteristic	Frequency	Percent
Age at the time of NCF testing, y		
Median	47	
Range	19–75	
Gender		
Male	154	66.1
Female	79	33.9
Education, y		
Median	15	
Range	8–20	
Histology[a]		
Grade 2	21	9.0
Grade 3	84	36.0
Grade 4	125	53.7
Unclassified	3	1.3
Steroid use at baseline		
No	100	42.9
Yes	123	52.8
Unknown	10	4.3
Antiepileptic drugs use at baseline		
No	52	22.3
Yes	173	74.3
Unknown	8	3.4
Treatment[b]		
No surgery	209	89.7
Biopsy	24	10.3
Tumor location (lobe with tumor)		
Frontal	106	45.5
Temporal	75	32.2
Parietal	36	15.4
Other (thalamus/ganglia, occipital, brainstem, cerebellum, or ventricular)	16	6.9
Hemisphere		
Right	80	34.3
Left	134	57.5
Other (bilateral, midline, multi-hemisphere, or other)	19	8.2

[a]Grade 4, glioblastoma, and gliosarcoma; grade 3: anaplastic oligodendroglioma and astrocytoma; grades 2: oligodendroglioma, not-otherwise-specified astrocytoma, and mixed glioma.
[b]These are presurgical cases.

Table 3. Descriptive characteristics of patients' NCF performance

	Memory (HVLT-R)	Processing Speed (TMTA)	Executive Function (TMTB)	Executive Function (COWA)
Patients, *n*	205	220	210	203
% Impaired[a]	51	27	34	20
Mean *z*-score	−1.76	−1.09	−1.44	−0.46
SD *z*-score	1.68	2.46	2.86	1.106
Median *z*-score	−1.51	−0.39	−0.80	−0.41
Range *z*-score	−6.00 to 1.63	−14.32 to 3.00	−16.00 to 1.89	−2.33 to 2.33

[a]All test scores for each cognitive test were converted to demographically adjusted z-scores using published normative data from healthy controls. Impairment defined as z-score ≤ -1.5.

including *PPARD* rs4713859 ($P = 3.4 \times 10^{-7}$; $P_{adjusted} = 0.0001$) in the inflammation pathway, *ERCC4* rs1573638 ($P = 3.4 \times 10^{-7}$; $P_{adjusted} = 0.0003$) in the DNA repair pathway, and *ABCC1* rs8187858 ($P = 6.6 \times 10^{-7}$; $P_{adjusted} = 0.0001$) and *SLC22A3* rs4708867 ($P = 1.8 \times 10^{-6}$; $P_{adjusted} = 0.0004$) in the metabolism pathway. For TMTB, additional strong associations were found with *IL16* rs1912124 ($P = 6.0 \times 10^{-7}$; $P_{adjusted} = 0.001$) and *MSR1* rs12680230 ($P = 6.0 \times 10^{-7}$; $P_{adjusted} = 0.001$) in the inflammation pathway, and *POLE* rs5744761 in the DNA repair pathway ($P = 6.0 \times 10^{-7}$; $P_{adjusted} = 0.001$).

Table 4. Genetic variants showing strong association with NCF in the single SNP analysis (FDR $P \leq 0.05$)

