Genetic Risk for Subsequent Neoplasms Among Long-Term Survivors of Childhood Cancer

Zhaoming Wang, Carmen L. Wilson, John Easton, Andrew Thrasher, Heather Mulder, Qi Liu, Dale J. Hedges, Shuoguo Wang, Michael C. Rusch, Michael N. Edmonson, Shawn Levy, Jennifer Q. Lancot, Eric Caron, Kyla Shelton, Kelsey Currie, Matthew Lear, Aman Patel, Celeste Rosencrance, Ying Shao, Bhavin Vadodaria, Donald Yergeau, Yadav Sapkota, Russell J. Brooke, Wonjong Moon, Evadnie Rampersaud, Xiaotu Ma, Ti-Cheng Chang, Stephen V. Rice, Cynthia Pepper, Xin Zhou, Xiang Chen, Wenan Chen, Angela Jones, Braden Boone, Matthew J. Ehrhardt, Matthew J. Krasin, Rebecca M. Howell, Nicholas S. Phillips, Courtney Lewis, Deokumar Srivastava, Ching-Hon Pui, Chimene A. Kesserwan, Gang Wu, Kim E. Nichols, James R. Downing, Melissa M. Hudson, Yutaka Yasui, Leslie L. Robison, and Jinghui Zhang

Author affiliations and support information (if applicable) appear at the end of this article.

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Z.W. and C.L.W. contributed equally to this work.

L.L.R. and J.Z. contributed equally as senior investigators.

Corresponding author: Jinghui Zhang, PhD, Department of Computational Biology, St Jude Children's Research Hospital, 262 Danny Thomas Pl, MS 1135, Memphis, TN 38105; e-mail: jinghui.zhang@stjude.org.

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ABSTRACT

Purpose

Childhood cancer survivors are at increased risk of subsequent neoplasms (SNs), but the germline genetic contribution is largely unknown. We assessed the contribution of pathogenic/likely pathogenic (P/LP) mutations in cancer predisposition genes to their SN risk.

Patients and Methods

Whole-genome sequencing (30-fold) was performed on samples from childhood cancer survivors who were ≥ 5 years since initial cancer diagnosis and participants in the St Jude Lifetime Cohort Study, a retrospective hospital-based study with prospective clinical follow-up. Germline mutations in 60 genes known to be associated with autosomal dominant cancer predisposition syndromes with moderate to high penetrance were classified by their pathogenicity according to the American College of Medical Genetics and Genomics guidelines. Relative rates (RRs) and 95% CIs of SN occurrence by mutation status were estimated using multivariable piecewise exponential regression stratified by radiation exposure.

Results

Participants were 3,006 survivors (53% male; median age, 35.8 years [range, 7.1 to 69.8 years]; 56% received radiotherapy), 1,120 SNs were diagnosed among 439 survivors (14.6%), and 175 P/LP mutations were identified in 5.8% (95% CI, 5.0% to 6.7%) of survivors. Mutations were associated with significantly increased rates of breast cancer (RR, 13.9; 95% CI, 6.0 to 32.2) and sarcoma (RR, 10.6; 95% CI, 4.3 to 26.3) among irradiated survivors and with increased rates of developing any SN (RR, 4.7; 95% CI, 2.4 to 9.3), breast cancer (RR, 7.7; 95% CI, 2.4 to 24.4), nonmelanoma skin cancer (RR, 11.0; 95% CI, 2.9 to 41.4), and two or more histologically distinct SNs (RR, 18.6; 95% CI, 3.5 to 99.3) among nonirradiated survivors.

Conclusion

The findings support referral of all survivors for genetic counseling for potential clinical genetic testing, which should be prioritized for nonirradiated survivors with any SN and for those with breast cancer or sarcoma in the field of prior irradiation.

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INTRODUCTION

The global number of survivors of childhood cancer is substantial and increasing according to a US estimate of 500,000 by the year 2020.¹ Childhood cancer survivors are at an increased risk for therapy-related health conditions.² Subsequent neoplasms (SNs) are of particular concern

because of their high incidence and associated morbidity and mortality.^{3,4} Risk of SN does not plateau with time,⁵⁻⁷ development of multiple SNs is common,⁸ and risk and histologic type of SN often are associated with specific therapies.⁹

Cancer predisposition genes are expressed by most cell types and typically are responsible for cellular activities that include DNA repair and cell cycle regulation.¹⁰ Pathogenic/likely pathogenic

