Glutathione *S*-transferase *M1* and *T1* polymorphisms may predict adverse effects after therapy in children with medulloblastoma

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Glutathione *S*-transferases (GSTs) are polymorphic enzymes that catalyze the glutathione conjugation of alkylating agents, platinum compounds, and free radicals formed by radiation used to treat medulloblastoma. We hypothesized that *GST* polymorphisms may be responsible, in part, for individual differences in toxicity and responses in pediatric medulloblastoma. We investigated the relationship between *GSTM1* and *GSTT1* polymorphisms and survival and toxicity in 42 children with medulloblastoma diagnosed and treated at the Texas Children's Cancer Center. We conducted Kaplan-Meier analyses to determine if the *GST* polymorphisms were related to progression-free survival (PFS) and performed logistic regression to explore associations between *GST* polymorphisms and occurrence of grade 3 or greater (≥Gr 3) myelosuppression, ototoxicity, nephrotoxicity, neurotoxicity, and intellectual impairment. Patients with at least one null genotype had a 4.3 (95% confidence interval, 1.1–16.8), 3.7 (1–13.6), and 6.4 (1.2–34) times increased risk for any ≥Gr 3 toxicity, any ≥Gr 3 toxicity excluding peripheral neuropathy, and any ≥Gr 3 toxicity requiring omission or cessation of chemotherapy, respectively. Compared with all others, patients with at least one null genotype had, on average, 27.2 ($p = 0.0002$), 29 ($p = 0.0004$), and 21.7 ($p = 0.002$) lower full-scale, performance, and verbal intelligence quotient (IQ) scores, respectively. *GSTM1* and *GSTT1* polymorphisms may predict adverse events, including cognitive impairment after therapy, in patients with medulloblastoma. A larger study to validate these findings is under way. *Neuro-Oncology 11, 292–300, 2009 (Posted to Neuro-Oncology [serial online], Doc. D07-00213, October 29, 2008. URL http://neuro-oncology.dukejournals.org; DOI: 10.1215/15228517-2008-089)*

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Medulloblastoma is the most common CNS malignancy in childhood and adolescence, accounting for approximately 20% of all primary pediatric brain tumors.[1] Surgical resection fol-

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lowed by craniospinal radiation and chemotherapy is an effective standard of care.[2–6] Overall, 60%–80% of these patients achieve long-term cure; however, many suffer from varying degrees of significant morbidity secondary to therapy, including intellectual impairment, hearing loss, and renal failure.[5–8] However, patients who will benefit from therapy and those who will experience significant side effects cannot be distinguished in advance, giving no opportunity for tailoring treatment to maximize outcome.

Glutathione *S*-transferases (GSTs) belong to a family of isoenzymes that catalyze the glutathione conjugation of a variety of electrophilic compounds, including carcinogens, mutagens, cytotoxic drugs and their metabolites, and products of reactive oxidation.[9] They catalyze detoxification of alkylating agents and platinum compounds that are used in medulloblastoma chemotherapy.[10–13] Furthermore, GSTs also detoxify free oxygen radicals formed spontaneously or by chemotherapy drugs and radiation and can sequester alkylating agents and steroids by direct binding.[9] They are highly heterogeneous proteins expressed in virtually all tissues, including the brain.[14] Polymorphism in *GSTM1* was reported by Board,[15] who described three alleles: *GSTM1*0*, *GSTM1*A*, and *GSTM1*B*. In the common *GSTM1*0* allele, *GSTM1* is deleted, and homozygotes (null genotype), comprising 42%–60% of the white population, do not express GSTM1 protein.[16,17] *GSTT1* is polymorphic, and 13%–26% of the white population has a homozygous deletion and thus lacks function.[16,18] Polymorphisms in the GST family of enzymes have been associated with survival and occurrence of toxicity in children and adults who have leukemia, lymphoma, or glioma; breast, lung, ovarian, gastric, or colorectal cancers; or germ cell tumors.[19–38]

