Title

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Journal

Cancer Epidemiology Biomarkers & Prevention, 31(9)

ISSN

1055-9965

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Publication Date

2022-09-02

DOI

10.1158/1055-9965.epi-22-0314

Peer reviewed

HHS Public Access

Author manuscript

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2023 March 02.

Published in final edited form as:

Cancer Epidemiol Biomarkers Prev. 2022 September 02; 31(9): 1858–1862.

doi:10.1158/1055-9965.EPI-22-0314.

Genome Wide Association Study of Pregnancy in Childhood Cancer Survivors: A Report from the Childhood Cancer Survivor Study

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Abstract

Background: Gonadotoxic treatment-related infertility has a significant impact on quality of life in childhood cancer survivors. Genome-wide association analyses to delineate the risk of infertility in childhood cancer survivors have not been previously reported.

Methods: Leveraging genotype data from a large survivor cohort, the Childhood Cancer Survivor Study (CCSS), we investigated the role of Single Nucleotide Polymorphisms (SNPs) on future pregnancy or siring a pregnancy in survivors without pelvic, testicular, or brain radiation who had ever been married. We calculated sex-stratified hazard ratios, using Cox proportional hazards modeling, adjusting for birth cohort (before 1965 vs. 1965 or later) and doses of relevant chemotherapies; replication was attempted in the independent St. Jude Lifetime Cohort study (SJLIFE).

Results: In the CCSS cohort, nine SNPs were found to be suggestive (p-value $<10^{-7}$) or statistically significantly (p-value $<5x10^{-8}$) associated with pregnancy, however, none of the SNPs were replicated in SJLIFE. Cohorts differed based on the overall pregnancy rate, frequency of sterilizing procedures and birth cohort.

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Conflict of Interest:
The authors declare no potential conflicts of interest

Conclusions: We were not able to replicate our findings of SNPs associated with pregnancy in childhood cancer survivors.

Impact: Future attempts at replication should be considered in cohorts treated in a comparable era. Additionally, understanding the role of genetics in fertility in childhood cancer survivors may be better approached using more advanced sequencing techniques.

Keywords

GWAS; pregnancy; cancer survivor; survivorship; fertility

Introduction

Infertility concerns have a significant impact on quality of life in survivors of childhood cancer.(1) Emerging technologies and greater awareness are expanding the pool of childhood cancer patients who may be able to preserve fertility despite intensive therapy, though predicting risk of infertility remains challenging.(1) Previous studies have found clear associations between gonadotoxic therapeutic exposures and subsequent risk of infertility.(2) Additionally, methods such as the cyclophosphamide equivalent dose (CED) for quantifying exposure to alkylating agents allow comparison of infertility risk across different treatment regimens, independent of primary cancer type.(3) In the general population, genome wide association studies (GWAS) have explored the individual risk of infertility.(4) However, genome-wide association analysis to delineate the likelihood of future pregnancy in childhood cancer survivors has not been previously attempted.

Materials and Methods

We performed a GWAS utilizing the Childhood Cancer Survivor Study (CCSS), to examine the association between single nucleotide polymorphisms (SNPs) and pregnancy or siring a pregnancy. The CCSS is a multicenter, retrospective cohort of 25,665 five-year survivors of childhood cancer diagnosed between 1970-1999, who are followed prospectively for the development of late-effects.(2) We attempted replication for SNPs with a p-value $<10^{-7}$, utilizing the St. Jude Lifetime Cohort (SJLIFE) that includes individuals treated for childhood cancer at St. Jude Children's Research Hospital who had survived ≥5 years after diagnosis between 1962-2012.(5) Participants were excluded if they received radiation to the pelvis, testes or brain, had a sterilizing surgical procedure prior to 5-year survival, were missing a CED, or were survivors of non-European genetic ancestry (Supplementary Figure 1).(2,3) If patients had a sterilizing procedure after 5-year survival, analysis was censored at the time of the sterilizing procedure.

The CCSS conducted genotyping and imputation on 4.1 million loci (Illumina [San Diego, CA] Infinium Human Omni5 Exome-4 v1.0 array with imputation using the 1000 Genomes Phase 3 data as reference).(6) SNPs passing quality control steps were included for analysis; SNPs were excluded if the minor allele frequency (MAF) was <0.05, were missing from >20% of subjects or showed extreme deviation from Hardy-Weinberg equilibrium (p-value $<10^{-6}$). For imputed SNPs the INFO score needed to be ≥0.5 and the certainty core ≥0.95.