ASSOCIATED CONTENT

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(P/LP) germline mutations in cancer predisposition genes have been shown to be present in 8.5% of children with newly diagnosed cancer.^{11,12} We hypothesize that these mutations contribute to SN risk among childhood cancer survivors because of the disruption of normal cellular processes, which may be further exacerbated by cancer treatment. Current data on the associations between cancer predisposition genes and SN risk are limited¹³ because available studies have focused on mutations in a single gene within a specific cancer subtype (eg, *RB1* in genetic susceptibility of subsequent sarcoma¹⁴ and *TP53* in leukemia¹⁵). Recent advances in whole-genome sequencing (WGS) technology have enabled comprehensive interrogation of genetic variations and, thereby, systematic investigation of the potential contribution of cancer predisposition genes to SN risk among survivors. We performed WGS to study the contribution of germline P/LP mutations in cancer predisposition genes to SN risk in 3,006 long-term (≥ 5 years) survivors of childhood cancer who were enrolled in the St Jude Lifetime Cohort Study (SJLIFE),¹⁶ with comprehensive longitudinal clinical follow-up evaluation.

PATIENTS AND METHODS

Participants in the SJLIFE study were eligible for the current analysis. SJLIFE is a retrospective cohort with prospective clinical follow-up and ongoing enrollment of childhood cancer survivors treated at St Jude Children's Research Hospital (SJCRH) beginning in 1962.^{16,17} Eligibility criteria, initially defined as surviving ≥ 10 years since diagnosis and 18 years of age or older, were modified in 2015 to include all SJCRH patients with cancer who survived ≥ 5 years since diagnosis. For the current analysis, survivors consented to an SJLIFE clinical assessment before April 2016 and provided a blood sample. Survivors who underwent allogeneic stem-cell transplantation were excluded. Survivors were followed until December 31, 2016. Vital status was monitored by periodic National Death Index searches. A control group (SJLIFE controls) of 341 individuals without a history of childhood cancer and frequency matched to survivors on the basis of sex, age, and race was included for comparison purposes. The SJLIFE protocol, biospecimen banking, and genomic study were approved by the SJCRH institutional review board. Consent for participants younger than 18 years of age was provided by a parent or legal guardian. All participants age 14 years and older provided written informed consent; participants between 7 and 13 years of age provided verbal assent.

Characterization of SNs

A detailed medical history was obtained from all participants as part of their comprehensive clinical evaluation. Reported SNs were verified pathologically and/or radiologically (97.1%), through clinical notes (2.7%), or from National Death Index reports (0.2%). The location of each SN was reviewed in conjunction with radiotherapy records and categorized as in or out of the radiation field.

Treatment Exposures and Survivor Characteristics

Chemotherapy exposures were abstracted from medical records. Region-specific radiation exposures were determined from radiation oncology treatment records.¹⁸ During SJLIFE clinical evaluations, each participant's family health history was reviewed by a clinician to ascertain medical conditions, including occurrence of cancer in family members, especially first-degree relatives.

DNA Sequencing

DNA was isolated from blood samples (Data Supplement). WGS was performed for 3,006 survivors at the HudsonAlpha Institute for Biotechnology

Genomic Services Laboratory (Huntsville, AL) by using the HiSeq X Ten System (Illumina, San Diego, CA). The average genome-wide coverage per sample was 36.8-fold. Whole-exome sequencing was performed for 2,996 survivors and 341 SJLIFE controls at SJCRH using the Illumina HiSeq 4000 platform. The average coverage per sample on coding exons was 72.9-fold (Data Supplement).

Cancer Predisposition Genes

Genes related to cancer predisposition were selected for mutation analysis (Data Supplement). Two sets of genes were defined a priori: 60 genes (SJCPG₆₀) with well-established associations with monogenic cancer risk inherited in an autosomal dominant fashion at moderate to high penetrance and 96 genes inherited in an autosomal dominant manner but with moderate to low penetrance, genes associated with autosomal recessive or X-linked inheritance, somatic mosaicism, and genes in which common variants increase cancer risk.

Variant Detection and Classification

Germline single-nucleotide variants, small insertions and deletions, and copy number variations (CNVs) were detected from WGS and/or whole-exome sequencing as previously described¹¹ (Data Supplement). Single-nucleotide variants and insertions and deletions were annotated, and pathogenicity was classified on the basis of American College of Medical Genetics and Genomics guidelines.¹⁹ To facilitate the review process, we developed the Pediatric Cancer Variant Pathogenicity Information Exchange (PeCan-PIE) Web portal, which provides interfaces for analysts to collect data systematically and calculate pathogenicity (Data Supplement). Preliminary classifications were reviewed and approved by a multidisciplinary committee. Pathogenicity classification was conducted similarly for all CNVs that passed the quality check and manual inspection. Promoter mutations in *APC*, *PTEN*, and *RB1* also were evaluated. Mutations classified as P/LP were experimentally verified by deep sequencing, with an average coverage of 14,000-fold using the MiSeq platform (Data Supplement).

