Study title: A genome-wide association study for frailty in adult survivors of childhood cancer

Working groups: Genetics – primary
Epidemiology/Biostatistics – secondary
Chronic Diseases - secondary

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Background and rationale

With improved treatment and supportive care, the number of childhood cancer survivors (CCS) living in the United States has increased exponentially, projected to exceed 500,000 by the year 2020.[1] However, this remarkable achievement is tempered by the recent discovery that many CCS are at increased risk for premature, accelerated aging and early onset frailty.[2-13] Two out of three CCS have at least one severe, disabling, or life-threatening chronic medical condition that increase with age, affecting 80% of survivors over 45 years old.[14] CCS are also more likely to report poor health, functional impairment, and activity limitations compared with sibling controls that increases with age.[4-6]

A steady increase in frailty accompanies healthy, physiologic aging, and is estimated to affect 9% of persons over 65 years of age[15] and 25-40% of persons over 80 years of age.[16] Frailty identifies individuals more vulnerable to adverse health outcomes[17] and predicts risk for early mortality.[18-20] One method for assessing frailty is to use the Fried criteria, a clinical assessment of weight loss, exhaustion, poor grip strength, slow gait speed, and low physical activity, with three out of five criteria considered 'frail,' and two of five considered 'pre-frail.'[16]

Two large studies have applied the Fried criteria to CCS. Both found a higher than expected frailty prevalence corresponding with features of accelerated aging. The first landmark study led by Dr. Ness objectively assessed frailty in-person for 1,922 CCS enrolled to the St. Jude Lifetime cohort study (SJLIFE). This study demonstrated a prevalence of pre-frailty (2/5 Fried criteria) in 31.5% of female survivors and 12.9% of male survivors, and of frailty (at least 3/5 Fried criteria) in 13.1% of female survivors and 2.7% of male survivors, mean age of 33.6 +/- 8.1 years, approaching the prevalence observed in non-cancer survivor populations that are at least three decades older.[7] For the second study, Dr. Ness and her team used longitudinal survey data collected from 10,899 survivors (mean age 37.6 years) and 2,097 sibling controls (mean age 42.9 years) participating in CCSS, to determine the prevalence of frailty in this cohort: 6.9% (95%

CI: 6.2-7.5) for females and 4.7% (95% CI:4.1-5.4) for males, compared with 1.8-2.1% of siblings.[21] Factors associated with frailty in this cohort were being female, age over 50 years at time of evaluation, sedentary lifestyle, and treatment that included cranial, pelvic, or abdominal radiation, cisplatin, amputation, or lung surgery. Survivors at highest risk for frailty were those with history of CNS tumors (9.5%), followed by bone tumors (8.1%), Hodgkin lymphoma (7.5%) and soft tissue sarcoma (7.0%). Presence of **chronic health conditions** augmented frailty risk, and when adjusting for presence of these conditions as well as **lifestyle factors**, relevant treatment factors were limited to **cranial and pelvic radiation** as well as **lung surgery**. There are potential limitations to this second study: survivors may have under-reported outcomes used to generate the estimated frailty status, and the constraints of self-reported data required modification of the criteria described by Fried. For example, objective measures such as grip strength, intended to be assessed by dynamometer, were instead assessed by a positive response to the question '"Have you ever been told by a doctor or other health care professional that you have, or have had, weakness or inability to move your arms?" Nevertheless, **the availability of estimated survivor frailty status represents a great opportunity within CCSS for investigating genetic associations with frailty in genotyped survivors.**

No genome-wide association studies (GWAS) for frailty have been conducted in survivors of childhood cancer, and it is important to note that no such GWAS has been conducted in elderly populations either. However, there have been GWAS conducted in the general population that have identified significant associations between multiple loci and components of frailty. For example, a GWAS of grip and lower body strength conducted in adults over 65 years old found a SNP association with proposed function in muscular repair.[22] Another GWAS identified 16 loci associated with grip strength in middle and older aged individuals.[23] Lead variants were located near or within genes implicated in skeletal muscle fiber structure and function, or neuronal pathways. Lastly, in the largest GWAS evaluating components of frailty conducted to date, 64 variants were associated with grip strength among individuals ages 40-69 years. For this study, the team used a genetic risk score method to determine the combined, weighted SNP association between grip strength and various outcomes related to frailty, showing positive associations with cardiorespiratory fitness, self-reported excellent health, and increased physical activity measured by accelerometer, and inverse associations with slow walking speed, self-reported fatigue, falls in the past year, weight loss, and reaction time.[24] With respect to sarcopenia, strong associations were observed between levels of lean or fat body mass (measured by DXA scan) and two SNPs in the thyrotropin-releasing hormone receptor.[25] In a second meta-analysis, five loci were identified in association with both whole body lean mass and appendicular lean mass, which is more specific to a diagnosis of sarcopenia.[26] Lastly, GWAS conducted in elderly community dwelling adults found several SNPs significantly associated with gait speed.[27]