For the SJLIFE cohort, whole-genome sequencing and quality control was performed as previously described.(7)

We created sex-specific multivariable Cox proportional hazard models to relate SNPs with pregnancy using attained age as the time axis, starting the at-risk time from five years post-diagnosis or at age 15 years, whichever was later, and ending at the earliest of pregnancy, death last survey, or age 44. The models included CED (0; >0), birth year (<1965; ≥1965), and the first three principle coordinates of genotypes. Due to the association of marital status on pregnancy we restricted the analysis to married subjects. Power analyses were conducted using the R package (survSNP) for the CCSS cohort.(8)

The data analyzed in this study were obtained from the Childhood Cancer Survivor Study (CCSS). https://ccss.stjude.org/develop-a-study/gwas-data-resource.html. Clinical Data is maintained in a database managed by the CCSS, and GWAS data was downloaded from dbGaP https://dbgap.ncbi.nlm.nih.gov/aa/wga.cgi?page=login. Childhood Cancer Survivor Study (CCSS) dbGaP Study Accession: phs001327.v2.p1 https://www.ncbi.nlm.nih.gov/projects/gap/cgi-bin/study.cgi?study_id=phs001327.v2.p1. SJLIFE data including genotype data are available through: https://www.stjude.cloud/research-domains/cancer-survivorship.

Results

The demographic and clinical characteristics of the two cohorts were generally similar with a few notable exceptions. CCSS participants were more likely to be born prior to 1965, less likely to have a sterilizing surgical procedure subsequent to 5-year survival, and had a higher rate of pregnancy/siring a pregnancy (Table 1). For male CCSS participants, our study had a power of 81% to detect a HR=1.61 for MAF=0.2 at a genome wide significance level of $5x10^{-8}$, assuming an additive model; the power was >90% for MAF >0.3. For female CCSS participants, our study had 81% power to detect a HR=1.52 for MAF=0.2.

In the CCSS cohort, using our GWAS model, one SNP had a p-value of $<5.0x10^{-8}$ and eight SNPs were in the range of $p<10^{-7}$ with hazard ratios ranging from 1.35-1.84 (Table 2). None of the nine SNPs was associated with a statistically significant impact on pregnancy/siring a pregnancy in the replication cohort.

Discussion

We performed a GWAS to determine the association of SNPs with the likelihood of pregnancy or siring a pregnancy in a large cohort of childhood cancer survivors. Although several SNPs were potentially associated with pregnancy in the discovery cohort, none was replicated in an independent cohort. We were unable to examine the risk of clinical infertility or biochemical markers of subfertility. Infertility is multifactorial, and our study may not have been able to incorporate all pertinent factors. Indeed the ability to conceive (if desired by the patient) is the outcome with the greatest clinical relevance; however, data on patient desires or clinical infertility were not available for analysis. Likewise, another limitation of the study was that the questionnaire asked participants if they had ever been married, but did not specify whether the marriage was heterosexual. Although models in both cohorts made adjustments for birth cohort and CED, the two cohorts differed in regards

to birth cohort, sterilizing procedures and overall rate of pregnancy. We did not include factors such as “desire to become pregnant” or use of assisted reproductive technology; these could have differed by birth cohorts. Overall, the SJLIFE cohort was born more recently and may have had greater access to assisted reproductive techniques compared to the CCSS cohort. Together these differences might explain why the findings in the CCSS cohort did not replicate. These limitations notwithstanding, our study demonstrates a need to continue to explore the role of genetic susceptibility in determining the risk of infertility among childhood cancer survivors.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Financial Support:

This work was supported by the National Cancer Institute (CA55727, G.T. Armstrong, Principal Investigator). Support to St. Jude Children’s Research Hospital also provided by the National Cancer Institute (NCI U01 CA195547, M.M. Hudson, Principal Investigator), Cancer Center Support (CORE) grant (CA21765, C. Roberts, Principal Investigator) and the American Lebanese-Syrian Associated Charities (ALSAC). Support to Seth Rotz from the National Center for Advancing Translational Sciences (2KL2TR002547, Raed Dweik, Principal Investigator).

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Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2023 March 02.

Table 1.

