Interindividual variation in ovarian reserve after gonadotoxic treatment in female childhood cancer survivors – a genome-wide association study: results from PanCareLIFE

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The data of this study are available on request. The use of health-related data for research is constrained by data protection legislation, the common law duty of confidentiality, and in many cases, requirements set by research ethics committees. Every effort is made to perform research on data that are as "de-identified" as possible. However, the present study required a very detailed data set comprising many variables about each person. Although it is unlikely that any of the variables in isolation could be used to identify a given individual, it is possible that a combination of variables could render a person's or persons' record(s) potentially identifiable. Data from the PanCareLIFE cohort are available to members of the consortium within the first 5 years after ending data collection. Data from the St Jude Lifetime Cohort are available on request via St Jude.
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Objective: To discover new variants associated with low ovarian reserve after gonadotoxic treatment among adult female childhood cancer survivors using a genome-wide association study approach.
Design: Genome-wide association study.
Setting: Not applicable.
Patients: A discovery cohort of adult female childhood cancer survivors from the pan-European PanCareLIFE cohort (n = 743; median age: 25.8 years), excluding those who received bilateral ovarian irradiation, bilateral oophorectomy, central nervous system or total body irradiation, or stem cell transplantation. Replication was attempted in the US-based St. Jude Lifetime Cohort (n = 391; median age: 31.3 years).
Exposure: Female childhood cancer survivors are at risk of therapy-related gonadal impairment. Alkylating agents are well-established risk factors, and the interindividual variability in gonadotoxicity may be explained by genetic polymorphisms. Data were collected in real-life conditions, and cyclophosphamide equivalent doses were used to quantify alkylation agent exposure.
Main Outcome Measure: Anti-Müllerian hormone (AMH) levels served as a proxy for ovarian function, and the findings were combined in a meta-analysis.
Results: Three genome-wide significant ($<5.0 \times 10^{-8}$) and 16 genome-wide suggestive ($<5.0 \times 10^{-6}$) loci were associated with log-transformed AMH levels, adjusted for cyclophosphamide equivalent dose of alkylating agents, age at diagnosis, and age at study in the PanCareLIFE cohort. On the basis of the effect allele frequency (EAF) (>0.01 if not genome-wide significant), and biologic relevance, 15 single nucleotide polymorphisms were selected for replication. None of the single nucleotide polymorphisms were statistically significantly associated with AMH levels. A meta-analysis indicated that rs78861946 was associated with borderline genome-wide statistical significance (reference/effect allele: C/T; effect allele frequency: 0.04, beta (SE): −0.484 (0.091).
Conclusion: This study found no genetic variants associated with a lower ovarian reserve after gonadotoxic treatment because the findings of this genome-wide association study were not statistically significant replicated in the replication cohort. Suggestive evidence for the potential importance of 1 variant is briefly discussed, but the lack of statistical significance calls for larger cohort sizes. Because the population of childhood cancer survivors is increasing, large-scale and systematic research is needed to identify genetic variants that could aid predictive risk models of gonadotoxicity as well as fertility preservation options for childhood cancer survivors. (Fertil Steril® 2024;122:514–24. ©2024 by American Society for Reproductive Medicine.)
El resumen está disponible en Español al final del artículo.

Key Words: Ovarian reserve, gonadotoxicity, childhood cancer, survivorship, GWAS

Over the past decades, the 5-year survival rate of pediatric cancer has increased tremendously and has currently reached almost 80% (1–3). Subsequently, the awareness of late effects of cancer treatment, including therapy-related gonadal damage, has increased and evolved into a well-established research field in pediatric cancer (4–8). Female childhood cancer survivors consider infertility to be one of the most concerning late effects (9, 10). Therefore, timely and adequate fertility care to prevent treatment-related gonadal damage is essential to ensure fertility preservation in patients with a high risk of gonadal damage (11). In addition to the well-known risk factors for gonadal damage, including alkylating agents and radiotherapy exposing the ovaries (12–15), an unexplained interindividual variation in treatment-related gonadal damage has been observed, suggesting a role for genetic susceptibility (15–17).

Genome-wide association study (GWAS) analyses in the general population have revealed multiple genetic polymorphisms influencing the age at natural menopause (18). Currently, known genetic variants may explain approximately 50% of the variation in age at natural menopause in the general population (19, 20). A GWAS in childhood cancer survivors identified a haplotype associated with premature menopause (21). Furthermore, a recent GWAS studying the association of genetic polymorphisms with the likelihood of pregnancy in the Childhood Cancer Survivor Study did not replicate findings (22). Because the likelihood of a successful pregnancy is determined by a large number of factors, including desire to have children, male fertility factors, and age, pregnancy likelihood may not be a reliable outcome measure to quantify gonadal damage in childhood cancer survivors.

Anti-Müllerian hormone (AMH) levels, as opposed to age at menopause or pregnancy likelihood, can be used as a proxy for ovarian function and reserve across an age spectrum. In previous candidate gene approach studies, single nucleotide polymorphisms (SNPs) in *BRSK1, CYP3A4,* and *CYP2B6* were shown to influence AMH levels (16, 23). However, a large proportion of the variability remains unexplained. Here, we aimed

to identify novel genetic polymorphisms associated with a lower ovarian reserve after treatment with alkylating agents among adult childhood cancer survivors using a GWAS approach. Specifically, we performed a GWAS in a discovery cohort of 743 adult female childhood cancer survivors in the pan-European PanCareLIFE cohort and aimed to replicate it in the US-based St. Jude Lifetime Cohort (SJLIFE) (n = 391).