NCF test and pathway	Gene	SNP ID[a]	Location	Chr: Position	Allele	Raw *P*	FDR *P*[b]	Estimate (β)	*In silico* prediction
HVLT-R									
DNA repair	*RAD51L1*	rs9323505^{D}	Intron	14: 67856540	C/T	3.8×10^{-5}	0.0794	−1.22	TBS
TMTA									
DNA repair	*ERCC4*	rs1573638^{R}	5′ Flanking	16: 13810814	A/G	3.4×10^{-7}	**0.0003**[b]	−11.50	Recombination hotspot, TBS
	NEIL3	rs11131792^{R}	3′ Flanking	4: 178534359	C/T	3.3×10^{-5}	0.0232	−4.29	Recombination hotspot, TBS
	XRCC5	rs207939^{R}	Intron	2: 216750743	A/C	6.8×10^{-5}	0.0285	1.56	TBS
	HUS1	rs3176565^{R}	Intron	7: 47976709	C/T	0.0001	0.0443	−6.37	TBS
	MGMT	rs12253191^{D}	5′ Flanking	10: 131062656	C/T	5.8×10^{-6}	0.0121	−1.60	TBS
Inflammation	*IRS1*	rs6725330^{R}	5′ Flanking	2: 227375101	A/G	2.5×10^{-10}	**1.2×10^{-6}**[b]	−6.58	Recombination hotspot, TBS
	PPARD	rs4713859^{R}	3′ Flanking	6: 35438376	C/T	3.4×10^{-7}	**0.0011**[b]	−11.49	
	MAP3K7	rs12660904^{R}	5′ Flanking	6: 92549554	A/G	1.8×10^{-6}	0.0044	−11.34	TBS
	EGFR	rs10488140^{R}	Intron	7: 55070695	C/T	3.0×10^{-5}	0.0466	−4.33	
Metabolism	*ABCC1*	rs8187858^{R}	Synonymous	16: 16069540	C/T	6.6×10^{-7}	**0.0001**[b]	−8.50	Regulatory region, TBS
	SLC22A3	rs4708867^{R}	Intron	6: 160762715	A/G	1.8×10^{-6}	**0.0004**[b]	−11.34	
	GSR	rs2551698^{R}	Intron	8: 30700119	C/T	5.0×10^{-5}	0.0064	−9.32	TBS
	ABCC1	rs2269800^{R}	Intron	16: 16104340	A/G	0.0002	0.0327	−4.33	TBS
	PPARG	rs2120825^{R}	Intron	3: 12388339	G/T	0.0003	0.0410	−5.81	TBS
Cognitive	*NCAM1*	rs4937786^{R}	5′ Flanking	11: 112258317	A/C	1.2×10^{-5}	0.0117	−3.44	
	DAOA	rs16951986^{R}	3′ Flanking	13: 105315831	A/G	0.0001	0.0443	−1.47	
	DAOA	rs1009697^{R}	5′ Flanking	13: 104775043	C/T	0.0002	0.0443	−4.36	TBS, Conserved element
	DRD1	rs265995^{R}	3′ Flanking	5: 174782552	C/T	0.0002	0.0443	−6.00	TBS
TMTB									
DNA repair	*POLE*	rs5744761^{R}	Intron	10: 131762012	C/T	6.0×10^{-7}	**0.001**[b]	−13.62	TBS
	WRN	rs4398867^{R}	Intron	8: 31139701	A/G	0.0001	0.0430	−12.72	TBS
	RTEL1	rs6011002^{R}	Intron	20: 61768246	A/G	0.0001	0.0430	−10.46	TBS
	UBE2B	rs11242213^{R}	Intron	5: 133747910	G/T	0.0001	0.0430	−8.29	TBS
	WRN	rs13269094^{R}	Intron	8: 31015693	G/T	0.0001	0.0430	−6.41	TBS
Inflammation	*NOS1*	rs11611788^{R}	Intron	12: 116222759	C/T	1.8×10^{-8}	**8.6×10^{-5}**[b]	−8.80	TBS
	IL16	rs1912124^{R}	Intron	15: 79286026	C/T	6.0×10^{-7}	**0.001**[b]	−13.62	TBS
	MSR1	rs12680230^{R}	3′ Flanking	8: 16104334	C/T	6.0×10^{-7}	**0.0012**[b]	−13.62	
	IGF1R	rs1980268^{R}	Intron	15: 97268929	C/T	1.7×10^{-5}	0.0272	−8.59	TBS
Cognitive	*DAOA*	rs323450^{R}	5′ Flanking	13: 104245314	C/T	9.1×10^{-6}	0.0042	−7.35	TBS
	DAOA	rs9300953^{R}	5′ Flanking	13: 104060135	A/G	0.0001	0.0273	−3.16	TBS
	DAOA	rs556281^{R}	5′ Flanking	13: 104072649	A/G	0.0001	0.0273	−3.32	Recombination hotspot, TBS
COWA									
Telomerase	*MCPH1*	rs6999296^{D}	5′ Flanking	8: 6158732	A/C	7.2×10^{-5}	0.0637	−0.69	TBS
	TANK	rs270952^{D}	5′ Flanking	2: 161435957	A/C	0.0002	0.0994	−0.58	TBS

Abbreviations: FDR-adjusted *P* value; TBS, Transfac-binding site.

[a]AIC was used to determine the genetic model for each SNP. D, dominant, R, recessive, genetic model.

[b]SNPs remained noteworthy at the very low FDR level of 0.001.

