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Antioxidant enzyme polymorphisms and neuropsychological outcomes in medulloblastoma survivors: a report from the Childhood Cancer Survivor Study

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Psychological or neurocognitive impairment is often seen in medulloblastoma survivors after craniospinal radiation; however, significant variability in outcomes exists. This study investigated the role of antioxidant enzyme polymorphisms in moderating this outcome and hypothesized that patients who had polymorphisms associated with lower antioxidant enzyme function would have a higher occurrence of impairment. From the Childhood Cancer Survivor Study (CCSS) cohort, 109 medulloblastoma survivors and 143 siblings were identified who completed the CCSS Neurocognitive Questionnaire (NCQ) and the Brief Symptom Inventory-18 (BSI-18) and who provided buccal DNA samples. Real-time polymerase chain reaction (PCR) allelic discrimination was used for *SOD2* (rs4880), *GPX1* (rs1050450), and *GSTP1* (rs1695 and rs1138272) genotyping and PCR for *GSTM1* and *GSTT1* gene deletions. Outcomes on NCQ and BSI-18 subscale scores were examined in association with genotypes and clinical factors, including age at diagnosis, sex, and radiation dose, using univariate and multivariate analysis of variance. Patients <7 years of age at diagnosis displayed more problems with task efficiency ($P < .001$) and fewer problems with somatic complaints ($P = .004$) than did patients ≥7 years of age. Female patients reported more organization problems than did male patients ($P = .02$). Patients with homozygous *GSTM1* gene deletion reported higher anxiety (mean null genotype = 47.3 ± 9.2, non-null = 43.9 ± 7.8; $P = .04$), more depression (null = 51.0 ± 9.8, non-null = 47.0 ± 9.4; $P = .03$), and more global distress (null = 50.2 ± 9.7, non-null = 45.2 ± 9.9; $P = .01$). All associations for the *GSTM1* polymorphism remained statistically significant in a multivariate model controlling for age, sex, and radiation dose. Homozygous *GSTM1* gene deletion was consistently associated with greater psychological distress in medulloblastoma survivors across multiple domains, suggesting that this genotype may predispose patients for increased emotional late effects.

Keywords: Childhood Cancer Survivor Study, glutathione S-transferase polymorphisms, medulloblastoma, neuropsychological impairment, radiation therapy.

Medulloblastoma is the most common malignant central nervous system (CNS) tumor seen in children and adolescents, accounting for approximately 20% of all new CNS tumor diagnoses.[1] With advances in chemotherapy and radiation therapy, 60–80% of patients can now achieve long-term

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survivorship.[2,3] However, a substantial number have significant late effects, including endocrinopathies, hearing loss, renal failure, second malignancies, and neuropsychological impairment.[3–6] Children with CNS tumors, particularly those treated with cranial radiation therapy (CRT) are at risk for development of serious neuropsychological problems. Progressive deficits in overall cognitive functioning (IQ), attention, memory, processing speed, and executive function are seen in 50%–60% of survivors.[7,8] Younger age at the time of treatment, radiation dose, volume of brain irradiated, longer time since treatment, and female sex are associated with increased risk for poor neuropsychological outcome.[7–9] In addition, survivors of childhood CNS tumors demonstrate higher levels of depression and psychological distress than do sibling control subjects.[10]

These residual neurocognitive and psychological effects can have a profound impact on a survivor's quality of life, school performance, and achievement of major adult milestones. For example, recent reports from the Childhood Cancer Survivor Study (CCSS) found that long-term survivors of a CNS tumor are at increased risk for lower educational attainment, unemployment, lower income, dependent living status (e.g., living with parents), and fewer marriages during adulthood.[11–15]