MATERIALS AND METHODS

Study participants: inclusion and exclusion criteria

Discovery cohort. The eligible study population of the discovery cohort consisted of adult female 5-year childhood cancer survivors who were diagnosed between 1970 and 2011 and treated with chemotherapy before the age of 25 years. Eligible participants were at least 18 years old at the time of study blood sampling and provided a blood sample to quantify AMH levels and for extraction of deoxyribonucleic acid (DNA). Some treatment modalities, such as radiotherapy to the gonads and high-dose chemotherapy used for stem cell transplantation, are associated with such extensive gonadal damage that little interindividual variability is observed, and the role of genetic susceptibility is considered negligible (12–15, 24). Because we aim to uncover genetic variants that may explain treatment-related interindividual variability, childhood cancer survivors for whom the influence of genetic variability is not expected to have any additional impact on gonadal function were excluded, and this included childhood cancer survivors who received bilateral ovarian (defined as bilateral irradiation of the abdomen below the pelvic/iliac crest), central nervous system, or total body irradiation, and those who underwent bilateral oophorectomy or hematopoietic stem cell transplantation. The same inclusion and exclusion criteria were applied to the replication cohort. The discovery cohort included participants of the pan-European retrospective PanCareLIFE study recruited from 28 institutions in 13 countries. There were no postbaseline exclusions. Demographic, disease, and treatment data were abstracted from medical records (14, 25, 26). Approval was obtained from all relevant local review boards in 13 countries, and written informed consent was obtained from all participants. The study was conducted in accordance with the Declaration of Helsinki.

Replication cohort. The replication cohort included female childhood cancer survivors enrolled in the St. Jude Lifetime Cohort (SJLIFE) study, which included childhood cancer survivors treated at St. Jude Children's Research Hospital (Memphis) between 1962 and 2012. SJLIFE participants included in this analysis were female childhood cancer survivors $\geq$10 years after cancer diagnosis and $\geq$18 years of age at clinical assessment, which included a comprehensive endocrinological evaluation (27). The SJLIFE protocol is approved by the SJCRH Institutional Review Board. Further details of the study protocol and both cohorts have been published previously (16, 25, 28, 29).

Outcome and genotype data from biological samples

The outcome of this study was serum level of AMH reflecting ovarian function or reserve, as previously described (16, 23, 25, 28). Blood samples were used to determine AMH levels using an automated, ultrasensitive Elecsys AMH assay (Roche Diagnostics GmbH). All samples from the PanCareLIFE and SJLIFE cohorts were processed in the same laboratory in the Netherlands to avoid interlaboratory variability. Genomic DNA was extracted from peripheral blood or saliva, and SNPs were genotyped using the Illumina Infinium Global Screening Array (Illumina). Standard quality control procedures, including testing deviation from Hardy-Weinberg equilibrium, were performed using PLINK, followed by imputations using the Michigan Imputation Server using default settings, with the Haplotype Reference Consortium (r1.1) as a reference panel (30). Quality control was performed, and details can be found in Supplemental Text 1 (available online) (28).

Alkylating agents

The cumulative cyclophosphamide equivalent dose (CED) of alkylating agents was calculated for all participants (31). To limit the effect of outliers, the CED scores exceeding 30,000 mg/m^2 were recoded to 30,000 mg/m^2. In the sensitivity analyses, the CED score was divided into 4 categories to distinguish the effects of no, little, medium, and high doses of alkylating agents (0; >0–4,000 mg/m^2; $\geq$4,000–8,000 mg/m^2; $\geq$8,000 mg/m^2) (16, 23, 28, 32, 33).

Statistical analysis for GWAS discovery and replication

A GWAS analysis was performed using linear mixed models of log-transformed AMH levels, adjusting for age at the time of the serum sample (because AMH values are age-dependent), age at diagnosis, the continuous CED score, and study-specific covariates (e.g., principal components, familial relationships, and study center) using rvtest (34). Further details on the choice of model can be found in Supplemental Text 2. Because of our small sample size, for GWAS standards, we analyzed variants with a minor allele frequency of $\geq$0.01 in our GWAS analysis. We judged the number of principal components sufficient when the addition of another principal component did not result in a significant difference in the association of the genetic data with AMH levels. We included the first 4 significant principal components.

P values of $<5.0 \times 10^{-6}$ were considered suggestive, and those $<5.0 \times 10^{-8}$ were considered statistically significant at the genome-wide level. Single nucleotide polymorphisms that were statistically significant in the discovery cohort were selected for replication analysis with the SJLIFE data. In addition, suggestive SNPs with biologic plausibility were assessed for replication. The replication analysis used the same model with the same adjustment variables as the discovery analysis.

A lack of statistically significant replication could occur in our study because of the use of small cohorts (i.e., limited power), especially in the SJLIFE cohort for replication analysis, which is not an issue in most GWAS analyses of general populations with a much larger sample size. Therefore, we performed a meta-analysis as suggestive evidence for potential importance. An inverse-variance weighted meta-analysis using a fixed effects model was performed using the data from the discovery and replication cohorts using R version 3.5.1,

package rmeta (35). The meta-analysis was performed using the results of PanCareLIFE and SJLIFE for each SNP, adjusting for the same variables as the original GWAS analyses: age at the time of the serum sample, age at diagnosis, the continuous CED score, and study-specific covariates. P values $<5.0 \times 10^{-8}$ were considered statistically significant at the genome-wide level. The size and direction of the SNP effects in the 2 studies were taken into account. Subsequently, we performed sensitivity analyses to evaluate subgroups and grouped CED scores instead of continuous CED scores on the effect of SNPs and a logistic regression comparing patients within their age group with AMH level in the upper tertile vs. the lower tertile. Descriptive statistics and sensitivity analyses were conducted using the Statistical Package for Social Sciences (version 26.0.0.1). Functional SNP annotations were applied using the Functional Mapping and Annotation web application as well as Locuszoom (36, 37).