The objective of this proposal is to investigate genetic associations with frailty and components of frailty in survivors of childhood cancer. Given that there are no frailty GWAS that have been conducted to date, we will first evaluate genetic risk for frailty-related outcomes using previously-identified variants that have been associated with components of frailty, such as weakness, low lean muscle mass, and slowness, comparing genetically-predicted risk with self-reported outcome data. We will then leverage survivor frailty classification derived from CCSS survey data, as well as the self-reported frailty-related outcomes investigated in Aim 1, to conduct a frailty GWAS to identify novel genetic associations with frailty-related outcomes and frailty classification. Lastly, we will use data obtained from Aims 1 and 2 to test if incorporation of genetic factors improves a risk prediction model that is based on demographic and treatment exposures.

Hypothesis and Specific Aims

We hypothesize that genetic variation influences the risk for frailty and components of frailty in survivors of childhood cancer. We will test this hypothesis in the following Aims.

Aim 1*: Evaluate the relationship between previously-published genetic variants associated with components of frailty and the risk for these frailty-related outcomes in survivors of childhood cancer*

- Aim 1a: Determine individual associations between previously identified grip strength variants and self-reported *weakness* in CCSS survivors.
- Aim 1b: Determine individual associations between previously identified sarcopenia variants and self-reported *low lean muscle mass* in CCSS survivors.
- Aim 1c: Determine individual associations between previously identified gait speed variants and self-reported *slowness* in CCSS survivors.
- Aim 1d: From these previously identified variants, determine the unweighted and weighted genetically-predicted risk for each self-reported outcome, including weakness, low lean muscle mass, and slowness.

Aim 2: *Identify novel genetic variants associated with frailty-related outcomes and frailty status classification in survivors of childhood cancer*

- Aim 2a: Determine the relationship between demographics, diagnosis and treatment factors, health behaviors, chronic health conditions, and frailty in CCSS survivors (Discovery Population)
- Aim 2b: Use a genome-wide approach to identify novel genetic variants associated with self-reported *weakness*, *low lean muscle mass*, and *slowness* as well as *frailty status* in CCSS survivors of European ancestry (Discovery Population)
- Aim 2c: Determine the role of gene-treatment interactions (e.g. CNS radiation, pelvic radiation, lung surgery) with frailty and frailty-related outcomes in CCSS survivors of European ancestry.
- Aim 2d: Replicate novel associations observed in an independent survivor cohort (CCSS expansion cohort).

Aim 3: *Develop an integrated clinical and genetic risk prediction model for frailty in survivors of childhood cancer*

- Aim 3a: Determine if the addition of relevant genetic factors (SNPs included in the polygenic risk score from Aim 1 and that were replicated in the GWAS from Aim 2) to a model that includes demographics, diagnosis and treatment factors, health behaviors, and number/duration of grade 3-4 chronic health conditions improves frailty risk prediction in CCSS survivors.
- Aim 3b: Validate the integrated risk prediction model in an independent population of survivors (CCSS expansion cohort).

Analysis Framework

This analysis will utilize existing data within the original CCSS cohort to address each specific aim. The proposed study population, variables of interest, and analytic plan for each aim are outlined below.

Study Population: This study will be conducted in the 5,324 childhood cancer survivors of European ancestry who are enrolled to the CCSS original cohort (diagnosed 1970-1986) and who have available genotype data (Discovery Population). Survivors treated with hematopoietic stem cell transplantation will be excluded (lack of genotype data). Of note, all CCSS participants (both original and expansion cohorts) who completed a follow up questionnaire by the end of 2016 and are at least 18 years old have been characterized for frailty (n=10,899). From the results of this study, we can estimate the number of frail participants in the original cohort by examining the incidence of frailty in the older age groups. For example, among survivors 40-49 years of age, 9.3% of females (n=1,590) and 4.7% of males (n=1,700) endorsed three or more frailty criteria (classified as frail). Among survivors 50+ years, this percentage increased to 10.6% of females (n=767) and 7.0% of males (n=646). Therefore, we anticipate the original cohort includes at least 350 frail survivors.

