Title: A genome-wide association study of hypertension in adult survivors of childhood cancer

Working Groups: Genetics- Primary Oversight
Chronic Diseases- Secondary Oversight
Epidemiology/Biostatistics- Secondary Oversight

Investigators:		
	Jeanne A. Pierzynski, PhD., MPH	jeanne.pierzynski@stjude.org
	Yadav Sapkota, PhD	yadav.sapkota@stjude.org
	Lindsay Morton, PhD	mortonli@mail.nih.gov
	Smita Bhatia, M.D., PhD.	sbhatia@peds.uab.edu
	Todd Gibson, PhD.	todd.gibson@stjude.org
	Eric Chow, M.D., MPH	ericchow@u.washington.edu
	Kevin Oeffinger, MD	kevin.oeffinger@duke.edu
	Zhaoming Wang, PhD.	zhaoming.wang@stjude.edu
	Yutaka Yasui, PhD.	yutaka.yasui@stjude.org
	Carmen Wilson, PhD.	carmen.wilson@stjude.edu
	Gregory T. Armstrong	greg.armstrong@stjude.edu
	Melissa M. Hudson, M.D.	Melissa.hudson@stjude.edu
	Les Robison, PhD.	les.robison@stjude.edu

BACKGROUND AND SIGNIFICANCE

Approximately 96% of long-term survivors of childhood cancer will develop at least one chronic health condition by the age of 45.[1] Chronic health conditions that affect survivors include second cancers, renal dysfunction, endocrinopathies, musculoskeletal problems, and cardiovascular disease.[2] Cardiovascular disease is the top non-cancer related morbidity and cause of mortality in childhood cancer survivors.[2-7] One of the main risk factors for cardiovascular disease is treatment-related exposures such as anthracyclines and chest-directed radiation.[8-11] The largest modifiable risk factor for cardiovascular disease is hypertension[12-15], and among St. Jude Lifetime Cohort Study (SJLIFE) participants more than 1 in 5 childhood cancer survivors had hypertension that developed at a younger age compared to the general population.[16] Previous research has shown that hypertension can exacerbate injury caused by cardiotoxic treatment in survivors. For example, a study published from the Childhood Cancer Survivor Study (CCSS) found that hypertension and cardiotoxic therapies were independently associated with cardiovascular disease with hypertension and anthracycline exposure resulting in an greater than additive 86-fold increased risk of heart failure.[17]

Common cancer therapies such as abdominal radiation and chemotherapy agents (e.g., ifosfamide, heavy metals) are risk factors for hypertension in childhood cancer survivors.[18] In addition to treatment-related risk factors, other risk factors for hypertension include body mass index (BMI) of greater than 25 kg/m^2, male sex, non-Hispanic black race, and older age at blood pressure assessment.[16,19,20] Even though much is known about the clinical and demographic risk factors for hypertension in childhood cancer survivors, SJLIFE investigators observed a higher prevalence of hypertension among survivors compared to the general population.[16] While treatment was a significant factor in this difference, as prevalence differed by cancer diagnosis, nephrectomy was the only significant treatment-related risk factor in the multivariable analysis.[16] This supports that hypertension risk is multifaceted and suggests there is a potential genetic component.

To date there have been two studies evaluating genetic susceptibility to hypertension in childhood cancer survivors. One candidate gene study did not identify an association between two variants in *ATP2B1* previously associated with hypertension in healthy populations and hypertension in 532 survivors.[21] The second study that examined rare and common variants in genes related to the methotrexate and corticoid metabolic pathways and cardiometabolic pathways also did not find an association between these variants and pre-hypertension in childhood acute lymphoblastic leukemia survivors .[22] Currently a large-scale genome wide association study (GWAS) to identify genetic variants associated with hypertension has not been undertaken in childhood cancer survivors. Identification of a genetic predisposition to hypertension in childhood cancer survivors will potentially help characterize survivors who may be at a higher risk of hypertension and its associated cardiovascular and renal sequelae. Therefore, in this study we aim to complete a GWAS using CCSS cohort to identify susceptibility markers of hypertension as well as identify variants that potentially modify treatment-related hypertension risk. We hypothesize that there are unique genetic loci that impact susceptibility to hypertension in childhood cancer survivors and will test this hypothesis in the following specific aims.

SPECIFIC AIMS

Specific Aim 1: To perform a GWAS to identify common genetic variants (MAF≥5%) associated with hypertension (as defined by patients self-reporting a physician diagnosis of hypertension and taking hypertension medication) in childhood cancer survivors using the imputed genetic data from the CCSS 1970-1986 baseline cohort and the genetic data from the CCSS expansion cohort.

Specific Aim 2: To identify variants that modify treatment-specific risk of hypertension in childhood cancer survivors using genetic data from the CCSS. Significant and borderline significant variants ($P<1x10^{-6}$) identified in Specific Aim 1 will be analyzed in a treatment subgroup analyses or a GxE analysis if sample size permits.