Institutional review board statement

The study was conducted according to the guidelines of the Declaration of Helsinki and approved by all relevant local institutional review boards (or ethics committees) in 13 countries, and written informed consent was obtained from all participants. Ethics Commission of the State Medical Association for Rhineland-Palatinate Reference: 837.437.14 (9676); Liguria Region Ethical committee, Reference: 507REG2014; Ethical Board of the University Hospital Brno Fakultní Nemocnice Brno; Etická komise pro multicentrická klinická hodnocení fakultní nemocnice v Motole (Ethics Committee for Multi-Centric Clinical Trials of the University Hospital Motol) Reference: EK-1447/14; Comite de protection des personnes sud-est i Reference: CPP 2015-23; Medisch Ethische Toetsingscommissie van de Stichting Nederlands Kankerinstituut–Het Antoni van Leeuwenhoek Ziekenhuis (METC AVL) METC15.1591./AVL; Regionale Komité for Medisinsk og Helsefaglig Forskningsetikk (REK sør-øst) (Ethical Committee Heath Region South-East) Reference: 2010/250; Medisch Ethische Toetsingscommissie VU medisch centrum Reference: 2006.249; Ethik-Kommission der ärztekammer Westfalen-Lippe und der Westfälischen Wilhelms-Universität Münster Reference: 2015-511-f-S; 2015-512-f-S; Medisch Ethische Toetsings Commissie Erasmus MC (METC) Reference : 05/047.

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

RESULTS

In total, 743 childhood cancer survivors from the PanCareLIFE cohort were included in the discovery cohort (Table 1). The alkylating agents received most frequently by childhood cancer survivors included cyclophosphamide, ifosfamide, and procarbazine (Table 1 and Supplemental Table 1, available online). The GWAS identified 3 genome-wide significant and 16 genome-wide suggestive loci associated with log-transformed AMH levels adjusted for CED score, age at diagnosis, and age at study in the discovery cohort (Fig. 1 and Supplemental Table 2). Notably, despite our minor allele frequency cut-off of >0.01, in the discovery cohort, the effect allele frequency (EAF) was low (<0.01) in some of the suggestive SNPs and one of the genome-wide significant hits. Fifteen SNPs, 3 genome-wide significant and 12 suggestive, were selected for replication on the basis of EAF (of $\geq$0.01 when not genome-wide significant), P value ($<5.0 \times 10^{-6}$), and biological plausibility (Tables 2 and 3 and Supplemental Tables 3 and 4).

Replication was performed in the independent US-based SJLIFE cohort (n = 391) (Table 1 and Supplemental Table 1). None of the SNPs was replicated in the SJLIFE cohort using the preestablished criteria for statistical significance, although the directions and effect sizes remained consistent for most of the SNPs (Table 2 and Supplemental Table 5).

A meta-analysis (Supplemental Table 6) indicated an association only at the borderline genome-wide statistical significance of 1 SNP located on chromosome 1, position 163167852 (GRCh37) (rs78861946; reference/effect allele: C/T) with lower log-transformed AMH levels (Beta (SE): −0.484 (0.091) per (T) allele, P value = 9.39×10^{-8}) (Supplemental Table 6). Sensitivity analyses (Supplemental Tables 7–18), including linear, logistic, and interaction models with CED in categories, showed associations with similar effect sizes and directions but without reaching convincing statistical significance.

A stratified analysis of the subcohort of childhood cancer survivors from the PanCareLIFE discovery cohort treated with a CED >8,000 mg/m^2 (n = 176) showed that the SNP rs78861946 was associated with a full-point decrease in AMH levels (AMH beta −1.061, P value .031) (Supplemental Table 18). A consistent decrease was observed in the replication cohort of SJLIFE but without statistical significance because of the small sample size of only 3 individuals with the genetic variant out of the subcohort of 93 childhood cancer survivors treated with a CED >8,000 mg/m^2 (AMH beta −1.217, P value .24) (Supplemental Table 18).

DISCUSSION

This is the first GWAS on ovarian reserve after gonadotoxic treatment in childhood cancer survivors, performed in a pan-European cohort of female childhood cancer survivors and an independent international replication cohort. This study found no genetic variants associated with lower ovarian reserve, as the findings of this GWAS were not statistically significant replicated in the replication cohort.

Because a lack of statistically significant replication could occur in our study due to the use of cohorts relatively small for GWAS (i.e., limited power), we performed a meta-analysis as suggestive evidence for potential importance. The meta-analysis of the 2 independent cohorts indicates a variant of interest (rs78861946), at borderline genome-wide statistical significance, associated with lower AMH levels in female survivors of pediatric cancer, potentially affecting susceptibility to alkylating agent-induced gonadal damage. Stratified analysis indicated that this variant may result in a decrease in AMH levels of 1–2 μg/L (depending on 1 or 2

TABLE 1

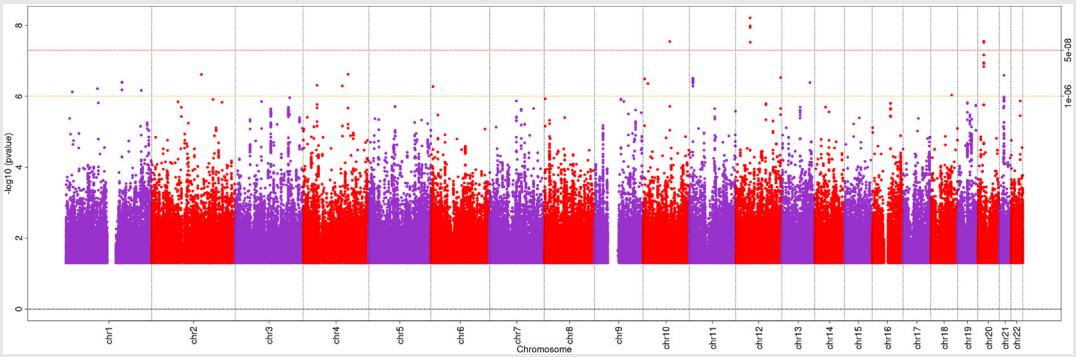
Characteristics of participating CCS of the discovery PanCareLIFE cohort and CCS of the replication St. Jude LIFE cohort (SJLIFE).