GWAS Cohort Stratified by Sex and Pregnancy/ Sired a Pregnancy

	CCSS Cohort						SJLIFE Cohort					
	Female			Male			Female			Male		
Variable	**Never Pregnant (N=192)**	**Any Pregnant (N=719)**	**p-value**	**Never Sired Pregnancy (N=262)**	**Any Sired Pregnancy (N=536)**	**p-value**	**Never Pregnant (N=142)**	**Any Pregnancy (N=347)**	**p-value**	**Never Sired Pregnancy (N=256)**	**Any Sired Pregnancy (N=267)**	**p-value**
Age at primary cancer diagnosis (years), median (min, max)	7.0 (0, 20)	8.0 (0, 20)	0.99 *b*	10 (0, 20)	8.0 (0, 20)	0.50 *b*	9.5 (0.0, 21)	7.5 (0.0, 19.5)	0.11 *b*	8.1 (0, 24)	9.4 (0, 20)	0.14 *b*
Age at last follow-up (years), median (min, max)	38 (21, 60)	40 (19, 62)	**0.039** *b*	42 (22, 62)	42 (22, 64)	0.56 *b*	30.9 (19, 58)	36.8 (19, 64)	**<0.001** *b*	34.8 (20, 61)	38.7 (19, 69)	**<0.001** *b*
Birth year prior to 1965, n (%)	41 (21%)	169 (24%)	0.53 *a*	62 (24%)	122 (23%)	0.78 *a*	5 (3.5%)	17 (4.9%)	0.50 *a*	14 (5.5%)	18 (6.7%)	0.54 *a*
Hispanic, n (%) *	8 (4.2%)	32 (4.7%)	0.80 *a*	6 (2.4%)	19 (3.7%)	0.31 *a*	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Diagnosis, n (%)			0.23 *a*			**<0.001** *a*			0.089 *a*			0.16 *a*
Leukemia	47 (24%)	216 (30%)		47 (18%)	119 (22%)		51 (36%)	114 (33%)		71 (28%)	63 (24%)	
CNS Tumor	21 (11%)	57 (7.9%)		9 (3.4%)	48 (9.0%)		4 (2.8%)	16 (4.6%)		7 (2.7%)	9 (3.4%)	
Hodgkin Disease	27 (14%)	77 (11%)		52 (20%)	52 (9.7%)		24 (17%)	41 (12%)		40 (16%)	43 (16%)	
Non-Hodgkin Lymphoma	14 (7.3%)	45 (6.3%)		27 (10%)	95 (18%)		8 (6%)	25 (7.2%)		25 (10%)	42 (16%)	
Renal Tumors	11 (5.7%)	55 (7.6%)		18 (6.9%)	35 (6.5%)		6 (4.2%)	29 (8.4%)		10 (3.9%)	19 (7.1%)	
Neuroblastoma	26 (14%)	71 (9.9%)		23 (8.8%)	39 (7.3%)		12 (8.5%)	29 (8.4%)		18 (7.0%)	14 (5.2%)	
Soft Tissue Sarcoma	14 (7.3%)	76 (11%)		30 (11%)	66 (12%)		5 (3.5%)	23 (6.6%)		7 (2.7%)	11 (4.1%)	
Bone Tumors	32 (17%)	122 (17%)		56 (21%)	82 (15%)		20 (14%)	26 (7.5%)		39 (15%)	26 (10%)	
Other Tumors	0 (0)	0 (0)		0 (0)	0 (0)		12 (8.5%)	44 (13%)		39 (15%)	40 (15%)	
CED, n (%)			0.31 *b*			**<0.001** *b*			0.90 *b*			**<0.001** *b*
<1 mg/m^2	102 (53%)	413 (57%)		109 (42%)	294 (55%)		77 (54%)	195 (56%)		102 (40%)	135 (51%)	
1-3,999 mg/m^2	19 (9.9%)	80 (11%)		16 (6.1%)	64 (12%)		13 (9.2%)	27 (7.8%)		28 (11%)	36 (14%)	