In this pilot study, we examined the relationship between polymorphisms in the *GST* genes and clinical outcomes in 42 patients with medulloblastoma. We hypothesized that patients who have *GST* genotypes that encode for high-activity enzymes would have poorer survival and decreased incidence of adverse effects compared with patients with genetically determined, low or nonfunctioning detoxification activity. We measured the association between *GSTM1* and *GSTT1* polymorphisms and the following clinical outcomes: progression-free survival (PFS), development of grade 3 or greater (≥Gr 3) toxicity requiring dose modification, and intellectual impairment. We observed a significant relation between combined *GSTM1T1* polymorphisms and development of any ≥Gr 3 toxicity requiring dose modification and intellectual decline. In the future, after further testing in a larger population, findings from this study and others are expected to form the foundation to develop tailored individualized treatment regimens and prevention strategies for adverse effects.

Materials and Methods

Patient Population and Data Collection

We identified 42 patients who were consecutively diagnosed and treated at the Texas Children's Cancer Center between 1996 and 2005 who were younger than 19 years at diagnosis, who had an available DNA sample isolated from peripheral blood mononuclear cells, and who consented to participate in the study, which had been approved by the Institutional Human Subjects Review Committee. All patients who were older than 3 years at diagnosis were treated with craniospinal radiation followed by systemic chemotherapy. Patients who were younger than 3 years were given systemic chemotherapy first, followed by craniospinal radiation when they reached 3 years of age or experienced relapse. Most patients received four cycles of high-dose chemotherapy followed by stem cell rescue (SJMB96 protocol, $n = 24$) or eight cycles of cisplatin, cyclophosphamide, and vincristine (A9961 regimen B protocol, $n = 9$). The details of each protocol have been previously published.[5,6] The remaining nine patients were treated with PBTC-001 ($n = 2$), POG9031 ($n = 3$), or POG9233 ($n = 4$).

We reviewed the medical records of participating patients and abstracted the following information: demographic characteristics at diagnosis; risk group (high risk, >1.5 cm^2 residual disease on MRI scan 24–72 h after surgery and/or presence of disseminated disease; average risk, ≤1.5 cm^2 residual disease on MRI scan 24–72 h after surgery and absence of dissemination by MRI or by cerebrospinal fluid cytology), treatment characteristics, date of progression, and date of death or date of last follow-up. In 41 of 42 patients, data were available for occurrence of ≥Gr 3 hematopoietic, audiological, neurological (peripheral neuropathy), and renal toxicity requiring dose modification. In 34 patients, consecutive hearing assessment results were available with a baseline completed prior to initiation of chemotherapy with cisplatin. Full-scale, verbal, and performance intelligence quotient (IQ) scores measured on the Wechsler Intelligence Scale for Children (WISC-III)[39] were also available in 21 patients ≥3 years of age, with at least two assessments including a baseline assessment prior to radiotherapy present in 10. (Only one assessment in 11, two assessments in 4, three assessments in 3, four assessments in 2, and five assessments in 1 patient.) Age requirement of ≥3 years was used because WISC-III is not an appropriate test to use in younger patients.

Multiplex GSTM1 *and* GSTT1 *Genotyping*

We used a multiplex polymerase chain reaction (PCR) technique to amplify both *GSTM1* and *GSTT1* simultaneously in a single PCR reaction.[17] Briefly, we amplified isolated DNA 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 inter-

