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GSTP1 Polymorphisms Sex-Specific Association with Cognitive Outcomes in Survivors of Pediatric Medulloblastoma Tumors

Rella J. Kautiainen

GSTP1 POLYMORPHISMS SEX-SPECIFIC ASSOCIATION WITH COGNITIVE OUTCOMES IN SURVIVORS OF PEDIATRIC MEDULLOBLASTOMA TUMORS

by

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Under the Direction of Tricia Z. King, Ph.D.

This study investigated specific single nucleotide polymorphisms (SNPs) and their association with attentional deficits and hippocampal volume in survivors of medulloblastoma brain tumors. The sample with neuropsychological assessment includes eighteen medulloblastoma survivors and eighteen age-and-sex-matched healthy controls. We hypothesized that medulloblastoma survivors with a GSTP1 polymorphism will have significantly greater deficits in attention span and smaller bilateral hippocampal volumes compared to survivors without a polymorphism and healthy controls. We did not establish the specificity of hippocampal volume loss, and our sample may have more global subcortical morphological alterations. When separating groups by sex, we found large effect sizes between males with a GSTP1 polymorphism and females with a GSTP1 polymorphism across measures of attention span, working memory span and processing speed. Females with a polymorphism performed significantly worse than females without a polymorphism on full-scale intelligence quotient (IQ) and verbal IQ. Sex-specific genetic risk may explain part of the variability in long-term cognitive outcomes for medulloblastoma survivors.

INDEX WORDS: Medulloblastoma survivors, Single-nucleotide polymorphism, GSTP1, Bilateral hippocampus, Long-term outcomes

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DEDICATION

This proposal is dedicated to my friends and family for their continued support of my graduate school endeavors. I am grateful for their kindness, patience and love as I pursue new opportunities and milestones.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

1 INTRODUCTION

Single nucleotide polymorphisms (SNPs) are associated with survival rates, recovery rates, and deficits in neurocognitive functioning of brain tumor patients (Ali-Osman et al., 1997; Barahmani et al., 2009; Okcu et al., 2004; Rednam et al., 2013). Glutathione S-transferase (GST) gene polymorphisms slow the production of crucial enzymes which detoxify foreign bodies and chemicals, such as chemotherapy agents. Null variants for GSTM1 and GSTT1 genes as well as the heterozygous or null variant for the GSTP1 gene cause a reduction or absence of enzyme activity (Hayes & Pulford, 1995). While there is a strong body of literature on the relationship of GSTM1, GSTT1, and GSTP1 polymorphisms with rates of survival and toxicity for brain tumor survivors (Ali-Osman et al., 1997; Kilburn et al., 2010; Okcu et al., 2004; Rednam et al., 2013), few researchers have focused on long-term outcomes for those who survive. A crucial next step in the field is to understand how brain tumor survivors with SNPs that inhibit protective enzyme activity against oxidative stress develop into adulthood.

The field has begun to examine the association of GST polymorphisms in brain tumor survivors and cognitive outcomes after treatment. Researchers examined cohorts of pediatric medulloblastoma survivors with GSTM1 and GSTT1 polymorphisms and found correlations with lower full-scale IQ, verbal IQ and performance IQ compared to age-matched peers (Barahmani et al., 2009). In 2011, Brackett and colleagues did not find significant evidence that GST polymorphisms cause significantly lower neurocognitive functioning based on self-report from the Childhood Cancer Survivor Study Neurocognitive Questionnaire (Brackett et al., 2012). However, self-report measures are distinct from cognitive performance measures and are influenced by insight and personal biases (Paulhus et al., 2003). Recently, Krull and colleagues discovered that GSTT1 and GSTP1 polymorphisms were correlated with reduced attention in

long-term survivors of leukemia compared to age-matched peers (Krull et al., 2013). In line with this body of research, we examined the impact of these polymorphisms on attention span performance of medulloblastoma survivors. We examined working memory and processing speed, in survivors and controls, to increase specificity and gain a greater understanding of how underlying core neurocognitive skills may be impacted.

In addition to cognitive outcomes, an important area to investigate is how genomics may influence structural changes in the brain that are associated with core cognitive processes. Structural loss in the bilateral hippocampus has been found in a cohort of pediatric medulloblastoma survivors (Nagel et al., 2004). Additionally, bilateral hippocampal volume loss is evident even after removing variance due to total brain volume or total intracranial volume (ICV) for childhood brain tumor survivors (Jayakar et al., 2015; Riggs et al., 2014). The hippocampus may be a particularly vulnerable structure due to treatment effects for survivors of childhood brain tumors. Jayakar and colleagues observed hippocampal and putamen volumetric differences between survivors of childhood brain tumors, on average 15.4 years past diagnosis, and age-matched controls (Jayakar et al., 2015). Survivors displayed significantly lower hippocampal volumes than healthy controls and this was associated with deficits in auditory attention span (Jayakar et al., 2015). Additionally, chemotherapy has been shown to decrease resting glucose metabolism and a 10-11% glucose metabolism decrease was shown in the bilateral hippocampi of non-small-cell lung cancer survivors (Horky et al., 2014). This study investigates the volume of the bilateral hippocampus in medulloblastoma survivors to determine if volumetric differences are associated with specific genetic polymorphisms.

In this study, we assessed the impact of GST polymorphisms on performance of attention span and hippocampal volume of medulloblastoma survivors. We wish to explore the interaction

between genetic factors and long-term treatment outcomes so individuals can be tested for SNP profiles to inform treatment and remediation. In line with precision medicine, a better understanding of underlying genetic factors may allow for the creation of individualized protocols based on risk.

1.1 Long-term Neurocognitive Effects of Childhood Brain Tumors

Childhood cancer is a tremendous public health issue and cancer is the second leading cause of death in children ages 1 to 14 (Siegel et al., 2017). As the childhood cancer survival rate has increased to approximately 83% (Siegel et al., 2017), researchers have shifted focus to the long-term quality of life and cognitive outcomes for survivors (Moore, 2005). Specifically for childhood brain tumor survivors, their development into adulthood can be hindered by adaptive and cognitive deficits (Beebe et al., 2005; Mulhern et al., 1992). Cognitive deficits include lower scores than expected or than peer controls on neuropsychological testing in the domains of IQ, academic achievement, working memory, executive functioning, processing speed and attention (King & Na, 2016; Palmer, 2008; Ris & Noll, 1994).

The most common type of posterior fossa childhood brain tumor is a medulloblastoma, which is a malignant and often high-risk tumor requiring chemotherapy, radiation, and surgical resection for treatment (Bartlett et al., 2013; Rood et al., 2004). Medulloblastoma tumors are located in the posterior fossa of the brain and arise from the cerebellum or fourth ventricle (Bartlett et al., 2013). Posterior fossa tumor survivors have declines in intellectual ability, academic achievement, educational attainment; and they often require utilization of special education resources (Mabbott et al., 2005; Mitby et al., 2003; Mulhern et al., 2005). Survivors of tumors of the central nervous system (CNS), which includes medulloblastoma survivors, were not as likely to complete college as their siblings, unlike all other groups of childhood tumor

survivors (Mitby et al., 2003). Since some survivors are not reaching their pre-treatment intellectual or academic capabilities, researchers have focused on the risk factors that contribute to long-term sequalae of treatment. Radiation is a key risk factor and a predictor of deficits in long-term neurocognitive skills; such as, poor processing speed and lower verbal memory in childhood posterior fossa tumor survivors (Kieffer-Renaux et al., 2000; Mabbott et al., 2008). Palmer and colleagues developed a model for risk-based management in pediatric posterior fossa survivors which includes all treatment risk factors and details on the levels of care for survivors (Palmer & Leigh, 2009). In order to capture the diversity in long-term outcomes for pediatric brain tumor survivors, the effects of radiation, chemotherapy, neurosurgery, hydrocephalus, seizures, and hormone deficiency should be measured cumulatively (Micklewright et al., 2008). All risk factors only account for a proportion of the variability in outcome for pediatric survivors, which suggests that additional unknown factors are influencing outcomes. We wish to examine GST polymorphisms as a genetic mechanism explaining variability in cognitive outcomes of medulloblastoma survivors.

1.2 Core Neurocognitive Deficits in Survivors of Childhood Brain Tumors

The foundational skills of attention, working memory and processing speed are considered by the field as three core neurocognitive deficits that contribute to poor long-term outcomes in posterior fossa tumor survivors (Mabbott et al., 2008; Palmer, 2008; Palmer et al., 2013). In a conceptual model developed by Palmer (**Figure 1**), for medulloblastoma survivors specifically, intellectual outcomes and academic achievement are viewed as distal markers which are secondary to attention, working memory, and processing speed (Palmer, 2008). Additionally, these three core neurocognitive markers influence each other, with processing speed exhibiting a cascading impact on attention and working memory (Palmer, 2008). Another model was

proposed by Wolfe and colleagues (2012) for posterior fossa tumor survivors, and contrary to the Palmer model, the three core neurocognitive skills had an equal influence on intelligence quotient (IQ) and academic achievement (Wolfe et al., 2012).

King and colleagues generated data-driven models to empirically test these competing models and to understand the relationships between risk factors, core skills and distal intelligence markers (King et al., 2019). From this study, the best-fitting neurodevelopmental model included components of Palmer and Wolfe's models, but also had novel significant contributions between risk factors, core skills and outcomes (King et al., 2019). In line with Palmer's model, processing speed had a robust relationship with intellectual outcomes, and lower processing speed was associated with lower attention span and lower memory. However, a cascade of weaknesses beginning with processing speed was not supported, and in line with Wolfe's model, all three neurocognitive skills had a unique relationship with lower IQ and academic achievement. Therefore, a new empirically-tested model that combined components of the previous models was developed by King and colleagues to elucidate the relationships between individual treatment factors, core neurocognitive skills, IQ and academic achievement (Figure 1). Recent studies have only tested associations between GST polymorphisms and self-report or neuropsychological measures for intelligence and they have not focused on the underlying core cognitive processes that influence these secondary outcomes. In line with the current neurodevelopmental model of long-term outcomes, this study can potentially distinguish which neurocognitive skills are the most vulnerable to genetic risk.

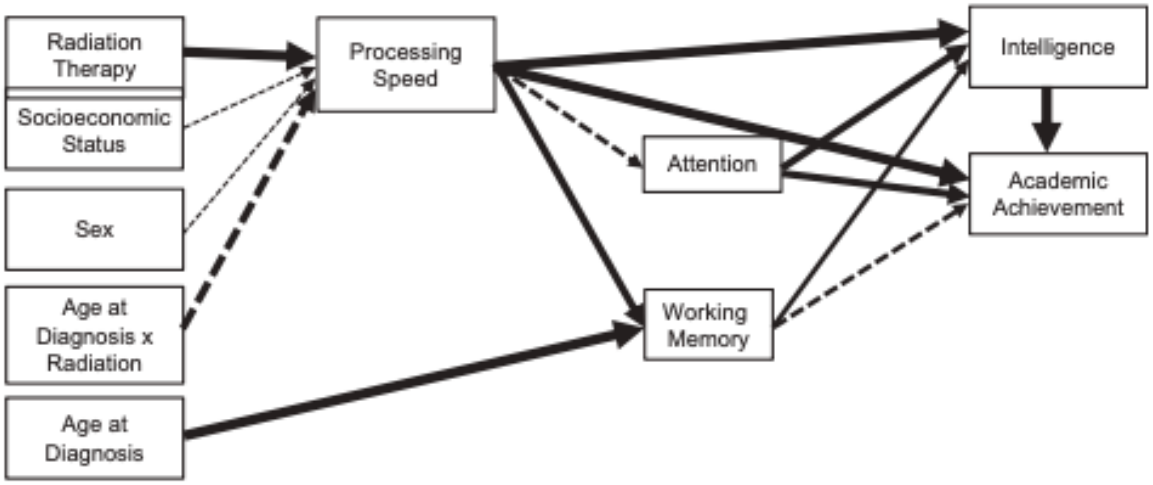

Figure 1 Conceptual model for core neurocognitive skills and the impact of diagnosis and treatment on pediatric brain tumor survivors (King et al., 2019).

1.2.1 Attentional Difficulties in Survivors of Childhood Brain Tumors

Attention, the ability to attend to stimuli presented, has been found to be significantly impaired for medulloblastoma survivors compared to healthy age-matched controls (Reddick et al., 2003; Reeves et al., 2006). Brière and colleagues conducted a study with a heterogenous group of brain tumor survivors and found that auditory attention deficits are significant and delayed in survivors (Briere et al., 2008). Palmer and colleagues utilized estimated trends for medulloblastoma survivors five years after diagnosis and projected that broad attention skills would fall in the low-average to low range, with worse declines for survivors with a higher baseline (Palmer et al., 2013). In 2004, Mulhern and colleagues conducted a review of studies that examined the long-term neurological sequelae of survivors of pediatric brain tumors and found that poor attention in medulloblastoma survivors was associated with young age at diagnosis, higher doses of radiotherapy, and increased time since radiotherapy (Mulhern et al., 2004). This illuminates the finding that attentional deficits may be delayed in survivors and researchers should follow medulloblastoma survivors' longitudinally to understand the full impact of treatment.

