Study title: Risk Prediction of <mark>Menopause-related Phenotypes</mark> in Childhood Cancer Survivors Using Polygenic Risk Scores and Clinical Predictors

Working groups: Primary: Genetics

Secondary: Biostatistics/Epidemiology; Cancer control; chronic disease

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1. Background and Rationale

Over 80% of children diagnosed with cancer are long-term survivors (survival for five years or more after cancer diagnosis) in the US and Canada due to advances in cancer treatment[1,2]. Improving the quality of life of long-term childhood cancer survivors is a priority since they face an increased risk of developing chronic health conditions due to their exposures to specific cancer treatments[3]. For female survivors, compromised reproductive function is a significant concern[4]. Studies have shown that specific cancer treatments can adversely affect ovarian function, leading to abnormal timing of menopause[5,6]. Among female childhood cancer survivors, menopause-related outcomes include primary ovarian insufficiency (POI, defined as naturally occurring menopause before 40 years old)[7], which has two subcategories: acute ovarian failure (AOF, or permanent cessation of menstruation within 5 years of cancer diagnosis or failure to achieve menarche by age 18 years) and non-surgical premature menopause (NSPM, defined as menopause that develops naturally before age 40, among those with normal ovarian function for at least 5 years following cancer diagnosis). An estimated 11% of female childhood cancer survivors develop POI[7], which is considerably higher than the estimated prevalence of POI in the general population (~1%)[8].

Extensive studies have been undertaken to identify treatment risk factors associated with compromised reproductive function following cancer treatment[3,9]. Chemotherapy agents, especially alkylating agents (such as busulfan, cyclophosphamide, lomustine, and procarbazine, etc.), can prevent cell division and growth by interacting with DNA and reduce the number of follicles for maturation and reproduction, increasing the risk for ovarian dysfunction[5]. Also, radiation to the ovary, abdominal or pelvic sites can induce genomic damage in oocytes and the surrounding granulosa cells, leading to either a decreased or exhausted ovarian follicle pool depending on the extent of the damage[5].

Although many fertility preservation options, such as oocyte and ovarian tissue cryopreservation, are available to preserve reproductive function[7,8], female childhood cancer survivors report that making fertility preservation decisions is difficult[10], especially since the individual risk for compromised reproductive function after treatment is unknown[11]. Well-established clinical risk factors and cancer treatments, including prescribed alkylating agents and radiation to abdomen, pelvis or total body irradiation[12], have been evaluated as risk factors to develop risk prediction models for compromised reproductive function in female survivors. For example, our research group has used clinical predictors such as cumulative alkylating drug dose[13], radiation exposure to the ovary, abdomen and pelvis, age at cancer diagnosis, and hematopoietic stem-cell transplant receipt to build models to predict AOF/NSPM risk for individual pediatric cancer patients at the time of cancer diagnosis[14,16]. The area under the ROC curve (AUCs) and average positive predictive value (APs) of the AOF clinical prediction model in the internal validation dataset are 0.82 and 0.50, respectively[14]. The AUC/AP of the NSPM clinical prediction model (at 15 years post cancer diagnosis) is 0.73/0.10 when evaluated in the CCSS cohort[15]. These results indicate that apart from the clinical predictors, the inclusion of other relevant predictors will improve the predictive performance of predictive models for menopause-related phenotypes, especially for the female survivors at risk for NSPM.

A limitation of risk prediction models for AOF/NSPM is that the age at cancer diagnosis was used as the time origin in the definition of AOF/NSPM, which implies these models are meant to be used to predict an individual's risk of menopause using age at diagnosis as the time origin. However, it is often of clinical interest in estimating female childhood cancer survivors' risk of menopause at pre-specified ages (e.g., age 25, 40, etc.) following their cancer treatment. By assessing the risk of developing menopause at different pre-specified ages, i.e., "age-specific risk", clinicians and female childhood cancer survivors can take actions to reduce the potential impact of the late effects of cancer treatment based on their age rather than by time since diagnosis. Therefore, it is important to assess both AOF and NSPM as POI status in predictive models to support clinical decision-making. Recently, our research group used clinical predictors to quantify a female survivor's POI risk at several pre-specified ages. Clinical predictors such as race/ethnicity, age at cancer diagnosis, chemotherapy exposure (e.g., Cyclophosphamide, Busulfan, Melphalan, Procarbazine, and Ifosfamide etc.), bone marrow transplantation, and radiation dosage to the ovarian, abdomen, and pelvis sites were evaluated.