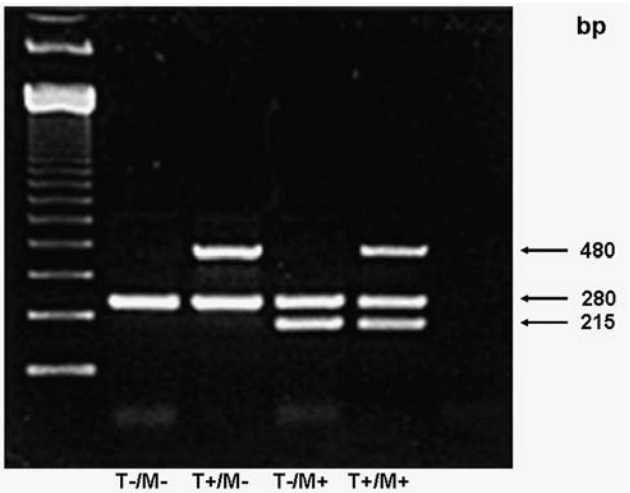

Fig. 1. Representative PCR products from coamplification of glutathione *S*-transferase T1 polymorphism (*GSTT1*; 480 bp), dihydrofolate reductase gene (*DHFR*; 280 bp), and glutathione *S*-transferase M1 polymorphism (*GSTM1*; 215 bp) viewed with an ethidium bromide–stained 2% agarose gel. Abbreviations: T–/+, absence or presence of *GSTM1*; M–/+, absence or presence of *GSTT1*.

nal control, we coamplified the dihydrofolate reductase gene (*DHFR*) using the primers 5'-GCA TGT CTT TGG GAT GTG GA-3' and 5'-GGA ATG GAG AAC CAG GTC TT-3'. The PCR conditions consisted of an initial melting temperature of 95°C (5 min) followed by 35 cycles of melting (95°C, 30 sec), annealing (58°C, 45 sec), and extension (72°C, 1 min). We then viewed the PCR products from coamplification of *GSTT1* (480 bp), *DHFR* (280 bp), and *GSTM1* (215 bp) with an ethidium bromide–stained 2% agarose gel for the presence or absence of *GSTM1* and *GSTT1* genes (Fig. 1). In 10% of the samples, for quality control, PCR was repeated. This is a robust technique that readily identifies presence or absence of the gene of interest. DHFR is used as an internal control to ensure that there is amplifiable DNA in the sample in the case of a double-null genotype. We compared patients with null genotypes with patients with nonnull genotypes. For combined *GSTM1T1* genotype, we compared patients with at least one null genotype with patients null for neither.

Statistical Analyses

We computed basic descriptive statistics for demographic and treatment characteristics and *GSTM1* and *GSTT1* variants. We used chi-square or Fisher's exact test for categorical variable comparisons. We applied the Kaplan-Meier procedure to estimate PFS and used the log-rank test for comparison. There is no effective salvage therapy for recurrent medulloblastoma; therefore, for assessing the effect of the genetic polymorphisms on survival, PFS is a more appropriate marker than overall survival. We calculated survival time from the date of registration to date of disease progression or relapse, date of death from any cause, or date of last follow-up visit. All survival estimates are reported in years ± 1 SEM unless otherwise noted. We included age at diagnosis as a continuous variable and used median age to dichotomize the group of patients who were ⩾3 years of age at diagnosis: <8 or ⩾8 years of age. Patients younger than 3 years of age are well known to have a significantly inferior survival because they are unable to receive the standard upfront radiation therapy. We performed bivariate analyses, adjusting for one variable at a time, for age at diagnosis, gender, race/ethnicity, and risk group. We did not perform multivariable analyses because our study sample is too small to adjust for all the necessary variables.

We calculated odds ratios (ORs) using logistic regression analysis to evaluate the relation between the genotypes and occurrence of ⩾Gr 3 treatment toxicity requiring dose modification, omission, or cessation, according to Common Terminology Criteria for Adverse Events, version 3.0 (CTCAE), for the following outcomes: myelosuppression, neurotoxicity, ototoxicity, and nephrotoxicity. We also used the Kaplan-Meier method to compare elapsed time to ⩾Gr 3 ototoxicity during chemotherapy among the *GST* variants.

For verbal, performance, and full-scale IQ comparisons, we performed *t*-tests to compare the within-subject slopes and mixed linear modeling to compare mean scores across time among the genotype groups. The within-subject slope was calculated by regression using the multiple measurements in each subject. Mixed linear modeling was performed with strong assumption that covariance structure was compound symmetry. If unstructured covariance was specified, analyses stopped because of too many likelihood evaluations. We compared IQ scores at single time points among the genotypes using the Wilcoxon rank sum test. We selected $p < 0.05$ as a statistical significance value, and all tests of statistical significance were two sided.