Statistical Analyses

Primary analyses used the SJCPG₆₀ genes. Analyses included the additional 96 genes associated with increased cancer risk, but because the prevalence of mutations in the 96 genes did not differ between survivors and SJLIFE controls or alter associations between mutation status and SN risk among survivors, the results are not presented.

Cumulative incidence of any SN as well as that of each specific SN (breast, sarcoma, thyroid, meningioma, and nonmelanoma skin cancer [NMSC]) was estimated for mutation carriers and noncarriers separately, stratified by radiation status, starting from study entry (5 years since diagnosis of childhood cancer), and taking into account death as a competing risk. Gray's method²⁰ was used to assess statistical significance of the differences in cumulative incidence by mutation status.

The association between carrying P/LP mutations in the SJCPG₆₀ genes and rate of any SN and each specific SN was assessed among survivors using multivariable piecewise exponential models that modeled SN incidence rates with piecewise constant time effects and enabled statistical inference on adjusted relative rates (RRs) for mutation status (Data Supplement). Follow-up of survivors started at study entry and was censored at their last date of contact (before or on December 31, 2016) or death. Initially, the risk of each SN type of interest was modeled on demographic and clinical variables (including treatment, sex, age at diagnosis of childhood cancer, and ancestry determined by principal components analysis). Mutation status was then added to the baseline models to assess the adjusted genetic effects on SN rates. Because of the well-established risk of SNs among survivors treated with radiation, we also performed analyses stratified by radiation exposure. To account for multiple events (SNs) per participant, models used modifications by generalized estimating equations.²¹ Analyses were performed using SAS 9.4 statistical software (SAS Institute, Cary, NC), and figures were generated using R-3.4.0. All tests were two-sided. Holm's method²² for multiple testing

correction was applied to the statistical hypothesis testing, with $\alpha = 0.05$ in the regression analysis of five SN types (breast cancer, sarcoma, thyroid cancer, meningioma, and NMSC) for overall and radiotherapy-stratified analyses.

RESULTS

Study Participants

In 3,155 eligible survivors, WGS was performed and passed quality check for 3,006 (95.3%; Fig 1). Survivors analyzed by WGS, including 35% with leukemia, 19% with lymphoma, 11% with CNS involvement, and 35% other solid tumors, did not differ significantly from non-sequenced survivors on demographic or childhood cancer diagnoses, except for a few specific treatment exposures; nor did they differ on the proportion with an SN (Table 1). The median ages at diagnosis and at last follow-up among the sequenced survivors were 7.1 and 35.8 years,

respectively. SJLIFE controls ($n = 341$) were a median age of 36.4 years (range, 19.9 to 72.6 years) at follow-up, and 46% were male, 89% were white, and 4% were Hispanic. Ancestry did not differ between survivors and SJLIFE controls (Data Supplement).

SNs

A total of 1,120 SNs were diagnosed after ≥ 5 years since childhood cancer diagnosis among 439 survivors (14.6%), of whom 91 (3.0%) developed two or more histologically distinct SNs. Median time to first SN since diagnosis of a childhood cancer was 25.6 years (interquartile range, 18.6 to 32.7 years), and the median age at the first SN was 35.1 years (interquartile range, 28.9 to 41.9 years). Fifty-six percent of survivors received radiotherapy. The majority (83%) of SNs developed within a radiation field. The most common SNs were NMSC (580 in 159 survivors), meningioma (233 in 102), thyroid cancer (67 in 67), and breast

Fig 1. Flow diagram of recruitment of childhood cancer survivors. QC, quality check; SJLIFE, St Jude Lifetime Cohort Study; WGS, whole-genome sequencing.

