1. STUDY TITLE: *Predicting Neurocognitive and Neuropsychological Impairment by using Genetic Polymorphisms in Medulloblastoma Survivors*.

2. WORKING GROUP AND INVESTIGATORS:

2.1. Working Group: Neuropsychology

2.2. Investigators:

Julienne Brackett	jxbracke@txccc.org
Michael Scheurer	mscheur@txccc.org
Kala Kamdar	kykamdar@txccc.org
Kevin R. Krull	kevin.krull@stjude.org
Leah Ellenberg	lellenberg@charter.net
Marilyn Hockenberry	mjhocken@txccc.org
Paul Nathan	paul.nathan@sickkids.ca
Ernest Fruge	edfruge@txccc.org
Wendy Leisenring	wleisenr@fhcrc.org
Marilyn Stovall	mstovall@mdanderson.org
Les Robison	les.robison@stjude.org
M. Fatih Okcu	mfokcu@txccc.org

3. BACKGROUND AND RATIONALE:

Medulloblastoma is the second most common malignant brain tumor seen in children and adolescents accounting for 20% to 25% of all new cases. With advances in chemotherapy and radiation therapy, 60-80% of patients can now be cured [1;2]. However, a substantial number of patients have significant long-term residual effects from radiation therapy, including endocrinopathies, hearing loss, renal failure, second malignancies, neuropsychological and cognitive impairments[2-5]. These residual effects can have a profound impact on survivors' quality of life, academic and professional achievement. Children who are treated for CNS tumors have been found to be at risk for development of serious neurocognitive problems, particularly those treated with cranial irradiation and intensive chemotherapy. Long-term effects include impairment in overall cognitive functioning (IQ), attention, memory, processing speed, executive functioning, and school performance. Fifty to 60 percent of patients develop progressive neurocognitive and neuropsychological deficits characterized by reduction in intelligence, impairment in executive function, memory and attention [6;7]. Younger age at the time of treatment is associated with increased risk for such outcome [6]. Almost all of the affected individuals live with learning disorders and require supplemental educational services. A recent study by Pang et al (2008) examined employment status among adult survivors in the Childhood Cancer Survivor Study (CCSS) and found that patients with a diagnosis of a central nervous system (CNS) tumor or patients who received cranial irradiation >30 Gy had the highest increased risk of unemployment of patients in the study[8]. Nevertheless, with current knowledge, patients who will experience significant side effects cannot be distinguished in advance. Studies of genetic polymorphisms may provide new tools for identifying patients at greatest risk.

Radiation therapy exerts its biological effects through the creation of free radicals. Irradiating eukaryotic cells in vitro results in a rapid burst of free oxygen and nitrogen radicals

generated primarily as a result of ionization of water molecules. Whole body irradiation leads to increased markers of lipid peroxidation, including thiobarbituric acid reaction products, 4-hydroxynonenal and hexane in animal models and in patients [9]. Enzymes including manganese superoxide dismutase, glutathione peroxidase, glutathione S-transferase belonging to antioxidant system scavenge such radicals to protect normal tissues from possible damage secondary to oxidative stress [10]. *Genetic polymorphisms in the antioxidant enzyme systems that scavenge free radicals may explain why some patients develop significant radiation induced neuropsychological and cognitive impairment while others do not.*

Other pathways that are relevant for development of neurocognitive impairment include dopamine/serotonin, radiation repair, folate and inflammation pathways. We are planning to explore these pathways in a second concept application with federal or private funding.

The Follow-Up 2 survey of the Childhood Cancer Survivor Study included a 25 item Neurocognitive Questionnaire (referred to as the CCSS-NCQ). On this questionnaire, participants rate the extent to which they have experienced problems over the past six 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 four neurocognitive factors: Task Efficiency, Memory, Emotional Regulation, and Organization. The Task Efficiency factor reflects skills related to attention and processing speed, while the Memory factor reflects both working and long-term memory processes. The Emotional Regulation and Organization factors reflect executive function skills related to control of emotional expression and organization of ones physical environment. As indicated above, these attention, processing speed, memory, and executive function processes are among those most commonly impacted in long-term survivors of childhood cancer.