Age at menopause is a heritable complex trait[16,17]. A meta-analysis of genome-wide association studies (GWAS) conducted in general population samples identified 54 single nucleotide polymorphisms (SNPs) at 44 genetic loci associated with age at menopause[18]. Studies have also shown that reproductive function phenotypes have overlapping genetic architectures. For example, a recent study indicated that reproductive performance, POI, and age at natural menopause share common genetic factors involved in DNA repair and maintenance[17]. Among survivors, Brooke *et al.* identified a novel haplotype associated with premature menopause risk among female childhood cancer survivors[19], indicating differences between the genetic architectures for premature menopause risk in survivors exist. Therefore, we hypothesize that including genetic variants associated with reproductive function phenotypes (e.g., menarche- and menopause-associated SNPs) identified in general population GWAS and genetic analyses in childhood cancer survivors may improve the model performance of existing clinical-predictor only predictive models for POI risk in female survivors.

Our proposal aims to improve the accuracy of risk prediction for menopause-related phenotypes, specifically AOF, NSPM, and POI, in female childhood cancer survivors using clinical and genetic information. To accomplish this goal, we propose to evaluate genetic risk in the form of a polygenic risk score (PRS), or a score that combines the estimated effects of many disease-associated genetic variants reported in published GWAS. PRSs have been proposed as a genetic risk prediction tool for a wide range of diseases[20–22]; a clinically-useful PRS would allow clinicians to identify individuals at elevated risk of disease, thus informing disease screening[23], therapeutic interventions[24], and life planning[25] to prevent or delay the onset of disease. To our knowledge, PRSs for reproductive function phenotypes have not been developed to predict risk for menopause-related phenotypes in survivors or in the general population. The evaluation of the general population PRS for these menopause-related phenotypes in survivors may not only have clinical utility for the survivor population, but can also provide further insights into the relative contribution of general population PRS to menopause phenotypes in childhood cancer survivors.

Therefore, we aim to: 1) construct and validate a PRS for menopause-related phenotypes based on results from published GWAS of complex reproductive function phenotypes conducted in the general population; and 2) develop a risk prediction model incorporating the validated PRS for the risk of developing menopause-related phenotypes and clinical risk factors associated with reproductive phenotypes in female childhood cancer survivors to generate a user-friendly clinical and genetic risk score system. This system could provide clinicians with the means to estimate a female survivor's risk for menopause-related phenotypes, thus informing clinical decision-making regarding fertility preservation.

2. Specific Hypotheses and Aims

Hypotheses:
H1: Common variants and/or low-frequency variants may contribute to changes in the reproductive function of female survivors of childhood cancer, affecting the timing of menopause (for example, acute ovarian failure [AOF], non-surgical premature menopause [NSPM], and primary ovarian insufficiency [POI]).

H2: Along with relevant clinical predictors (e.g., cancer treatments), incorporating genetic risk profiles in the form of polygenic risk scores (PRS) may improve the performance of predictive models for menopause-related phenotypes (specifically AOF, NSPM, and POI) among female childhood cancer survivors.

Aims:
Aim 1: Identify existing PRS for menopause-related phenotypes, if available, or develop and validate PRS using susceptibility variants identified to date for menarche-/menopause-related phenotypes in genome-wide association studies (GWAS) conducted in the general population.

Aim 2: Develop and validate predictive models that include both the validated PRS and clinical predictors to accurately predict the risk of menopause-related phenotypes among childhood cancer survivors and translate the predicted risk of menopause-related phenotypes into a clinical and genetic risk score system.

2a) Use existing baseline predictive models for menopause-related phenotypes if available, or develop and validate new predictive models that only include the clinical predictors (e.g., demographic information, cancer treatment, etc.), if none exists;

2b) Examine the discrimination ability of the validated PRS (derived from Aim 1) to discriminate: 1) AOFs vs. non-AOFs, 2) NSPMs versus survivors whose menopause age is greater than 40, and 3) POIs versus survivors whose menopause age is greater than 40. Compare the utility of the PRS in these three menopause-related phenotypes, as well as compare the predictive power of the PRS to the clinical predictive models derived from Aim 2a).