Outcomes of Interest (dependent variable): The primary outcome of interest is frailty status, as previously determined by Hayek et al[21] and based on self-reported data obtained from CCSS questionnaires (Long Term Follow Up 5, LTFU5). For this analysis, estimated frailty status will be dichotomized (yes/no). Frailty status has already been estimated for CCSS participants using survivor responses to questions representing each of the five criteria for frailty,[21] 1) *Low lean muscle mass* – body mass index (BMI) of <18.5 kilograms per square meter (kg/m^2) or unintentional weight loss of at least 10 pounds in the past year; 2) *Self-reported exhaustion* - a score of ≤40 on the Vitality subscale of the Medical Outcomes Survey Short Form-36 (SF-36); 3) *Low energy expenditure (LEE)* – expending <383 kilocalories per week (kcal/wk) for males and <270 kcal/wk for females based on conversion of reported frequency and duration of low, moderate, and vigorous activities into kilocalories; 4) *Slowness* – indicating "limited for more than 3 months" in response to either "Over the last 2 years, how long has your health limited you in walking uphill or climbing a few flights of stairs?" or "Over the last 2 years, how long has your health limited you in walking one block?"; and 5) *Weakness* – answering "yes and the condition is still present" to "Have you ever been told by a doctor or other health care professional that you have, or have had, weakness or inability to move your arms?" Participants who endorsed three or more of these criteria were classified as frail.[21]

Independent Variables: As outlined in the analytic approach for each specific aim, the primary independent variables are genotypes obtained from Illumina HumanOmni5Exome array. We will only use genetic data considered representative of constitutional survivor DNA, i.e. survivors with history of bone marrow transplant will only be included if genotypes were estimated from buccal DNA obtained from mouthwash kits.

Additional covariates considered in the analysis will include those associated with frailty as determined by Hayek et al. and related variables, including:

- Primary cancer diagnosis: leukemia, CNS tumor, Hodgkin lymphoma, non-Hodgkin lymphoma, neuroblastoma, Wilms tumor, rhabdomyosarcoma, bone tumor
- Age at cancer diagnosis: Date of diagnosis – date of birth
- Cumulative chemotherapy dose: alkylating agent score, total platinum dose
- Radiation: maximum tumor dose to the following body regions: cranial, chest, abdomen/pelvis, other
- Surgery: lung surgery yes/no, amputation yes/no
- Age at baseline assessment: Date of baseline survey completion
- Age at LTFU 5 assessment: Date of LTFU5 survey completion
- Sex

- Genetically determined ancestry (calculated ancestry–specific principal components)
- Health behaviors (assessed at LTFU5)
  - Sedentary behavior i.e. report of no physical activity in the past month (yes/no)
  - Smoking history (yes/no)
  - BMI ≥30 kg/m^2
- Grade 3-4 chronic health conditions (assessed at baseline and LTFU5, defined by the Common Terminology Criteria for Adverse Events version 5)
  - Category: cardiac, respiratory, endocrine, subsequent neoplasm, renal, musculoskeletal, neurologic disease
  - Number of conditions
  - Duration of each condition: continuous variable measured in years. The most recent chronic health condition data will be used if data are not available from the LTFU 5 survey.

Analytic Approach: Descriptive statistics will be generated and compared between survivors of childhood cancer with and without the outcome of interest (frailty, and components of frailty). For each aim, we will conduct regression diagnostics to evaluate the assumptions and overall goodness of fit for the most significant findings. Appropriate steps will be taken to address multiple comparisons (i.e. Bonferroni-corrected p-values), influential observations, and violations of the regression model assumptions. Study cohort characteristics will be displayed in tables such as proposed Table 1.