Since Mulhern's review, researchers have delved deeper into how underlying cognitive deficits impact survivors. Using neuropsychological testing; such as, Connors Continuous Performance Test (CPT 3) and the California Verbal Learning Test-II (CVLT-II) Trial 1 subtest, researchers found long-term attentional deficits in survivors of childhood medulloblastoma (Maddrey et al., 2005). Along with the CPT 3 and CVLT-II Trial 1, Digit Span Forward is a validated measure for assessing auditory attention span. A group of 100 pediatric brain tumor survivors exhibited significantly worse performance ($p = .02$) on Digit Span Forward than age-normed population means (McCurdy et al., 2016). Digit Span has been evaluated in a study by Cole and colleagues that found an association between GSTP1 and lower mean Digit Span performance in survivors of leukemia (Cole et al., 2015). However, the study did not separate Digit Span Forward and Backward which is an important distinction between attention and working memory. Therefore, the current study aims to build on previous research and administer the Digit Span Forward subtest and CVLT-II Trial 1 as evaluations of attention span in survivors of pediatric tumors.

1.2.2 Working Memory Deficits in Survivors of Childhood Brain Tumors

Working memory is the ability to keep information in mind while performing complex reasoning, comprehension or a learning task (Baddeley, 1992). While research has established brain tumor survivors experience working memory declines after treatment (Conklin et al., 2012; Dennis et al., 1992; King et al., 2015; Palmer et al., 2013) some studies indicate survivors still score within normative limits on neuropsychological measures (Knight et al., 2014; Mabbott et al., 2008). For example, Mabbott and colleagues studied posterior fossa tumor survivors, mean age of 11.44, who received cranial radiation and found that they scored within normative limits on measures of working memory (Mabbott et al., 2008). One limitation of the study, coinciding

with Cole and colleagues' study, is that Digit Span Backward and Forward were not separated in analyses.

Knight and colleagues (2014) studied medulloblastoma survivors five years past diagnosis and found a statistically significant increase in parent-reported working memory concerns on the BRIEF-Working Memory scale. However, on a neurocognitive measure of working memory (Woodcock-Johnson-III, Working Memory Composite) the group remained within normative limits (1 SD) of same-aged peers (Knight et al., 2014). In contrast, a study with heterogenous brain tumor survivors who received radiation found that brain tumor survivors performed significantly worse than non-CNS childhood tumor survivors and sibling controls on the Digit Span Backward task (Conklin et al., 2012). The performance of non-CNS tumor survivors and brain tumor survivors' siblings were not significantly different, indicating specific CNS-tumor or treatment variables were associated with working memory decline (Conklin et al., 2012).

A study conducted by King and colleagues (2015) examined both the behavioral and functional differences in working memory performance between posterior fossa tumor survivors and demographically-matched controls. Survivors performed significantly worse than healthy controls on the Auditory Consonants Trigram (ACT), a working memory task in which participants have to recall three letters, after a 36-second delay, with a distractor task in between. Similarly, on a letter n-back paradigm, survivors demonstrated increased BOLD signals for the frontal and parietal lobe compared to controls during a working memory 2-back task relative to a 0-back task. Compared to healthy controls, survivors are performing significantly worse and recruiting additional cognitive control regions to assist with working memory task demands (King et al., 2015). The field has found some mixed results in terms of the magnitude of

survivors' working memory deficits compared to normative means. Nonetheless, working memory is an important construct to measure because there is variability in outcome which could increase the specificity and focus on attention in this study.

1.2.3 Processing Speed Deficits and Survivors of Childhood Brain Tumors

Processing speed refers to the rate at which individuals are capable of processing perceptual or cognitive information (Gontkovsky & Beatty, 2006). The processing speed abilities of posterior fossa survivors are significantly slower than normative controls (Briere et al., 2008; Stargatt et al., 2007). Brière and colleagues conducted a study with a heterogenous group of brain tumor survivors and found that the average processing speed index for survivors was 1.7 standard deviations below normative means (Briere et al., 2008). Spiegler and colleagues (2004) examined thirty-four posterior fossa tumor survivors, thirty with medulloblastoma, across time on intelligence and neurocognitive measures. The survivors' decline in the processing speed index (PIQ) on the Wechsler Intelligence Scale for Children-III (WISC-III) had a steep decline which leveled off over the course of 16 years (Spiegler et al., 2004). Finally, both attention and processing speed, assessed by the WISC-III and Tests for Attention Performance (TAP) were found to be impaired in 79% of a sample of eighteen medulloblastoma survivors (Ribi et al., 2005). Of the three core neurocognitive skills, researchers have found processing speed to be the most vulnerable to decline in posterior fossa tumor survivors (Mabbott et al., 2008; Palmer et al., 2013). Mabbott and colleagues (2008) discovered that posterior fossa survivors treated with cranial radiation had significantly lower processing speed scores than survivors treated by surgery only, and this deficit was only exacerbated by neurological complications (i.e., hydrocephalus). The researchers concluded that deficits in processing speed may be the first evident neurocognitive difficulties in posterior fossa survivors (Mabbott et al., 2008). Based on

these studies, processing speed is an important primary marker for cognitive decline in long-term survivors and it may influence performance on additional cognitive measures. The relationship between processing speed and attentional deficits will be important to conceptualize for interpretation of results.

1.3 Hippocampal Volume and the Relationship to Attention

Chemoradiation inhibits hippocampal neurogenesis, the generation of new neurons which occurs throughout the lifespan, by dramatically reducing the production of immature neurons in medulloblastoma survivors (Monje et al., 2007). Neuroimaging studies have shown that survivors of childhood brain tumors display abnormal hippocampal development and lower hippocampal volumes than healthy controls (Jayakar et al., 2015; Nagel et al., 2004; Seibert et al., 2017). Researchers have observed attentional deficits associated with the following brain regions: the bilateral hippocampus, right frontal white matter, and right prefrontal white matter (Ailion et al., 2017; Jayakar et al., 2015; Mulhern et al., 2004). Long-term survivors of pediatric brain tumors have reduced volume of normal-appearing white matter and these changes are associated with impaired attentional abilities (Reddick et al., 2003). Nagel and colleagues studied 33 medulloblastoma survivors and found that right and left hippocampal volume loss occurs predominantly in the posterior regions of the hippocampus (Nagel et al., 2004). Jayakar and colleagues discovered an association between lower hippocampal volume and worse performance on the CVLT-II Trial 1 subtest, which measures auditory attention, for long-term childhood brain tumor survivors (Jayakar et al., 2015). There were no additional associations between hippocampal volume of survivors and performance on the short and long-term CVLT-II memory indices after controlling for attention, which suggests a specific relationship between auditory attention and hippocampal volume (Jayakar et al., 2015). The second aim of the current

study is to examine bilateral hippocampal volume differences between survivors with GST polymorphisms and those without. We compared the hippocampal volumes of these groups to age-matched healthy controls to ensure the volumetric differences between controls and our sample of survivors is consistent with the literature.

1.3.1 Hippocampal Volume and Single Nucleotide Polymorphisms

Recently, researchers have joined a collaborative effort to identify genetic contributions to variance in the brain volume of healthy individuals and individuals with various disorders through the Enhancing NeuroImaging Genetics through Meta-Analysis (ENIGMA) consortium (consortium, 2011). ENIGMA has led to the discovery of genes and polymorphisms linked to hippocampal volume differences (consortium, 2011; Hibar et al., 2017). Hibar and colleagues performed a genome-wide association study (GWAS) on 33,536 healthy individuals and discovered six loci within genes HRK, MSRB3, SHH, ASTN2, DPP4, and MAST4 that are associated with hippocampal volume (Hibar et al., 2017). Additionally, the researchers found that SNPs on these genes accounted for 18.76% of the variance in hippocampal volume, and those with decreased hippocampal volume have an increased risk for Alzheimer's disease (AD) (Hibar et al., 2017). While GST SNPs were not implicated in the previous study, researchers measured the expression of GST SNPs in hippocampal tissue and found an association between GST SNP expression and age-of-onset in AD and Parkinson's disease (Li et al., 2003). According to the National Human Genome Research Institute (NHGRI) GWAS catalog, SNP-trait association studies have not been published on childhood brain tumors or medulloblastoma (MacArthur et al., 2016). Additionally, ENIGMA has formed a Cancer and Chemotherapy Working group to investigate how chemotherapy is related to cognitive impairment; however, their first published study only utilized non-central nervous system cancer survivors (Shiroishi et

al., 2018). With the assistance of the ENIGMA consortium, the oncology research field is beginning to conduct GWAS on cancer survivors which will hopefully reveal the influence of genetic polymorphisms on subcortical structures.

1.4 GST Genes

Glutathione S-transferases (GST) protect the human body from dangerous chemicals through enzymes involved in detoxification. GSTs work by binding glutathione (GSH) to exogenous chemicals that have an electrophilic center, such as chemotherapy agents, carcinogens, pesticides and oxidative-damage products, as a mechanism to protect the cells (Hayes & Pulford, 1995; Hollman et al., 2016). GSH conjugation not only removes the harmful chemicals, but also reduces the half-life of hydrophobic xenobiotics (Hayes & Pulford, 1995). Ultimately, the GST family provides several lines of defense for the body against foreign xenobiotics; such as, anti-cancer drugs. Human GSTs are active towards alkylating agents used in anti-cancer therapy; such as, triethylenemelamine and cyclophosphamide, and they may protect against the cytotoxicity of anti-cancer drugs (Hayes & Pulford, 1995). GSTs have at least twenty isoenzymes, but the three genes of interest, GSTM1, GSTT1 and GSTP1, have established roles in taking care of cells through enzymatic activity or production of the enzymes that detoxify xenobiotics (Hayes & Pulford, 1995). A summary of the potential roles that GST genes play in enzyme activity is illustrated in **Figure 2**. Before diving into the changes caused by a GST polymorphism, it is essential to describe how the GST gene interacts upstream and downstream with other genes to carry out cell protection.

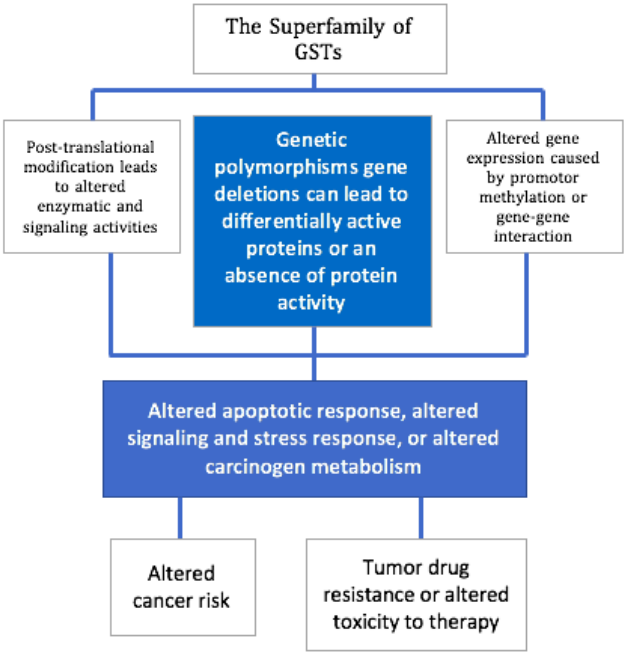

Figure 2 The GST Superfamily.
Figure adapted from (Lo & Ali-Osman, 2007)

1.4.1 GST Pathway

GST genes play a vital role in altering protein and enzyme response to carcinogens and agents of oxidative stress, which will alter cancer risk and drug resistance (Lo & Ali-Osman, 2007). Researchers have found that elevated expression of GSTP1 during cancer treatment can alter the balance of regulation of signaling pathways that influence cell proliferation and apoptosis, which grants tumor cells the ability to evade death (Laborde, 2010). The pathway from oxidative stress to evading apoptosis and genomic damage is digitally illustrated through the Kyoto Encyclopedia of Genes and Genomes (KEGG). KEGG is a genomic database that outlines the molecular functions of genes and proteins and their relationship to each other within various pathways (Kanehisa et al., 2016). One of the notable pathways to cancer begins with oxidative stress directly inhibiting the KEAP1 gene which in turn inhibits the NRF2 gene. This inhibition causes oxidative stress to alter HO-1 and GST gene expression. GST genes work indirectly to evade apoptosis. Specifically, the GSTP1 gene works to inhibit carcinogens that

lead to genomic damage. Additionally, this inhibition pathway is altered by whether or not there is a mutation on the GSTP1 gene. These pathways are visualized as Pathway 1 and 2 in **Figure 3** (Kanehisa et al., 2017). The genes functioning upstream from GSTs are the first to respond and change due to oxidative stress. Heme oxygenase-1 (HO-1) plays a role in maintaining homeostasis and is induced by oxidative stress (Choi & Alam, 1996). The genes that are inhibited by oxidative stress, KEAP1 and NRF2, have established roles with chemotherapy resistance and poor survival rates in adenocarcinoma, gallbladder cancer, and non-small-cell lung cancer (Li et al., 2011; Shibata et al., 2008; Yamadori et al., 2012). Ohta and colleagues have conceptualized the KEAP1 and NRF2 pathway as a "double-edged sword," aiding in growth and development of cancer cells while protecting the body from oxidative stress and carcinogens (Ohta et al., 2008). Similar to KEAP1 and NRF2, GSTP1 can also act as a double-edged sword in protecting the cell from carcinogens and removing cancer treatment agents at the same time (Chatterjee & Gupta, 2018; Kanehisa et al., 2017).