Results

Demographic and Clinical Characteristics of the Study Population

The mean and median ages for the overall group were 7.3 and 6.8 years, respectively (range, 1.6–18 years). Six (14%) patients were younger than 3 years at diagnosis. Thirty-four (81%) patients were male, and 21 (50%) were non-Hispanic white. Table 1 summarizes descriptive data for all patients, patients who were older than 3 years of age at diagnosis, and patients who were treated with high-dose chemotherapy followed by stem cell rescue. The proportions of the selected *GST* polymorphisms were similar to published population values, suggesting that *GST* polymorphisms were not potential risk factors for development of medulloblastoma.

Survival Analyses

The median follow-up for the 29 survivors was 3.7 years (range, 2–8.6 years; Table 2). Fourteen patients had progressed, and 13 died, resulting in an estimated 4-year

Table 1. Demographic and clinical characteristics, including glutathione *S*-transferase genotype, for all patients, patients older than 3 years at diagnosis, and patients treated with high-dose chemotherapy and stem cell rescue (HDCSCR)

	All Patients (n = 42)		Patients >3 years of Age at Diagnosis (n = 36)		Patients Treated with HDCSCR (n = 24)	
Variable	***n***	**%**	***n***	**%**	***n***	**%**
Age at diagnosis						
<8 years	23	55	17	47	11	46
>8 years	19	45	19	53	13	54
Gender						
Male	34	81	28	77.8	19	79.2
Female	8	19	8	22.2	5	20.8
Ethnicity/race						
Hispanic	14	33.3	14	38.9	10	41.7
Non-Hispanic white	21	50.0	16	44.4	10	41.7
African American	5	11.9	4	11.1	3	12.5
Other	2	4.8	2	5.6	1	4.2
Risk group (age >3 years)						
Average risk	NA	NA	22	61.1	15	62.5
High risk	NA	NA	14	38.9	9	37.5
GSTM1						
Null	17	40.5	13	36.1	9	37.5
Nonnull	25	59.5	23	63.9	15	62.5
GSTT1						
Null	12	28.6	11	30.6	5	20.8
Nonnull	30	71.4	25	69.4	19	79.2
GSTM1T1 combined						
⩾1 null	25	59.5	20	55.6	12	50
Nonnull	17	40.5	16	44.4	12	50

Abbreviations: *GSTM1*, glutathione *S*-transferase M1 polymorphism; *GSTT1*, glutathione *S*-transferase T1 polymorphism.

PFS of 68% ± 7% and 4-year overall survival rate of 76% ± 7%. In univariate analyses, patients <8 years of age (p = 0.12) and with high-risk disease at diagnosis (p = 0.01) had shorter PFS. In patients >3 years of age at diagnosis, patients with average-risk disease (p = 0.16) and *GSTM1* nonnull genotype (p = 0.21) had longer PFS. In patients treated with high-dose chemotherapy followed by stem cell rescue (n = 24), those with the *GSTM1* null genotype had an estimated 4-year PFS of 44% ± 16% compared with 79% ± 10% in patients with the *GSTM1* nonnull genotype (p = 0.12). Adjustment for age at diagnosis, gender, ethnicity, or risk group did not change this result.

Treatment-Related Toxicity and GST *Genotypes*

Twenty-seven (66%) of the 41 patients (data not available for one patient) experienced ⩾Gr 3 treatment toxicity according to CTCAE, requiring dose modification, omission, or cessation of therapy. Patients with *GSTT1* null were 8.9 times (95% confidence interval, 1.1–79) more likely to have any ⩾Gr 3 toxicity than were patients with the *GSTT1* nonnull genotype (Table 3). In combined genotype analyses, patients with at least one null genotype had a 4.3 (1.1–16.8), 3.7 (1–13.6), and 6.4 (1.2–34) times increased risk for any ⩾Gr 3 toxicity, any ⩾Gr 3 toxicity excluding peripheral neuropathy, and any ⩾Gr 3 toxicity requiring omission or cessation of chemotherapy, respectively.

Serial hearing evaluations (at least two, including a pretreatment assessment) were available in 34 patients. Nineteen (56%) developed ⩾Gr 3 ototoxicity, requiring hearing aids. There was no relation between development or time to development of ⩾Gr 3 ototoxicity and the study variables, including the *GST* polymorphisms. There was also no relation between any toxicity outcome and PFS.