Among patients treated with similar protocols, there is significant variation in the occurrence and severity of neuropsychological late effects. We hypothesized that genetic polymorphisms affecting a patient's ability to respond to radiation-induced oxidative stress and subsequent tissue injury may explain, in part, differences in susceptibility to these late effects. In this study, we investigated several common polymorphisms that may affect antioxidant enzyme activity and have been implicated in increased risk for treatment toxicities due to presumed increased oxidative damage. Manganese superoxide dismutase (encoded by *SOD2*) acts as one of the most effective intracellular antioxidants by converting superoxide anions to hydrogen peroxide and oxygen.[16,17] A single nucleotide polymorphism (SNP) affecting the targeting sequence of the gene leads to greater enzyme activity (rs4880, *47T > C*).[18,19] Glutathione peroxidase 1 (encoded by *GPX1*) is a major enzyme in the metabolism of hydrogen peroxide generated by superoxide dismutase. A well-characterized SNP (rs1050450, *599C > T*) results in an amino acid change from proline to leucine, which is associated with greater enzyme activity.[20,21] Glutathione-S-transferases (GSTs) are a group of enzymes involved in the metabolism of free radicals, products of lipid oxidation, and chemotherapy agents used in the treatment of medulloblastoma.[22–25] In the case of *GSTM1* and *GSTT1,* deletion of the gene is common, leading to no functional enzyme. *GSTP1* is characterized by 2 different SNPs resulting in amino acid substitutions that lead to steric changes at the substrate-binding site of the enzyme (rs1695, *1404A > G [exon 5]*, rs1138272, *2294C > T [exon 6]*).[26] On the basis of the function of these enzymes, we aimed to examine associations between these antioxidant polymorphisms and neuropsychological impairment and psychological distress, measured by 2 validated questionnaires in childhood medulloblastoma survivors participating in the CCSS.

Methods

Participants

Subjects for the current study are participants in the Childhood Cancer Survivor Study (CCSS). The complete cohort and study design have been previously described.[27,28] In brief, the CCSS is a multi-institutional retrospective cohort of children and adolescents treated for cancer from 1970 through 1986 who have been followed up longitudinally since 1992. Eligibility criteria included diagnosis of childhood cancer prior to age 21 years and survival of at least 5 years after diagnosis. The CCSS protocol was reviewed and approved by the institutional review boards of the participating institutions. Participants provided informed consent for survey data collection, medical record abstraction, and banking of DNA. The current study was approved by the Baylor College of Medicine Institutional Review Board, where the genotyping was performed.

The current study was limited to CCSS participants who met the following eligibility criteria: (1) diagnosis of medulloblastoma; (2) availability of buccal cell DNA sample; (3) receipt of craniospinal radiation therapy, with treatment records available for radiation dosimetry; (4) no history of relapse or second malignancy requiring additional cranial radiation therapy; and (5) completion of the neurocognitive and psychological outcome measures as described below and included in the 2003 follow-up survey. Participants in the CCSS sibling cohort, selected from a sample of participating survivors, who had (1) provided buccal cell DNA samples and (2) completed the 2003 follow-up survey, were also included in this study as a comparison group. On the basis of the aforementioned criteria, 109 medulloblastoma survivors and 143 siblings were eligible to be included in the current study.

Demographic and Treatment Information

Sex, race, age at survey completion, employment status, and highest educational level were obtained from participant self-report. Medical records were abstracted using a standardized protocol for treatment information including: age at diagnosis, chemotherapy agents, and radiation therapy. Radiation dosage was quantified specifically for 4 segments of the brain: (1) posterior fossa, (2) temporal lobes, (3) frontal lobes, and (4) parietal/occipital lobes. Maximum radiation to each segment was determined by central review of radiation oncology records from the treating facility.[29] All questionnaires and data abstraction forms are available on the CCSS Web site (http://ccss.stjude.org).

Buccal Cell DNA Collection, Extraction, and Storage

Methods for DNA collection, extraction, and storage have been described previously.[30,31] Collection kits were mailed to eligible participants, who were instructed on how to collect and return mouthwash samples in the provided container. DNA was extracted using the Gentra Puregene kit (Qiagen). Extracted DNA was stored in Qiagen AE buffer at −80°C until used for genotyping.