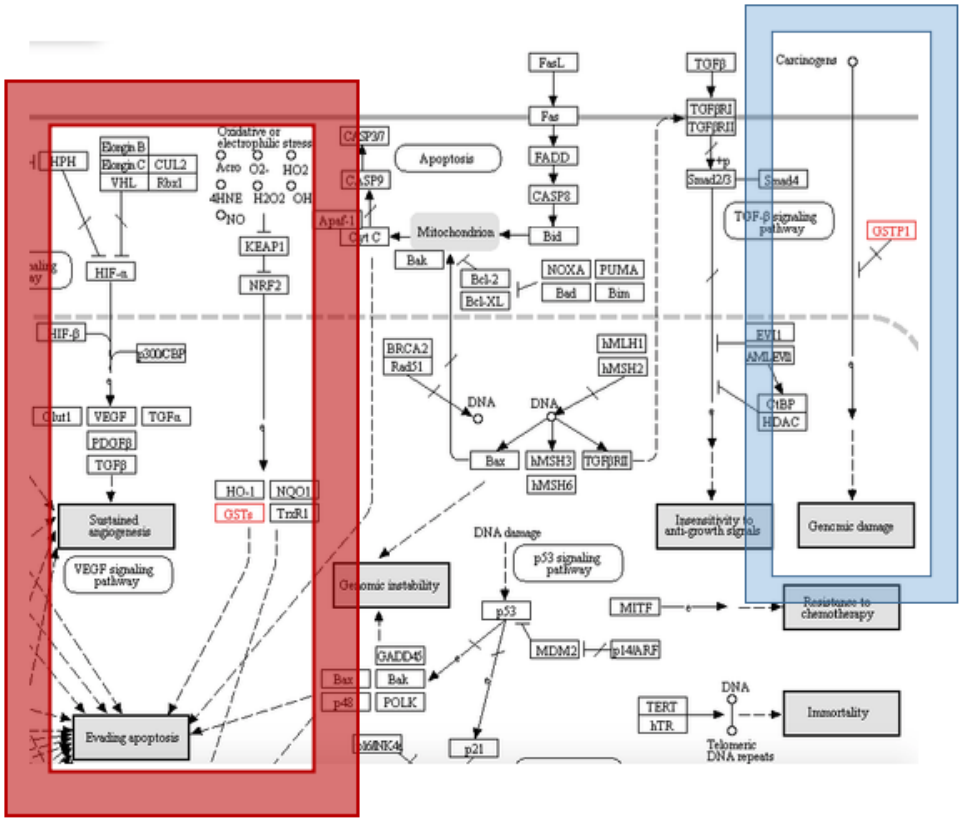

Figure 3 GST pathways in cancer (Kanehisa & Goto, 2000)

Pathway 1 (red outline): *Upstream oxidative stress directly inhibits KEAP1 which inhibits NRF2 and directly interacts with HO-1 and GST gene expression. GST genes indirectly work downstream to evade apoptosis*
Pathway 2 (blue outline): *GSTP1, which can have a mutation or SNP, inhibits carcinogens that lead to genomic damage*

1.5 GST Polymorphisms

A single-nucleotide polymorphism (SNP) is a single base change in a genome sequence that alters the genetic code. SNPs within the GST family have been associated with reduced enzyme activity and reduced detoxification of chemical agents (London et al., 2000). The presence of a GST polymorphism increases chemotherapy-induced cytotoxicity in the tumor location and is associated with significant risk of post therapy complications; such as, developing a secondary cancer in leukemia survivors (Allan et al., 2001). Hollman and colleagues published a 2016

review on the public health risk of GST polymorphisms and their role as a significant biomarker for cancer treatment and prevention (Hollman et al., 2016). Additionally, in a review by Chatterjee and Gupta, evidence is provided for the GST class to be considered as a biomarker, with emphasis on the GSTP1 gene for preventive and therapeutic intervention (Chatterjee & Gupta, 2018). The GSTP1 gene has a unique role in cancer development, inhibition of kinases that signal apoptosis within the cell, and interaction with dietary agents (Chatterjee & Gupta, 2018). GSTP1 has strong associations with caretaking of cells and polymorphisms impact the efficiency of the gene. Given the results of our preliminary analyses (***2.2.1 SNP Calling)*** for frequency of GST SNPs in our sample, we chose to focus on GSTP1 polymorphisms and their potential role in long-term cognitive outcomes and hippocampal volume.

1.5.1 GSTP1 Polymorphisms

Although there are inconsistencies in the literature, GSTP1 has been significantly associated with drug resistance, tumor development, decreased survival rates, and changes in cytotoxicity. Oguztunzan and colleagues observed breast cancer tissue and found that significantly stronger GSTP1 expression is found in tumor epithelium than healthy epithelium (Oguztuzun et al., 2011). Significant associations between the level of GSTP1 expression and response to chemotherapy treatment was found in 60 cancer patients with acute non-lymphoblastic leukemia, with a high expression correlating with poor response and survival rates (Tidefelt et al., 1992). Overexpression of GSTP1 is associated with drug resistance, advanced tumor stage and poor survival in a study of 61 patients with primary glioma (Ali-Osman et al., 1997). These studies pertain to gene expression within the tumor tissue, which indicates the role of GSTP1 and treatment, but does not provide evidence of how the host's GSTP1 polymorphisms interact with treatment factors.

GSTP1 contains two single nucleotide polymorphisms, at exon 5 and exon 6 which produce Ile105Val (G313A) and Al114Val (C341T) amino acid substitutions (Lecomte et al., 2006). Having a heterozygous or null variant of the GSTP1 gene occurs in about 50% of the population (Sun et al., 2010). A GSTP1 (rs1695 or G313A) polymorphism occurs in 48% for populations of African descent, 33% in populations of European descent, and 17% in populations of East Asian descent (The Genomes Project, 2015). A GSTP1 polymorphism (rs1138272 or C341T) occurs in 1% for individuals of African descent, 7% for individuals of European descent and less than 1% for individuals of East Asian descent (The Genomes Project, 2015). If an individual has either variant allele, then the enzyme activity will be reduced compared to the wild type gene.

Jiao and colleagues found that GSTP1 polymorphisms, which cause lower levels of metabolizing activity toward anticancer agents, provide a significant survival advantage for patients with pancreatic cancer receiving 5-flouracil (Jiao et al., 2007). GSTP1 polymorphism Ile105Val has been shown to significantly increase chemotherapy response in a cohort of 113 patients with non-small cell lung cancer (Sun et al., 2010). The same polymorphism was also associated with better chemotherapy response, due to lower GSTP1 activity and increased chemotherapy-induced cytotoxicity in target tumor tissue, for breast cancer participants and this led to better overall prognosis (Sweeney et al., 2000). However, improved prognosis and long-term survival after therapy increases the prevalence of a second therapy-related cancer for acute myeloid leukemia patients with the GSTP1 polymorphism (Allan et al., 2001). Additionally, in a large study of pediatric cancer patients, including a cohort of medulloblastoma patients, researchers found a large confidence interval for the association between GSTP1 rs1695 and cisplatin ototoxicity (Olgun et al., 2016). The GSTP1 gene has an important role in binding with

c-Jun N-terminal kinases (JNK) to repress the signaling of cell apoptosis regulated by the kinase (Adler et al., 1999). GSTP1 polymorphisms do not bind and inhibit JNK as effectively as the wild type, non-polymorphic, gene (Chatterjee & Gupta, 2018; Yin et al., 2000). Either polymorphism on the GSTP1 gene is unable to provide the same level of protection as a wild-type variant against cell death. Therefore, a reduction of detoxification enzyme activity, caused by a GSTP1 polymorphism, may allow for chemotherapeutic agents to stay longer in a patient's system, causing ototoxicity, cell apoptosis and potentially long-term consequences for healthy tissue.

While survival is the ultimate goal of cancer treatment, researchers have just begun to investigate the long-term outcomes of individuals with variant alleles for the GSTP1 gene. Survivors with GSTP1 SNPs will have a prolonged cytotoxic effect (Sun et al., 2010) and cytotoxic effects from intrathecal chemotherapy have been correlated with significantly lower Digit Span Forward scores for survivors of ALL (Ashford et al., 2010). This was further highlighted in Cole and colleagues study of the lower mean Digit Span combined scores in leukemia patients who have one GSTP1 polymorphism (Cole et al., 2015). Given this body of evidence, we predict that our sample of medulloblastoma survivors will have long-term structural and functional changes if they have a GSTP1 polymorphism.

1.6 Aims of the Proposed Study

The oncology field has recently pushed for individualized treatment based on genetic risk factors. This study is among the first on how GSTP1 polymorphisms may be associated with core cognitive difficulties and structural volume loss in long-term survivors of medulloblastoma. Studies to date have focused on rates of survival and neurotoxicity for cancer patients with a GST polymorphism. Therefore, while cancer patients with GST SNPs may have difficulty

removing foreign toxins efficiently, research is just beginning to focus on the long-term consequences for cognitive skills development. We hypothesize that long-term medulloblastoma survivors with a GSTP1 polymorphism have significantly greater deficits in attention and smaller bilateral hippocampal volumes compared to survivors without a polymorphism. We determined whether an individual has a GSTP1 polymorphism by analyzing variants through R and Golden Helix Browser. Additionally, we used FMRIB's Integrated Registration and Segmentation Tool (FIRST) to quantify hippocampal and putamen volume in survivors. We ran independent t-tests to compare the group of medulloblastoma survivors with a GSTP1 polymorphism to the group without a polymorphism on structural volume. We assessed group differences on neuropsychological performance, hippocampal and putamen volume, and utilize a healthy age and sex-matched control group for comparison. Finally, we tested if GSTP1 polymorphism status is a moderator between hippocampal volumes and core cognitive difficulties in medulloblastoma survivors.

1.6.1 Specific Aim 1: Medulloblastoma survivors who have GSTP1 polymorphisms will have long-term deficits in attention.

Hypothesis 1: *Survivors with a GSTP1 polymorphism will perform worse on measures of attention span compared to survivors without a GSTP1 polymorphism.*

1.6.2 Specific Aim 2: GSTP1 polymorphisms are associated with reduced bilateral hippocampal volume in medulloblastoma survivors.

Hypothesis 2: *Survivors with a GSTP1 polymorphism will have lower volume in their bilateral hippocampus than survivors without a GSTP1 polymorphism.*

1.6.3 Specific Aim 3: GSTP1 polymorphisms moderate the relationship between bilateral hippocampal volume and attention.

Hypothesis 3: *The relationship between bilateral hippocampal volumes and attentional deficits will be significantly stronger in survivors with a GSTP1 polymorphism.*

2 METHODS

2.1 Procedures

2.1.1 Participants and Screening and Recruitment

Participants were survivors of medulloblastoma childhood brain tumors or age and sex-matched controls. This study was approved by the local institutional review boards. Survivors were recruited through the following sources a) a previous longitudinal childhood brain tumor study b) an advertisement in an annual newsletter from the Brain Tumor Foundation of Georgia in which survivors were encouraged to call in and inquire about the study c) survivors from a large southeastern hospital system database. Participants were excluded from the study if they did not indicate fluency in English, have had a traumatic brain injury, a pervasive developmental disorder, neurofibromatosis, or have a diagnosis for Attention Deficit Hyperactivity Disorder (ADHD) prior to cancer treatment. Participants were excluded if hearing loss was not corrected by a hearing aid or if they did not complete a full battery of testing due to hearing accommodations. Healthy control participants were recruited by four sources a) an undergraduate psychology participant pool at a large Southeastern University, b) a research imaging center, c) friends and family of survivors, and d) a community flier. Control participants were not significantly different from the survivor group with regard to sex or age.

Ninety-five brain tumor survivors were contacted by mail about participating in MRI testing or asked for follow-up testing with a blood draw. Thirty-nine participants scheduled

appointments over the phone and twenty-two medulloblastoma participants completed the blood draw. Neuroimaging data was not used if the survivors had poor brain registration or segmentation due to movement in the scanner. Eighteen medulloblastoma survivors completed the blood draw and neuropsychological testing. Fifteen medulloblastoma survivors completed the blood draw, MRI scan and neuropsychological testing. Survivors were on average 20.72 (6.26) years at examination and an average of 12.42 (6.96) years post-diagnosis.

Healthy controls were recruited through the undergraduate psychology participant pool at Georgia State University (GSU), the Center for Advanced Brain Imaging (CABI), friends of survivors, and community flyers. Controls were excluded if they met criteria for Major Depressive Disorder, substance abuse, or a psychiatric disorder based on the Structured Clinical Interview of the DSM-IV. Eighteen controls with both neuropsychological testing and an MRI scan were matched with survivors. Controls were on average 20.61 (2.25) years at examination. Participant demographic information is listed in **Table 1**.