GST *Genotypes and Intellectual Impairment*

Data were available from 21 patients for at least one time point after diagnosis. At baseline comparisons, there were no statistically significant differences for all three IQ scores for the selected polymorphisms (Table 4). The mean slopes for full-scale, performance, and verbal IQ scores were, –8.4, –11.1, and –4, respectively, in patients with the *GSTM1* null genotype, compared with mean slopes of 4.5, 6.6, and 2.6, respectively, in patients with

Table 2. Univariate comparisons for the study variables for progression-free survival in patients >3 years old at diagnosis and patients treated with high-dose chemotherapy and stem cell rescue (HDCSCR)

	Patients >3 Years Old at Diagnosis ($n = 36$)			Patients Treated with HDCSCR ($n = 24$)		
Variable	***n* (Event)**	**4-Year PFS (SE)**	**HR (95% CI)**	***n* (Event)**	**4-Year PFS (SE)**	**HR (95% CI)**
Age at diagnosis						
<8 years	17 (5)	71 (11)	1.5 (0.4–5.6)	11 (5)	55 (15)	2.4 (0.6–10)
>8 years	19 (4)	76 (11)	1	13 (3)	71 (14)	1
Gender						
Male	28 (6)	78 (8)	1	19 (6)	68 (11)	1
Female	8 (3)	50 (22)	2.3 (0.6–9.3)	5 (2)	40 (30)	1.6 (0.3–8)
Risk group						
High risk	14 (5)	64 (13)	2.5 (0.7–9.3)	9 (4)	56 (17)	2.2 (0.6–8.9)
Average risk	22 (4)	80 (9)	1	15 (4)	70 (13)	1
GSTM1						
Null	13 (5)	61 (14)	2.3 (0.6–8.5)	9 (5)	44 (17)	3 (0.7–13)
Nonnull	23 (4)	82 (8)	1	15 (3)	79 (11)	1
GSTT1						
Null	11 (2)	81 (12)	1	5 (1)	75 (22)	1
Nonnull	25 (7)	70 (10)	1.6 (0.3–7.6)	19 (7)	62 (12)	1.8 (0.2–15)
GSTM1T1 combined						
⩾1 null	20 (6)	68 (11)	1.7 (0.4–6.8)	12 (5)	55 (15)	1.8 (0.4–7.7)
Nonnull	16 (3)	81 (10)	1	12 (3)	75 (13)	1

Abbreviations: PFS, progression-free survival; HR, hazard ratio; CI, confidence interval; *GSTM1*, glutathione *S*-transferase M1 polymorphism; *GSTT1*, glutathione *S*-transferase T1 polymorphism. "Event" refers to relapse or progression.

Table 3. Distribution of glutathione *S*-transferase *M*1 (*GSTM1*) and glutathione *S*-transferase T1 (*GSTT1*) genotypes and calculated odds ratios (ORs) for occurrence of grade 3 or greater (⩾Gr 3) treatment toxicity requiring dose modification, omission, or cessation for myelosuppression, neurotoxicity, ototoxicity, and nephrotoxicity

	GSTM1			***GSTT1***			***GSTM1T1* Combined**		
Toxicity	**Null (*n*)**	**Nonnull (*n*)**	**OR (95% CI)**	**Null (*n*)**	**Nonnull (*n*)**	**OR (95% CI)**	**⩾1 Null (*n*)**	**Nonnull (*n*)**	**OR (95% CI)**
Any ⩾Gr 3 toxicity									
Yes	11	16	1.2	11	16	8.9 (1.1–79.0)	19	8	4.3 (1.1–16.8)
No	5	9	(0.3–4.7)	1	13		5	9	
Any ⩾Gr 3 toxicity excluding peripheral neuropathy									
Yes	9	13	1.2	10	12	7.1 (1.3–38.0)	16	6	3.7 (1–13.6)
No	7	12	(0.3–4.1)	2	17		8	11	
Any ⩾Gr 3 toxicity requiring omission or cessation of chemotherapy									
Yes	7	6	2.5	5	8	1.8 (0.5–7.5)	11	2	6.4 (1.2–34.0)
No	9	19	(0.6–9.6)	7	21		13	15	
Any ⩾Gr 3 toxicity requiring omission or cessation of chemotherapy excluding peripheral neuropathy									
Yes	5	3	3.3	4	4	3.1 (0.6–15.4)	8	0	—[a]
No	11	22	(0.7–17.0)	8	25		16	17	$p = 0.01$
Any ⩾Gr 3 ototoxicity requiring dose modification									
Yes	8	11	0.9	6	13	1.1	13	6	1.9 (0.5–7.7)
No	6	9	(0.2–3.6)	5	10	(0.3–4.6)	8	7	

