Background & Rationale:
Advances in treatment have caused 5-year survival of childhood cancer to exceed 80%\(^1\); however, many of these individuals are susceptible to persistent neurocognitive impairment as adults\(^2\). Survivors face the potential for lower academic, professional, and economic achievements, as well as greater demand for supplementary educational and medical support. Neurocognitive impairment can affect individuals of all ages who have undergone cancer treatment, but pediatric patients are particularly susceptible. Young age at diagnosis, female sex, and treatment with cranial radiation therapy, intrathecal methotrexate, high-dose intravenous methotrexate or cytarabine, and corticosteroids\(^3\)-\(^6\) are known to increase risk of neurocognitive impairment. However, these factors do not fully explain the interindividual variability for this outcome. Since some childhood cancer survivors experience disproportionate neurocognitive impairments not explained by the known risk factors, there may be an underlying genetic susceptibility to this late effect of cancer treatment.

We hypothesize that genetic loci influence the risk of neurocognitive impairment among long-term survivors of childhood cancer. Our specific aims outline an approach to identify the underlying genetic variation that may ultimately be used to predict which children with cancer are more likely to develop neurocognitive impairments secondary to their cancer treatments. We will first use a discovery genome-wide association study in survivors from the CCSS cohort to identify variants associated with self-reported neurocognitive measures, followed by replication in the St. Jude Lifetime Cohort Study (SJLIFE) of childhood cancer survivors. CCSS survivors who did not participate in SJLIFE and who completed the Neurocognitive Questions (NCQ) will comprise the discovery set. Survivors who were treated at St. Jude and who completed the NCQ as part of the SJLIFE will serve as the validation set. Since the discovery set and validation set will not overlap and since the NCQ was conducted under different circumstances (i.e. CCSS Questionnaire vs SJLIFE Questionnaire), we will consider these independent data sets. We will then use clinician-assessed neurocognitive functioning measured in the SJLIFE cohort to further describe neurocognitive functioning in survivors with the genetic variants. The variants identified in this analysis are expected to occur in regulatory regions, and we plan to annotate our findings for their relationship to transcriptional regulation using well-established, publicly-available databases and advanced statistical techniques. Given this approach our specific aims are to:
Primary Aims:
   1.a. Conduct a genome-wide association study for overall neurocognitive functioning measured on the Childhood Cancer Survivor Study-Neurocognitive Questionnaire (CCSS-NCQ) among the CCSS cohort.
   1.b. Conduct genome-wide association studies using NCQ subscales (task efficiency, memory, organization, and emotional regulation) among the CCSS cohort.
   1.c. Conduct exploratory analyses to investigate if 1) cranial radiation therapy, 2) intrathecal methotrexate, 3) intravenous methotrexate, and 4) corticosteroids modify the genetic risk of neurocognitive impairment on NCQ measures.

   2.a. Assess the novel neurocognitive functioning-associated polymorphisms from the CCSS cohort using the St Jude Life as a replication cohort.
   2.b. Evaluate the functional roles of replicated genetic variants in silico using publicly available data relating genetic variants to gene transcription and regulation (e.g., HaploReg, GTEx, RegulomeDb).

Analysis Framework: This analysis will use existing data within the CCSS and St Jude Life. The proposed analysis is outlined below and may be modified with input from CCSS and St Jude Life collaborators.

Outcome: The primary outcome will be neurocognitive functioning measured on the NCQ assessment. The NCQ is a questionnaire designed within the CCSS to assess neurocognitive functioning specifically among childhood cancer survivors. Briefly, the NCQ measures neurocognitive functioning in the following domains: task efficiency, emotional regulation, organization, and memory. For regression modeling purposes the four domains of the NCQ may be treated as continuous outcomes (i.e., age-adjusted Z-scores) or dichotomized as impaired/non-impaired using previously described thresholds. Descriptive statistics will be presented within the dichotomous groups.

Study Population: The study population will consist of the 5,324 childhood cancer survivors of European ancestry enrolled in the Original CCSS Cohort (diagnosed 1970-1986) with available genotype data. In this eligible population, there are 3,592 survivors with at least one measurement on the NCQ as of the June 1, 2017 data release. The replication study population of European ancestry are 2,478 individuals enrolled in St Jude Life with available genotype data, NCQ and/or clinical neurocognitive measures, and covariates. Since more detailed data are available on survivors in the St Jude Life cohort, any overlapping survivors with the Original CCSS cohort will be retained in the replication cohort for analyses. Individuals who received allogeneic bone marrow transplant were not genotyped/sequenced in the CCSS or SJLIFE; therefore, they will not be included in this analysis. However, patients who underwent autologous transplant will be included.

Exploratory Variables: The primary exploratory variables are genotypes obtained from the Illumina HumanOmni5Exome array. Directly genotyped SNPs will be imputed using 1000 Genomes phase 3 or the Haplotype Reference Consortium (HRC) r1.1 reference panel. Additional covariates that may be considered in analysis:

- Cancer diagnosis
- Year of cancer diagnosis
- Age at cancer diagnosis (±6 years of age)
- Age at follow-up
- Sex
Genetically determined ancestry (principal components)
Radiation therapy field (any, brain, abdominal, total body) and dose
Corticosteroid exposure (yes/no)

Additional covariates that will be considered for effect modification:

- Grade 2+ chronic conditions (e.g., cardiac, pulmonary, endocrine) previously shown to impact neurocognitive outcomes
- Measures of anxiety, depression, and vitality (which may impact cognitive measurements)

**Analytic Approach:** The long-term childhood cancer survivors will be characterized according to each covariate within groups of impaired and non-impaired neurocognitive domains (as determined using standard cutpoints). These study characteristics will be displayed similarly to Example Table 1, with each covariate stratified by each of the four NCQ domains.