The Brief Symptom Inventory – 18 (BSI-18) is an 18 item screening questionnaire designed to assess psychosocial symptoms of depression, anxiety, and somatic complaints[11]. With the BSI-18, participants describe the extent to which they have been distressed or bothered by each symptom in the previous seven days. Possible responses were presented using a Likert scale ranging from 1 ("Not at all") to 5 ("Extremely"). This measure was collected in the CCSS follow-up survey at the same time the CCSS-NCQ was obtained. Recent literature has suggested that psychosocial symptoms may not be a direct outcome of cancer therapy, but are related to adjustment and social functioning[12]. Psychosocial functioning, as reported on the BSI-18, is also related to the CCSS-NCQ Task Efficiency and Emotional Regulation factors.

The major goal of this proposed research is to investigate the relationship between genetic variations in free radical scavenging enzymes and neurocognitive and neuropsychological functioning as assessed by the CCSS-NCQ and BSI-18 in children who survived after medulloblastoma. If a significant relationship is found between a genotype and these outcomes, existing or new interventions can be administered in the identified at risk patients.

4. SPECIFIC AIMS/OBJECTIVES/RESEARCH HYPOTHESES:

4.1. Primary Aim:
To explore the relationship between genetic variations in free radical scavenging enzymes and neurocognitive and psychological functioning as assessed by the CCSS-NCQ and BSI-18 in medulloblastoma survivors.

4.2. Objectives:
4.2.1. To determine frequencies of germline free radical scavenging enzyme polymorphisms from buccal DNA samples of 130 survivors of childhood medulloblastoma and 146 siblings who are participants of the CCSS.

4.2.2. To assess whether germline free radical scavenging enzyme polymorphisms are correlated with greater neurocognitive and neuropsychological impairment determined by the NCQ and BSI-18 surveys in long term survivors of childhood medulloblastoma.

4.2.3. To assess whether germline free radical scavenging enzyme polymorphisms are correlated with neurocognitive and neuropsychological function determined by the NCQ and BSI-18 surveys in healthy siblings of childhood cancer survivors.

4.3. Hypotheses:
4.3.1. We hypothesize that survivors with low activity genotypes will have higher scores (suggesting impairment) for neurocognitive and neuropsychological functioning compared to patients with high activity polymorphisms.

5. ANALYSIS FRAMEWORK:

5.1. Outcome(s) of interest: The primary outcome of interest is the association between selected genotypes and neurocognitive and neuropsychological function test scores based on CCSS-NCQ and BSI-18. Specifically, we plan to conduct the following analyses:

5.1.1. We will compute basic descriptive statistics for patient characteristics, radiation therapy dose, frequencies for enzyme variants and the mean scores for task efficiency, emotional regulation, organization and memory based on NCQ and, depression, anxiety and somatic complaints based on BSI-18. Mean scores will be compared between the polymorphic variants (wild type, compared to heterozygous and homozygous variant alleles combined, dominant model) by two sample t-test. Four factor (NCQ) and three factor (BSI-18) scores will be generated from the weighted responses. Three separate analyses will be done: Case-case, case-sibling and siblings only comparisons. Odds ratios will be created based how often a rating of '3' (NCQ, i.e. "Often a Problem") or “5” (BSI-18, extremely) occurred. Multivariable logistic regression analyses will then be conducted for adjustment for potential confounding variables including age at radiation therapy, gender, race/ethnicity, and whole brain radiation dose. A two-sided p-value of 0.05 will be considered as statistically significant in all analyses. Based on the available functional alteration data due to the polymorphism heterozygous genotype group will be combined with either so-called wild type or homozygous variant group to create 2 categories for each genotype. A genetic risk score will be created using all polymorphisms by assigning 1 to “at risk” low activity genotype and 0 to “low risk” high activity genotype. Analyses will be done

to test if the composite risk score is associated with neurocognitive and neuropsychological outcomes. Mock tables that we will present the results of the analyses are shown below.