Specific Aim 3: To replicate significant findings associated with hypertension in an independent set of childhood cancer survivors in the SJLIFE cohort.

ANALYSIS FRAMEWORK

Population: The study population will be 5,324 survivors with European ancestry in the original CCSS cohort (diagnosed 1970-1986) who completed the Baseline, Follow-up 4, or Follow-up 5 survey and have available imputed genotype data, along with approximately 3,000 survivors who completed the Expansion Baseline survey and have available genetic data in the expansion cohort.

Outcome of interest: Survivors were considered to have hypertension if they had a CTCAE hypertension grading of 2 or above (this includes only patients that reported use of an anti-hypertensive medication).

Exploratory variables: Covariates to be included in the analysis:

Sociodemographic characteristics:

- Age at most recent questionnaire
- Sex: Female, Male (Baseline: A.2 and Baseline Expansion: A2)

Health behaviors:

- Smoking status: defined as never smoker if smoke less than 100 cigarettes in lifetime (Baseline questionnaire: N.1-N.1f and Baseline questionnaire: O1-O6).
- Obesity: "obese" defined as participants with a BMI≥30, "overweight" defined as participants with a BMI≥25 and BMI<30, "normal weight" defined as BMI<25 and BMI≥18.5, and "underweight" defined as BMI<18.5. (Baseline Questionnaire Item: A10 and A11; Expansion Baseline Questionnaire Item: A3 and A4)

Clinical variables at first primary neoplasm diagnosis:

- Time since diagnosis: Current date (Questionnaire) – date at initial diagnosis
- Age at cancer diagnosis
- Treatment within five years of cancer diagnosis:
  Any chemotherapy:
  - Anthracyclines – Doxorubicin equivalent dose (mg/m^2)
  - Alkylating agents – Cyclophosphamide equivalent dose (CED): Categorical – 0, 1-3999, 4000-7999, ≥8000 (mg/m^2)
  - Platinum agent – Cisplatin (dose)
  - Platinum agent – Carboplatin (dose)
  - Antimetabolites – Methotrexate (Dose) (IT/IV)
  - Antimetabolites – Oral methotrexate

  Radiation: maximum tumor dose (max TD) to the following body regions in cGY:
  - Chest
  - Abdomen/Pelvis

  Surgery
  - Nephrectomy (yes/no)

- CCSS GWAS genotyping data using the Illumina HumanOmni5Exome microarray
- Whole genome sequencing (WGS) genotyping data
- Genetically determine ancestry (calculated ancestry-specific principal components)

<u>Analytic Approach:</u>

Overall: In this proposed GWAS for hypertension to explore how variants may modify treatment-related risk of hypertension, standard quality control will be performed for both populations (the baseline CCSS and expansion CCSS cohorts). Individuals with >5% missingness, outlying per-sample heterozygosity, sex discordances, and cryptic relatedness (pi-hat>0.2) will be excluded. Also, SNPs will be excluded if they have a missingness >5%, a Hardy-Weinberg Equilibrium (HWE) $P<10^{-6}$ among those without hypertension, are in non-autosomal chromosomes, or a minor allele frequency (MAF) of <5%. Additional quality control measures will be completed on the WGS data as appropriate.

Power: The following power calculation is for the baseline CCSS cohort. Data regarding hypertension in the expansion cohort will be available in the next few months. Assuming a multiplicative model, disease prevalence of 11% and a statistical significance of $5x10^{-8}$, our discovery sample size has 63%, 79% and 89% power to detect alleles of frequency 0.05, 0.1 and 0.2 contributing to genotype relative risk of 1.75, 1.60 and 1.50, respectively.

Specific Aim 1: A single-variant approach using an additive genetic model will be used to test the association between QC-passed genetic variants and hypertension in childhood cancer survivors. Two GWAS analyses will be completed, the first will use the imputed baseline CCSS cohort and the second will use the expansion CCSS cohort. Two separate analyses will be completed because the baseline cohort was genotyped on a different platform than the expansion cohort which makes combining populations difficult. Both analyses will use multivariable logistic regression with the outcome of hypertension dichotomized as yes/no. Odd ratios (ORs), 95% confidence intervals (CIs), and P-values will be calculated. For both GWASs, two multivariable models will be generated. First, the previously-published clinical model will be fit that adjusts for sex, age at questionnaire, age at diagnosis, sex, BMI, smoking status, cranial radiation, chest radiation, abdominal/pelvic radiation, anthracycline exposure, alkylating agent exposure, platinum compounds, and nephrectomy[16]. The second model will include the same covariates as the first and include the top 10 principle components (PCs) and a genetic variant. A GWAS meta-analysis will be completed using both cohorts and genetic variants with a $P< 5x10^{-8}$ will be considered statistically significant. Bioinformatics analysis (e.g., chromatin state, eQTL, in-silico analysis) utilizing ENCODE and other bioinformatics databases will be conducted for discovered SNPs for biological/functional insights.