Genotyping

The polymorphisms investigated are listed in Table 1. For *SOD2, GPX1,* and *GSTP1* genotyping, real-time polymerase chain reaction (PCR) TaqMan-based 5′nuclease allelic discrimination assays (Applied Biosystems) were used. Commercially predesigned assays were used for the genotyping of *SOD2* (Assay ID C_8709053_10) and *GSTP1* (Assay IDs C_3237198_20 and C_1049615_20). For the *GPX1* assay, validated primer and minor groove binder probe sequences available on the National Cancer Institute's SNP500Cancer Database (http://variantgps.nci.nih.gov/cgfseq/pages/snp500project.do) were custom manufactured (Applied Biosystems). Sequences of the primers and probes used were as follows: 5′-CATCGAAGCCCTGCTGTCT-3′ (forward primer), 5′-CACTGCAACTGCCAAGCA-3′ (reverse primer), 5′ VIC- ACAGCTGGGCCCTT-MGB-3′ (C-specific allele probe), and 5′ FAM- ACAGCTGAGCCCTT-MGB-3′ (T-specific allele probe). Each 25 μL reaction included 5 ng of genomic DNA, 12.5 μL of 2× TaqMan Genotyping Master Mix, and 1.25 μL of the respective 20× genotyping assay mix (primer concentration 18 μM and probe concentration 4 μM), and DNase-free water. All DNA samples were diluted to a uniform concentration prior to genotyping. Genotyping was performed according to manufacturer-recommended thermocycling conditions on a CFX96 Real-Time PCR Detection System (Bio-Rad). Samples were run in duplicate with no template, positive, and negative controls on each 96-well plate. Genotype for each sample was determined by allelic discrimination with review of the scatter plots of major allele relative fluorescent units versus minor allele relative fluorescent units.

Multiplex PCR was used to amplify *GSTM1* and *GSTT1* simultaneously.[32] In brief, 50 μg of DNA was amplified using *GSTM1* primers 5′-GAA CTC CCT GAA AAG CTA AAG C-3′ and 5′-GTT GGG CTC AAA TAT ACG GTG G-3′ and *GSTT1* primers corresponding to the 3′ coding region of human cDNA: 5′-TTC CTT ACT GGT CCT CAC ATC TC-3′ and 5′-TCA CCG GAT CAT GGC CAG CA-3′. As an internal control, the dihydrofolate reductase gene (*DHFR*) was co-amplified using the primers 5′-GCA TGT CTT TGG GAT GTG GA-3′ and 5′-GGA ATG GAG AAC CAG GTC TT-3′. These PCRs were performed on a GeneAmp2700 system (Applied Biosystems). The PCR conditions consisted of an initial melting temperature of 95°C (5 min), followed by 35 cycles of melting (95°C, 30 s), annealing (58.3°C, 45 s), and extension (72°C, 1 min), followed by a final extension step at 72°C for 10 min. We then viewed the PCR products from co-amplification of *GSTT1* (480 bp), *DHFR* (280 bp), and *GSTM1* (215 bp) with ethidium bromide–stained 10% polyacrylamide gels for the presence or absence of *GSTM1* and *GSTT1* genes. *DHFR* was used as an internal control to ensure that there was amplifiable DNA in the sample in the case of a double-null genotype.

Genotyping was inconclusive in 1 subject for *SOD2*, *GSTM1,* and *GSTT1* and 10 subjects for *GPx1*. For all assays, a 10% random sample of subjects were repeated for quality control with agreement between the initial results and the results of the samples repeated for quality control with the exception of 1 subject for *GSTM1* and *GSTT1*.

Neuropsychological Functioning

Two validated, self-report questionnaires administered in the 2003 follow-up survey to survivors and siblings were used to assess level of neurocognitive impairment and psychological distress.

The CCSS Neurocognitive Questionnaire (CCSS-NCQ) consists of 25 questions designed to assess self-report of neurocognitive function. Participants rated the extent to which they had experienced problems over the previous 6 months using a Likert scale ranging from 1 ("never a problem") to 3 ("often a problem"). Recent analysis of the CCSS-NCQ reveals it to reliably assess 4 neurocognitive factors: task efficiency (attention and