Aim 1 approach: The primary focus of Aim 1 is to test the association in CCSS of genetic variants previously identified by GWAS as related to components of frailty (see citations above). Of the five frailty components, this Aim will be limited to investigation of associations with sarcopenia, grip strength, and gait speed, as there have been no relevant GWAS conducted to date for frailty in general, nor for exhaustion or low energy expenditure. For previously-described variants with a minor allele frequency (MAF) ≥1%, we will calculate an odds ratio (OR), 95% confidence interval (CI), and p-value for the association between each SNP and each of the three outcomes using multivariable logistic regression. A log-additive model of inheritance will be used. For this analysis, statistical significance will be defined as $p<0.05$, as these variants have all been previously linked to frailty-related outcomes in the general population. The direction of effect and effect size will be compared with previous assessments. Results will be displayed in tables such as Table 2. For Aim 1d, we will estimate the OR, 95% CI and *P* value for the association between each individual SNP identified in Aims 1a-1c and estimated frailty status. We will then use the previously published effect sizes to estimate the OR for frailty using the weighted sum of all SNPs significantly associated with frailty. An un-weighted genetic risk score (GRS) will be determined for each participant from the number of risk alleles present (0, 1, or 2), and a weighted GRS will be generated using the beta estimate for each effect allele. Covariates will be based on the work of Hayek et al, and include sex, cranial radiation exposure, pelvic radiation exposure > 33Gy, and history of lung surgery. The false discovery rate will be used to account for multiple comparisons. Results will be shown in tables such as Table 3.

Aim 2 approach: The primary focus of Aim 2 is to identify novel genetic variants associated with frailty in CCSS. We will calculate an OR, 95% CI, and p-value for the association between each imputed genetic variant and frailty using SNPTEST v2.5.4, assuming a log-additive model of inheritance. Quality control of the imputed data set will remove data with a MAF <1% or imputation quality score (R2) <0.30. In secondary analyses, we will utilize the MH test statistic

for less frequent variants (MAF <1%). In each analysis, we will evaluate potential confounding due to primary cancer diagnosis, age at cancer diagnosis, sex, age at time of last follow-up or time of death, radiation site and dose, and number of chronic health conditions. Therapeutic subgroup analyses will be restricted to the high-risk survivor populations previously identified by Hayek et al (those exposed to cranial radiation, pelvic radiation >33 Gy, lung surgery).[21] Genome-wide statistical significance for the discovery cohort will be defined as $p < 5x10^{-7}$ Replication of these findings will be attempted in the CCSS expansion cohort, and statistical significance for the combined discovery and replication cohorts will be defined as $p < 5x10^{-8}$. Results will be displayed as shown in Table 4.

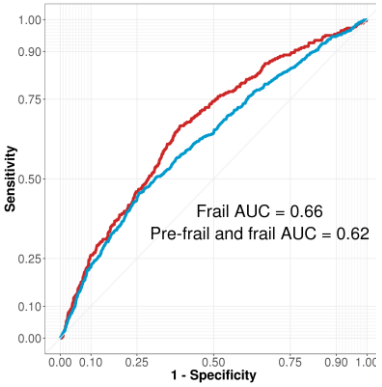

Figure 1: Clinical risk prediction of frail and pre-frail in the CCSS.

Aim 3 approach: For this Aim, we will include any self-reported chronic health conditions diagnosed prior to the LTFU5 survey time point. We will calculate ORs and 95% CIs to predict frailty at LTFU5 using logistic regression models. To determine the most parsimonious model using these variables, we will begin our multivariable modeling by including significant genetic findings from Aims 1 and 2 (retaining variables with $p<0.05$). By adding these variables to a model determined from data obtained by Hayek et al, (demographic and lifestyle factors, as well as treatment exposures only), we will evaluate the extended model's ability to discriminate between survivors with and without frailty by calculating the area under the curve (AUC) statistic. The current AUC statistic is poor (0.62-0.66) (**Figure 1**). Our objective will be to achieve a minimum AUC of 0.70, but we will have power to detect larger improvements in risk prediction. Cross-validation will be performed to avoid over-fitting and correcting "over optimism" of AUC. We will compare performance of the extended risk prediction model to the clinical model by evaluating the added predictive ability of the new markers using integrated discrimination improvement (IDI), or the difference in mean predicted probabilities between frail and not-frail in nested models (IDI = improvement in average sensitivity weighted by the average 1 minus specificity).[28] Results will be modeled graphically as shown in Figure 1.

Power: We used the easyROC webtool (http://www.biosoft.hacettepe.edu.tr/easyROC/) to calculate power to show non-inferiority of the GRS-extended prediction model compared to the clinical risk prediction model. We have >99% power to show non-inferiority of a GRS-extended model with AUC of 0.70 compared to the clinical frailty model with AUC of 0.66 given our available sample size in the CCSS. For validation of the clinical and GRS-extended prediction models, we need at least 33 cases and 495 controls to have 99% power to show AUC of 0.7, assuming a similar frailty risk in our validation population.