Specific Aim 2: Based on the results from Specific Aim 1, we will test if treatment modifies the effect of genetic variants on hypertension in childhood cancer survivors. Genetic variants that reach or are close to reaching genome-wide significance ($P<1x10^{-6}$) in the meta-analysis will be further analyzed in a treatment subgroup analysis with the outcome hypertension in the imputed baseline and expansion CCSS cohorts. We will focus on four groups that have been previously associated with hypertension in childhood cancer survivors: abdominal radiation, heavy metal chemotherapy, alkylating agents, and antimetabolites (specifically methotrexate)[18]. Categories within treatment groups will be determined based on sample size. ORs, 95% CI, and P-values will be calculated with multivariable logistic regression, adjusting for the same covariates used in the discovery analysis. A $P <0.05$ will be considered significant. A GxE analysis will be completed if sample size permits.

Specific Aim 3: Any variants that are found to pass genome-wide significance in Specific Aim 1, will be evaluated for replication in the SJLIFE cohort. Further, any variants that are found to be significant in the subgroup analysis ($P<0.05$) will be assessed for replication in the SJLIFE cohort, using the same model and treatment groups that were used in Specific Aim 2.

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Table 1. Characteristics for the discovery study population from the baseline and expansion CCSS cohort GWAS

		Baseline Cohort: By hypertension			Expansion Cohort: By hypertension	
	Total	No	Yes	Total	No	Yes
Characteristic	N (%)	N (%)	N (%)	N (%)	N (%)	N (%)
Total						
Sex						
Male						
Female						
Year of primary cancer diagnosis						
1970-1975						
1976-1981						
1982-1986						
Age at primary cancer diagnosis, years						
<5						
5 - <10						
10 - <15						
15+						
Age at assessment, years						
<20						
20-29.9						
30-39.9						
40-49.9						
Time since diagnosis, years						
5-9						
10-14						
15-19						
20+						
Primary diagnosis						
Leukemia						
CNS malignancy						
Hodgkin lymphoma						
Non-Hodgkin lymphoma						
Wilms tumor						
Neuroblastoma						
Soft tissue sarcoma						
Bone tumor						
BMI, kg/m^2						
<18.5						
18.5-24.9						
25.0-29.9						
≥30						
Smoking status						

Never smoker	
Former smoker	
Current smoker	
Unknown	
Anthracyclines	
No	
<250 mg/m^2	
≥250 mg/m^2	
Alkylating agents (CED)	
No	
≤8000 mg/m^2	
>8000 mg/m^2	
Platinum Compounds (Cisplatin/Carboplatin)	
No	
Yes	
Antimetabolites (methotrexate)	
No	
Yes	
Nephrectomy	
No	
Yes	
Chest radiation	
No	
<20 Gy	
≥20 Gy	
Abdomen/pelvis radiation	
No	
<20 Gy	
≥20 Gy	
Cranial radiation	
No	
<20 Gy	
≥20 Gy	

Abbreviations: BMI, body mass index

Table 2. Clinical and model showing non-genetic risk factors and their associations with hypertension among survivors of European ancestry

	Clinical Model Baseline Cohort		Clinical Model Expansion Cohort	
Variables	OR (95% CI)	P-Value	OR (95% CI)	P-Value
Age (y)				
Age at diagnosis (y)				
Sex				
Female	1.00 (Ref)		1.00 (Ref)	
Male				
BMI (kg/m^2)				
<18.5	1.00 (Ref)		1.00 (Ref)	
18.5-24.9				
25.0-29.9				
≥30				
Smoking status				
Never smoker	1.00 (Ref)		1.00 (Ref)	
Former smoker				
Current smoker				
Unknown				
Anthracyclines				
No	1.00 (Ref)		1.00 (Ref)	
<250 mg/m^2				
≥250 mg/m^2				
Alkylating agents (CED)				
No	1.00 (Ref)		1.00 (Ref)	
≤8000 mg/m^2				
>8000 mg/m^2				
Platinum Compounds (Cisplatin/Carboplatin)				
No				
Yes				
Antimetabolites (methotrexate)				
No	1.00 (Ref)		1.00 (Ref)	
Yes				
Nephrectomy				
No	1.00 (Ref)		1.00 (Ref)	
Yes				
Chest radiation				
No	1.00 (Ref)		1.00 (Ref)	
<20 Gy				
≥20 Gy				
Abdomen/pelvis radiation				
No	1.00 (Ref)		1.00 (Ref)	
<20 Gy				
≥20 Gy				
Cranial radiation				

No	1.00 (Ref)	1.00 (Ref)
<20 Gy		
≥20 Gy		

Abbreviations: BMI, body mass index

Table 3. Association between common genetic variants and hypertension

		CCSS Cohort (Meta-Analysis)			Replication Cohort	
Gene: SNP	EA	OR (95% CI)	P Value	EA	OR (95% CI)	P Value

Abbreviations: EA, effect allele; OR, odds ratio; CI, confidence interval