Characteristics	Discovery PanCareLIFE cohort (n = 743) (n (%))[a]	Replication SJLIFE (n = 391) (n (%))[a]
Age at time of study (y) (median (IQR))	25.8 y (22.1–30.6)	31.3 y (26.3–37.4)
Age at diagnosis (y) (median (IQR))	8.3 y (3.3–14.0)	6.9 y (3.1–13.4)
Time since diagnosis (y) (median (IQR))	18.3 y (13.2–22.9)	23.7 y (18.3–29.3)
Diagnosis		
Leukemia	221 (29.7)	121 (30.9)
Hodgkin lymphoma	136 (18.3)	48 (12.3)
Non-Hodgkin lymphoma	70 (9.4)	22 (5.6)
Brain tumor	17 (2.3)	28 (7.2)
Neuroblastoma	46 (6.2)	36 (9.2)
Renal tumor	72 (9.7)	27 (6.9)
Carcinoma (hepatic, thyroid, colon liver, and other)	7 (0.9)	9 (2.3)
Osteosarcoma	33 (4.4)	22 (5.6)
Ewing sarcoma	31 (4.2)	12 (3.1)
Soft tissue sarcoma	49 (6.6)	28 (7.2)
Germ cell tumor	34 (4.6)	13 (3.3)
Skin cancer (including melanoma)	3 (0.4)	1 (0.3)
Retinoblastoma	5 (0.7)	20 (5.1)
Other	12 (1.6)	3 (0.8)
Nonmalignant	0	1 (0.3)
Radiotherapy		
No	479 (64.5)	269 (68.8)
Yes[b]	264 (35.5)	122 (31.2)
Thorax	110 (14.8)	67 (17.1)
Spine	5 (0.7)	0 (0.0)
Abdomen, not pelvic	15 (2.0)	29 (7.4)
Unilateral pelvis	9 (1.2)	6 (1.5)
Other	78 (10.5)	121 (30.9)
CED score		
Median mg/m^2 (IQR)	3,000 mg/m^2 (0–7,350)	0 mg/m^2 (0–7,731)
0	266 (35.8)	198 (50.6)
>0–4,000	183 (24.6)	19 (4.9)
≥4,000–8,000	118 (15.9)	78 (19.9)
≥8,000	176 (23.7)	93 (23.8)
Exposed with an unknown dose		3 (0.8)
Alkylating agent		
Cyclophosphamide	362 (48.7)	176 (45)
Busulfan[c]	15 (2.0)	2 (0.5)
Carmustine[c]	3 (0.4)	2 (0.5)
Chlorambucil[c]	12 (1.6)	0 (0)
Ifosfamide	139 (18.7)	23 (5.9)
Lomustine[c]	0	3 (0.8)
Mechlorethamine	27 (3.6)	4 (1.0)
Melphalan	28 (3.7)	0 (0)
Procarbazine	70 (9.4)	26 (6.6)
Thiotepa[c]	8 (1)	1 (0.3)
Unilateral surgery of the ovary		
No	740 (99.6)	391 (100.0)
Yes	3 (0.4)	0
Anti-Müllerian hormone level in μg/L		
Median (IQR)	2.33 μg/L (1.02–4.03)	1.84 μg/L (0.68–3.28)
Age category 18–25 (IQR)	2.70 μg/L (1.41–4.39)	2.79 μg/L (1.68–4.14)
Age category ≥25–32 (IQR)	2.62 μg/L (1.37–4.24)	2.55 μg/L (1.44–3.90)
Age category ≥32–40 (IQR)	1.22 μg/L (0.41–2.58)	1.69 μg/L (0.70–2.55)
Age category ≥40 (IQR)	0.27 μg/L (0.13–0.52)	0.09 μg/L (0.01–0.47)

Note: Values represent the number (%) of women unless indicated otherwise. IQR = 25–75 percentile.
CCS = childhood cancer survivors; CED score = cyclophosphamide equivalent dose score; IQR = interquartile range; SJLIFE = St. Jude LIFE cohort.
[a] Numbers are given as n (%) unless specified otherwise.
[b] Not mutually exclusive.
[c] Frequency below 3.5%.

Perk. Gonadotoxicity GWAS in female CCSs. Fertil Steril 2024.

FIGURE 1



Manhattan plot for the PanCareLIFE cohort (n = 743) for logAMH levels in childhood cancer survivors adjusted for cyclophosphamide equivalent dose score (linear), age at diagnosis, age at serum sampling, and study-specific covariates (including 4 principal components). AMH = anti-Müllerian hormone.

Perk. Gonadotoxicity GWAS in female CCSs. Fertil Steril 2024.

affected alleles) in survivors treated with high levels of alkylating agents. Despite this possible clinical significance, larger cohorts are needed to assess this variant because the power in the replication cohort was too low to reach statistical significance for this variant. This variant, rs78861946, is located in a part of noncoding ribonucleic acid in the intronic region of the gene *RGS5* (regulator of g protein signaling 5) and is close to genes *RSG4* (regulator of g protein signaling 4) and *NUF2* (NUF2 component of the NDC80 kinetochore complex). The SNP rs78861946 has not been previously associated with premature ovarian insufficiency, menopause, or infertility. However, noncoding ribonucleic acid may regulate surrounding genes, including transcription of *RGS5*, *RGS4,* and/or *NUF2*. These genes are involved in follicle-stimulating hormone receptor signaling, cell cycle regulation, and apoptosis, pathways known to be involved in natural age at menopause (38–40). The age of onset of natural menopause has previously been associated with genetic variants in genes associated with genome stability and DNA repair, immune function, and mitochondrial biogenesis (19). An expression quantitative trait locus has been reported in the tibial nerve for *RGS4* and, albeit less clearly, also in the prostate and brain (41) (Supplemental Text 3).