**Example Table 1.** Characteristics of long-term childhood cancer survivors who do and do not show neurocognitive impairment within each of the four NCQ domains

<table>
<thead>
<tr>
<th>NCQ Task Efficiency domain</th>
<th>Impaired n = _____</th>
<th>Non-impaired n = _____</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at primary cancer diagnosis, n (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 6 years</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥ 6 years</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Aim 1 proposes to identify genetic variants associated with neurocognitive functioning among long-term childhood cancer survivors in the CCSS using a genome-wide association study approach. We will use linear regression models to assess the association of dosage-imputed variants with each of the neurocognitive domains. Models will be adjusted for risk factors and genetic principal components (PCs) of ancestry, as necessary. The threshold to define discovery loci will be any SNP with \( P < 5 \times 10^{-8} \), additionally loci with \( P < 1 \times 10^{-7} \) will also be explored in the replication cohort to limit the possibility of false-negative results in the discovery. We will first assess unadjusted models of the SNP main effect on neurocognitive functioning, then further refine effect estimates using adjusted models. We will adjust for covariates known to affect neurocognitive functioning in childhood cancer survivors, as described in the above list of covariates. Adjusted effect estimates will be reported for each SNP.

Some individuals may be genetically more susceptible to neurocognitive impairment based on treatment exposures. We will therefore perform stratified analyses to explore genetic effect modification by treatment characteristics. Linear regression models, as described in Aim 1, will be stratified by cranial radiation therapy, intrathecal methotrexate, high-dose intravenous methotrexate or cytarabine, corticosteroid treatments, and psychosocial factors that may impact neurocognitive functioning. As these are exploratory analyses, treatment-specific effects within a stratum will be considered suggestive using \( P < 1 \times 10^{-5} \). Additionally, a chi-square test of homogeneity between the two strata of a treatment will be performed.

For Aim 2, we will use similar modeling strategies for NCQ in St Jude Life as was used for Aim 1, but only analyze discovery loci rather than genome-wide variants. We will employ inverse variance-weighted meta-analysis to combine the effect estimates from CCSS and St Jude Life. We will use two thresholds to declare SNPs associated with neurocognitive functioning: 1) \( P < 0.05 \) in St Jude Life after Bonferroni correction for the number of loci identified in Aim 1, or 2) \( P < 5 \times 10^{-8} \) in the meta-analysis of CCSS and St Jude Life.

Genetic loci that meet either of these criteria will be followed-up for functional annotation using publicly available databases that relate genetic variation to transcriptional regulation such as HaploReg, GTEx, and RegulomeDb. Using these databases, we will annotate each index variant and SNPs in high linkage
disequilibrium with each index variant (1000 Genomes EUR $r^2>0.8$) for location within regions shown to regulate gene transcription. Results will be displayed similarly to Example Table 2, with sections for each of the four NCQ domains.

**Example Table 2.** Results from meta-analyses of NCQ efficiency impairment in meta-analysis of CCSS and St Jude Life

<table>
<thead>
<tr>
<th>SNP</th>
<th>Chr</th>
<th>Pos</th>
<th>Gene</th>
<th>Functional annotation</th>
<th>EA</th>
<th>EAF</th>
<th>N</th>
<th>OR</th>
<th>95% CI</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs6909</td>
<td>1</td>
<td>1,111,111</td>
<td>ABC</td>
<td>eQTL</td>
<td>A</td>
<td>0.10</td>
<td>5324</td>
<td>2.5</td>
<td>1.6-3.4</td>
<td>1x10^-8</td>
</tr>
</tbody>
</table>

SNP = single nucleotide polymorphism; Chr = chromosome; Pos = chromosomal position; EA = effect allele; EAF = averaged effect allele frequency in study populations

For Aim 3, we will leverage a subset of SJLIFE participants who have neurocognitive functioning measured on both the NCQ and through clinical assessments. We will perform genetic modeling similar to Aim 2 using the clinical measures of impairment to characterize the SNPs that are associated with self-reported neurocognitive functioning (from Aim 2).

**Power:** Power at alpha=5x10^-8 was explored with the following inputs: 3,592 subjects (non-overlapping patients between CCSS and St Jude Life), log-additive allele effects, and minor allele frequency of 0.5% using Quanto software, version 1.2.4. In this model, we have >80% power to detect a locus that explains 2% of the variance in a normally-distributed neurocognitive outcome. In the evaluation of genetic risk among those with high-risk treatment exposures (Aim 1.c), assuming a conservative estimate of 20% of individuals are exposed, we would have >80% power to detect a locus that explains 4% of the variance in that sub-group.

**Special Considerations:**
**Replication and Validation:** Neurocognitive impairment is a long-term consequence that many childhood cancer survivors face. In this proposal, we have sought replication within St Jude Life using both data from the same questionnaire and complementary measures of clinical neurocognitive functioning. Approximately 20-25% of survivors in CCSS and St Jude Life report neurocognitive deficits by the NCQ. In St Jude Life, 35-50% of survivors show impairment based on direct clinical testing. This collaboration strengthens our ability to identify true genetic associations of these cancer treatment sequelae. We have also proposed to seek both *in silico* functional annotations as another type of validation for our findings. With a multi-faceted approach to replication and annotation, we hope to best characterize the biological etiology of cancer treatment-associated neurocognitive impairment.

**Future Directions:** We have established collaborations with investigators at MD Anderson Cancer Center (David Groshans) and Texas Children’s Cancer Center (Waleed Gaber) who can assist with further functional evaluation of replicated genetic variants using *in vitro* cell line models and *in vivo* using animal models, respectively. Given the expense associated with these validation assays, we will seek additional funding in the future to more fully characterize the effects of any identified and replicated variants on neurocognitive impairment.

**References**