Table 1. Characteristics of Participants in the St Jude Lifetime Cohort Study

Characteristic	Eligible Survivors, No. (%)	Sequenced Survivors, No. (%)	Not Sequenced Survivors, No. (%)	P*
Total	3,155	3,006	149	
Sex				
Male	1,659 (53)	1,584 (53)	75 (50)	.57
Female	1,496 (47)	1,422 (47)	74 (50)	
Race†				
White	2,641 (84)	2,512 (83)	129 (87)	.61
Black	468 (14)	450 (15)	18 (12)	
Other	46 (2)	44 (2)	2 (1)	
Ethnicity†				
Hispanic	64 (2)	58 (2)	6 (4)	.08
Non-Hispanic	3,091 (98)	2,948 (98)	143 (96)	
Diagnosis				
Leukemia				.56
Acute lymphoblastic leukemia	1,024 (34)	966 (32)	58 (39)	
Acute myeloid leukemia	84 (3)	80 (3)	4 (3)	
Other leukemia	4 (< 1)	4 (< 1)	—	
CNS tumors				
Astrocytoma or glioma	165 (5)	158 (5)	7 (5)	
Medulloblastoma or PNET	85 (3)	81 (3)	4 (3)	
Ependymoma	39 (1)	36 (1)	3 (2)	
Other	61 (2)	59 (2)	2 (1)	
Lymphoma				
Hodgkin lymphoma	393 (13)	369 (12)	24 (16)	
Non-Hodgkin lymphoma	224 (6)	217 (7)	7 (5)	
Sarcoma				
Ewing sarcoma	96 (3)	90 (3)	6 (4)	
Osteosarcoma	119 (4)	115 (4)	4 (3)	
Rhabdomyosarcoma	109 (4)	103 (3)	6 (4)	
Nonrhabdomyosarcoma	87 (3)	85 (3)	2 (1)	
Embryonal				
Wilms tumor	216 (7)	210 (7)	6 (4)	
Neuroblastoma	142 (5)	140 (5)	2 (1)	
Germ cell tumor	77 (2)	75 (2)	2 (1)	
Other				
Retinoblastoma	103 (3)	99 (3)	4 (3)	
Hepatoblastoma	21 (1)	19 (1)	2 (1)	
Melanoma	19 (1)	18 (1)	1 (1)	
Carcinomas	44 (1)	43 (1)	1 (1)	
Other	43 (1)	39 (1)	4 (3)	
Radiation				
Any	1,767 (56)	1,670 (56)	97 (65)	.02
Cranial	1,008 (32)	952 (32)	56 (38)	.13
Neck	1,407 (44.5)	1,328 (44)	79 (53)	.03
Breast	466 (15)	441 (15)	25 (17)	.48
Abdomen	632 (20)	597 (20)	35 (24)	.28
Pelvis	561 (18)	531 (18)	30 (20)	.44
Chemotherapy				
Alkylating agent	1,836 (58)	1,757 (58)	79 (53)	.19
Anthracyclines	1,825 (58)	1,737 (58)	88 (59)	.76
Epipodophyllotoxins	1,145 (36)	1,078 (36)	67 (45)	.02
Mercaptopurine	1,171 (37)	1,106 (37)	65 (43)	.09
Methotrexate	1,563 (50)	1,481 (49)	82 (55)	.17
Platinum agents	401 (13)	378 (13)	23 (15)	.31
Vinca alkaloids	2,290 (73)	2,171 (72)	119 (80)	.04
Corticosteroids	1,484 (47)	1,401 (47)	83 (56)	.03
Median age at diagnosis, years (range)	7.1 (0.1-24.8)	7.1 (0.1-23.6)	7.5 (0.1-24.8)	
Median age at follow-up, years (range)	35.9 (7.1-69.8)	35.8 (7.1-69.8)	36.8 (9.9-59.1)	
Median length of follow-up, years (range)	28.0 (6.1-54.7)	28.0 (6.1-54.7)	28.0 (8.4-48.4)	

Abbreviation: PNET, primitive neuroectodermal tumor.

* χ^2 test for a comparison between sequenced and nonsequenced survivors.

†Race/ethnicity was based on self-report.

cancer (60 in 53; Data Supplement). Among SJLIFE controls, six individuals developed 15 neoplasms, including 14 NMSCs and one meningioma.

Prevalence of Mutations

A total of 175 P/LP mutations in 32 genes were detected in 175 survivors (prevalence, 5.8%; 95% CI, 5.0% to 6.7%). They

comprised 27 missense mutations, 136 truncation mutations, one in-frame protein insertion, nine CNVs, and three promoter mutations (Data Supplement). The most frequently mutated genes among survivors were *RB1* ($n = 43$), *NF1* ($n = 22$), *BRCA2* ($n = 14$), *BRCA1* ($n = 12$), and *TP53* ($n = 10$; Data Supplement). Eight mutations that affected *BRCA1*, *NF1*, *RB1*, and *TP53* had low variant allele frequency (VAF; range, 0.074 to 0.243); thus, these were considered germline mosaicism (Data Supplement). Two SJLIFE controls (0.6%) had P/LP mutations (Data Supplement). The mutation prevalence was approximately 10-fold higher among survivors than SJLIFE controls (5.8% ν 0.6%; prevalence ratio, 9.9; 95% CI, 2.5 to 39.6; two-sample binomial proportion test $P < .001$).

Cumulative Incidence of SN

Curves for cumulative incidence of any SN, breast cancer, sarcoma, and thyroid cancer among survivors by mutation status are shown in Figure 2. The overall cumulative incidence of developing any SN was similar between survivors with and without P/LP mutation who were treated with radiotherapy ($P = .24$). In contrast, the overall cumulative incidence of developing any SN was significantly higher among mutation carriers who were treated without radiotherapy ($P < .001$; Fig 2A). In subset analyses of major SNs, higher cumulative incidence of breast cancer ($P = .02$) and sarcoma ($P < .001$) was associated with P/LP mutation among irradiated survivors and breast cancer ($P = .001$) among non-irradiated survivors (Figs 2B and C).