Abbreviation: CI, confidence interval.

[a]OR cannot be calculated with one cell containing 0 value.

Table 4. Average full-scale, performance, and verbal intelligence quotient (IQ) scores by *GSTM1* and combined *GSTM1T1* genotypes for baseline (prior to radiation) and first follow-up assessment

		GSTM1			*GSTM1T1* Combined		
IQ Scores	**Assessment**	**Null**	**Nonnull**	***p*-Value**	**⩾1 Null**	**Nonnull**	***p*-Value**
Full-scale IQ	1	81.2	91.1	0.4	79.7	93.9	0.15
	2	65.3	90.8	0.048	64.3	100.2	0.002
Performance IQ	1	80.6	85.6	0.5	78.5	88.5	0.2
	2	65.5	92.9	0.08	63.8	103.7	0.004
Verbal IQ	1	84.8	92.4	0.4	84.2	94.3	0.2
	2	70.8	90.6	0.046	70.5	97.5	0.002

Abbreviations: *GSTM1*, glutathione *S*-transferase M1 polymorphism; *GSTM1T1*, glutathione *S*-transferase T1 and M1 polymorphisms combined.

Table 5. Comparison of scores for full-scale, performance, and verbal intelligence quotient (IQ) (Wechsler Intelligence Scale for Children) over time following radiation therapy in 21 children with medulloblastoma by glutathione *S*-transferase genotype

	Full-Scale IQ		**Performance IQ**		**Verbal IQ**	
Genotype	**Score**	***p*-Value**	**Score**	***p*-Value**	**Score**	***p*-Value**
GSTM1						
Mean slope	–8.4	0.001[a]	–11.1	0.004[a]	–4.0	0.009[a]
Null	4.5	0.16[b]	6.6	0.18[b]	2.6	0.3[b]
Nonnull mean difference[b]	–12.5		–12.4		–8.7	
GSTM1T1 combined						
Mean slope	–8.9	0.01[a]	–11.5	0.02[a]	–4.5	0.02[a]
⩾1 null	4.5	0.0002[b]	6.6	0.0004[b]	2.6	0.002[b]
Nonnull mean difference[b]	–27.2		–29.0		–21.7	

Abbreviations: *GSTM1*, glutathione *S*-transferase M1 polymorphism; *GSTM1T1*, glutathione *S*-transferase T1 and M1 polymorphisms combined.

[a]Comparison of slopes by *t*-test.

[b]Comparison by linear mixed model.

the *GSTM1* nonnull genotype ($p = 0.001, 0.004, 0.009$, respectively; Table 5). Combined *GSTM1T1* genotype analyses showed statistically significant differences for comparisons of average slope values (*t*-test) and mean IQ scores (linear mixed model). IQ scores for the patients with at least one null genotype significantly declined in time (negative slope) compared with patients with no null genotypes (Fig. 2, Table 5). Age at diagnosis (<8 years vs. >8 years) and risk group did not correlate significantly with the genotypes and were not associated significantly with IQ change in time. All patients with at least one null genotype had a negative slope for all three IQ scales after radiation.