Table 5. Multivariate analysis and dose effect on NCF performance

	Memory (HVLT-R)		Processing Speed (TMTA)		Executive Function (TMTB)		Executive Function (COWA)	
Characteristic	**Estimate (β)**	***P***	**Estimate (β)**	***P***	**Estimate (β)**	***P***	**Estimate (β)**	***P***
Education	0.18	0.00001	0.15	0.005	0.18	0.008	0.05	0.06
Age	−0.01	0.56	−0.03	0.009	−0.002	0.88	−0.01	0.03
Gender								
Male	Ref.		Ref.		Ref.		Ref.	
Female	0.49	0.03	0.57	0.05	0.57	0.11	−0.21	0.14
Tumor location								
Frontal	Ref.		Ref.		Ref.		Ref.	
Temporal	−0.59	0.76	−0.05	0.87	−0.54	0.15	−0.02	0.94
Parietal	−0.09	0.59	−0.71	0.08	−0.31	0.53	−0.05	0.80
Other	−1.58	0.006	−2.50	3.4×10^{-5}	−1.88	0.03	−0.01	0.97
Hemisphere								
Right	Ref.		Ref.		Ref.		Ref.	
Left	−0.90	0.0001	0.12	0.66	−0.52	0.15	−0.57	0.0001
Other	−0.33	0.49	−0.50	0.39	−0.04	0.95	−0.14	0.66
Histology								
Grade 2	Ref.		Ref.		Ref.		Ref.	
Grade 3	−0.07	0.16	−0.42	0.39	−0.22	0.71	−0.06	0.81
Grade 4	−0.90	0.88	−0.48	0.31	−0.99	0.09	−0.20	0.40
SNP dose effect[a]								
0 at-risk allele	Ref.		Ref.		Ref.		Ref.	
1 at-risk allele	−1.22	3.8×10^{-5}	−1.00	0.01	−2.77	0.0001	−0.61	4.1×10^{-5}
2 at-risk alleles	—	—	−1.78	3.7×10^{-5}	−2.43	0.0041	−1.39	9.5×10^{-7}
≥3 at-risk alleles	—	—	−4.42	6.1×10^{-16}	−10.93	2.0×10^{-16}	—	—

[a]At-risk alleles were defined as the minor allele of the risk SNPs and the common allele of the protective SNPs.

Joint SNP dose effects on NCF

We next assessed the dose-effect of the SNPs from the main effect analysis (Table 3) that were associated with processing speed (TMTA) and executive function (TMTB). We treated the minor allele of each of the risk SNPs (OR > 1) and the common allele as the protective SNPs (OR < 1) as at-risk alleles. Joint effect analysis found significant gene-dosage effects for TMTA ($P_{trend} = 9.4 \times 10^{-16}$) and TMTB ($P_{trend} = 6.6 \times 10^{-15}$), the NCF scores and β estimate values progressively decreased as the number of at-risk genotypes increased (Table 5).

Multivariate model of NCF performance

Table 5 summarizes the results of multivariate regression models and lists estimates of the effect size (β) for each variable on NCF performance. Generally, patients with less education, female gender, older age, temporal or parietal lobe tumor, left hemisphere tumor, higher grade histology, and carriers of more at-risk alleles tended to have worse NCF. Specifically, education and at-risk SNPs effects were seen in significant association with all of the four NCF tests. Gender was a significant predictor for HVLT-R ($P = 0.03$) and TMTA ($P = 0.05$); age was a significant predictor for processing speed (TMTA test $P = 0.009$) and executive function (COWA test $P = 0.03$); however, left hemisphere tumors was significantly associated with impairment of memory (HVLT-R test $P = 0.0001$) and executive function (COWA test $P = 0.0001$). Although not significant, higher tumor grade is correlated with the risk of all the NCF impairments.

Discussion

In our comprehensive pathway-based evaluation of genetic variants associated with glioma patients' neurocognitive performance before surgical resection, we found that NCF was mediated by polymorphisms in genes related to inflammation, DNA repair, and metabolism pathways. For processing speed (TMTA), of the five strongest signals, two were in the inflammation pathway (*IRS1* rs6725330 and *PPARD* rs4713859), two in the metabolism pathway (*ABCC1* rs8187858 and *SLC22A3* rs4708867), and one in the DNA repair pathway (*ERCC4* rs1573638). For executive function (TMTB), of the four the strongest associations, three were in the inflammation pathway (*NOS1* rs11611788, *IL16* rs1912124, and *MSR1* rs12680230), and one in the DNA repair pathway (*POLE* rs5744761). Furthermore, our joint effect results suggest that NCF risk is not only dependent on the effect size of individual SNP but also on the number of "at-risk" alleles.