2c) Include both the PRS (derived from aim 1) and clinical predictors (identified in aim 2a) in the predictive models for menopause-related phenotypes and compare the model performance of prediction models constructed in 2a) and 2b).

Aim 3: Assess whether PRS that include or reconcile treatment-specific SNP effects identified in previous GWAS conducted in female cancer survivors[19] improve the prediction performance of the predictive models for menopause-related phenotypes in comparison to PRS constructed from the general population.

3. Analysis Framework

Outcomes of Interest

There are four possible outcomes (ovarian status): AOF, NSPM, surgical premature menopause (SPM, defined as long-term female childhood cancer survivors who had bilateral oophorectomy procedure after cancer treatment), and normal. SPM is treated as a competing risk, since once a female childhood cancer survivors experienced SPM, she is no longer at risk of developing other conditions that are of research interest.

Base on the proposed aims, the outcomes of interest in this study include:

1) AOF (Yes/No)

2) NSPM (Yes/No) at specific time after the cancer diagnosis (e.g., NSPM status at 15 years following cancer treatment)

3) POI (Yes/No) and age at POI onset

The definitions of outcomes of interest:

AOF is defined as the permanent cessation of menstruation within five years of cancer diagnosis for individuals who had menarche before cancer treatment, or failure to achieve menarche by age 18 years for individuals who did not have menarche before cancer treatment[26].

NSPM is defined as menopause that develops naturally before age 40, among those with normal ovarian function for at least 5 years following cancer diagnosis[4].

POI is a combination of AOF and NSPM, defined as either: (1) experiencing menopause naturally before the age of 40 years for individuals who had menarche before cancer treatment, or (2) never experiencing menarche by the age of 18 years for individuals who did not have menarche before cancer treatment[26,27].

Two variables are associated with the outcomes of interest:

1. The ovarian status;
2. The age at AOF/NSPM/POI onset

The ovarian status has been determined by two means: 1) Using the above-established definition, on the basis of patients' self-reported menstrual history information; or 2) manual review by endocrinologists (Drs. Sogol Mostoufi-Moab and Charles A. Sklar) for ambiguous cases, using the patient responses for menstrual history questions in the baseline and follow-up 1, 4 and 5 questionnaires. Age at menopause is available via the CCSS surveys. The questions on menstrual history and age at menopause in these questionnaires are provided below.

CCSS survey prompts for menstrual history:

- Have you ever had a menstrual period naturally?
  - If yes, at what age did you have your first menstrual period?
- Have you ever taken birth control pills or female hormones to regulate your periods?
- Are you currently experiencing menstrual periods?
  - If no, at what age did you last have a menstrual period naturally?
  - If no, what type of menopause? (normal or early menopause, surgical)

Subject Population

The data source for the primary analysis is from the Childhood Cancer Survivor Study (CCSS). CCSS is a multi-institutional retrospective cohort study of over 20,000 childhood cancer survivors, of which 11,336 are female[13] and 2958 females childhood cancer survivors of European ancestry have provided GWAS data in the CCSS original cohort. We will include expansion cohort GWAS data if it is available.

European ancestry subpopulation with GWAS data

The numbers of AOF and NSPM cases in the subpopulation that have GWAS data are estimated assuming that the distribution of ovarian status is the same as the parent CCSS study sample (i.e., the event rates of AOF, NSPM, SPM and normal are the same as they are in the original study CCSS study sample).

Subpopulation 1: To examine the ability of PRS to discriminate those at risk for AOF from those at risk of NSPM and normal female childhood cancer survivors, we have subpopulation 1 with an estimate of 208 AOF cases.

Subpopulation 2: To examine the ability of PRS to discriminate those at risk for NSPM from normal female childhood cancer survivors, we will exclude the female survivors with AOF. We estimated that approximately 2750 female childhood cancer survivors would be included, with about 161 NSPM cases.