Table 1 Comparison of demographic information

Participant Characteristics	*Medulloblastoma Survivors*	*Controls*
N (# of participants with genotyping and neuropsychological assessment)	18	X
N (# of participants with genotyping, neuropsychological assessment and neuroimaging)	15	X
N (# of participants with neuropsychological assessment and neuroimaging)	18	18
Sex (% Female)	61.1	61.1
Ethnicity (n, %)		

Caucasian	14 (77.8)	14 (77.8)
African-American	2 (11.1)	2 (11.1)
Asian	1 (5.6)	1 (5.6)
Hispanic	1 (5.6)	1 (5.6)
Age at Diagnosis (SD)	8.22 (3.89)	X
Age at testing (SD)	20.72 (6.26)	20.61 (2.25)
Range	12-35	18-25
Mean years education (SD)	11.55 (2.57)	13.67 (1.08)
WASI Full Scale IQ	92.11 (12.60)	109.61 (9.51)
N (# of participants with a GSTP1 Polymorphism)	12	X

2.1.2 Genetic Parameters and Processing

Whole genome DNA-sequencing was performed on blood samples from twenty-two pediatric medulloblastoma survivors. A certified nurse obtained a blood sample. DNA samples were normalized to 1,000ng of DNA in 50ul of water. Following normalization, samples were acoustically sheared via Covaris LE-220 instrument to a final fragment size of ~350-400bp. The sheared DNA was then transformed into a standard Illumina paired-end sequencing library via standard methods. The sheared DNA was end-repaired and A-tailed using New England Biolabs End-Repair and A-Tailing kits, respectively under the manufacturer's recommended conditions. Following each step, the library was purified via Agencourt AMPure XP beads and eluted in water. Standard Illumina paired-end adaptors were ligated to the A-tailed DNA via New England BioLabs Rapid Ligation kit. Following ligation, the reactions were purified using AMPure XP beads. The purified ligated DNA was amplified via PCR using KAPA Biosystems HIFI PCR kit

using 6 cycles of PCR. The primers were standard Illumina primers with a custom 7-base sample barcode in the i7 position to allow sample identification/demultiplexing following sequencing. The final library was quality controlled using size verification via PerkinElmer LabChip GX and real-time PCR using the KAPA SYBR FAST qPCR Master Mix, primers and standards according to the manufacturer's directions. Libraries were normalized to 2.5 nM stocks for use in clustering and sequencing. All sequencing was performed on the Illumina HiSeq X platform by loading a single sample per flowcell lane. Following sequencing all base-calling was performed using standard Illumina software to generate the final FASTQ files for each sample.

The quality of raw reads generated from Illumina sequencing were assessed using FastQC (Andrews, 2012). Reads were filtered and trimmed using the Trimmomatic tool (Bolger et al., 2014). BWA aligner was used to map post-quality filtered reads against the human reference genome (hg19) (Li & Durbin, 2010). The alignment quality was evaluated using SAMtools (Li et al., 2009) and Picard-Tools (http://picard.sourceforge.net). The assembly, or human genome reference build, utilized was GRCh37 or Genome Reference Consortium Human Build 37. A genome reference build is compiled from reference sequences of different individuals to better reflect the genetic variation of subpopulations and ethnicities worldwide. GRCh37 was used to call our variants by searching the genome 30 times to provide a percentage of how likely a single nucleotide polymorphism is present. The mean target coverage was 30X and 95% of the targeted bases have a coverage of 10X or greater. Potential PCR duplicates were removed with Picard-tools (http://picard.sourceforge.net). Somatic variants (SNV and Indel) were called using SAMTools (Li et al., 2009) with Varscan2 (Koboldt et al., 2012) and annotated using ANNOVAR (Wang et al., 2010). Variants with low quality read depth (<6X) were excluded from the analysis. A variant proportion was estimated for each gene variant for each

sample. Here variant proportion is defined as the reads supporting the variants divided by the total number of reads supporting the variant and the reference allele, hence ranging from 0 to 1. A value of 0 means no reads supporting the variant have been identified, a value of 0.5 means half of the reads support variant and half support reference allele, and a value of 1 means all reads are supporting the variant allele.

We used custom scripts, included in the Appendix, within R software to merge the whole genome of participants with gene variants of interest by exact position on the chromosome to obtain the variant information (i.e., variant chromosomal position, genotype and variant allele frequency). Variants were visually conformed using Golden Helix Browser. This is expressed as percent read or the percentage of times the assembly read an alternate allele instead of a reference one. A reference allele means there was no polymorphism, while an alternate allele can be for a heterozygous or mutant (null) genotype. If an individual possesses a GST polymorphism this information was coded as present (1) or absent (0) creating two groups among the survivors. Two graduate students in the King lab independently processed the variant call format using the programming software R. Interrater reliability was 100%.

2.1.3 Imaging Parameters and Processing

The Siemens Trio 3T scanner was used to collect high resolution T1-weighted structural images for each of the participants. T1-weighted structural images were acquired through 176 sagittal slices. A 3D magnetization prepared rapid gradient echo imaging (MPRAGE) sequence was used with the following parameters: acquisition matrix = 256 X 256, repetition time (TR) = 2,250 milliseconds, echo time= 3.98 milliseconds, field of view (FOV) = 256 milliseconds, slice thickness = 1.0 millimeters and flip angle = 9 degrees. Volumetric analysis and segmentation of the hippocampus and putamen was processed by the research team using FMRIB's Integrated

Registration and Segmentation Tool (FIRST). FIRST is used empirically to quantify hippocampal volumes and correlate these volumes with neuropsychological performance (Jayakar et al., 2015; Turner et al., 2012). FIRST accomplishes segmentation by transforming the 3D T1 image into an MNI 152 standard space. A subcortical mask is used to locate the hippocampus, which is composed of the dentate gyrus, ammonic subfields, presubiculum, and subiculum. Hippocampal volumes were recorded in millimeters and entered into SPSS 23.0 (Corp, 2015) as a dependent variable. The right and left putamen volumes were acquired using the same methods and software. The volume of the putamen was utilized as a control region, since attentional deficits have not been reported to be associated with the putamen. Additionally, we acquired total intracranial volume (ICV) using FreeSurfer v.5.3 software. FreeSurfer transforms a T1-image into MNI 305 standard space and normalizes the image to correct for voxel intensities (Fischl, 2012). We checked the ICV segmentation for errors using the Tkmedit tool and none were found. The segmented images were transformed back to native space and the estimates for the ICV include the brain, meninges, and CSF and are measured in mm^3. We acquired the total volume of the participants' brains to ensure that lower volumes of the bilateral hippocampus do not only occur in those survivors with significantly lower brain volumes.

2.1.4 Assessment of Attention Span

To measure attention, we compared the Digit Span Forward performance of survivors with GSTP1 polymorphisms to those without and to age and sex-matched controls. For the Digit Span Forward subtest, a participant must repeat a list of digits in the order that the examiner reads them. Digit Span Forward is a test of auditory attention span and performance on this subtest is used as a dependent variable in our study. We utilized the raw scores of participants and covary with age. Digit Span Backward, which measures working memory, should be

considered separately from Digit Span Forward and results on these two tests cannot be combined (Rosenthal et al., 2006). All three sections of the Digit Span have internal consistency coefficients, across all ages, of 0.81- 0.83 (Kaufman & Lichtenberger, 2009). The Digit Span has a Standardization sample of 2,200 individuals with ethnicities representing the 2005 US Census, and it has a split-half reliability of 0.93 and test-retest reliability of 0.83 (Kaufman & Lichtenberger, 2009).

As a second measure of auditory attention span, we compared the CVLT-II and CVLT-C Trial 1 performance of survivors with GSTP1 polymorphisms to those without and to age and sex-matched controls. The CVLT-II and CVLT-Children's version are measures of attention span, learning and memory (Delis, 1994; Strauss et al., 2006). The Trial 1 subtest measures auditory attention span and requires participants to immediately recall words from a word list that is read to them. The Standardization for the CVLT-II included 1,087 individuals chosen to represent 1999 US Census data. The split-half reliability of the CVLT-II on the Trials 1-5 subtests is 0.94 and the internal consistency coefficient is 0.82 (Strauss et al., 2006). The Standardization of the CVLT-C included 920 children in twelve age ranges and the test was stratified based on the 1988 U.S. Census data (Strauss et al., 2006). The CVLT-C has good internal consistency and the test-retest reliability for Trial 1 ranges from 0.6 to 0.79 (Strauss et al., 2006). We utilized age-normed z-scores for statistical analyses of the CVLT-II/CVLT-C.

2.1.5 Assessment of Working Memory

For working memory, we utilized the Auditory Consonant Trigrams (ACT) in which participants are asked to remember three consonants (e.g., B-D-T) that are read by the examiner. Next, participants are asked to count backwards from a given number, and after 18 seconds the participants must recall the three consonants. Test-retest reliability for the ACT was .71 (Shura et

al., 2015). Performance was converted into z-scores for both the adult and child versions of the tests based on normative data (Paniak et al., 1997; Stuss et al., 1987). Additionally, we used the Digit Span Backward raw scores as a second working memory measure. We covaried the raw scores with age and compare performance across the three groups.

2.1.6 Assessment of Processing Speed

Processing speed was assessed by the Oral Symbol Digit Modality Test (Smith, 1982). This is a timed task in which the examiner hands the participant a piece of paper with a sequence of symbols and underneath each symbol is a box. At the top of the page, there is a key which indicates each symbol corresponds with a number. Participants have 90 seconds to say the number that corresponds with the symbol in each box. The test-retest reliability for the Oral Symbol Digit Modality Test is 0.76 (Smith, 1982). All raw scores were computed into age-normed z-scores.

2.2 Preliminary Analyses and Results

2.2.1 SNP Calling

Analysis on SNPs in our sample was conducted to determine the polymorphism status of survivors based on predetermined genes of interest. These analyses were run by two graduate students independently to ensure interrater reliability. The results are listed on **Tables 2, 3** and **4**. Based on these analyses, the greatest variation in our population was for the GSTP1 gene and SNPS, G313A (rs1695) and C341T (rs1138272). One of our SNPs of interest, G313A, also known as rs1695, was expressed at higher rates than control populations. Based on a meta-analysis of healthy non-cancerous controls, 49.3% of the Caucasian population has an amino acid substitution resulting in rs1695 (Garte et al., 2001). For our Caucasian survivors, 71.4% had

a rs1695 SNP. For our African American participants, 50% had the rs1695 SNP, our Asian participant had the rs1695 SNP and our Hispanic participant did not have the rs1695 SNP. Since a higher chance of survival is associated with rs1695 SNPs for multiple cancer types (Jiao et al., 2007; Sun et al., 2010; Sweeney et al., 2000), investigators have called for larger scale studies of the rs1695 polymorphism and pediatric cancer survivors (Olgun et al., 2016).

Table 2 Frequency of alleles for all participants sequenced

Gene Symbol	Genomic Variation	RSID	Wild Type	%	Heterozygous	%	Homozygous	%
GSTP1	G313A	1695	8	36%	13	59%	1	5%
GSTP1	C341T	1138272	17	77%	5	23%	0	0%
GSTM1	GSTM1 0	2071487	22	100%	0	0%	0	0%
GSTT1	GSTT1 0	2266637	21	95%	1	5%	0	0%

Table 3 Frequency of alleles for sample with neuropsychological data

Genomic Variation	RSID	Wild Type	%	Homozygous + Heterozygous	%
G313A	1695	6	33%	12	67%
C341T	1138272	14	78%	4	22%

Table 4 Frequency of alleles for sample with neuropsychological and neuroimaging data

Genomic Variation	RSID	Wild Type	%	Homozygous + Heterozygous	%
G313A	1695	5	33%	10	67%
C341T	1138272	12	80%	3	20%

2.2.2 Potential Confounds

We chose to compare sex, age at testing, and ethnicity across survivors and controls to increase specificity in the present study. Independent samples t-test ($p<.05$) were used to compare groups on continuous variables (i.e., age at examination). Age at examination was not

significantly different between groups (p=.944). Next, chi-squared tests of independence (p<.05) were used to compare groups on categorical variables (i.e., sex and ethnicity). Controls and survivors had the same breakdown between males (n=7) and females (n=11); and therefore, there was not a significant association between sex and group. Also, controls and survivors had the same breakdown for ethnicities: Caucasian (n=14), African American (n=2), Hispanic (n=1), and Asian (n=1). When we split the groups into Caucasian (n=14) and Other (n=4), there was not a significant association between ethnicity and group (χ^2=.64, p =.42). Overall, we will not be utilizing any demographic variables as covariates in our study, with the exception of controlling for age on Digit Span raw scores.

2.2.3 Potential Covariates

A covariate for our study is ICV because the diversity in brain size for each individual will impact the average hippocampal and putamen volume for the group. Since individuals under eighteen are included in this study, the disparity in ICV will be larger between minors and the rest of the adult cohort. We conducted bivariate correlations between ICV, GSTP1 polymorphism status, bilateral hippocampal volume, and bilateral putamen volume. ICV was significantly related to the volume of the bilateral hippocampus (r=.521, p<.05). Therefore, ICV will be utilized as a covariate for neuroimaging analyses. Since ICV was not significantly related to polymorphism status, the IV, it is not a confounding variable.

Given evidence that covarying for IQ overcorrects and leads to counterintuitive neurocognitive findings in neurodevelopmental disorders, we decided not to utilize IQ as a potential covariate for our sample (Dennis et al., 2009). Additionally, the effects of chemoradiation likely impact our outcomes of interest. While our sample is homogenous in cerebellar tumor location, our survivors do not receive identical treatment protocols and will be

influenced by the overall effects of aggressive chemoradiation treatment for medulloblastoma. Therefore, we will not explore the direct impact of genetic influences on chemotherapy because diverse protocols cannot be separated into groups with adequate power. Previous studies on the relationship between genetic polymorphisms and neurocognitive outcomes for brain tumor survivors have not separated the effects of chemotherapy and radiation for long-term outcomes (Barahmani et al., 2009; Brackett et al., 2012).

2.3 Planned Analyses

2.3.1 Tests of Data Assumptions

We conducted tests of normality, homogeneity of variance, heterodescascitiy, non-independence of residuals, and normality of residuals on the survivor sample to determine the influence that polymorphism status had on dependent variables.