5.2. Subject population: By searching CCSS databases we have identified 130 childhood medulloblastoma survivors from the patient cohort and 146 subjects from the sibling cohort with an available buccal cell DNA sample and completed CCSS-NCQ and BSI-18 data.

5.2.1. Inclusion criteria

- Medulloblastoma survivors who completed CCSS-NCQ and BSI-18 at Follow-Up 2 with an available germline DNA sample.
- Siblings who completed CCSS-NCQ and BSI at Follow-up 2 with an available germline DNA sample.

5.2.2. Exclusion criteria

- Stroke (F9)
- Cerebral Palsy (J1)
- Epilepsy (J4)

5.2.3. Requested Variables

- Cancer diagnosis
- Tumor location
- Radiation Therapy Variable (localization and dose details required, whole brain dose to be used as the main covariate)
- Chemotherapy protocol and list of agents
- Age at diagnosis
- Age at follow up-2 (A1)
- Sex (A2)
- Ethnicity –Race (A4 – A4a)
- Income (S1-S3)
- Highest grade level of schooling (1)
- Current employment status (4)

5.3. Genotyping: PCR based genotyping methods for the selected polymorphisms have already been established in our laboratory for the glutathione S-transferases. The remaining 3 free radical scavenging enzyme genotyping methods have been published by many groups. We will establish these methods in our laboratory. Table 1 demonstrates the list of enzyme polymorphisms that we will study. For all genotyping assays we request a total of 1 microgram of DNA extracted from buccal cells. 10% of all genotyping will be repeated for quality control. All costs will be incurred by Texas Children's Cancer Center.

6.0 Future Plan: If we successfully show an association with the selected genotypes and the neurocognitive outcomes using the survey data, we will plan to apply for funding to formally assess full neurocognitive function in these survivors and siblings in a multicenter study and replicate the genotype-phenotype correlation.

Table 1. Characteristics of the study genetic polymorphisms.

Gene	SNP rs number	Polymorphism	Variant Allele or Haplotype Prevalence in *Whites*	Expected At Risk Genotype
Free Radical Scavenging Enzyme Polymorphisms				
SOD2	rs4880	*47C>T*		*C47T and T47T*
GPx	rs1050450	*599C>T*	0.19 - 0.25	*C599T and T599T*
GSTM1	na	*Gene Deletion*	0.42 – 0.60	*GSTM1 Null*
GSTT1	na	*Gene Deletion*	0.13 – 0.26	*GSTT1 Null*
GSTP1	rs1695	*1404A>G (exon 5)*	A 0.68 – B 0.26	*GSTP1 Not AA*
	rs1138272	*2294C>T (exon 6)*	AA – 0.44	

6. REFERENCES:

(1) Gajjar A, Chintagumpala M, Ashley D, Kellie S, Kun LE, Merchant TE et al. Risk-adapted craniospinal radiotherapy followed by high-dose chemotherapy and stem-cell rescue in children with newly diagnosed medulloblastoma (St Jude Medulloblastoma-96): long-term results from a prospective, multicentre trial. *Lancet Oncol.* 2006; 7(10):813-820.

(2) Packer RJ, Gajjar A, Vezina G, Rorke-Adams L, Burger PC, Robertson PL et al. Phase III study of craniospinal radiation therapy followed by adjuvant chemotherapy for newly diagnosed average-risk medulloblastoma. *J Clin Oncol.* 2006; 24(25):4202-4208.

(3) Duffner PK. Long-term effects of radiation therapy on cognitive and endocrine function in children with leukemia and brain tumors. *Neurologist.* 2004; 10(6):293-310.

(4) Mulhern RK, Merchant TE, Gajjar A, Reddick WE, Kun LE. Late neurocognitive sequelae in survivors of brain tumours in childhood. *Lancet Oncol.* 2004; 5(7):399-408.