We have been able to perform a GWAS in one of the largest cohorts of childhood cancer survivors, in which AMH levels as a proxy for ovarian reserve were available. Although AMH is currently the best proxy to measure gonadal function and remaining ovarian reserve, it should be noted that in addition to ovarian reserve, fertility is influenced by numerous factors, including but not limited to female and male fertility potential, desire to have children, and availability of a partner. Additionally, variability in AMH levels occurs, e.g., during the menstrual cycle (42). We acknowledge that excluding patients receiving radiotherapy to the gonads and stem cell transplantation may have led to some selection bias or even collider bias, but we do not think this bias will be (clinically) relevant because additional genetic factors influencing gonadal damage are negligible for these patients. For this study, we cannot rule out a form of selection bias as a result of nonresponse and consequent absence of informed consent in a specific group of survivors, but minor allele frequencies were consistent with known minor allele frequencies across populations. We expect a substantial number of variants with small effects on AMH levels could be identified when larger cohorts of childhood cancer survivors can be included. Although the sample size of both the discovery and replication cohorts is large for childhood cancer survivor cohorts, it is very small compared with GWASs in the general population. The limited power is likely one of the reasons the identified SNPs were not statistically significant in our replication cohort, SJLIFE, and why only rs78861946 reached borderline genome-wide significance in the meta-analysis.

CONCLUSION

In conclusion, we performed the first GWAS on female childhood cancer survivor patients in the PanCareLIFE cohort with an independent international replication cohort (the St. Jude Lifetime Cohort). We found no genetic variants associated with ovarian reserve after gonadotoxic treatment because the findings of this GWAS were not statistically significant replicated in the replication cohort.

A meta-analysis of the 2 cohorts suggests involvement of a new variant, rs78861946, in proximity to genes associated with follicle-stimulating hormone-receptor signaling regulation, follicular atresia, and the ovulation process. Suggestive evidence for the potential importance of this variant is briefly discussed, but the lack of statistical significance on a genome-wide level calls for larger cohort sizes. Identification of risk-contributing variants may be used in future polygenic risk scores for individualized counseling regarding the treatment-related risk of gonadotoxicity and fertility preservation options for the growing population of childhood cancer survivors.

TABLE 2

Associations of log-AMH values with SNPs identified in the discovery analysis with the results of the replication analysis.

							Discovery PanCareLIFE			Replication SJLIFE		
SNP	CHR	Position (GRCh37)	Reference allele	Effect allele	EAF PanCareLIFE	EAF SJLIFE	Beta	SE	*P* value	Beta	SE	*P* value
rs78861946	1	163167852	C	T	0.03777	0.038	−0.503	0.0993	4.01×10^{-07}	−0.387	0.222	.082
rs141161580	1	91893136	C	T	0.02187	0.015	−0.756	0.152	6.07×10^{-07}	0.666	0.371	.073
rs115555674	1	219535948	G	A	0.0169	0.013	−0.642	0.129	6.80×10^{-07}	−0.384	0.393	.33
rs35180975	4	40397018	G	A	0.1998	0.165	−0.224	0.0446	4.88×10^{-07}	−0.116	0.117	.322
rs75994832	4	113869892	G	A	0.01789	0.023	−0.494	0.0984	5.11×10^{-07}	−0.21	0.295	.477
rs116855361	10	5112398	A	G	0.01889	0.027	−0.575	0.113	3.22×10^{-07}	0.025	0.276	.929
rs116926206	10	14829454	G	A	0.02584	0.024	−0.541	0.107	4.38×10^{-07}	−0.173	0.289	.549
rs11001828	10	78342816	G	A	0.0159	0.013	−0.985	0.178	**2.86×10^{-08}**	−0.271	0.393	.491
rs2923109	11	10378309	A	G	0.3042	0.308	0.178	0.0348	3.13×10^{-07}	−0.1	0.099	.311
rs549344146	12	41988673	C	T	0.00497	0.006	−0.896	0.154	**6.15×10^{-09}**	−0.241	0.553	.664
rs73150874	12	130873577	G	A	0.01392	0.004	−0.816	0.159	2.94×10^{-07}	0.701	0.713	.326
rs9557491	13	101397599	C	T	0.06262	0.051	−0.503	0.0993	4.10×10^{-07}	−0.387	0.222	.645
rs71353373	18	60623035	C	T	0.01193	0.013	−0.746	0.152	9.29×10^{-07}	−0.05	0.393	.899
rs6034839	20	17487409	G	A	0.1193	0.105	−0.264	0.0475	**2.81×10^{-08}**	0.222	0.144	.125
rs111650415	21	28073771	G	A	0.1173	0.147	−0.255	0.0495	2.55×10^{-07}	−0.102	0.12	.397

Note: P values of $<5.0 \times 10^{-6}$ were considered suggestive, and those $<5.0 \times 10^{-8}$ were considered statistically significant at the genome-wide level (in bold). CHR = chromosome; EAF = effect allele frequency; GRCh37 = Genome Reference Consortium Human Build 37; SE = standard error; SJLIFE = St. Jude Lifetime Cohort; SNP = single nucleotide polymorphism.

Perk. Gonadotoxicity GWAS in female CCSs. Fertil Steril 2024.