	CCSS Cohort						SJLIFE Cohort					
	Female			Male			Female			Male		
Variable	**Never Pregnant (N=192)**	**Any Pregnant (N=719)**	**p-value**	**Never Sired Pregnancy (N=262)**	**Any Sired Pregnancy (N=536)**	**p-value**	**Never Pregnant (N=142)**	**Any Pregnancy (N=347)**	**p-value**	**Never Sired Pregnancy (N=256)**	**Any Sired Pregnancy (N=267)**	**p-value**
4,000-7,999 mg/m^2	33 (17%)	79 (11%)		40 (15%)	78 (15%)		30 (21%)	62 (18%)		48 (19%)	52 (20%)	
8,000-11,999 mg/m^2	14 (7.3%)	67 (9.3%)		32 (12%)	51 (9.5%)		20 (14%)	51 (15%)		51 (20%)	30 (11%)	
≥12,0000 mg/m^2	24 (13%)	80 (11%)		65 (25%)	49 (9.1%)		2 (1.4%)	12 (3.5%)		27 (11%)	14 (5.2%)	
Sterilizing procedure, n (%) *	16 (8.4%)	101 (14%)	**0.038** *a*	4 (1.5%)	25 (4.7%)	**0.027** *a*	16 (11%)	94 (27%)	**<0.001** *a*	20 (7.8%)	34 (13%)	0.06 *a*

CED: Cyclophosphamide Equivalent Dose

P-values:

a=Pearson's chi-square test,

b=Wilcoxon Rank Sum test.

*Data not available for all subjects. Missing values in CCSS males: Hispanic = 37; Sterilizing procedure = 6. Missing values in CCSS females: Hispanic = 36; Sterilizing procedure = 5. Missing values in SJLIFE males: sterilizing procedure = 6. Missing values in SJLIFE females: sterilizing procedure = 6

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Table 2.

Suggestive and Significant GWAS SNPs

										CCSS Cohort			SJLIFE Cohort				
#	Pregnancy/ Sired a Pregnancy	rsID	Chr	Position	Risk Allele	Location	Nearest Gene	Nearest Gene Function	Potential Role in Fertility/ Pregnancy	RAF	HR	P-Value	RAF	HR	P-Value	Power (α=0.05)	Power (α=0.05/9)
1	Pregnancy	rs61784815	1	77166140	G	Intergenic	*ST6GALNAC3*	Transfer sialic acids to terminal positions of carbohydrate groups in glycoproteins and glycolipids	Unclear	0.117	1.48	5.3E-07	0.123	1.21	0.111	0.87	0.62
2	Pregnancy	rs6773487	3	93640771	T	Intronic	*PROS1*	Protein S: vitamin K-dependent plasma protein that functions as a cofactor for the anticoagulant protease, activated protein C	Venous thromboembolism risk during pregnancy	0.195	1.38	7.7E-07	0.180	1.09	0.374	0.80	0.51
3	Pregnancy	rs4857343	3	93645047	T	Intronic				0.195	1.38	7.7E-07	0.180	1.09	0.374	0.80	0.51
4	Pregnancy	rs6866644	5	118619993	C	Intronic	*TNFAIP8*	Negative mediator of apoptosis. Suppresses the TNF-mediated apoptosis by inhibiting caspase-8 activity	Upregulated in endometrial tissue	0.163	1.48	2.2E-08	0.155	1.00	0.960	0.91	0.71
5	Pregnancy	rs62375089	5	118633916	A	Intronic				0.165	1.40	6.3E-07	*	*	*	*	*
6	Pregnancy	rs62375091	5	118637291	A	Intronic				0.169	1.40	7.4E-07	0.174	1.02	0.884	0.83	0.56
7	Pregnancy	rs6595183	5	118649282	T	Intronic				0.254	1.35	3.6E-07	0.246	0.98	0.811	0.79	0.50
8	Siring Pregnancy	rs2405853	5	51733627	T	Intergenic	*PELO*	Encodes a protein which contains a conserved nuclear localization signal	Role in spermatogenesis, cell cycle control, and in meiotic cell division	0.061	1.84	3.6E-07	0.075	1.19	0.296	0.94	0.77
9	Siring Pregnancy	rs2405832	5	51737908	T	Intergenic				0.060	1.82	5.6E-07	0.074	1.17	0.350	0.93	0.75

Suggestive (p-value $<10^{-7}$) and Significant (p-value $<5x10^{-8}$) GWAS SNPs from CCSS Cohort with attempted replication in the SJLife Cohort; p-value calculated by multivariable Cox regression adjusting for cyclophosphamide equivalent dose, birth year, and three principle coordinates.

* SNP #5 did not pass quality control in the SJLife Cohort for Analysis;

Note: SNP: Single Nucleotide Polymorphism; rsID: reference SNP identification; Chr: Chromosome Number; GRCh37: Genome Reference Consortium Human Build 37; RAF: Risk Allele Frequency; HR: Hazard Ratio