RR of SN

Twenty-eight mutation carriers developed 81 SNs, and 411 noncarriers developed 1,039 SNs. In multivariable analyses (Table 2), after adjusting for sex, age at diagnosis of the primary cancer, and treatment, mutation carriers had a significantly higher rate of developing any SN (RR, 1.8; 95% CI, 1.2 to 2.6). The rate of subsequent breast cancer was increased among females who carried a P/LP mutation (RR, 9.4; 95% CI, 4.8 to 18.2) and those treated with radiation to the chest (RR, 7.9; 95% CI, 4.0 to 15.5) or higher cumulative exposure of anthracyclines (third tertile consisting of $> 204 \text{ mg/m}^2$; RR, 2.4; 95% CI, 1.2 to 4.6). The rate of subsequent sarcoma was significantly increased for mutation carriers (RR, 10.9; 95% CI, 4.7 to 25.2) and those treated with higher cumulative exposure of alkylating agents (third tertile consisting of a cyclophosphamide equivalent dose of $> 10,000 \text{ mg/m}^2$; RR, 3.8; 95% CI, 1.4 to 10.8). Among survivors who developed two or more histologically distinct SNs, the rate was significantly increased for mutation carriers (RR, 3.1; 95% CI, 1.5 to 6.3; Data Supplement). No increased rate of thyroid cancer, meningioma, or NMSC was found among mutation carriers by Holm's method of multiple testing correction.

The analysis also was stratified by radiation exposure (Fig 3). Among irradiated survivors, the rates of breast cancer (RR, 13.9; 95% CI, 6.0 to 32.2) and sarcoma (RR, 10.6; 95% CI, 4.3 to 26.3) were significantly increased among survivors who carried a P/LP mutation, whereas the rates of thyroid cancer, meningioma, and NMSC were not. Nonirradiated survivors who carried a mutation had significantly increased rates of developing any SN (RR, 4.7; 95% CI, 2.4 to 9.3), breast cancer (RR, 7.7; 95% CI, 2.4 to 24.4),

NMSC (RR, 11.0; 95% CI, 2.9 to 41.4), and two or more histologically distinct SNs (RR, 18.6; 95% CI, 3.5 to 99.3). SNs stratified by location relative to the radiation field also were analyzed. When considering only SN outside the radiation field, a significantly increased rate of developing sarcoma (RR, 18.8; 95% CI, 6.0 to 59.1) was found for mutation carriers; otherwise, the set of association results was similar to those for nonirradiated survivors (Data Supplement).

With recognition that retinoblastoma survivors have a well-established genetic predisposition to sarcoma as a result of the presence of germline *RB1* mutations, sensitivity analyses were performed that excluded retinoblastoma survivors. The findings demonstrated that statistically significant associations between mutation status and SN risk remained (Data Supplement).

Genetic Heterogeneity in SN Risk

Substantial genetic heterogeneity and pleiotropic effect were observed because mutations that affect multiple genes contributed to the same SN, and mutations in the same gene may result in different SNs (Fig 4). For example, breast cancers developed in *BRCA1*, *BRCA2*, *TP53*, and *PTEN* mutation carriers; subsequent sarcomas occurred in carriers of P/LP mutations in *RB1*, *BRCA2*, and *SDHB*; and thyroid cancer developed in *SUFU*, *PTCH1*, *TP53*, *BRCA2*, and *RB1* mutation carriers. Of note, two survivors who carried mosaic pathogenic mutations developed an SN, a retinoblastoma survivor who carried a mosaic *RB1* p.L199Ffs mutation (VAF = 0.22) developed sarcoma, and a soft tissue sarcoma survivor with a mosaic *BRCA1* p.T276Afs mutation (VAF = 0.24) developed breast cancer (Data Supplement).

DISCUSSION

We used WGS to systematically assess the contribution of germline mutations in well-established cancer predisposition genes to the risk of SNs among cancer survivors. Within the SJLIFE cohort, approximately 6% of childhood cancer survivors carried a P/LP mutation in a cancer predisposition gene, whereas among non-irradiated survivors with SN, the prevalence of mutation was much higher at 18% (Data Supplement). Mutation status was significantly associated with increased rates of subsequent breast cancer and sarcoma among irradiated survivors and any SN, breast cancer, NMSC, and two or more histologically distinct SNs among non-irradiated survivors. In contrast, we did not observe an increased rate of meningioma or thyroid cancer among mutation carriers; the occurrences of these cancers among survivors were primarily driven by radiation exposure. Of note, eight mosaic mutations (5% of all) also were identified among survivors, two of whom developed an SN, which suggests that germline mosaicism also may contribute to SN.