Table 1. Study polymorphisms

Gene	SNP rs number	Polymorphism	Amino Acid Change	Predicted At-Risk Genotype
SOD2	rs4880	*47T > C*	*Val16Ala*	*C47T and T47T*
GPX1	rs1050450	*599C > T*	*Pro198Leu*	*C599T and T599T*
GSTM1	N/A	*Gene Deletion*		*GSTM1 Null* (homozygous deletion)
GSTT1	N/A	*Gene Deletion*		*GSTT1 Null* (homozygous deletion)
GSTP1	rs1695	*1404A > G (exon 5)*	*Ile105Val*	*GSTP1 Not AA*[a]
	rs1138272	*2294C > T (exon 6)*	*Ala114Val*	

[a]*GSTP1* characterized by 2 SNPs with 4 recognized alleles, *GSTP1*A* is *Ile* at position 105 and *Ala* at position 114.

processing speed), memory (working and long-term), emotional regulation (emotional lability), and organization (organization of one's environment).[33] These 4 factors accurately discriminated survivors who were considered to be at high risk for neurocognitive dysfunction, from healthy low-risk survivors and siblings in a prior CCSS study[33] and have been shown to be sensitive to cranial radiation in a dose-dependent fashion.[34]

The Brief Symptom Inventory–18 (BSI-18) is an 18-item screening questionnaire designed to assess acute symptoms of depression, anxiety, and somatic complaints. Participants described the extent to which they have been distressed or bothered by each symptom in the previous 7 days using a Likert scale ranging from 1 ("not at all") to 5 ("extremely"). Responses to all 18 items were summed to yield the Global Severity Index (GSI).[35]

Statistical Analysis

For each polymorphism, the allele associated with decreased enzyme activity in the literature was considered to be the at-risk allele. With use of a dominant model, the genotypes predicted to be at risk are shown in Table 1. Outcome measures were the continuous subscale scores from the CCSS-NCQ and BSI-18. Analysis of variance (GLM procedure, SAS, version 9.2; SAS Institute) was used for univariate and multivariate analyses, comparing mean CCSS-NCQ subscale scores, BSI-18 subscale scores, or GSI T scores. Independent variables included genotype, age at diagnosis (<7 years of age vs. ≥ 7 years of age), sex, and segment dose level (<36 Gy vs. ≥ 36 Gy for temporal, frontal, and parietal segments). Because segment exposures were highly correlated, we fitted the multivariable model for one segment at a time. Posterior fossa radiation doses were not included in these multivariate analyses because of the homogeneity of exposure doses, with only 5 survivors (4.9%) receiving <36 Gy to the posterior fossa. A 2-sided P value of $<.05$ was considered to be statistically significant in all analyses.

Results

Characteristics of the Study Population

Demographic, treatment, and genotypic characteristics of the childhood medulloblastoma survivors and their siblings are shown in Table 2. The subjects were predominantly white. The sibling group was older ($P < .001$) and more educated ($P = .03$) than the survivor group. Genotype frequencies for *SOD2, GPx1, GSTM1, GSTP1,* and *GSTT1* polymorphisms were not different between survivors and siblings.

Neurocognitive Impairment

Data for the CCSS-NCQ were available for 108 survivors and for 142 siblings. Survivors had significantly higher mean scores (worse functioning) on both Task Efficiency and Memory subscales ($P < .001$), compared with siblings, but no difference was found in Emotional Regulation or Organization subscales between survivors and siblings. There were no statistically significant differences in mean scores between genotypes on any of the CCSS-NCQ subscales for the survivors. However, survivors <7 years of age at diagnosis had higher mean scores (worse functioning) on the Task Efficiency subscale than did survivors ≥ 7 years of age at diagnosis (mean $\pm$ SD: 18.6 ± 4.32 vs. 14.1 ± 4.49, $P < .001$). This association was still significant ($P < .001$) in multivariate models controlling for genotype, sex, and radiation dose to each segment. Female survivors reported worse functioning on the Organization subscale, compared with male survivors (5.0 ± 1.87 vs. 4.3 ± 1.41, $P = .02$). In multivariate models, sex was still significant ($P < .01$) after controlling for age, genotype, and radiation dose to each segment.

For the sibling group, those with the *GSTM1* null genotype had higher scores on the Emotional Regulation subscale than did those with *GSTM1* non-null genotype (5.4 ± 1.77 vs. 4.8 ± 1.46, $P = .03$). There were no statistically significant differences in mean scores between any other genotypes on any of the CCSS-NCQ subscales for the siblings. In addition, there were no genotype variables associated with scores on the other 3 CCSS-NCQ subscales.