2.3.2 Analyses for Specific Aim 1

Hypothesis 1: *Survivors with a GSTP1 polymorphism will perform worse on measures of attention span compared to survivors without a GSTP1 polymorphism.*

Using IBM SPSS 23.0, the group of medulloblastoma survivors with a GSTP1 polymorphism were compared to medulloblastoma survivors with no polymorphism and control participants on measures of attention span. The dependent variables, scores on the Digit Span and scores on the CVLT-II/CVLT-C Trial 1, are continuous. We ran Pearson's bivariate correlations to see if polymorphism status is associated with Digit Span Forward and CVLT-II/CVLT-C Trial 1 performance. The raw scores on the Digit Span Forward are covaried with age. We ran a one-way between-groups analysis of variance (ANOVA) to determine differences between the two groups of survivors and healthy controls on auditory attention performance. We

ran independent t-tests to compare the two survivor groups on attentional, working memory and processing speed performance. One individual was removed from working memory analyses because they did not complete the ACT during their neuropsychological testing session. Since the groups with a polymorphism, without a polymorphism and controls are uneven we utilized Levene's Test to determine if equal variances can be assumed.

If an individual's score on the CVLT-II/CVLT-C Trial 1 is greater than 1.5 standard deviations below the age-normed performance mean, then the individual meets criteria for impairment. We quantified the percentage of survivors that met impairment criteria and ran a Pearson chi-squared test, or Fisher's exact test if the group is less than 5 survivors, to determine if the difference between groups is significant. It was hypothesized that the group of survivors with a polymorphism will have significantly lower mean scores than any other group.

2.3.3 Analyses for Specific Aim 2

Hypothesis 2: *Survivors with a GSTP1 polymorphism will have lower volume in their bilateral hippocampus than survivors without a GSTP1 polymorphism.*

Using IBM SPSS 23.0, the group of medulloblastoma survivors with a GSTP1 polymorphism were compared to medulloblastoma survivors without a GSTP1 polymorphism and healthy controls to determine whether there are significant differences in their bilateral hippocampal or putamen volume. The dependent variable, volumes of the bilateral hippocampus and putamen, are continuous. First, we tested if there are significant group differences between the two groups of survivors and healthy controls in hippocampal and putamen volume using a one-way between-groups analysis of covariance (ANCOVA) with ICV as the covariate. Due to unequal group sizes we utilized Levene's Test to determine if equal variances can be assumed. Next, we focused on differences between the two groups of survivors, with and without a

polymorphism, and ran correlations to see if polymorphism status is associated with hippocampal or putamen volume. Finally, we ran an ANCOVA using polymorphism status as the independent variable and hippocampal and putamen volumes as dependent variables, with ICV as a covariate. We predicted that survivors with a polymorphism will have lower hippocampal volumes than survivors without a polymorphism and healthy controls. We utilized bilateral putamen volume as a control region which will increase specificity of our study.

2.3.4 Analyses for Specific Aim 3

Hypothesis 3: *The relationship between bilateral hippocampal volumes and attentional deficits will be significantly stronger in survivors with a GSTP1 polymorphism*

The next analysis explored whether the relationship between bilateral hippocampus volume and attentional deficits is moderated by GSTP1 polymorphisms. Moderation analysis is used when researchers are interested in the magnitude of a moderating variable's influence on the independent variables' relationship with the outcome variable (Hayes, 2012). We wanted to examine whether having a polymorphism strengthens the relationship between hippocampal volume and attentional outcomes. Previous research has found an association between lower bilateral hippocampal volume and attentional deficits (Jayakar et al., 2015). However, research has not yet examined if the nature of this relationship partially depends on GSTP1 polymorphism status. First, we ran a linear regression to test whether hippocampal volume predicts attentional deficits in our sample of medulloblastoma survivors. We tested for an indirect effect of hippocampal volume on attentional deficits moderated by GSTP1 polymorphism status. We used the PROCESS macro for SPSS (Hayes, 2012) to create a moderation model. PROCESS calculates indirect effects by constructing confidence intervals based on resampling of the data with a replacement bootstrapping method. We resampled the data 10,000 times to approximate

the sampling distribution, which resolves the issue of a skewed distribution in smaller samples. PROCESS allows for the inclusion of covariates within the moderation model and ICV was included as a predictor variable along with bilateral hippocampal volume.

2.3.5 Analyses for Supplementary Aim 1

Losses and gains of segments of genomic DNA contribute to the expression of oncogenes. The mapping of these losses and gains, also called copy number variations (CNVs) allows for the detection of critical genes (Pinkel et al., 1998). While the primary focus of our study has been on SNPs, analyzing CNVs allows for a greater understanding of the genetic abnormalities in our sample. CNVs in high-grade neuroblastoma tumors were associated with clinical outcomes and may be related to the aggressiveness of the tumor (Carén et al., 2010). CNVs can be used to differentiate the four major subgroups of medulloblastoma, each subgroup with distinct genetic abnormalities and clinical outcome (Hovestadt et al., 2013; Northcott et al., 2011). Researchers discovered that copy gains on Chromosome 6q and 17q in pediatric medulloblastoma survivors leads to poor prognosis (Pfister et al., 2009). We choose to map the CNVs in our sample to locate genetic abnormalities, and to ensure there are no genomic losses or gains at the locations of GSTP1 polymorphisms. To map CNVs, a control (normal) sample is generally needed; however, the Control-FREEC tool (Boeva et al., 2011) does not necessitate a matched normal sample for analysis. Control-FREEC allows for automatic calculation of copy number variants and predicts regions of losses and gains of genomic DNA (Boeva et al., 2011). Control-FREEC operates by calculating and segmenting copy number profiles, then calculating and segmenting smoothed B-allele frequency profiles, and finally predicting final genotype status (i.e., copy number) (Boeva et al., 2011). Genotype status is predicted for each segment independently by choosing the allelic content that corresponds to the maximal log-likelihood (Boeva et al., 2011).

A normalized copy number profile can be visualized utilizing Linux commands and the Control-FREEC downloadable files. A value of two on the normalized copy number output indicates that there is no change, and this corresponds with the color green on the copy number visualizations (Boeva et al., 2011). On the visualizations, red represents a copy number gain (>2), and blue represents a copy number loss (<2). Visualizations for each participant were provided to interpret the copy number variants in our sample.

2.3.6 Analyses for Supplementary Aim 2

A previous study of GST polymorphisms found a relationship between one null genotype on GSTM1 and GSTT1 and lower full-scale IQ, verbal IQ, and performance IQ in survivors of medulloblastoma (Barahmani et al., 2009). Although we did not have enough representation of GSTT1 and GSTM1 polymorphisms in our sample, we chose to replicate Barahmani's findings with GSTP1. Full-scale IQ is a distal marker associated with genetic risk, demographic variables (i.e., sex), and treatment variables (i.e., radiation, chemotherapy, hydrocephalus). Therefore, we tested for a main effect of sex by splitting our sample into four groups: males with GSTP1 polymorphisms (n=6), males without GSTP1 polymorphisms (n=1), females with GSTP1 polymorphisms (n=6), and females without GSTP1 polymorphisms (n=5). We ran independent t-tests to determine if group differences exist for the survivor sample, and male and female groups on the Weschler Abbreviated Scale Intelligence (WASI) full-scale IQ, verbal IQ, and performance IQ scales. The independent t-tests were uncorrected due to limited power to detect an effect.

3 RESULTS

3.1 Tests of Data Assumptions

Tests of normality, linearity, heteroscedasticity, and independence were not violated, and the sample was normally distributed. Outlier testing was conducted to determine the influence that polymorphism status has on dependent variables. One survivor violated outlier testing on the CVLT-II/CVLT-C Trial 1 subtest, based on a studentized residual of higher than 2.5. The individual had acceptable scores on the other attention, working memory and processing speed measures and excluding them reduces the power of our findings. Their score was winsorized to reflect the closest acceptable score (z-score change from 2 to 1). A different survivor violated outlier testing on the O-SDMT, based on a studentized residual lower than -2.5. The individual had acceptable scores on attention and working memory. Their score was also winsorized to reflect the next lowest acceptable score (z-score change from -4.99 to -4.47). No individuals in the survivor sample violated outlier testing for the influence of polymorphisms status on hippocampal or putamen volume. For controls, tests of normality were not violated for attention and processing speed. The control group had two outliers for working memory performance, and these scores were winsorized to the next acceptable z-score (z=-.84).

3.2 Aim 1

Pearson's bivariate correlations were run to see if polymorphism status was associated with Digit Span Forward and CVLT-II/CVLT-C Trial 1 performance. GSTP1 status was not associated with scores on the CVLT-II/CVLT-C Trial 1 (r= .061, p=.81). The raw scores on the Digit Span Forward were covaried with age, and the associations were not significant (r= -.108, p=.831). A one-way between-groups analysis of variance (ANOVA) was conducted to determine differences between survivors with a polymorphism, survivors without a polymorphism, and

healthy controls on auditory attention performance. Results indicated that there was a significant difference in group mean performance ($F(2,33)= 5.963$, $p = 0.006$). However, this significant difference only existed between healthy controls compared to all survivors. On average, survivors scored lower than controls. We ran an ANOVA with working memory performance (ACT/CCT), and a significant difference was found between groups ($p <.001$). Finally, we also ran an ANOVA with processing speed performance (O-SDMT), and a significant difference was found between groups ($p =.001$). Descriptive statistics including the mean performance and standard deviation are listed for each group in Table 5.

We also conducted the Games-Howell (HSD) post hoc test to further probe any significant omnibus effects. This post-hoc analysis was chosen because population variances were not always equal in our sample, and Games-Howell can be utilized when sample sizes are small and unequal (Field, 2013). A significant difference was found between the survivors without polymorphism and controls ($p=.003$). A significant difference was also found between the survivors with a polymorphism and controls ($p=.013$). However, there was not a significant difference in mean performance on the CVLT/CVLT-C between survivors with a polymorphism and those without ($p=.945$).

We ran independent t-tests to compare the two survivor groups, with a GSTP1 polymorphism and without a GSTP1 polymorphism, on attention span, working memory and processing speed performance. Since the groups with a polymorphism and without a polymorphism are uneven, we utilized Levene's Test to determine if equal variances can be assumed. For the attention span, on the CVLT-II/CVLT-C, equal variances can be assumed ($F(1,15)= 4.267$, $p = 0.055$), although the difference in standard deviations is approaching significance ($p=.055$). This resulted in the standard deviation for the survivors with a

polymorphism being almost three times the size of the standard deviation for survivors without a polymorphism. The variability in attention span performance is represented in a boxplot in Figure 4. There was not a statistically significant difference between the group with and without a polymorphism (p =.690). Since the sample size was small, we chose to use Hedges g over Cohen's d as a measure of effect size (Goulden, 2006). The effect was small for the difference between groups on attention span performance (g = 0.21). Additionally, a one-way ANOVA was run to determine if polymorphism status predicted a secondary auditory attention span measure, Digit Span Forward, controlling for age at examination. After controlling for age, GSTP1 status did not significantly predict auditory attention span performance (p=.831).

For working memory, measured by the ACT/CCT, equal variances cannot be assumed ($F(1,15)= 14.877$, $p = 0.002$). The variability in performance between both groups is represented as a boxplot in Figure 5. The difference in mean performance on working memory was not statistically significant (p =.095). Since our sample size is small, and the mean differences were approaching significance, we measured the magnitude of the effect using Hedge's g. The effect was medium to large (g = .702). In contrast to our hypothesis, the mean performance on the ACT/CCT for the survivors with a polymorphism was a z-score of -.8467, and the mean performance for the survivors without a polymorphism was worse, with a z-score of -1.562. As a secondary measure of working memory, a one-way ANOVA was run to determine if polymorphism status predicted Digit Span Backward performance, controlling for age at examination. After controlling for age, GSTP1 status did not significantly predict working memory span performance (p=.808).

For processing speed, measured by the Oral SDMT, equal variances can be assumed ($F(1,15)= 1.742$, $p = 0.221$). The variability in performance between both groups is represented

as a boxplot in Figure 6. There was not a statistically significant difference between the survivors with a polymorphism and the survivors without a polymorphism (p =.511). The effect was small to medium for the difference between groups on processing speed performance (*g* = .336).

If an individual's score on attention, working memory or processing speed measures is greater than 1.5 standard deviations below the age-normed performance mean (z > -1.5), then the individual meets criteria for impairment. We assessed proportion of impairment in our sample by running Chi-Square analyses. The difference between the proportion of impairment in the survivors with a polymorphism compared to the survivors without a polymorphism was not significant (p = .109). The group without GSTP1 polymorphisms had no individuals that met impairment criteria, out of six total individuals. The group with GSTP1 polymorphisms had four individuals that met criteria for impairment out of twelve total survivors. We also tested the level of impairment for working memory and processing speed. For working memory, the difference in number of participants in the impaired level for groups was not statistically significant (p= .149). Four individuals without a polymorphism met impairment on the ACT/CCT compared to five individuals with a polymorphism. Of note, the group without a polymorphism fell in the impaired range on average (mean=-1.56). In contrast, for processing speed, the group with a polymorphism were overall impaired with a mean z-score of -1.87. The difference between impairment levels was not statistically significant (p=.317). Four individuals without a polymorphism met impairment on the O-SDMT compared to five individuals with a polymorphism. Impairment of core cognitive skills for both groups of survivors is described in Table 6.