The key finding of a substantially elevated risk of SN among survivors who carried a P/LP mutation in a cancer predisposition gene supports a recommendation of referral for genetic counseling of all childhood cancer survivors to discuss potential benefits, risks, and harms of clinical genetic testing. In the context of genetic counseling, several factors inform whether a survivor is likely to harbor a harmful germline mutation. These factors are the type of primary cancer; a previous diagnosis or presence of phenotypic

Fig 2. Cumulative incidence of subsequent neoplasms. The x-axis shows time in years since childhood cancer diagnosis, starting at 5 years. The plots show cumulative incidence curves for survivors with and without a pathogenic/likely pathogenic mutation in an *SJCGP60* gene by radiation exposure. Numbers of survivors at risk are shown for mutation carriers (Mu⁺) and non-carriers (Mu⁻). (A) Any SN. (B) Breast cancer using chest radiation. (C) Sarcoma. (D) Thyroid cancer using neck radiation. Meningioma and nonmelanoma skin cancer are shown in the Data Supplement.

characteristics of an underlying genetic syndrome; a positive family history of cancer; and, now on the basis of the current findings, a diagnosis of a subsequent breast cancer or sarcoma in the field of

prior irradiation or a history of any SN in a field not previously exposed to therapeutic radiation. Knowledge of whether a cancer survivor harbors an underlying genetic mutation has significant

Table 2. Multivariable Analysis of RR of Any and Specific SNs, Including Mutation Status and Diagnostic and Treatment Variables

Variable	Any SN		Breast*		Sarcoma	
	RR (95% CI)†	P	RR (95% CI)†	P	RR (95% CI)†	P
Gene mutation						
No	Reference		Reference		Reference	
Yes	1.8 (1.2 to 2.6)	.003	9.4 (4.8 to 18.2)	< .001	10.9 (4.7 to 25.2)	< .001
Age at diagnosis, years						
0-4	Reference		Reference		—	
5-9	0.6 (0.5 to 0.8)	< .001	0.8 (0.2 to 4.3)	.840	—	
10-14	0.8 (0.7 to 1.1)	.150	3.0 (0.9 to 10.3)	.080	—	
≥ 15	0.7 (0.5 to 0.9)	.004	3.1 (0.8 to 11.6)	.090	—	
Sex						
Male	Reference		—		Reference	
Female	1.6 (1.3 to 1.8)	< .001	—		1.1 (0.5 to 2.4)	.870
RT regions						
Cranial no	Reference		—		—	
Cranial yes	2.4 (2.0 to 3.0)	< .001	—		—	
Neck no	—		—		—	
Neck yes	—		—		—	
Breast no	Reference		Reference		—	
Breast yes	2.5 (2.0 to 3.2)	< .001	7.9 (4.0 to 15.5)	< .001	—	
Abdomen no	Reference		—		—	
Abdomen yes	1.6 (1.3 to 2.0)	< .001	—		—	
Pelvis no	—		—		—	
Pelvis yes	—		—		—	
Alkylating agents, tertiles‡						
None	—		—		Reference	
First	—		—		1.0 (0.2 to 4.2)	.980
Second	—		—		0.8 (0.1 to 4.1)	.750
Third	—		—		3.8 (1.4 to 10.8)	.010
Anthracyclines, tertiles‡						
None	—		Reference		—	
First	—		0.9 (0.3 to 3.5)	.930	—	
Second	—		1.5 (0.7 to 3.0)	.320	—	
Third	—		2.4 (1.2 to 4.6)	.010	—	
Epipodophyllotoxin, tertiles‡						
None	Reference		—		—	
First	1.6 (1.2 to 2.0)	< .001	—		—	
Second	1.1 (0.8 to 1.6)	.530	—		—	
Third	1.1 (0.8 to 1.5)	.700	—		—	

Abbreviations: RR, relative rate; RT, radiotherapy; SN, subsequent neoplasm.

*Analyses used females only.

†All regression models adjusted for attained age as cubic splines and significant eigenvectors of race.