Psychological Impairment

Data for the BSI-18 were available for all 109 survivors and for 143 siblings. No statistically significant difference was identified in depression, anxiety, and somatic complaints or global severity between survivors and siblings. Results from the univariate analyses are shown in Fig. 1. Survivors with the *GSTM1* null genotype had higher mean scores for Anxiety (Mean $\pm$ SD: 47.3 ± 9.17 vs. 43.9 ± 7.76, $P = .04$), Depression (51.0 ± 9.83 vs. 47.0 ± 9.36, $P = .03$), Global Severity (50.2 ± 9.73 vs. 45.2 ± 9.87, $P = .01$), and Somatic Complaints (51.1 ± 8.64 vs. 48.3 ± 8.69, $P = .1$), compared with *GSTM1* non-null survivors.

The results of the multivariate model including radiation exposure to the temporal lobe, which was the only significant segment in univariate analyses among all segments, are shown in Table 3. Radiation doses to the other segments were not significant predictors of BSI-18 subscale scores at the univariate level and were, therefore, not examined in multivariate models. The *GSTM1* null genotype remained a significant predictor of increased anxiety, depression, and global distress scores after controlling for age, sex, and radiation dose to the temporal lobes in a multivariate model. There were no interactions found between *GSTM1* genotype and age, sex, or radiation dose. No other genotypes demonstrated statistically significant differences in mean scores on any BSI-18 subscale. For the sibling group, there were no statistically significant associations

Table 2. Descriptive characteristics of the medulloblastoma survivors and sibling participants

Characteristic	Medulloblastoma Survivors ($n = 109$)	Siblings ($n = 143$)	P value[a]
Gender			
Male	56 (51.4%)	65 (45.5%)	NS
Female	53 (48.6%)	78 (54.5%)	
Age at time of questionnaire completion (yrs)			
Mean (SD)	30.9 (6.06)	34.3 (9.00)	.0004
Range	18.6–43.7	18.9–54.6	
Ethnicity			
Caucasian	98 (89.9%)	131 (91.6%)	NS
Other	11 (10.1%)	5 (3.5%)	
Missing	0 (0%)	7 (4.9%)	
Highest education level			
Less than 12th grade	7 (6.4%)	5 (3.5%)	.0002
High school graduate	32 (29.4%)	15 (10.5%)	
Further education	68 (62.4%)	123 (86.0%)	
Unknown	2 (1.8%)	0 (0%)	
Age at diagnosis (yrs)			
Mean (SD)	8.0 (0.41)	N/A	
Range	0.6–18.3		
Time since diagnosis at time of questionnaire completion (yrs)			
Mean (SD)	22.8 (4.22)	N/A	
Range	16.5–32.5		
Received Chemotherapy			
Yes	53 (48.6%)	N/A	
No	51 (46.8%)		
Unknown	5 (4.6%)		
Radiation Doses (Gy) Mean (SD)			
Posterior Fossa (Segment 1)	50.4 (6.01)	N/A	
Temporal (Segment 2)	43.1 (9.53)		
Frontal (Segment 3)	36.9 (7.64)		
Parietal/Occipital (Segment 4)	40.1 (8.21)		
SOD2 (47T > C)			
TT	21 (19.3%)	34 (23.8%)	NS
CT	50 (45.9%)	78 (54.5%)	
CC	37 (33.9%)	31 (21.7%)	
Missing	1 (0.9%)	0	
GPX1 (599C > T)			
CC	50 (45.9%)	71 (49.7%)	NS
CT	47 (43.1%)	58 (40.5%)	
TT	8 (7.3%)	8 (5.6%)	
Missing	4 (3.7%)	6 (4.2%)	
GSTP1(1404A > G and 2294C > T)			
AA	45 (41.3%)	60 (42.0%)	NS
Non AA	64 (58.7%)	83 (58%)	
GSTM1 (Gene deletion)			
Null	51 (46.8%)	71 (49.6%)	NS
Non-null	57 (52.3%)	72 (50.4%)	
Missing	1 (0.9%)	0	
GSTT1 (Gene deletion)			
Null	19 (17.4%)	21 (14.7%)	NS
Non-null	89 (81.7%)	122 (85.3%)	
Missing	1 (0.9%)	0	

[a]*P*-value was based on those participants without missing data.

found between any of the polymorphisms and the BSI-18 subscales.