Table 5 Aim 1 Results: Descriptive of core cognitive skill performances for each group (z-score)

	Polymorphism *(n=12)*	No Polymorphism *(n= 6, n=5 for ACT/CCT)*	Control *(n=18)*	F	p
Attention Span (CVLT/CVLT-C)	-1.167 (.807)	-1.25(.274)	.028(1.32)	5.963	.006**
Working Memory* (ACT/CCT)	-.8467 (1.15)	-1.56(.502)	.222(.676)	6.182	.0001**
Processing Speed (O-SDMT)	-1.873 (1.65)	-1.3667(1.12)	.114(.778)	8.247	.001**

Note. Percent impaired defined as $\geq$ 1.5 standard deviation below the mean based on normative scores
*n=5 for No Polymorphism group on ACT/CCT
******Significant at 0.01 level

Table 6 Impairment of core cognitive skills for both groups of survivors

	Polymorphism (*n= 12)* % impaired	No Polymorphism (*n*= 6) % impaired	Hedge's *g*	χ^2/Fisher's exact
Attention Span (CVLT/CVLT-C)	33.3%	0%	.12	χ^2 (1) =.109
Working Memory* (ACT/CCT)	41.67%	80%	.702	χ^2 (1) =.149
Processing Speed (O-SDMT)	41.67%	66.67%	.336	χ^2 (1) =.317

Note. Percent impaired defined as $\geq$ 1.5 standard deviation below the mean based on normative scores
*n=5 for No Polymorphism group on ACT/CCT

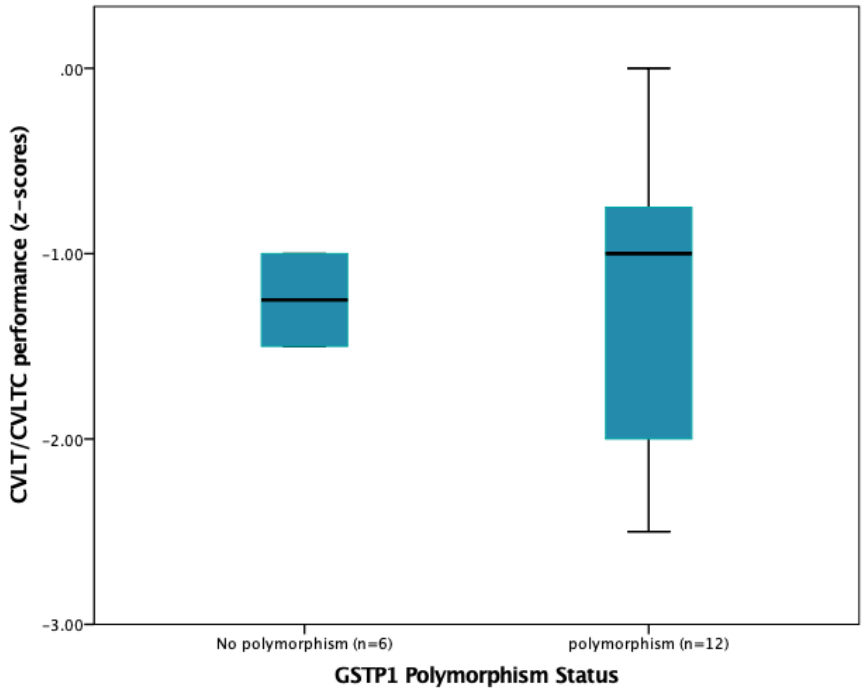

Figure 4 Boxplot of attention span performance by two groups of survivors

Note: Center line represents the median of the data

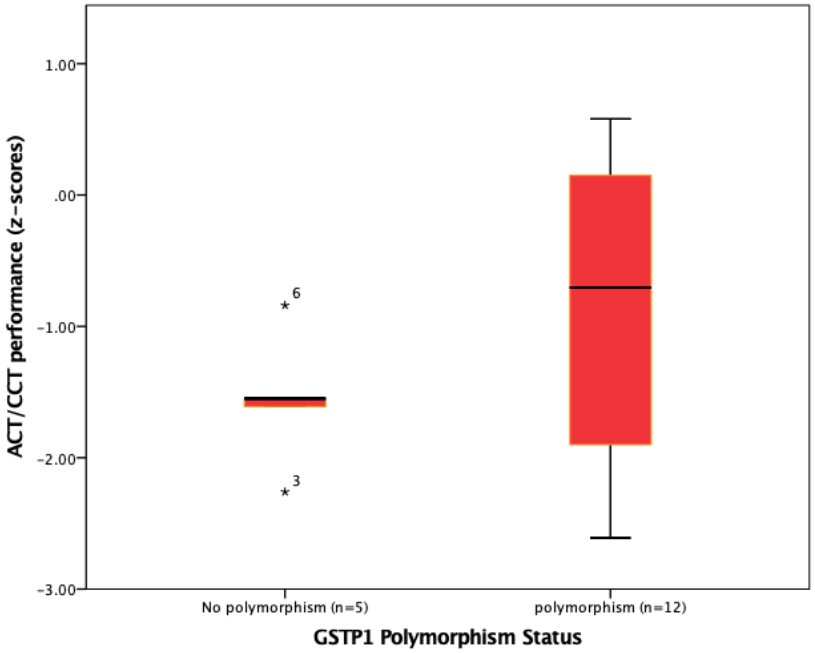

Figure 5 Boxplot of working memory performance by two groups of survivors

Note: Center line represents the median of the data

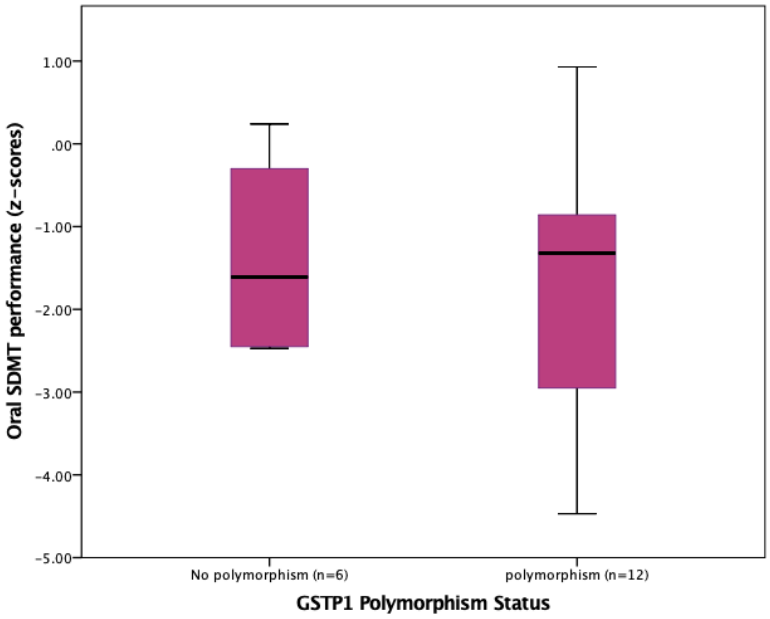

Figure 6 Boxplot of processing speed performance by two groups of survivors
Note: Center line represents the median of the data

3.3 Aim 2

We ran partial correlations to determine if polymorphism status was related to hippocampal or putamen volume while controlling for intracranial volume. There were no significant correlations between polymorphism status and the bilateral hippocampus, left hippocampus, right hippocampus, bilateral putamen, left putamen, or right putamen. Table 7 displays the correlation coefficients and significance values.

Table 7 Partial correlations between polymorphism status and subcortical volumes

Structure	r	p
Right Hippocampus	-.165	.574
Left Hippocampus	-.009	.976
Bilateral Hippocampus	-.094	.75
Left Putamen	-.191	.512

Right Putamen	-.356	.212
Bilateral Putamen	-.285	.323

Note: All partial correlations control for ICV

We ran an ANCOVA to test for significant group differences between the two groups of survivors and healthy controls in hippocampal and putamen volume with ICV as the covariate. Equal variances can be assumed ($F(2,26)= 1.214$, $p = .313$). There is a significant group difference between the controls, and both survivor groups on volume of the bilateral hippocampus ($F(2,26)= 6.45$, $p = .005$). The effect of these differences is large ($\eta^2= .332$, $R^2=.396$). There is a significant group difference between the controls, and both survivor groups on volume of the bilateral putamen ($F(2,26)= 9.611$, $p = .001$). Equal variances can also be assumed ($F(2,26)= .455$, $p = .639$). The effect of these differences is large ($\eta^2= .425$, $R^2=.435$). We ran another ANCOVA to test for significant group differences between both groups of survivors, excluding controls, on hippocampal and putamen volume with ICV as a covariate. This allows us to determine if GSTP1 polymorphism status is related to hippocampal and putamen volumes. Equal variances can be assumed ($F(1,14)= 1.608$, $p = .227$). There was no significant group difference between survivor groups on volume of the bilateral hippocampus ($F(1,14)= .106$, $p = .75$). The effect of these differences is small ($g=.2$, $R^2=.158$). There was no significant group difference between survivor groups on volume of the bilateral putamen ($F(1,14)= 1.06$, $p = .323$). Equal variances can also be assumed ($F(1,14)= .809$, $p = .385$), and the effect of these differences is small ($g=.17$, $R^2=.134$). The group difference results, including descriptive statistics, are represented in Tables 8 and 9.

Table 8 Aim 2 Results: Group differences in subcortical volumes controlling for ICV

	Polymorphism *(n=10)*	No Polymorphism *(n= 5)*	Control *(n=15)*	Adjusted R^2	p	η^2
Bilateral Hippocampal Volume	7031.9(1086.9)	6838.8(373)	7949.5(752.9)	.396	.005	.332
Bilateral Putamen Volume	9258.3(1078.4)	9427.6(587.5)	10642.7(887.6)	.435	.001	.425

Note: η^2 = Eta squared, hippocampal and putamen volumes were recorded in millimeters (mm)

Table 9 Aim 2 Results: Survivor group differences in subcortical volumes controlling for ICV

	Polymorphism *(n=10)*	No Polymorphism *(n= 5)*	Adjusted R^2	p	Hedge's *g*
Bilateral Hippocampal Volume	7031.9(1086.88)	6838.8(373)	.158	.75	.2
Bilateral Putamen Volume	9258.3 (1078.4)	9427.6(587.5)	.134	.323	.17

Note: hippocampal and putamen volumes were recorded in millimeters (mm)

3.4 Aim 3

We tested for an indirect effect of hippocampal volume on attentional deficits moderated by GSTP1 polymorphism status. First, we ran a regression analysis to see if there was a direct effect of hippocampal volume on attention span performance, controlling for ICV. The direct effect did not exist (t=.257, p=.801). Although it is unlikely for an interaction to be present if there are no main effects, a crossover interaction is possible (Wang et al., 2010). Therefore, we ran our moderation using the PROCESS macro for SPSS (Hayes, 2012). ICV was included in this model as a predictor variable along with bilateral hippocampal volume. The moderation model was not

significant, and our results indicated that a crossover interaction does not exist in our sample. The non-significant result was determined by the bootstrap confidence interval crossing over zero [LLCI-ULCI: .000-.0005].

3.5 Planned Supplemental Analysis 1

We ran Control-FREEC on aligned files of each participant's genomic data. Using a configuration file that was generated based on the parameters for our sample, Control-FREEC generates normalized copy number profiles (Boeva et al., 2011). Using custom Linux commands and R scripts, we visualized the normalized copy number profile for each chromosome of each participant. Table 10 displays a key for interpreting the copy number visualizations. Using the Integrative Genomic Viewer (IGV) tool (Robinson et al., 2011), we were able to magnify the visualizations of the normalized copy number profiles within our regions of interest. We imported the genomes of our twelve participants with GSTP1 polymorphisms into the IGV tool. We can then specify our region of interest (Chromosome 11: Position 67352689- 67353579) and the IGV browser provides the copy number estimated value at this position. The copy number value for each participant (labeled 1-12) is represented in Table 11. No significant gains or losses were seen at the site of GSTP1 polymorphisms in our survivors (M=2.036, Range =1.998-2.123).

Table 10 Copy Number Variant Key

Color	Genomic Alteration	Copy Number Segment Mean
Red	Gain	>2.0
Blue	Loss	< 2.0
Green	Neutral	Approx. 2.0

Table 11 Copy Number at GSTP1 position on chromosome 11

Participant	Genomic Alteration	Copy Number

Survivor 1	Neutral	2.079
Survivor 2	Neutral	2.012
Survivor 3	Neutral	2.073
Survivor 4	Neutral	1.998
Survivor 5	Neutral	2.051
Survivor 6	Neutral	2.023
Survivor 7	Neutral	2.004
Survivor 8	Neutral	1.981
Survivor 9	Neutral	2.051
Survivor 10	Neutral	2.018
Survivor 11	Neutral	2.019
Survivor 12	Neutral	2.123

3.6 Planned Supplemental Analysis 2

There was not a significant group difference for survivors with a polymorphism and survivors without a polymorphism for WASI full-scale IQ (p=.194). While we did not replicate Barahmani's overall findings with our full sample, we did find a sex-specific main effect of polymorphism status on full-scale IQ. Females with a polymorphism performed worse on the WASI full-scale IQ than females without a polymorphism (p=.048). The mean performance of females without a polymorphism (n=5) was a z-score of -.013 and the mean performance of females with a polymorphism (n=6) was a z-score of -.933. Equal variances can be assumed, and the effect size was large, Hedge's g = 1.2652, as the difference between the two means is larger than one standard deviation. Additionally, there was a significant group difference for survivors with a polymorphism and without a polymorphism for WASI verbal IQ (p =.026). The effect size was large, Hedge's g = 1.22, and survivors with a polymorphism performed worse on average than survivors without a polymorphism on verbal intelligence. Furthermore, when probing for a sex effect, female survivors with a polymorphism performed significantly worse than female

survivors without a polymorphism (p=.005). The effect size was very large, Hedge's g = 2.24. Finally, for performance IQ, there was not a significant difference between groups based on polymorphism status (p=.738) or based on female sex and polymorphism status (p =.551). However, on average, survivors with a polymorphism (M= -.64) performed worse than survivors without a polymorphism (M= -.47), and females with a polymorphism (M= -.74) performed worse than females without a polymorphism (M= -.42). These results are displayed in Tables 12 and 13.