Subpopulation 3: There are approximately 2,958 female childhood cancer survivors of European ancestry with GWAS data in CCSS, with 369 POI cases.

Inclusion and exclusion criteria

Inclusion criteria:

Long-term (≥5-year) female survivors who:

- Were diagnosed before the age of 21 years with eligible cancer types;
- Provided biospecimens for DNA genotyping;
- Are of European genetic ancestry;
- Had complete treatment exposure data, including chemotherapy radiation therapy;
- Provided menstrual history information, including age at menarche, age at last menstrual period, current menstrual status, and the causes of menopause (surgical or non-surgical), if applicable.

Exclusion criteria:

Long-term (≥5-year) female survivors who:

- Were exposed to a cranial or pituitary radiation dose higher than 30 Gy;
- Had a history of tumors in the hypothalamus or pituitary region;
- Had a history of Turner or Down's Syndrome;
- Had a secondary malignancy within 5 years of primary cancer diagnosis.

Exploratory Variables

a) Baseline variables
- Date of Birth (age at follow-up)
- Date of Diagnosis (age at diagnosis)
- Cancer diagnosis
    o Leukemia, central nervous system (CNS) cancers, Hodgkin lymphoma, non-Hodgkin lymphoma, Wilms' tumor, neuroblastoma, soft-tissue sarcoma, or bone tumors
- Smoking status (Current/Ever/Never/Unknown)
- Alcohol history (Yes/No)

b) Genetic factors

Polygenic risk score (constructed using susceptibility variants identified to date for menarche-/menopause-related phenotypes in GWAS conducted in the general population)

c) Cancer Treatment
- Chemotherapy
    o Any chemotherapy exposure (Yes/No)
    o Type and dose of chemotherapy agent: Methotrexate, BCNU (Carmustine), Bleomycin, Busulfan, Carboplatin, Cis-Platinum, Cyclophosphamide (Cytoxan), Daunorubicin (Daunomycin), Doxorubicin (Adriamycin), Epirubicin, Idarubicin, Ifosfamide, Melphalan, Mitoxantrone, Nitrogen Mustard, Thiotepa, VM-26 (Teniposide), VP-16 (Etoposide), CCNU (Lomustine), Chlorambucil, Myeleran, Procarbazine.
    o Route of administration: intramuscular (IM), Intrathecal (IT), Intravenous (IV), IV, IM or Intra-arterial (IA), oral, sub-Q.
    o Dosage (mg/m^2)
    o Cyclophosphamide-equivalent dose[28]
- Radiation therapy
    o Radiation exposure to the total body, abdominal, and pelvic body regions (Yes/No)
    o Maximum prescribed radiation dose to the total body, abdominal, and pelvic body regions
    o Average radiation dose to the pituitary gland
    o Minimum and maximum radiation dose to the right and left ovaries

- o Hematopoietic stem-cell transplant (Yes/No)
- o Bone marrow transplantation (Yes/No)

Method

To achieve the proposed specific aims, we will: 1) construct the PRS for POI risk, and 2) use the constructed PRS and the clinical predictors to build prediction models for POI risk using the CCSS data.

Aim 1: Construction of PRS for menopause-related phenotypes using findings from general population GWAS
The PRSs that will be evaluated in survivor data require two components: (1) external GWAS/ meta-analyses results; and (2) an external process for PRS construction and validation.

Step 1: GWAS selection
1) GWAS results: Summary statistics from GWAS and meta-analyses recently conducted in the general population for menarche- and menopause-related phenotypes are publicly available. A summary table describing these GWAS is available in the Appendix. To construct PRS, we will extract the following summary statistic information for each of the selected GWAS:

- SNP/variant identifiers (e.g., chromosome, base pair position, human genome assembly/build)
- Effect allele (allele corresponding to the direction of effect)
- Reference allele (non-effect allele)
- Effect allele frequency
- Regression coefficient (SNP effect size)
- Standard error (of the regression coefficient)
- Sample size
- P-value

Any GWAS/meta-analysis conducted without appropriate standard sample/variant quality control procedures or incomplete summary statistic information (e.g., unspecified reference/effect alleles) will be excluded. Below are inclusion criteria for the reference GWAS that will be used to inform PRS:

- GWAS conducted in general population sample(s) of predominantly European ancestry
- Sample size ≥10,000
- Phenotype definition in GWAS/meta-analyses is relevant for study of menopause-related phenotypes in survivors

  Since the genetic architectures of primary ovarian insufficiency and other reproductive function phenotypes in survivors may substantially overlap, reference GWAS for multiple menarche-/menopause-related phenotypes may be evaluated. In this case, we will consider multi-trait GWAS methods (e.g., GenomicSEM[29], MTAG[30]) to re-estimate effect sizes before constructing the PRS. Phenotypes considered in the reference GWAS include:

- o Primary ovarian insufficiency;
- o Early menopause: Natural cessation of menopause before age 45 years;
- o Age at natural menopause;
- o Age at menarche;
- o Reproductive lifespan: the difference between age at menopause and age at menarche.

Step 2: PRS construction

In this study, we plan to use the phenotype and genomic data from the UK Biobank resource that has been centrally quality-controlled[31] for PRS construction and validation. The UK Biobank Study is a prospective cohort study consisting of approximately 500,000 individuals from across the United Kingdom[32].

Construction: UK Biobank data for participants that do not overlap with selected reference GWAS will be partitioned into training and test datasets. Using self-reported data for age at menarche and menopause in the UK Biobank, we will define individuals who experience menopause naturally before the age of 40 years as POI. The training dataset will be used for PRS construction. We will employ several approaches to construct the PRS. The pruning and thresholding approach will be used as a benchmark; popular Bayesian approaches, such as LDpred[33], PRS-CS[34], and penalized regression-based methods like lassosum[35] will also be considered. The UK Biobank test dataset will be used for internal PRS validation before evaluation in survivor data. We will use a logistic regression model with appropriate model covariates (e.g., age, ancestry) to evaluate the PRS (continuous or quantile) as a predictor of menopause-related phenotypes in the test dataset.

PRS Evaluation: We will evaluate the calibration, discrimination, and prediction ability of the candidate PRS using the calibration curve, AUC, and AP. Finally, we will select the PRS generating the best overall performance for further evaluation in survivors.

Aim 2

Statistical models

Logistic regression for AOF prediction

AOF is defined as the permanent loss of ovarian function within five years of the cancer diagnosis or no menarche after cancer diagnosis by age 18 years. The AOF status for all included survivors has been determined due to the inclusion criteria: survival for five years or more after cancer diagnosis who were at least 18 years old at their most recent follow-up. We will use logistic regression, a popular prediction technique for binary outcome variables.

Weighted logistic regression for NSPM/POI prediction

For NSPM: The NSPM risk at a specific time post cancer treatment is of clinical interest. To determine the NSPM status (Yes/No) at a specific time after cancer treatment, we need to know the menstrual history (ovarian status) and age at menopause. For binary outcomes, we can employ the logistic regression model. However, the outcome status is not always observable due to censoring. For example, assuming we are interested in the NSPM risk after 15 years of cancer treatment, a survivor's NSPM status would be censored if she was in her ninth year after cancer

treatment at her last follow-up and had normal menstrual function. Censoring is a concern in the analysis as the censored individuals can develop the outcomes of interest. To account for the censored observations, we will employ the inverse-probability-of-censoring weighting (IPCW)[36] method. The IPCW weights are obtained by modeling the censoring process using the same set of covariates, such as age at diagnosis and radiation dosage to the ovary, for modeling the NSPM status. Censored individuals will thus contribute to the risk model through the IPCW weights. Individuals with known NSPM status will be given weights in the estimation of the logistic regression model. Therefore, we call this model "weighted logistic regression".

For POI: Similarly, we need to collect the menstrual history (ovarian status) and age at menopause to determine if a survivor developed POI (Yes/No). Censoring is also a concern when modeling the POI risk. For example, a survivor's POI status was censored if she was 27 at her last follow-up and had normal menstrual function. A similar analysis framework used for modeling the NSPM risk at a specific time post the cancer treatment will be used. i.e., the IPCW method will be used to account for censoring, and weighted logistic regression will be used to model the POI risk.