For the Somatic Complaints subscale, survivors aged ≥ 7 years at diagnosis had higher T scores than did those aged <7 years (51.5 ± 8.62 vs. 46.6 ± 8.13, $P = .004$). This association between Somatic Complaints and age remained statistically significant ($P < .01$) across models controlling for sex and radiation dose to each segment.

Discussion

Currently, age at the time of radiation therapy, radiation dose, volume of brain irradiated, longer time since treatment, and female sex are the main identified risk factors for neuropsychological impairment following radiation therapy in survivors of CNS malignancies. However, even among patients with these known risk factors, significant variation in levels of impairment remains and little is known to more precisely identify patients at greater risk for radiation-induced late effects at the time of diagnosis when treatment modifications or early interventions might reduce or prevent such

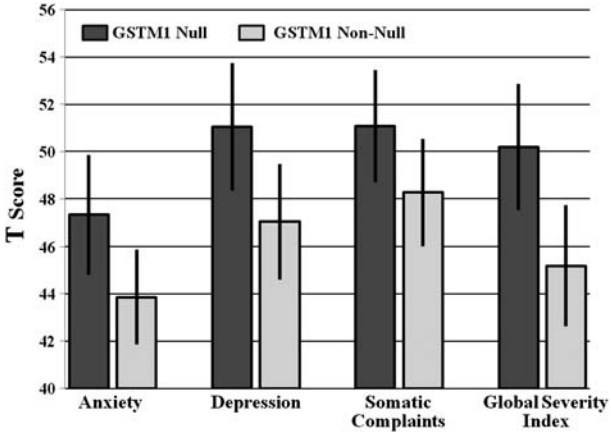

Fig. 1. BSI-18 subscale scores by *GSTM1* genotype.

Table 3. Multivariate results for selected domains for BSI-18 scores and radiation dose to the temporal lobes

Variable	BSI-18 Scale					
	Anxiety		Depression		Global Severity Index	
	Estimate	*P*	Estimate	*P*	Estimate	*P*
GSTM1						
Non-null	−3.29	.05	−3.85	.04	−4.53	.02
Null	Referent		Referent		Referent	
Age						
<7 years	−0.64	.71	0.51	.79	−2.61	.20
≥7 years	Referent		Referent		Referent	
Gender						
Male	1.00	.56	0.55	.77	0.10	.96
Female	Referent		Referent		Referent	
Radiation dose						
≥36 Gy	−2.21	.26	−4.50	.04	−3.19	.16
<36 Gy	Referent		Referent		Referent	

effects. Prior studies have found that genetic polymorphisms leading to enzyme functioning differences in GSTs[36] and catechol-O-methyltransferase[37] are associated with neurocognitive changes following cancer therapy, suggesting that enzymes that clear reactive species or are involved in neurotransmitter metabolism may explain some of this variation in occurrence of late effects.

The most notable finding from this study was a consistent association of *GSTM1* null genotype with increases in psychological distress across the domains of anxiety, depression, and global distress scores, compared with patients with the *GSTM1* non-null genotype. Although the effects were modest, *GSTM1* null genotype remained a significant independent predictor of increased distress in a multivariate model controlling for age at diagnosis, sex, and radiation dosage. This association is particularly interesting because it complements the findings of Barahmani et al., which demonstrated that *GSTM1* null genotype was associated with significant declines in full-scale, performance, and verbal intelligence quotient (IQ) scores, compared with *GSTM1* non-null genotype in childhood medulloblastoma patients following radiation therapy.[36] These results taken together suggest that *GSTM1* null genotype may potentially be a predictor for increased risk of neuropsychological impairment resulting from medulloblastoma therapy.