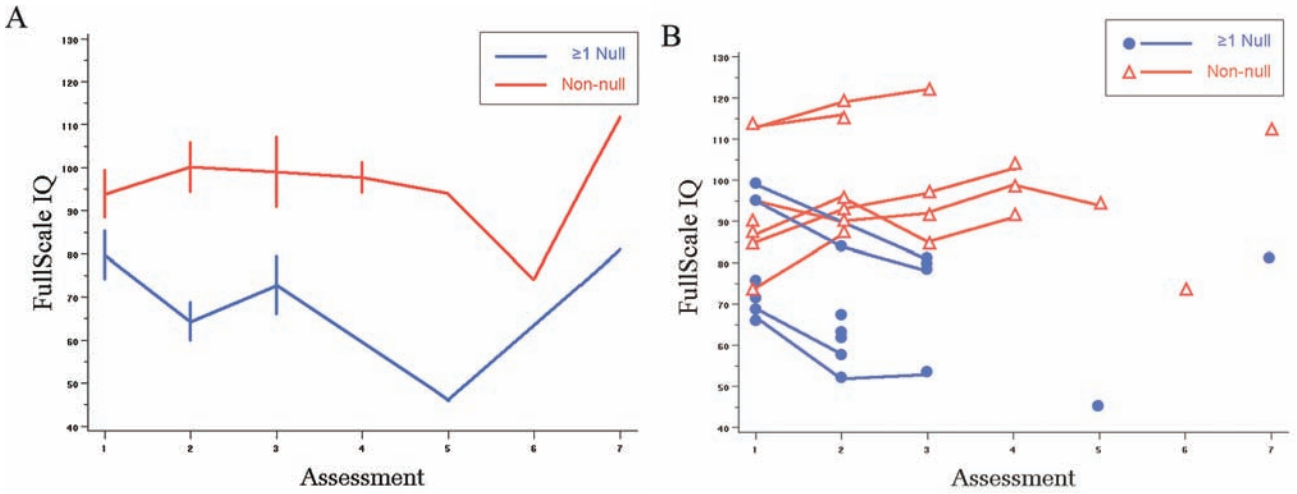

Fig. 2. Comparisons for serial full-scale intelligence quotient (IQ) measurements by *GSTM1T1* combination genotype. (A) Mean slope comparison. (B) All observations. The first assessment was conducted before radiation therapy.

Discussion

In an attempt to discover potential markers to identify patients with medulloblastoma who are at risk of treatment failure and development of adverse effects, we conducted a pilot study in 42 children and adolescents. Most remarkable was the association between *GSTM1T1* polymorphism and multiple adverse events, including intellectual impairment and ≥Gr 3 toxicity due to treatment. Neurocognitive deficits usually become evident within 1–2 years after radiotherapy and are progressive in nature. Affected children may experience information-processing deficits and attention and memory impairment, leading to academic failure in the areas of reading, mathematics, and language. Progressive loss of IQ scores in children diagnosed with brain tumors and leukemia following cranial radiotherapy has been reported in a number of studies.[8,40–42] In medulloblastoma, the rate of decline for full-scale, performance, and verbal IQ scores ranged from 2 to 4.3 points per year in three separate reports.[8,40,41] While the most likely cause for neurocognitive impairment is radiation therapy, chemotherapy also has been associated with this outcome.[43]

While young age at diagnosis and higher dose of radiation have been associated with development of intellectual impairment after radiation therapy, we were not able to adjust for these markers appropriately in our small study sample. However, neither factor was statistically significantly related to cognitive impairment. In current care of children with brain tumors, we are unable to predict who will develop significant intellectual impairment after radiation therapy. Our findings suggest that *GSTM1* and *GSTT1* polymorphisms may be a potential marker to identify such patients in advance. The mean scores for full-scale, performance, and verbal IQ at the baseline evaluation (prior to radiation) were slightly lower than expected in patients with no null (combined *GSTM1T1*) genotype, but this difference was not statistically significant. Three patients had all three scores less than 80 points at this evaluation. Their tests were repeated within 1 month of their initial evaluation, and the repeat scores were very similar. The neuropsychologist who performed the tests commented that these scores reflected the patients' true performance and were not influenced by any health impairment due to surgery or medication at the time. It is also important to emphasize that our analyses compared the slopes of cognitive evaluations at consequent time points, not at one single time point. There are no data regarding whether *GST* polymorphisms may be related to cognitive function differences in the normal population.