TABLE 3

Fifteen SNPs identified by GWAS in the discovery analysis were selected for replication on the basis of biologic plausibility.

rsID	Genes	Function and biologic relevance
rs78861946	*RGS4; RGS5; NUF2*	Cell division, apoptosis, drug interaction
rs141161580	*CDC7; HFM1*	Cell division, meiosis 1, premature ovarian failure
rs115555674	*IARS2; RAB3GAP2; LYPLAL1; ZC3H11B*	Hormones, sex hormone-binding globulin
rs35180975	*CHRNA9; RBM47; RHOH; N4BP2*	Cell growth, maturation, survival, DNA repair
rs75994832	*C4orf21; ANK2*	DNA repair, carmustine interaction
rs116855361	*AKR1C3; AKR1C1; AKR1C2; AKR1E2; AKR1C8P; AKR1C4*	CYP450, sex hormone-binding globulin, hormone biosynthesis/metabolism, cell growth/differentiation
rs116926206	*FAM107B; CDNF; HSPA14; SUV39H2; DCLRE1C; MEIG1; OLAH*	DNA repair, apoptosis, cell cycle, HSP
rs11001828	*LRMDA*	Response antineoplastic agents, sex hormone-binding globulin
rs2923109	*ADM; AMPD3; SBF2; MTRNR2L8; LYVE1; IRAG1; RNF141*	Hormones, interaction mustard gas, cyclophosphamide, cell differentiation/growth, detected in follicles
rs549344146	*PDZRN4*	Low EAF. Cell proliferation
rs73150874	*PIWIL1; RIMBP2; FZD10*	Gene specific for tissue of interest, germ cell proliferation, meiosis, apoptosis, impacts reproductive function, follicle maturation
rs9557491	*GGACT; PCCA; TMTC4*	Enzyme metabolism
rs71353373	*PHLPP1; BCL2*	Apoptosis, ovarian follicle development, interaction mustard gas, carmustine, cyclophosphamide, ifosfamide, thiotepa
rs6034839	*BFSP1; PCSK2; DSTN; RRBP1; BANF2*	Menarche, androgen receptor signaling pathway, sex hormone-binding globulin, AMH, ER stress
rs111650415	*CYYR1; ADAMTS1; ADAMTS5*	Fertility, follicular rupture, premature ovarian failure

AMH = anti-Müllerian hormone; EAF = effect allele frequency; ER stress = endoplasmic reticulum stress; GWAS, genome-wide association study; HSP = heat shock protein; SNP = single nucleotide polymorphism.

Perk. Gonadotoxicity GWAS in female CCSs. Fertil Steril 2024.

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CRediT Authorship Contribution Statement

M.E.M.v.d.P., A.-L.L.F.v.d.K., L.B., and M.M.v.d.H.-E. designed the study and wrote the manuscript. M.M.v.d.H.-E. and M.E.M.v.d.P. had full access to all the data in the study and take responsibility for the integrity of the data and the accuracy of the data analysis. M.E.M.v.d.P, A.-L.L.F.v.d.K., L.B., J.L.B., F.W., and Y.Y. performed the analyses. F.E.v.L., Y.Y., J.L.B., F.W., J.S.E.L., G.J.L.K., A.O., C.C.M.B., C.B., C.B.L., S.D.F., J.B., J.J.L., K.K.N., M.M.H., O.Z., J.F.W., L.L.R., and A.M.E.B. made suggestions to improve the analyses and contributed to writing the manuscript. L.B., J.S.E.L., and A.G.U. provided their genetic expertise and contributed to writing the manuscript. All other co-authors were involved in the conception and/or data collection of VEVO, PanCare-LIFE, or the St. Jude Lifetime Cohort. All co-authors reviewed the final article for intellectual content. In all, this document represents a fully collaborative work.

Declaration of Interests

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REFERENCES

1. Ward E, DeSantis C, Robbins A, Kohler B, Jemal A. Childhood and adolescent cancer statistics, 2014. CA Cancer J Clin 2014;64:83–103.
2. Botta L, Gatta G, Capocaccia R, Stiller C, Canete A, Dal Maso L, et al. Long-term survival and cure fraction estimates for childhood cancer in Europe