(5) Palmer SL, Reddick WE, Gajjar A. Understanding the cognitive impact on children who are treated for medulloblastoma. *J Pediatr Psychol.* 2007; 32(9):1040-1049.

(6) Mulhern RK, Palmer SL, Merchant TE, Wallace D, Kocak M, Brouwers P et al. Neurocognitive consequences of risk-adapted therapy for childhood medulloblastoma. *J Clin Oncol.* 2005; 23(24):5511-5519.

(7) Ris MD, Packer R, Goldwein J, Jones-Wallace D, Boyett JM. Intellectual outcome after reduced-dose radiation therapy plus adjuvant chemotherapy for medulloblastoma: a Children's Cancer Group study. *J Clin Oncol.* 2001; 19(15):3470-3476.

(8) Pang JW, Friedman DL, Whitton JA, Stovall M, Mertens AC, Robison LL et al. Employment status among adult survivors in the Childhood Cancer Survivor Study. *Pediatr Blood Cancer.* 2008; 50(1):104-110.

(9) Zhao W, Diz DI, Robbins ME. Oxidative damage pathways in relation to normal tissue injury. *Br J Radiol.* 2007; 80 Spec No 1:S23-S31.

(10) Robbins ME, Diz DI. Pathogenic role of the renin-angiotensin system in modulating radiation-induced late effects. *Int J Radiat Oncol Biol Phys.* 2006; 64(1):6-12.

(11) Derogatis LR. *The Brief Symptom Inventory-18 (BSI-18): Administration, scoring and procedures manual.* Minneapolis, MN: National Computer Systems, 2000.

(12) Zebrack BJ, Gurney JG, Oeffinger K, Whitton J, Packer RJ, Mertens A et al. Psychological outcomes in long-term survivors of childhood brain cancer: a report from the childhood cancer survivor study. *J Clin Oncol.* 2004; 22(6):999-1006.

7. Mock tables for analyses

	Mean scores by NCQ (Comparison by t-test)			
	Task Efficiency	Emotional regulation	Organization	Memory
GSTM1 and T1				
Homozygous Null Patients				
Intact gene (Non null)				
P value				
For all other genotypes				
Wild type genotype				
Heterozygous/Homozygous Polymorphic Genotype				
P value				

	Mean scores by BSI-18 (Comparison by t-test)			
	Depression	Anxiety	Somatic Complaints	
GSTM1 and T1				
Homozygous Null Patients				
Intact gene (Non null)				
P value				
For all other genotypes				
Wild type genotype				
Heterozygous/Homozygous Polymorphic Genotype				
P value				

NCQ measures	Calculation of Odds ratios			
	GSTM1 and T1		All other genotypes	
	Homozygous Null	Intact (Non null)	Wild Type	Heterozygous/Homozygous variant
Frequency of "Often a Problem"				
> 10 questions	a	b	a	b
<= 10 questions	c	d	c	d

BSI-18 measures	Calculation of Odds ratios			
	GSTM1 and T1		All other genotypes	
	Homozygous Null	Intact (Non null)	Wild Type	Heterozygous/Homozygous variant
Frequency of "Extremely"				
> 10 questions	a	b	a	b
<= 10 questions	c	d	c	d

Genetic risk score will also be used in these tables to cross-tabulate patients with risk scores 4-5 and 0-3 with the NCQ and BSI-18 outcomes

Multivariable Analyses for the NCQ and BSI-18 outcomes that ORs were calculated

	Odds Ratio	95% CI
GSTM1 and T1		
Homozygous Null Patients		
Intact gene (Non null)	Ref	
*		
For all other genotypes		
Wild type genotype	Ref	
Heterozygous/Homozygous Polymorphic Genotype		
*		
Genetic Risk Score 4-5 0-3	 Ref	
Age at Radiation		
Gender Male Female	 Ref	
Ethnicity White Hispanic African American	 Ref	
Whole Brain Radiation Dose 23.4 Gy 36 Gy	 Ref	

***** this is just an example of how either genotype comparisons would be done. We will not a large enough sample to include more than one genotype in one model