Table 12 Polymorphism status and WASI full-scale, verbal, and performance IQ

	Polymorphism *(n=12)*	No Polymorphism *(n= 6)*	t	p
WASI full-scale IQ	-711(.916)	-.156(.552)	1.355.	.194
WASI verbal IQ	-.643(.779)	.247(.599)	2.45	.026*
WASI performance IQ	-.645(1.1)	-.477(.684)	.34	.738

*****Significant at 0.05 level

Table 13 Polymorphism status and WASI full-scale, verbal, and performance IQ for females

	Females with Polymorphism *(n=6)*	Females without Polymorphism *(n= 6)*	t	p
WASI full-scale IQ	-.933(.782)	-.013(.479)	2.29	.048*

WASI verbal IQ	-.92(.765)	.456(.346)	3.95	.005**
WASI performance IQ	-745(.92)	-.426(.753)	.620	.551

******Significant at 0.01 level
*****Significant at 0.05 level

Based on these results, we decided to conduct new independent t-tests of neurocognitive performances using the two groups of females and excluding the males (n=7). For attention span, CVLT-II/CVLT-C performance, equal variances cannot be assumed and on average females with a polymorphism (M= -1.5) performed worse than females without a polymorphism (M = -1.3). The difference between groups was not significant (p=.575). As an alternate measure of attention span, Digit Span Forward raw scores were covaried with age at examination. We conducted a one-way ANCOVA and found that GSTP1 polymorphism status was not significantly related to attention span (p=.315). However, similar to performances on the CVLT-II/CVLT-C, girls with a polymorphism had lower mean and median scores than girls without a polymorphism.

Equal variances can be assumed for working memory, and females without a polymorphism (n=4) performed slightly worse on average (M= -1.56) than females with a polymorphism (n=6; M= -1.42). The difference between groups is not significant (p=.776). For Digit Span Backward, the opposite was found with lower average scores for girls with a polymorphism (M =4.33) compared to girls without a polymorphism (M =6.2). When covarying for age, the relationship between GSTP1 polymorphism status and Digit Span Backward performance is approaching significance (p=.094). One more individual without a polymorphism was included in Digit Span Backward analyses compared to ACT/CCT analyses. For processing speed, females with a polymorphism performed on average worse than those without a

polymorphism, and this result was trending toward significance (p=.082). Equal variances can be assumed, and the effect size was large, Hedge's g = 1.185.

We visualized the differences in sex and neurocognitive performance for males without a polymorphism, males with a polymorphism, females without a polymorphism, and females with a polymorphism using boxplots. Differences in attention span, CVLT-C/CVLT-II performance, for each group can be viewed in Figure 11. The boxplot displays a trend of females performing worse than males on attention span, and females with polymorphisms having a lower median performance than the other groups and higher variability. The difference between males with a GSTP1 polymorphism and females with a GSTP1 polymorphisms' performance on attention span was not significant (p=.162). The effect size of the differences in performance between males and females with GSTP1 polymorphisms was large, Hedge's g = .877.

The differences in working memory span, ACT/CCT performance, can be viewed in Figure 12. Females performed worse than males with a large effect size, Hedge's g = 1.12. The difference in working memory span performance for males with a GSTP1 polymorphism and females with a GSTP1 polymorphism approached significance (p=.082). Finally, sex differences in processing speed performance can be viewed in Figure 13. Processing speed displayed a similar trend as attention span, with females having the lowest median and larger variability than the other groups. The difference in performance between males with a GSTP1 polymorphism and females with a GSTP1 polymorphism was significant (p=.024), and the effect size was very large, Hedge's g = 1.53.

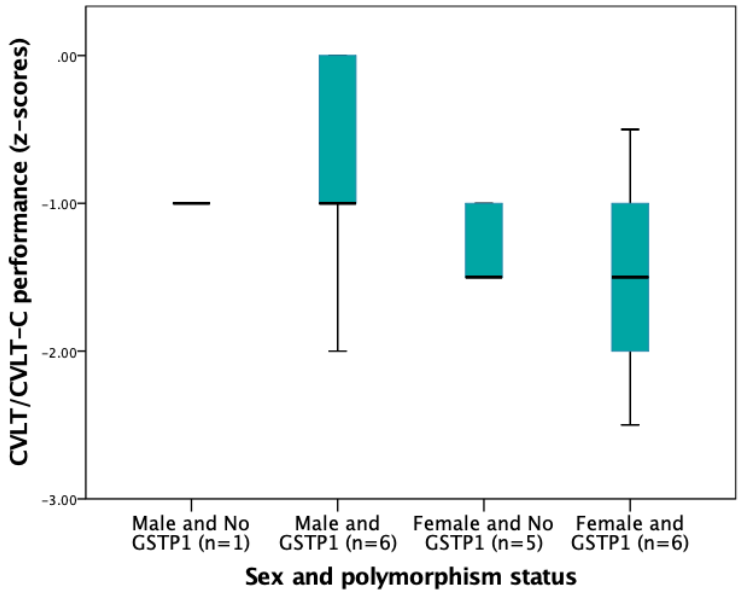

Figure 7 Sex differences in survivors with and without GSTP1 polymorphisms and attention span performance

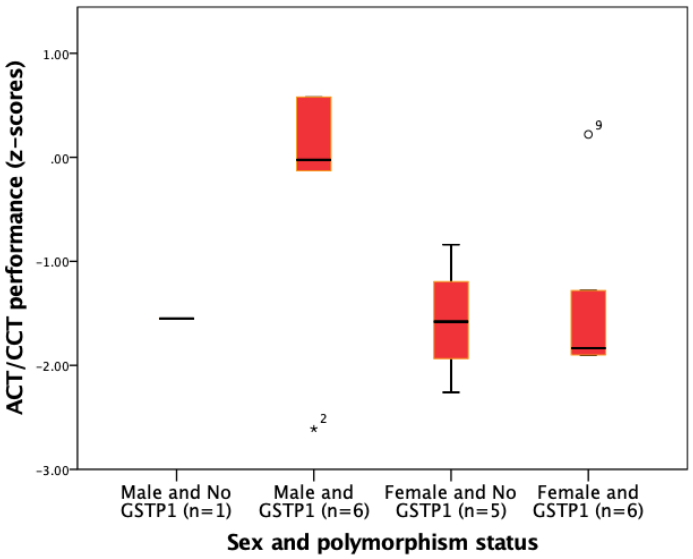

Figure 8 Sex differences in survivors with and without GSTP1 polymorphisms and working memory span performance

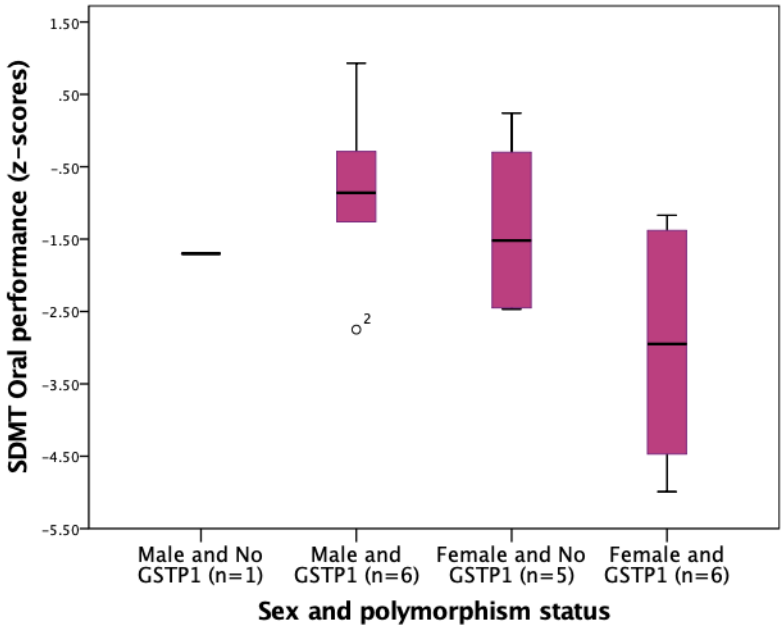

Figure 9 Sex differences in survivors with and without GSTP1 polymorphisms and processing speed performance

4 DISCUSSION

4.1 Discussion of Aim 1 Results

The purpose of this aim was to examine possible associations of GSTP1 polymorphisms and attention span performance. We also tested the relationship between GSTP1 polymorphism status and working memory and processing speed performance to establish specificity of our findings. We confirmed that survivors had significantly worse performance on attention span, working memory, and processing speed than controls which is consistent with previous analyses on the data of long-term outcomes for pediatric brain tumor survivors (Jayakar et al., 2015; King et al., 2019; King et al., 2015).

Based on our correlations, GSTP1 polymorphism status was not associated with both measures of auditory attention. We obtained a small effect for differences between survivor groups on attention span performance. Our data did not support our hypothesis that survivors

with a polymorphism would perform worse on measures of auditory attention than survivors without a polymorphism. Survivors without a polymorphism had a slightly lower mean attention span z-score performance (M=-1.25) than survivors with a polymorphism (M=-1.167). The standard deviation for the survivors with a polymorphism was almost three times the size of the standard deviation for survivors without a polymorphism, resulting in a Levene's Test approaching significance (p=.055). Likely the differences in variances are due to the small sample size, yet across all three core neurocognitive skills, survivors with a polymorphism had a larger range in performance. This leads us to speculate that with a larger sample we may be able to parse apart sub-groups of individuals with a GSTP1 polymorphism that are impaired at a higher rate than the rest of the survivors. While there were no significant differences in impairment between both survivor groups, none of the survivors without a polymorphism were impaired. Four of the survivors with a polymorphism, out of twelve, met criteria for impairment. This result suggests that a subset of the survivors with a polymorphism may be particularly vulnerable to genetic alterations that impact their efficiency with oxidization of chemotherapy agents and free radicals. Further analyses should be conducted to determine the demographic variables or additional genetic risk associated with this impaired sub-group.

While not a statistically significant difference (p=.095), there was a medium to large effect size for differences in working memory performance, suggesting that the survivors without a polymorphism had reduced capabilities at working memory tasks. Survivors without a polymorphism had a group mean performance of greater than 1.5 SD below the normative mean, suggesting that the group is impaired on average. This result is also contrary to our hypotheses; however, the difference between groups was not statistically significant, and five survivors with a polymorphism fell into the impaired range as well. One individual did not complete the ACT

during their neuropsychological testing; therefore, the group without a GSTP1 polymorphism only had five individuals. The group of survivors with a polymorphism were 2.4 times greater in size than those without a polymorphism; therefore, the differences in proportion of impairment should be interpreted with caution. The medium to large effect is likely inflated by how small the group size is for survivors without a polymorphism. The standard deviation for the survivors with a polymorphism was over three times the size of the standard deviation for survivors without a polymorphism, resulting in a significant Levene's Test ($p=0.002$). This increase in range and variability in performance for survivors with a polymorphism is similar to the trend seen with attention span. Notably, three of the four survivors with a polymorphism who were significantly impaired on attention span were also impaired on working memory.

The effect size was small to medium for the difference between groups on processing speed performance. In line with our hypothesis, the survivors with a polymorphism performed worse than survivors without a polymorphism, and their overall mean performance was greater than 1.5 SD below the normative mean ($M =-1.87$). However, the difference between impairment levels was not statistically significant, and four individuals without a polymorphism also met impairment criteria. The result that over half of both groups of survivors are impaired on this skill is consistent with the literature, as processing speed is the first of the neurocognitive skill difficulties for medulloblastoma survivors (Mabbott et al., 2008; Palmer et al., 2013). However, the sub-group of survivors with a polymorphism who have impaired performances on attention, working memory, and processing speed represent a vulnerable group, and additional risk factors may explain the variability in our sample.

Our results indicate that GSTP1 polymorphism status is not associated with statistically significant differences in mean performances on three core neurocognitive skills. However,

variability in performance, measured by Levene's test and standard deviations was robust for working memory and attention. A primary objective of this study was to understand additional biological mechanisms that may contribute to the variance in long-term cognitive outcomes that researchers observe in medulloblastoma survivors. While this pilot study was underpowered to isolate the genetic mechanisms underlying long-term cognitive functioning in a sample of medulloblastoma survivors, genes do not function independently. It is possible, particularly for studies with smaller sample sizes, that the additive effect of genes working within a network is needed to elucidate long-term deficits. Kamdar and colleagues have highlighted the additive effect of SNPs on attention and processing speed in 72 leukemia survivors. They assessed the impact folate path polymorphisms on cognition, and they found that the combined effect of multiple polymorphisms best-predicted individuals who are at risk for cognitive impairment (Kamdar et al., 2011). Survivors with over six risk alleles performed significantly worse on measures of attention and processing speed and often were clinically impaired (Kamdar et al., 2011). Beyond these analyses, it is important to consider polymorphisms on genes located within GST's pathway; such as HO-1, KEAP-1, and NRF2. The additive impact of KEAP-1 and NRF2 aiding in the growth of cancer cells while protecting cells from oxidative stress has been studied, but not along with GST polymorphisms (Ohta et al., 2008). While examining pathways of genes was not the focus of this pilot study, it is a crucial consideration for further understanding of the variance in outcomes for medulloblastoma survivors. Our sub-group of survivors with a polymorphism who were significantly impaired across multiple core neurocognitive skills may have additional genetic risk associated with other genes involved in responding to and eliminating agents of oxidative stress.