Competing risk
Apart from censoring, the competing risk event of surgical premature menopause (had bilateral oophorectomy) needs to be considered. Female childhood cancer survivors who had bilateral oophorectomy before the age of 40 would not develop menopause naturally (i.e., are no longer at risk of natural menopause). The competing risk is considered in the IPCW method[37], where there is an indicator variable for the event (menopause, menstruation, or surgical premature menopause).

Other modern machine learning methods can be similarly modified to model the risk of NSPM/POI by using weighted observations.

Aim 2a) Clinical predictor models: We have used logistic regression and random forest to estimate the AOF risk, and weighted logistic regression and random forest for NSPM risk prediction using clinical predictors in our previous investigations[14,15]. We are currently using the weighted logistic regression method and XGBoost[38] that include clinical predictors, such as age at diagnosis, type of diagnosis, Cyclophosphamide-equivalent dose[28] and radiation dosage to the ovary, to build models for age-specific POI risk. We intend to use these clinical models for the menopause-related phenotypes that we have built under a previously approved CCSS (separate) project as the benchmark.

Aim 2b) PRS model (constructed from SNP effects identified in the general population): To examine the discrimination ability of the PRS (on its own) for different menopause-related phenotypes, we will build a logistic regression model for AOF risk prediction and separate weighted logistic regression models for estimating the NSPM risk at a specific time post cancer treatment and POI risk. We will use PRS as a continuous or categorical (e.g., PRS quantiles) variable to investigate the association separately. We will compare the performance of the PRS in different menopause-related phenotypes using metrics such as AUC, AP, and scale Brier score.

***Aim 2c) PRS constructed from SNP effects identified in the general population + clinical predictor models*:** Similarly, we will build a logistic regression/weighted logistic regression prediction model that includes both the constructed PRS and clinical predictors, including age at cancer diagnosis, chemotherapy exposure (e.g., Cyclophosphamide, Busulfan etc.), bone marrow transplantation, and radiation dosage to the ovary, abdomen, and pelvis to predict the risk for menopause-related phenotypes. We will deal with censoring and competing risk as in Aim 2a). We will compare the prediction model performance (Aim 2a, 2b, and 2c) by computing the incremental improvements in AUC, AP, and scaled Brier score[39].

Aim 3: *PRS including treatment-specific SNP effects + clinical predictor models*
We will build appropriate logistic regression models that include clinical predictors and PRS which incorporate treatment-specific SNP effects identified in previous GWAS in survivors[19] to predict the risk of menopause-related phenotypes. We will compare the incremental improvement in evaluation metrics as illustrated in Aim 2b) and Aim 2c)[39]

Performance assessment
Model selection for all Aims described above is based on prediction performance, including calibration[40] and discrimination. The AUC[41], the AP[37], and the scaled Brier score[42] will be used to assess prediction ability. A model-based framework, including calibration slope, will be used to evaluate calibration. We will compare the prediction model performance by computing the incremental values in AUC, AP, and scaled Brier scores[39].

Conceptual Figures and Tables

Aim 1: Odds ratio vs PRS quantiles vs PRS quantiles for menopause-related phenotypes

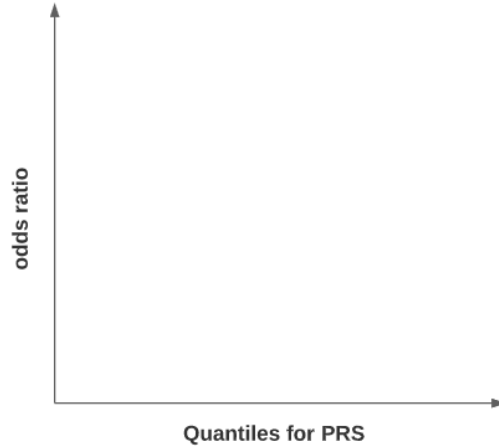   1) Odds ratio vs PRS quantiles, evaluated in **the general population**

Figure 1: Odds ratio and 95% CI for PRS quantiles in the general population test data.