A major finding in this study was the consistent association of the inflammation pathway genes, *IRS1* rs6725330 and processing speed problems, and *NOS1* rs11611788 and executive dysfunction in glioma patients. *IRS1* (insulin receptor substrate 1) plays crucial roles in the regulation of cognitive performance, and neuroprotection. Aberrant expression of *IRS1* has been associated with pathogenesis and progression of breast cancer and prostate cancer (31–33). *IRS1* dysregulation is highly associated with cognitive decline (negative relationship to episodic and working memory) in Alzheimer's disease patients, and has been proposed as a new therapeutic target for Alzheimer's disease (34). Given these parallel sources of evidence, we suggest that it is likely that this *IRS1* variant exerts an effect on NCF, although more evidence is required. *NOS1* (nitric oxide synthase 1) synthesizes nitric oxide in both the central and peripheral nervous system. Human and animal (35) studies have implicated *NOS1* in both cognition and mental disorders, including schizophrenia susceptibility. The *NOS1* rs6490121 variant identified in a GWAS of schizophrenia has recently been associated with variation in general intelligence, working memory and executive function in both patients and healthy participants (36, 37). Our findings of the association with polymorphisms in executive function are consistent with cognitive studies in both animal models and humans of *NOS1* where a general rather than specific effect on cognition is suggested.

Other promising findings are the association between NCF and DNA repair genes (*ERCC4* and *POLE*) and metabolism genes (*ABCC1* and *SLC22A3*) in glioma patients. *ERCC4* is involved in nucleotide excision repair (NER), and participates in removal of DNA inter-strand cross-links and DNA double-strand breaks. *ERCC4* has been implicated in neurodegeneration and progressive cognitive impairment (38, 39). *POLE* encodes the catalytic subunit of DNA polymerase epsilon, one of the four nuclear DNA polymerases in eukaryotic cells. *POLE* mutations have been recently identified in familial colorectal cancer patients (40) and high-grade glioma (41). *ABCC1 is* involved in the oxidative stress defense and also known as multidrug resistance protein 1 (MRP1) from the brain in many diseases, including stroke, epilepsy, and brain cancer (42). Similarly, *SLC22A3* plays a significant role in the disposition of cationic neurotoxins and neurotransmitters in the brain (43).

In silico analysis using the SNP Function Portal server (44) revealed that both the *IRS1* rs6725330 and *ERCC4* rs1573638 variants are located in recombination hotspots (typically 1–2 kb wide). Recombination is important for evolution and is also highly associated with genome instability, and hotspots are the main contributor of the block-like pattern of LD (haplotype blocks). A SNP in the recombination hotspot region could affect hotspot activity, disrupt the motifs of the hotspot, and lead to chromosomal rearrangements, many of which have been associated with diseases (45–47).

A number of studies have investigated putative associations between cognitive gene polymorphisms and NCF (11–13). However, the number of patients in those studies is often small, and only a very limited number of candidates SNPs have been studied as predictors of NCF. The major strengths of our study are the comprehensive pathway-based approach, the large sample size, and the fact that cases were from a single treatment center with objective, standardized NCF testing prior to surgical resection. The present analysis focuses on the relationship between germline SNPs and presurgical NCF performance (before surgery and adjuvant therapy), which helps us to understand the variability in presentation of patients with glioma and may similarly provide insights into patients at risk for different responses to therapy. To begin to address this possibility, we are conducting a separate analysis of longitudinal NCF outcomes (patients were assessed prior to surgery and during/after adjuvant therapy) in a smaller subset of patients, to reflect changes in NCF over time for each patient.

The primary limitation to our study is the inability to confirm associations for all of the significant polymorphisms. Recruitment of an independent cohort will be necessary to validate the associations we observed in this study, in particular, the inflammation pathways genes. We have no *a priori* reason to believe that germline genetic polymorphisms may be differentially associated with NCF in patients with different tumor histologies. However, our sample is composed primarily of patients with glioblastoma and thus the results may not generalize as well to patients with lower grade tumor. In addition, our models did not include tumor size, which may have an impact on cognitive function. However, we did control for other potential demographic (age, education) and clinical confounders (tumor location, histology) and even with controlling for these factors still found robust genetic associations with NCF. Future studies have the opportunity to resequence and fine map the haplotype blocks for these interesting gene regions followed by functional characterization studies to identify the causal variants to further our understanding of the influence of these genes on NCF in glioma patients. Moreover, a more agnostic approach to the genotyping and risk prediction analysis not based on a pathway approach may reveal previously unknown genetic associations with NCF that could further explain variation in NCF. Our findings of genetic variants associated with NCF in glioma patients have implications for clinical practice and could allow for the development of new neuroprotective therapies to reduce neurocognitive dysfunction, and improve QOL.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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