(EUROCARE-6): results from a population-based study. Lancet Oncol 2022; 23:1525–36.
3. Ellison LF, Xie L, Sung L. Trends in paediatric cancer survival in Canada, 1992 to 2017. Health Rep 2021;32:3–15.
4. Bhakta N, Liu Q, Ness KK, Baassiri M, Eissa H, Yeo F, et al. The cumulative burden of surviving childhood cancer: an initial report from the St Jude lifetime cohort study (SJLIFE). Lancet 2017;390:2569–82.
5. Geenen MM, Cardous-Ubbink MC, Kremer LCM, van den Bos C, van der Pal HJH, Heinen RC, et al. Medical assessment of adverse health outcomes in long-term survivors of childhood cancer. J Am Med Assoc 2007;297:2705–15.
6. Mostoufi-Moab S, Seidel K, Leisenring WM, Armstrong GT, Oeffinger KC, Stovall M, et al. Endocrine abnormalities in aging survivors of childhood cancer: a report from the childhood cancer survivor study. J Clin Oncol 2016;34:3240–7.
7. Oeffinger KC, Mertens AC, Sklar CA, Kawashima T, Hudson MM, Meadows AT, et al. Chronic health conditions in adult survivors of childhood cancer. N Engl J Med 2006;355:1572–82.
8. Overbeek A, van den Berg MH, Kremer LCM, van den Heuvel-Eibrink MM, Tissing WJE, Loonen JJ, et al. A nationwide study on reproductive function, ovarian reserve, and risk of premature menopause in female survivors of childhood cancer: design and methodological challenges. BMC Cancer 2012;12:363.
9. Lee SJ, Schover LR, Partridge AH, Patrizio P, Wallace WH, Hagerty K, et al. American Society of Clinical Oncology recommendations on fertility preservation in cancer patients. J Clin Oncol 2006;24:2917–31.
10. Skinner R, Wallace WH, Levitt GA, UK Children's Cancer Study Group Late Effects Group. Long-term follow-up of people who have survived cancer during childhood. Lancet Oncol 2006;7:489–98.
11. van der Perk MEM, van der Kooi ALF, van de Wetering MD, IM IJ, van Dulmen-den Broeder E, Broer SL, et al. Oncofertility care for newly diagnosed girls with cancer in a national pediatric oncology setting, the first full year experience from the Princess Maxima Center, the PEARL study. PLOS ONE 2021;16:e0246344.
12. Lie Fong S, Laven JS, Hakvoort-Cammel FG, Schipper I, Visser JA, Themmen AP, et al. Assessment of ovarian reserve in adult childhood cancer survivors using anti-Mullerian hormone. Hum Reprod 2009;24:982–90.
13. van Beek RD, van den Heuvel-Eibrink MM, Laven JSE, de Jong FH, Themmen AP, Hakvoort-Cammel FG, et al. Anti-Mullerian hormone is a sensitive serum marker for gonadal function in women treated for Hodgkin's lymphoma during childhood. J Clin Endocrinol Metab 2007;92:3869–74.
14. van den Berg M, van Dijk M, Byrne J, Campbell H, Berger C, Borgmann-Staudt A, et al. Fertility among female survivors of childhood, adolescent, and young adult cancer: protocol for Two Pan-European Studies (PanCareLIFE). JMIR Res Protoc 2018;7:e10824.
15. van Dorp W, van den Heuvel-Eibrink MM, Stolk L, Pieters R, Uitterlinden AG, Visser JA, et al. Genetic variation may modify ovarian reserve in female childhood cancer survivors. Hum Reprod 2013;28:1069–76.
16. van der Kooi ALF, van Dijk M, Broer L, van den Berg MH, Laven JSE, van Leeuwen FE, et al. Possible modification of BRSK1 on the risk of alkylating chemotherapy-related reduced ovarian function. Hum Reprod 2021;36:1120–33.
17. van Santen HM, van de Wetering MD, Bos AME, Vd Heuvel-Eibrink MM, van der Pal HJ, Wallace WH. Reproductive complications in childhood cancer survivors. Pediatr Clin North Am 2020;67:1187–202.
18. Ruth KS, Day FR, Hussain J, Martinez-Marchal A, Aiken CE, Azad A, et al. Genetic insights into biological mechanisms governing human ovarian ageing. Nature 2021;596:393–7.
19. Laven JSE, Visser JA, Uitterlinden AG, Vermeij WP, Hoeijmakers JHJ. Menopause: genome stability as new paradigm. Maturitas 2016;92:15–23.
20. Perry JRB, Hsu YH, Chasman DI, Johnson AD, Elks C, Albrecht E, et al. DNA mismatch repair gene MSH6 implicated in determining age at natural menopause. Hum Mol Genet 2014;23:2490–7.
21. Brooke RJ, Im C, Wilson CL, Krasin MJ, Liu Q, Li Z, et al. A high-risk haplotype for premature menopause in childhood cancer survivors exposed to gonadotoxic therapy. J Natl Cancer Inst 2018;110:895–904.
22. Rotz SJ, Worley S, Hu B, Bazeley P, Baedke JL, Hudson MM, et al. Genome-wide association study of pregnancy in childhood cancer survivors: a report from the childhood cancer survivor study. Cancer Epidemiol Biomarkers Prev 2022;31:1858–62.
23. van der Perk MEM, Broer L, Yasui Y, Robison LL, Hudson MM, Laven JSE, et al. Effect of genetic variation in cyp450 on gonadal impairment in a European cohort of female childhood cancer survivors, based on a candidate gene approach: results from the PanCareLIFE Study. Cancers (Basel) 2021;13: 4598.
24. Green DM, Sklar CA, Boice JD Jr, Mulvihill JJ, Whitton JA, Stovall M, et al. Ovarian failure and reproductive outcomes after childhood cancer treatment: results from the childhood cancer survivor study. J Clin Oncol 2009;27:2374–81.
25. Byrne J, Grabow D, Campbell H, O'Brien K, Bielack S, Am Zehnhoff-Dinnesen A, et al. PanCareLIFE: the scientific basis for a European project to improve long-term care regarding fertility, ototoxicity and health-related quality of life after cancer occurring among children and adolescents. Eur J Cancer 2018;103:227–37.
26. Kaatsch P, Byrne J, Grabow D, PanCare LC. Managing a Pan-European Consortium on late effects among long-term survivors of childhood and adolescent cancer-the pancarelife project. Int J Environ Res Public Health 2021;18:3918.
27. Howell CR, Bjornard KL, Ness KK, Alberts N, Armstrong GT, Bhakta N, et al. Cohort profile: the St. Jude Lifetime Cohort Study (SJLIFE) for paediatric cancer survivors. Int J Epidemiol 2021;50:39–49.
28. van der Kooi ALF, Clemens E, Broer L, Zolk O, Byrne J, Campbell H, et al. Genetic variation in gonadal impairment in female survivors of childhood cancer: a PanCareLIFE study protocol. BMC Cancer 2018;18:930.
29. Hudson MM, Ehrhardt MJ, Bhakta N, Baassiri M, Eissa H, Chemaitilly W, et al. Approach for classification and severity grading of long-term and late-onset health events among childhood cancer survivors in the St. Jude Lifetime Cohort. Cancer Epidemiol Biomarkers Prev 2017;26:666–74.
30. Das S, Forer L, Schonherr S, Sidore C, Locke AE, Kwong A, et al. Next-generation genotype imputation service and methods. Nat Genet 2016;48:1284–7.
31. Green DM, Nolan VG, Goodman PJ, Whitton JA, Srivastava D, Leisenring WM, et al. The cyclophosphamide equivalent dose as an approach for quantifying alkylating agent exposure: a report from the childhood cancer survivor study. Pediatr Blood Cancer 2014;61:53–67.
32. Mulder RL, Font-Gonzalez A, Hudson MM, van Santen HM, Loeffen EAH, Burns KC, et al. Fertility preservation for female patients with childhood, adolescent, and young adult cancer: recommendations from the PanCareLIFE Consortium and the International Late Effects of Childhood Cancer Guideline Harmonization Group. Lancet Oncol 2021;22:e45–56.
33. van den Berg MH, van Dijk M, Byrne J, Berger C, Dirksen U, Winther JF, et al. Treatment-related fertility impairment in long-term female childhood, adolescent and young adult cancer survivors: investigating dose-effect relationships in a European case-control study (PanCareLIFE). Hum Reprod 2021;36:1561–73.
34. Zhan X, Hu Y, Li B, Abecasis GR, Liu DJ. RVTESTS: an efficient and comprehensive tool for rare variant association analysis using sequence data. Bioinformatics 2016;32:1423–6.
35. R: A language and environment for statistical computing. R Foundation for Statistical Computing; 2019. Available at: https://www.R-project.org/. Accessed June 3, 2024.
36. Watanabe K, Taskesen E, van Bochoven A, Posthuma D. Functional mapping and annotation of genetic associations with FUMA. Nat Commun 2017;8:1826.
37. Boughton AP, Welch RP, Flickinger M, VandeHaar P, Taliun D, Abecasis GR, et al. LocusZoom.js: Interactive and embeddable visualization of genetic association study results. Bioinformatics 2021;37:3017–8.
38. Louwers YV, Visser JA. Shared genetics between age at menopause, early menopause, POI and other traits. Front Genet 2021;12:676546.
39. Stolk L, Zhai G, van Meurs JBJ, Verbiest MMPJ, Visser JA, Estrada K, et al. Loci at chromosomes 13, 19 and 20 influence age at natural menopause. Nat Genet 2009;41:645–7.
40. Perry JRB, Corre T, Esko T, Chasman DI, Fischer K, Franceschini N, et al. A genome-wide association study of early menopause and the combined impact of identified variants. Hum Mol Genet 2013;22:1465–72.
41. GTex. GTex portal rs78861946 [19-8-2022]. Available at: https://gtexportal.org/home/snp/chr1_163198062_C_T_b38. Accessed October 10, 2023.
42. Sowers M, McConnell D, Gast K, Zheng H, Nan B, McCarthy JD, et al. Anti-Mullerian hormone and inhibin B variability during normal menstrual cycles. Fertil Steril 2010;94:1482–6.