    2) Odds ratio vs. PRS quantiles, evaluated in **the female childhood cancer survivors**
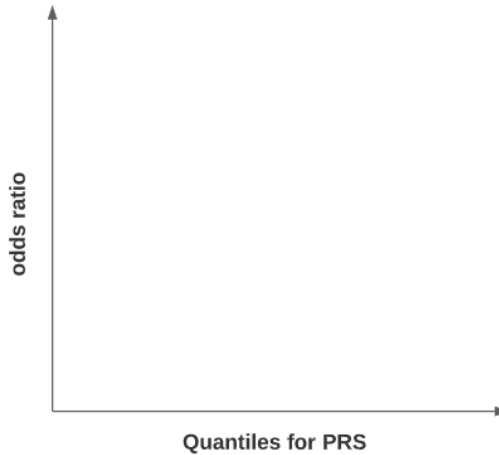

Figure 2: Odds ratio and 95% CI for PRS quantiles among the female childhood cancer survivors.

    3) Summary of estimated performance for PRS constructed from **the general population data**

Table 1[*]: PRS prediction performance in the general population test data for menopause-related phenotypes

Metric	Pruning and thresholding	LDpred	PRS-CS	Lassosum
AUC (95% Confidence interval)				

Metric	Pruning and thresholding	LDpred	PRS-CS	Lassosum
AP (95% Confidence interval)				
Scaled Brier score (95% Confidence interval)				

Aims 2 and 3: Risk prediction performance of selected models for PRS in female survivors in CCSS

Table 2[*]: Risk prediction performance of selected models for PRS in female survivors in CCSS

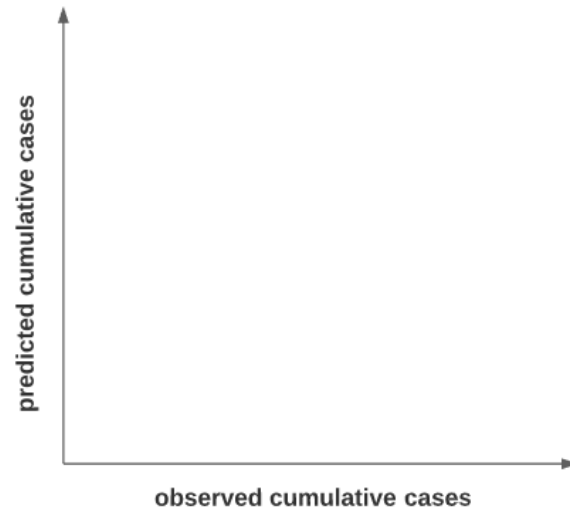|	Aim 2a) Clinical predictor model	Aim 2b) PRS model	Aim 2c) PRS conducted from the general population+ predictor model	Aim 3) PRS including the treatment-specific SNP effects + clinical predictor model
AUC (95% Confidence interval)				
AP (95% Confidence interval)				
Scaled brier score (95% Confidence interval)				

Calibration curves

Figure 3: Calibration curves for the best models in Aims 2, 3

Appendix

Table 1 Summary of reference GWAS studies or meta-analyses for menarche-/menopause related phenotypes conducted in the general population

	Phenotype: Age at menarche	**Phenotype: age at natural menopause**
Study	Elks,et al.,2013 (Nat Genet)	Day, et al., 2015 (Nat Genet)
Meta-analysis component	yes	Yes
Number SNP associations	30 loci	54
Discovery cohort	87,802 Caucasian women	38,968 European women
Replication cohort	14,731 Caucasian women	14,435 European women
Genotyping platform	Affymetrix and Illumina	Illumina iSelect array (iCOGs)
Number of QCed SNPs	~2.5 million	~2.6 million
Whether imputed	yes	Yes
Participant inclusions or exclusions	women of European ancestry with a valid age at menarche between 9 and 17 years were included	Inclusion: women with age at natural menopause 40–60 years. Exclusion: women with menopause induced by hysterectomy, bilateral ovariectomy, radiation or chemotherapy, and those using hormone replacement therapy (HRT) before menopause
Phenotype measurement	Recalled by the participants	Questionnaire: self-report
Phenotype transformation or case definition	Age at the first menstrual period(questionnaire can be found here)	Age at last menstrual period
SNP genetic effect model	additive	Additive
Adjustment covariates	birth year	study, seven principal components

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