In contrast to the findings of Barahmani et al., we did not find significant associations between genetic polymorphisms and neurocognitive functioning on the CCSS-NCQ. However, we feel that it would be premature to conclude that there are no effects from the investigated polymorphisms on neurocognitive functioning, given the relatively small sample size and homogeneity of cranial radiation doses. In the prior study, patients received risk-adapted radiotherapy and global IQ was used as the outcome measure. In this study, all patients received high doses of craniospinal irradiation and neurocognitive functioning was assessed on a self-report measure that assesses attention, memory, and executive functions (CCSS-NCQ). The specific neurocognitive functions may demonstrate lower thresholds for radiation-induced impairment compared to global IQ. These factors could have overshadowed any effect of the polymorphisms on neurocognitive functioning. Thus, such associations should be further explored in a contemporary sample of medulloblastoma patients receiving risk-adapted radiotherapy, and outcomes should include variables of global and specific abilities.

It is unknown which antioxidant enzymes are most significant in the metabolism of reactive species generated by radiation. Glutathione S-transferases (GSTs) belong to a family of enzymes that catalyze the glutathione conjugation of a variety of compounds, including cytotoxic drugs, their metabolites, and reactive species generated by radiation.[38] In particular, these enzymes catalyze the detoxification of alkylating agents and platinum compounds that are used in medulloblastoma chemotherapy.[39–42] In 42%–60% of the white population, *GSTM1* is deleted and affected individuals do not have expression of the GSTM1 enzyme.[32,43] It is possible that this enzyme is of greater importance in the protection of the brain against damage from free radicals, compared with the other investigated enzyme systems, because of its function in processing both radiation- and chemotherapy-induced free radicals. Previous studies have suggested a neuroprotective role of *GSTM1*, such as a direct antioxidant effect against reactive metabolites from endogenous and exogenous toxins, which damage dopaminergic cells, leading to neurodegenerative processes.[44–46] These findings offer support for *GSTM1* null genotype as a marker of increased susceptibility to neurotoxins, suggesting that functional GSTM1 enzyme is important in the protection of the brain, whether the toxins are generated from radiation, chemotherapy, or a combination of the 2.

Results from this study were consistent with prior studies demonstrating that younger age and female sex are risk factors for worse functioning. On the CCSS-NCQ, patients who were younger at diagnosis reported greater difficulties with task efficiency and female patients reported greater difficulties with organization.

In a larger sample of all CNS tumor survivors from the CCSS cohort, Armstrong et al. demonstrated that radiation exposure to the temporal lobe is related to neurocognitive and social deficits.[34] Therefore, our finding that only radiation dosage to the temporal lobes was associated with differences in Depression scores on the Brief Symptom Inventory was expected. Although radiation dose to the temporal lobes was significantly associated with Depression scores, there was considerable homogeneity in radiation dosages in this small sample, which limits the conclusions that can be drawn from this finding. However, it does suggest that exposure of the temporal lobes to radiation may affect one's functioning.

There are limitations of this present study, which may affect interpretation of the findings. In 22% of survivors,

the questionnaires were completed by a proxy. Second, there are no data on the survivors' neuropsychological functioning prior to diagnosis, which would allow evaluation of the patients' cognitive reserve prior to therapy and change in functioning after therapy. Third, survivors in the CCSS with a college education were more likely to return DNA specimens; therefore, it is possible that our sample was biased towards more educated survivors who most likely did not have as significant impairment following therapy.[31] In addition, factors such as neurological deficits from surgery and post-operative cerebellar mutism, could be important risk factors, but such information is not available from the CCSS data.

Although limited by small sample size, findings from this pilot study, in association with prior findings, suggest the *GSTM1* null genotype may be a significant risk factor for neuropsychological impairment in patients being treated for medulloblastoma. A validation study is needed to examine the antioxidant pathways in a larger sample of a more recently treated cohort of patients who underwent formal neuropsychological testing. After at-risk polymorphisms are identified, testing at the time of diagnosis could provide an opportunity to intervene starting at the time of diagnosis. Through the identification of markers of increased susceptibility, it will hopefully be possible to prevent some of the late effects affecting medulloblastoma survivors through individualized treatment approaches, including possible radiation dose reductions, treatment modifications, or prevention strategies, such as treatment with free radical scavenging agents,[47] cognitive-behavioral therapy, or educational interventions.

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