‡Cut points for tertiles of chemotherapeutic agent doses were alkylating agents, 6,617 mg/m² and 10,500 mg/m²; anthracyclines, 114 mg/m² and 204 mg/m²; and epipodophyllotoxin, 1,588 mg/m² and 6,704 mg/m².

and often immediate clinical implications. First, cancer screening recommendations and risk reduction strategies are available for certain cancer predisposition syndromes.²³⁻²⁵ Implementation of these strategies can significantly improve outcomes among at-risk populations.^{26,27} Second, current SN screening guidelines for survivors focus on identifying SNs that developed in or adjacent to an irradiated site. However, survivors who carry cancer predisposing mutations may be at increased risk of developing cancer in organs/tissues that lie outside the field of radiation exposure. Hence, genetic information may augment screening recommendations for some mutation carriers, which would lead to early detection and treatment of cancers that might otherwise go unnoticed. With advancing technologies, these high-risk patients also may be eligible for surveillance programs aimed at early cancer detection because new approaches that involve deep sequencing of blood samples are shown to be effective.²⁸ Finally, for survivors with SN who carry a cancer predisposition gene mutation, genetic information may

guide SN treatment (ie, avoidance of radiotherapy), provide valuable information with regard to cancer risk for other family members, and inform reproductive decisions among this young population.

This study had several limitations. First, because WGS was only available to participants who were alive at recruitment, mutations associated with increased cancer mortality may be under-represented. Thus, prevalence of and associations between mutation carrier status and SN risk are likely to be underestimated. Nevertheless, recent studies of the prevalence of mutations in cancer predisposition genes among newly diagnosed children have ranged from 6% to 8% after correction for cohort bias.²⁹ Second, incomplete ascertainment of family history limited the ability to assess whether identified P/LP mutations were enriched among survivors with a family history of cancer or trace segregation of these mutations using family history. Third, our cohort size limited more rigorous analyses of specific SN-gene associations and formal testing for possible interactions between gene

Fig 3. Relative rate (RR) of subsequent neoplasm (SN) by mutation status. Multivariable piecewise exponential regression was used to calculate the RR of SNs and 95% CIs for mutation status, stratified by (A) exposure to radiation and (B) no exposure to radiation. Mutation in the *SJCPG₆₀* gene was analyzed for overall and specific SNs. Both RR and 95% CI are plotted along the x-axis in log10 scale. NA represents analyses that were limited by small number of events. The dotted line represents an RR of 1. NA, not applicable; NMSC, nonmelanoma skin cancer.

mutation and specific treatments. Fourth, sequenced and non-sequenced survivors had statistically significant differences with respect to a few specific treatment exposures, the influence of which on the mutation-effects estimates of SN rates is not

estimable from our data but are expected to be small because the mutation status is largely uncorrelated with these treatment exposures. Finally, the SJLIFE cohort is relatively young, with a median age of 36 years. Hence, the risk of SNs among

Fig 4. (A) to (F) Genetic heterogeneity in subsequent neoplasm (SN) risk. Each circle represents one specific type of SN. Each colored sector within a circle is sized in proportion to the number of SN occurrences among mutation carriers in one of the *SJCPG₆₀* genes. NMSC, nonmelanoma skin cancer.

mutation carriers may change with increasing age and extended follow-up.

The findings have immediate implications for the growing population of childhood cancer survivors. Survivors who carried P/LP mutations in cancer predisposition genes had significantly higher risks of developing one or more SNs. Although all survivors are candidates for genetic counseling, elevated genetic risk supports prioritization of clinical genetic testing of non-irradiated survivors with any SN and those with breast cancer or sarcoma in the field of prior irradiation. The findings also exemplify the utility of genomics-driven precision medicine in facilitating the identification of survivors at high risk for developing SNs and in providing opportunities for personalized cancer surveillance and prevention strategies, which over time may reduce the significant morbidity and mortality associated with these outcomes.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Disclosures provided by the authors are available with this article at jco.org.

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AUTHOR CONTRIBUTIONS

Conception and design: Zhaoming Wang, Carmen L. Wilson, James R. Downing, Melissa M. Hudson, Yutaka Yasui, Leslie L. Robison, Jinghui Zhang

Financial support: Leslie L. Robison

Administrative support: Leslie L. Robison

Provision of study materials or patients: Ching-Hon Pui, Leslie L. Robison

Collection and assembly of data: Zhaoming Wang, Carmen L. Wilson, John Easton, Heather Mulder, Shawn Levy, Kyla Shelton, Ying Shao, Bhavin Vadodaria, Donald Yergeau, Xin Zhou, Angela Jones, Braden Boone

Data analysis and interpretation: Zhaoming Wang, Carmen L. Wilson, Andrew Thrasher, Qi Liu, Dale J. Hedges, Shuoguo Wang, Michael C. Rusch, Michael N. Edmonson, Jennifer Q. Lanctot, Eric Caron, Kelsey Currie, Matthew Lear, Aman Patel, Celeste Rosencrance, Yadav Sapkota, Russell J. Brooke, Wonjong Moon, Evadnie Rampersaud, Xiaotu Ma, Ti-Cheng Chang, Stephen V. Rice, Cynthia Pepper, Xiang Chen, Wenan Chen, Matthew J. Ehrhardt, Matthew J. Krasin, Rebecca M. Howell, Nicholas S. Phillips, Courtney Lewis, Deokumar Srivastava, Ching-Hon Pui, Chimene A. Kesserwan, Gang Wu, Kim E. Nichols, Melissa M. Hudson, Yutaka Yasui, Leslie L. Robison, Jinghui Zhang