Replication and Validation: Replication (Aim 2) and validation (Aim 3) will be first conducted in the CCSS expansion cohort (diagnosed 1987-1999). Expansion cohort participants have also submitted survey data, permitting frailty classification for those that have been genotyped. Within the expansion cohort, a smaller number of participants are classified as frail due to their younger age distribution. Specifically, the frailty study conducted by Dr. Ness in CCSS shows that ~3.5% of survivors between the ages of 18 and 29 years are frail, and 5-6% of survivors between the ages of 30 and 39 are frail, so that in this age group that approximates the expansion cohort age group, about 300 out of 6,326 are frail. Secondary replication and validation will be sought in SJLIFE, a cohort that has been directly assessed for frailty in person, rather than assessed using survey data. Although we suspect frailty-related outcome phenotypes may differ when assessed

by survey compared with direct assessment, replication/validation in SJLIFE would further strengthen the findings of our study, and therefore is worth attempting.

Proposed Figures and Tables

Table 1: Demographic and treatment exposure characteristics of survivors in CCSS and SJLIFE

	Discovery		Replication / Validation	
	CCSS Original (N=)		CCSS Expansion (N=)	
Variable	**No.**	**%**	**No.**	**%**
Sex				
Male				
Female				
Age at evaluation (years)				
18-25				
26-35				
36-45				
46-55				
>55				
Body mass index (kg/m^2)				
<25				
≥25 and <30				
≥30 and <35				
≥35 and <40				
≥40				
Physical activity				
Sedentary				
Not sedentary				
Age at diagnosis (years)				
0-4				
5-9				
10-14				
>14				
Year of diagnosis				
1970-1975				
1976-1980				
1981-1986				
Diagnosis				
Leukemia				
Hodgkin lymphoma				
Non-Hodgkin lymphoma				
Central nervous system malignancy				
Kidney				
Neuroblastoma				
Soft tissue sarcoma				
Bone tumor				
Other malignancy				
Relevant cancer therapies				
Alkylating score				
0				
1				
2				
3				
Cranial irradiation (any)				
Pelvic irradiation (>33 Gy)				
Lung surgery (any)				
Number of grade 3-4 chronic health conditions				
0				
1				
2				
3 or more				
Frailty status				
Frail				
Not frail				

Table 2 (Aim 1): Top genetic variant association results (P<0.05) for frailty-related outcomes in CCSS

Previously-identified genetic variant						CCSS (N=)			
Chr.	Pos.	Nearest gene	RSID	Ref.	Alt.	Freq.	RR / Beta	SE	P
Slowness									
Weakness									
Low lean muscle mass									

Abbreviations: Chromosome (Chr.), genomic position (Pos.), SNP identifier (RSID, if available), reference allele (Ref.), alternative allele (Alt.), alternative allele frequency in sample (Freq.), standard error (SE).

Table 3 (Aim 1): Risk for frailty in CCSS associated with previously-established SNPs for frailty-related outcomes

RSID	Chr	Nearby gene	Ref.	Alt.	Freq. in EUR*	RR/beta	P value for frailty-related outcome in CCSS	Reference	Unadjusted HR (95% CI)	P value for frailty	Adjusted HR[&] (95% CI)	P value for frailty
Un-weighted Genetic Risk Score												
Weighted Genetic Risk Score												

Abbreviations: Chromosome (Chr.), SNP identifier (RSID, if available), reference allele (Ref.), alternative allele (Alt.), alternative allele frequency in sample (Freq.), hazard ratio (HR)

Table 4 (Aim 2): Top genetic variant association results (P<$5x10^{-8}$) for frailty in discovery and replication cohorts

Genetic variant						CCSS Original (Discovery, N=)				CCSS Expansion (Replication, N=)				Joint (N=)			
Chr.	Pos.	Nearest gene	RSID	Ref.	Alt.	Freq.	RR / Beta	SE	P	Freq.	RR / Beta	SE	P	Freq.	RR / Beta	SE	P

Abbreviations: Chromosome (Chr.), genomic position (Pos.), SNP identifier (RSID, if available), reference allele (Ref.), alternative allele (Alt.), alternative allele frequency in sample (Freq.), standard error (SE).

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