Variación interindividual en la reserva ovárica tras el tratamiento gonadotóxico en mujeres supervivientes de cáncer infantil: un estudio de asociación de genoma completo: resultados de PanCareLIFE.

Objetivo: Descubrir nuevas variantes asociadas con una baja reserva ovárica tras el tratamiento gonadotóxico entre mujeres adultas supervivientes de cáncer infantil mediante una aproximación de estudio de asociación de genoma completo.

Diseño: Estudio de asociación de genoma completo.

Lugar: No aplicable.

Pacientes(s): Una cohorte de descubrimiento de mujeres adultas supervivientes de cáncer infantil de la cohorte pan-Europea PanCareLIFE (n = 743; mediana de edad: 25.8 años), excluyendo a aquellas que recibieron irradiación ovárica bilateral, ooforectomía bilateral, irradiación del sistema nervioso central o corporal total, o con trasplante de células madre. Se intentó replicar en la cohorte de St. Jude Lifetime, con sede en EE. UU. (n = 391; mediana de edad: 31.3 años).

Exposición: Las mujeres supervivientes de cáncer infantil corren el riesgo de sufrir deterioro gonadal relacionado con la terapia. Los agentes alquilantes son factores de riesgo bien establecidos, y la variabilidad interindividual de la gonadotoxicidad puede explicarse por polimorfismos genéticos. Los datos se recopilaron en condiciones de la vida real y se utilizaron dosis equivalentes de ciclofosfamida para cuantificar la exposición al agente alquilante.

Principal(es) medida(s) de resultado(s): Los niveles de hormona antimülleriana (AMH) sirvieron como indicador de la función ovárica, y los hallazgos se combinaron en un meta-análisis.

Resultado(s): Se asociaron tres loci significativos ($<5.0 \times 10^{-8}$) y 16 loci sugestivos ($<5.0 \times 10^{-6}$) en todo el genoma con los niveles de AMH transformados logarítmicamente, ajustados por la dosis equivalente de ciclofosfamida de agentes alquilantes, la edad en el momento del diagnóstico, y la edad en el momento del estudio en la cohorte PanCareLIFE. Sobre la base del efecto de la frecuencia alélica (EAF) (>0.01 si no es significativo para el genoma completo) y la relevancia biológica, se seleccionaron 15 polimorfismos de un solo nucleótido para su replicación. Ninguno de los polimorfismos de un solo nucleótido se asoció de forma estadísticamente significativa con los niveles de AMH. Un metaanálisis indicó que rs78861946 estaba asociado con una significatividad estadística límite en el genoma completo (alelo de referencia/efecto: C/T; frecuencia del alelo de efecto: 0.04, beta (SE): −0.484 (0.091).

Conclusión(es): Este estudio no encontró variantes genéticas asociadas con una reserva ovárica más baja después del tratamiento gonadotóxico, ya que los hallazgos de este estudio de asociación de genoma completo no fueron reproducidos de forma estadísticamente significativa en la cohorte de replicación. Se discute brevemente la evidencia sugestiva de la potencial importancia de una variante, pero la falta de significación estadística requiere tamaños de cohorte más grandes. Debido a que la población de supervivientes de cáncer infantil está aumentando, se necesita investigación sistemática y a gran escala para identificar variantes genéticas que puedan ayudar a los modelos de riesgo predictivo de gonadotoxicidad, así como a las opciones de preservación de la fertilidad para los supervivientes de cáncer infantil.