Manuscript writing: All authors

Final approval of manuscript: All authors

Accountable for all aspects of the work: All authors

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Affiliations

Zhaoming Wang, Carmen L. Wilson, John Easton, Andrew Thrasher, Heather Mulder, Dale J. Hedges, Shuoguo Wang, Michael C. Rusch, Michael N. Edmonson, Jennifer Q. Lanctot, Eric Caron, Kyla Shelton, Kelsey Currie, Matthew Lear, Aman Patel, Celeste Rosencrance, Ying Shao, Bhavin Vadodaria, Donald Yergeau, Yadav Sapkota, Russell J. Brooke, Wonjong Moon, Evadnie Rampersaud, Xiaotu Ma, Ti-Cheng Chang, Stephen V. Rice, Cynthia Pepper, Xin Zhou, Xiang Chen, Wenan Chen, Matthew J. Ehrhardt, Matthew J. Krasin, Rebecca M. Howell, Nicholas S. Phillips, Courtney Lewis, Deekumar Srivastava, Ching-Hon Pui, Chimene A. Kesserwan, Gang Wu, Kim E. Nichols, James R. Downing, Melissa M. Hudson, Yutaka Yasui, Leslie L. Robison, and Jinghui Zhang, St Jude Children's Research Hospital, Memphis, TN; Qi Liu, University of Alberta, Edmonton, Alberta, Canada; Shawn Levy, Angela Jones, and Braden Boone, HudsonAlpha Institute for Biotechnology, Huntsville, AL; and Rebecca M. Howell, The University of Texas MD Anderson Cancer Center, Houston, TX.

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Genetic Risk for Subsequent Neoplasms Among Long-Term Survivors of Childhood Cancer

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Zhaoming Wang

No relationship to disclose

Carmen L. Wilson

No relationship to disclose

John Easton

No relationship to disclose

Andrew Thrasher

No relationship to disclose

Heather Mulder

No relationship to disclose

Qi Liu

No relationship to disclose

Dale J. Hedges

No relationship to disclose

Shuoguo Wang

Employment: Bristol-Myers Squibb

Michael C. Rusch

No relationship to disclose

Michael N. Edmonson

No relationship to disclose

Shawn Levy

No relationship to disclose

Jennifer Q. Lancot

Employment: Covidien (I), Medtronic (I)

Stock or Other Ownership: Covidien (I), Medtronic (I)

Eric Caron

No relationship to disclose

Kyla Shelton

No relationship to disclose

Kelsey Currie

No relationship to disclose

Matthew Lear

No relationship to disclose

Aman Patel

No relationship to disclose

Celeste Rosencrance

No relationship to disclose

Ying Shao

No relationship to disclose

Bhavin Vadodaria

No relationship to disclose

Donald Yergeau

No relationship to disclose

Yadav Sapkota

No relationship to disclose

Russell J. Brooke

No relationship to disclose

Wonjong Moon

No relationship to disclose

Evadnie Rampersaud

No relationship to disclose

Xiaotu Ma

No relationship to disclose

Ti-Cheng Chang

No relationship to disclose

Stephen V. Rice

No relationship to disclose

Cynthia Pepper

No relationship to disclose

Xin Zhou

No relationship to disclose

Xiang Chen

Stock or Other Ownership: Gilead Sciences, Medtronic, Pfizer, Juno Therapeutics, Cytokinetics

Wenan Chen

No relationship to disclose

Angela Jones

No relationship to disclose

Braden Boone

No relationship to disclose

Matthew J. Ehrhardt

No relationship to disclose

Matthew J. Krasin

No relationship to disclose

Rebecca M. Howell

No relationship to disclose

Nicholas S. Phillips

No relationship to disclose

Courtney Lewis

No relationship to disclose

Deokumar Srivastava

No relationship to disclose

Ching-Hon Pui

No relationship to disclose

Chimene A. Kesserwan

No relationship to disclose

Gang Wu

No relationship to disclose

Kim E. Nichols

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James R. Downing

No relationship to disclose

Melissa M. Hudson

Consulting or Advisory Role: Coleman Supportive Oncology Initiative for Children with Cancer, Oncology Research Information Exchange Network, Princess Máxima Center

Yutaka Yasui

No relationship to disclose

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