4.2 Discussion of Aim 2 Results

Our results are similar to recent studies that have found smaller hippocampal volumes in pediatric brain tumor survivors compared to typically developing children (Decker et al., 2017) and adults (Jayakar et al., 2015). Hippocampal volumetric differences between controls and long-term survivors have also been observed in acute lymphoblastic leukemia (Monje et al., 2013; Zeller et al., 2013). Long-term volumetric differences are essential to track, as the hippocampus in typical controls continues to develop from childhood into early adulthood with hippocampal volume increasing in later childhood and early adolescence followed by a slight decrease in volume in young adulthood (Tamnes et al., 2018; Wierenga et al., 2014). In contrast, medulloblastoma survivors have significant volume loss in the hippocampus for the first two years after diagnosis and treatment before they shift to growth and volume increases (Nagel et al., 2004). Comparing subcortical structures in childhood survivors may capture this initial decrease, while long-term studies can demonstrate subcortical differences that persist. Jayakar and colleagues discovered that not only hippocampal volume of long-term pediatric brain tumor survivors was reduced compared to controls, but the bilateral putamen volume was less than controls with a medium effect size (Jayakar et al., 2015). In line with Jayakar's research, group differences were not specific to the hippocampus, and this may indicate the disruption to brain structure development is more global than specific. Research that focuses on specific structure and function relationships may underestimate the widespread loss of volume for brain tumor survivors with extensive treatment regimens. Therefore, this exemplifies the importance of a control sample and control region to ensure the effects are limited to the structure of interest.

Counter to our predictions, there was not a significant group difference between the survivor groups on volume of the bilateral hippocampus. The effect was small, but our results

show that survivors without a polymorphism have smaller mean hippocampal volumes than survivors with a polymorphism. There was also not a significant group difference between the survivor groups on volume of the bilateral putamen. The effect size was also small and in the opposite direction of the hippocampal results, as survivors with a polymorphism have smaller mean putamen volumes than survivors without a polymorphism. The survivors with a polymorphism group had twice the number of individuals compared to the survivors without a polymorphism, and given our small sample for imaging analyses, the mean volume differences between survivors should be considered with caution. Overall, survivor's bilateral hippocampal and putamen volumes were significantly smaller than controls supporting the research around abnormal development of these subcortical structures even after long-term recovery from treatment and subsequent maturation.

4.3 Discussion of Aim 3 Results

Our sample size was underpowered for multiple regression; however, given the theoretical background for the hypotheses, testing for an indirect effect within our sample was a worthy exploration to characterize the data. Since the direct effect between hippocampal volume and attention span performance was not significant, it was unlikely that deficits would be significantly moderated by GSTP1 polymorphism status. Ultimately, the moderation model was not significant. We encourage that this hypothesis is probed further in a larger sample of medulloblastoma survivors, as it is not indicative that this relationship is non-existent given limitations in sample size.

4.4 Discussion of Planned Supplementary Analysis 1

The primary purpose of this analysis was to ensure that our genomic positions of interest for GSTP1 (Chromosome 11: Position 67352689- 67353579) do not contain significant copy number variation. The removal of copy number gains and losses leads to higher-quality data and lends support to identified SNPs in whole-genome association studies (Korn et al., 2008). If our results had shown gains or losses that would diminish the support to the GSTP1 polymorphisms in our samples. However, with neutral copy number, our copy number visualizations lend confidence to our read depth and coverage for SNPs of interest. Instilling checks within a plan for analyses is critical when making decisions around personalized medicine and rehabilitation for survivors of pediatric brain tumors. Therefore, we can present our results with greater certainty, and for future studies, identify additional SNPs of interest to aggregate genetic risk and test possible associations

4.5 Discussion of Planned Supplementary Analysis 2

The aims of these supplementary analyses were to, first, examine if we could replicate the association between GST polymorphisms and IQ found by Barahmani and colleagues, and second, to examine if sex differences accounted for the variability, we saw in our survivors with a GSTP1 polymorphism. Earlier we highlighted a group of survivors with GSTP1 polymorphisms who performed in the impaired range for working memory and attention span tasks, and we wished to assess if sex was associated with worse outcomes. Our key finding was a main effect of sex for overall full-scale IQ. Female survivors with a polymorphism represent a particularly vulnerable group with significantly worse full-scale IQ scores compared to females without a polymorphism. All survivors with a GSTP1 polymorphism had significantly lower verbal IQ score than survivors without a GSTP1 polymorphism. Additionally, we found a very

large effect of females with a GSTP1 polymorphism performing significantly worse than females without a polymorphism, indicating that both genetic risk and sex interact and are associated with long-term verbal IQ. This vulnerability was not significant for performance IQ, although females with a GSTP1 polymorphism had the worst average performance of the four groups. Barahmani and colleagues studied the combined risk of either a null allele on GSTT1 or GSTM1, and our results highlight the risk of new polymorphisms within the GST family. Since GSTP1 polymorphisms produce higher levels of enzyme activity than the null genotypes, a null genotype may not be necessary for long-term deleterious consequences for intelligence, particularly verbal intelligence (Sun et al., 2010).

Long-term studies of pediatric brain tumor survivors have shown that female sex predicts more impairment on executive functioning tasks, processing speed performance, and adaptive functioning (Ellenberg et al., 2009; Panwala, in press). Additionally, female sex was related to significant drops in verbal IQ, three points per year, in a longitudinal study that tested medulloblastoma survivors over four years (Ris et al., 2001). Four years post-diagnosis is still relatively recent to treatment, and our study highlights the long-term vulnerability of the combined risk of GSTP1 polymorphisms and female sex.

The combined risk was further supported when we ran analyses of neurocognitive skills and separated groups by sex. For attention and processing speed, there was a trend of females with GSTP1 polymorphisms performing on average worse than the other three groups, males without a polymorphism, males with a polymorphism and females without a polymorphism. Additionally, for survivors with polymorphisms, the effect size was large for the difference in attention span performance between the sexes (g=.877). For processing speed, the effect size was very large (g=1.53), and there was a significant difference between survivors' performance with

GSTP1 polymorphisms based on sex (p=.024). Also, there was a large effect size (*g*=1.18) for the difference in processing speed performance for females without a polymorphism and females with a polymorphism. Females with a polymorphism had a mean z-score falling far below the cut-off of impairment (M=-2.9), representing a particularly vulnerable and impaired group. Although the difference between females with a polymorphism and females without a polymorphism was not significant for working memory span, the effect size of the difference between males with the polymorphism and females with the polymorphism was large (g =1.12) and approached significance.

These results help demystify the large standard deviations in performance for the survivors with a polymorphism compared to the restricted range of the survivors without a polymorphism. Females with a polymorphism are obtaining the lowest scores, often in the impaired range, and males with a polymorphism are bringing up the group's average for processing speed, attention span and working memory span. Overall, sex differences between survivors with a GSTP1 polymorphism had large effect sizes for all three neurocognitive skills. The trends in our results appear to be consistent with the long-term neurodevelopmental model developed by King and colleagues, although they would need to be tested in a larger sample (King et al., 2019). King and colleagues did not find large sex-specific effects on neurocognitive skills, and it would be interesting to add genetic risk into the model as a risk factor interacting with sex. Our finding that sex differences are associated with genetic risk has been established in the literature for other neurological diseases and disorders. For example, in a large-scale genome-wide association study, sex-specific associations were found between female sex and the APOE ε4 allele (Hohman et al., 2018). The APOE ε4 allele causes higher levels of cerebrospinal fluid tau and neurofibrillary tangles, the hallmarks of Alzheimer's diseases, and may modulate risk for

neurodegeneration. This sex-specific effect pinpoints a particularly vulnerable group, who experience impairment at higher rates, yet would not be noticed unless analyses were separated by sex. Similar to Hohman and colleagues, we were not able to explain the large standard deviations in our GSTP1 polymorphism group until we separated the groups by sex. Females with a GSTP1 polymorphism are experiencing impairment many years after diagnosis, rendering them particularly vulnerable to cognitive difficulties in adulthood. With replication, these results can be utilized to help clinicians and researchers in remediation plans of neurocognitive skills for females with genetic risk factors.

4.6 Limitations and Strengths

This study is novel for the field; and therefore, utilizes pilot study data which was intended to lay the groundwork for future studies with larger sample sizes. While our study was underpowered, a study including neuropsychological and genetic data for medulloblastoma survivors has been published in the field with a comparable sample size (n=21) (Barahmani et al., 2009). Additionally, given that genetic, neuroimaging and neuropsychological data were retrieved for this sample, the sample size is reasonable compared to neuroimaging studies of medical populations that analyze multiple domains of functioning (Davidson et al., 2000; Nolen et al., 2016). A strength of this study is that we utilized neuropsychological assessment instead of questionnaires to more accurately and precisely measure attentional performance impairment in the brain tumor survivor population. We increased the specificity of our study by assessing three core neurocognitive skills and utilizing a control region for our neuroimaging analyses. Additionally, we included an age-and-sex-matched control group for neuropsychological

assessment and neuroimaging to better capture impairment in our sample compared to same-age peers.

Since this design is cross-sectional, longitudinal studies are needed to fully understand how treatment and genetics influence brain structure and cognitive development for pediatric tumor survivors. This study may be susceptible to recruitment bias as survivors chose to participate. Survivors who are higher functioning may be more capable of participation in neuropsychological testing. On the other hand, survivors who are more impaired may be looking for clinical research studies to understand the long-term difficulties that they experience. Given that we conducted tests of data assumptions, this should not bias the study in one direction. The participants in this study received treatment over a decade ago which may call to question the generalizability of the findings. However, until we understand the mechanism of action between GST polymorphisms and the deleterious effects of chemoradiation, it is essential to measure the long-term cognitive outcomes of survivors. Since the neuro-oncology field allocates treatment and intervention resources based on risk, increased understanding of GSTP1 biomarkers, and their interaction with sex, can only improve the risk-adaptive care model (Gragert & Ris, 2011). Overall, sex-specific neurocognitive impairment that is associated with polymorphisms can be utilized as a benchmark for identifying risk to create individualized protocols and remediation plans for survivors of childhood brain tumors.

4.7 Conclusions and Further Directions

This study contributes to the gap in research on how GST polymorphisms influence long-term neurocognitive outcomes for pediatric medulloblastoma survivors. While the significant associations found in this study are underpowered, this pilot study lays the groundwork for future

investigation of sex-specific effects of GST polymorphisms and long-term neurocognitive outcomes. We found significant differences in attention span, working memory, processing speed, hippocampal and putamen volumes between survivors and controls. We did not establish specificity of hippocampal volume loss, and our sample may have more global subcortical morphological alterations, which will be important to replicate in a larger sample. The ENIGMA Cancer and Chemotherapy Working group, developed in 2017, investigates brain volume abnormalities that underlie cognitive impairment associated with cancer. However, to date, the group has only studied non-central nervous system cancer survivors (Shiroishi et al., 2018). With the development of this group and the collaboration of cancer researchers around the world, high-powered neuroimaging studies may elucidate the nature of morphometric abnormalities for medulloblastoma survivors.

We chose to look at one family of genes and focused our analyses on GSTP1 because of the prevalence of the two GSTP1 polymorphisms in our sample. However, looking at one gene family may not adequately isolate genetic risk. In a recent GWAS study, non-coding DNS regions, with unknown regulatory function, within the host genome have been associated with neurocognitive outcome in medulloblastoma survivors (Siegel, in press). Siegel and colleagues' results indicate that researchers need to look beyond individual gene families and consider the contributions of non-coding regions to genetic risk. Hohman and colleagues research on genetic risk for Alzheimer's disease supports the theory that a single marker analysis is not enough to understand the complex genetic makeup of disorders and epistatic relationships (Hohman et al., 2013). SNPs on the *APOE* gene have also been assessed in adult patients with brain tumors. Correa and colleagues utilized a backward selection regression analysis, which included a total of nine APOE SNPs, and found a significant association in their final model between seven

SNPs and attention (Correa et al., 2014). Utilizing additive genomic risk factors may be useful for explaining variance in outcomes, yet it is also essential to isolate the demographic and treatment factors associated with each added SNP.

Due to the robust variance in performance for survivors with GSTP1 polymorphisms on measures of attention, working memory, and processing speed, we chose to examine sex as a demographic factor that may interact with genetic risk. We found large sex-specific effect sizes across all measures of neurocognitive skill performance, with females with a GSTP1 polymorphism performing significantly worse than other groups. Additionally, we replicated the results of Barahmani and colleagues for verbal IQ and found very large sex-specific effects for verbal IQ and full-scale IQ. Researchers should consider the role of sex when assessing for genetic risk, as this interaction has also been found in large-scale GWAS studies for APOE polymorphisms in Alzheimer's disease (Hohman et al., 2018). Females may have a specific vulnerability to genetic risk, and additional SNPs should be studied for sex-specific main effects and their association with cognitive outcomes. Our study highlights a group that may endure long-term cognitive problems, both with proximal and distal cognitive markers. Replication studies which would assess the impact of sex-specific polymorphisms can include the interaction between sex and polymorphism status as a risk factor in the current long-term neurodevelopmental model (King et al., 2019). As more groups cross-validate important biomarkers and identify genetic risk, these findings inform precision medicine and remediation plans for long-term medulloblastoma survivors

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APPENDIX

R Script:

```
total <- merge(RSIDNEW_Sheet1,SNP_X , by="POS", all.x=TRUE, sort=FALSE)
write.csv(total, "practice_data.csv")
```