GST enzymes are known for their free-radical–scavenging function in addition to xenobiotic metabolism. In a recent study exploring the relation between homocysteine metabolism polymorphisms and changes in IQ scores over the 4 years following diagnosis of pediatric acute lymphoblastic leukemia, endothelial nitric oxide synthase (eNOS) *894T* homozygosity was associated with a change in IQ scores ($p = 0.007$).[44] Almost two-thirds of the patients were treated with cranial radiation. Together with our finding, this result further supports the premise that enzymes such as GSTs and eNOS that are involved in cellular protection from free radicals may help predict which patients will develop neurocognitive toxicity from radiation.

In addition to neurocognitive adverse events, the combined *GSTM1T1* polymorphism was also significantly related to other adverse events after medulloblastoma therapy. These consistent observations support the potentially important implication of this genotype in modifying the effects of cancer therapy. The trends for the main genotype effect for the toxicity outcome were in the direction of our hypotheses for each gene individually. Existence of synergy and substrate overlap between the *GST* genotypes have been described before and are a possible explanation for why the statistical comparisons became significant with the combined genotype analyses.[9]

We observed a trend for a possible relation with *GSTM1* polymorphisms and PFS. In contrast to our hypothesis, patients with the *GSTM1* null genotype who did not have a functional enzyme had outcomes inferior to outcomes of patients with the *GSTM1* nonnull genotype. This could occur simply because of the small sample size. While patients with *GSTM1* null genotype experienced more frequent, but statistically insignificant, toxicity requiring omission or cessation of a chemotherapy agent compared with patients with the *GSTM1* nonnull genotype, this difference was not related to PFS. In a larger study, we will explore whether this observation persists, and if it does, we will investigate whether excess toxicity and dose modification may have led to inferior outcome in the *GSTM1* null patients. The *GSTM1* null genotype has been related to increased toxicity requiring dose reduction in adults with glioma treated with nitrosourea-based regimens.[26]

Clearly, our study is small and our results should be interpreted with caution. These preliminary findings are promising but require validation in a larger study that will enable us to perform multivariate analyses, adjusting for age at diagnosis, radiation dose, and other potential confounding variables. A follow-up study in a larger patient group treated with high-dose chemotherapy followed by stem cell rescue is under way. GSTs are not the only mechanisms that modify chemotherapy and radiation therapy effects. Alkylating agents are initially activated by phase 1 cytochrome P450 enzymes. Free radicals created by radiation can possibly be cleared by other enzymes, including superoxide dismutase, glutathione peroxidase, and eNOS. All of these enzymes have polymorphisms, and a large study is needed to explore whether combined effects of these polymorphisms can predict survival and the toxicities examined in our study better than GSTs alone.

Individual variation in the responses to chemotherapeutic agents at the host level is an understudied clinical problem. Studies have found that this variability contributes to widely disparate outcomes, including complete responsiveness, toxic effects (which can be severe), drug withdrawal, and, in the worst cases, therapeutic failure.[45,46] This variability in drug response is in part determined genetically.[45–47] Combined modality

treatment with surgery, radiation, and chemotherapy achieves reasonable survival rates in children and adolescents with medulloblastoma. However, cure rates are still unacceptably low for high-risk patients, and such permanent adverse effects as intellectual impairment significantly reduce quality of life. Because there is no successful salvage therapy for patients who relapse, identification of patients at risk of treatment failure in advance could perhaps promote development of more aggressive treatment options. Similarly, if we were able to identify patients who were at increased risk for intellectual impairment or other toxicities, we could examine whether they could be treated with lower doses of radiation and or chemotherapy. The current national Children's Oncology Group medulloblastoma protocol is indeed investigating whether reduced-dose craniospinal radiation therapy (18 Gy instead of 24 Gy) is effective in average-risk medulloblastoma patients. Moreover, in patients who are at increased risk of cognitive impairment, early implementation of prevention strategies such as cognitive remediation or methylphenidate therapy could be explored.

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