

# Pathogenic Germline Mutations in DNA Repair Genes in Combination With Cancer Treatment Exposures and Risk of Subsequent Neoplasms Among Long-Term Survivors of Childhood Cancer

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## abstract

**PURPOSE** To investigate cancer treatment plus pathogenic germline mutations (PGMs) in DNA repair genes (DRGs) for identification of childhood cancer survivors at increased risk of subsequent neoplasms (SNs).

**METHODS** Whole-genome sequencing was performed on blood-derived DNA from survivors in the St Jude Lifetime Cohort. PGMs were evaluated in 127 genes from 6 major DNA repair pathways. Cumulative doses of chemotherapy and body region–specific radiotherapy (RT) were abstracted from medical records. Relative rates (RRs) and 95% CIs of SNs by mutation status were estimated using multivariable piecewise exponential models.

**RESULTS** Of 4,402 survivors, 495 (11.2%) developed 1,269 SNs. We identified 538 PGMs in 98 DRGs (*POLG*, *MUTYH*, *ERCC2*, and *BRCA2*, among others) in 508 (11.5%) survivors. Mutations in homologous recombination (HR) genes were significantly associated with an increased rate of subsequent female breast cancer (RR, 3.7; 95% CI, 1.8 to 7.7), especially among survivors with chest RT  $\geq$  20 Gy (RR, 4.4; 95% CI, 1.6 to 12.4), or with a cumulative dose of anthracyclines in the second or third tertile (RR, 4.4; 95% CI, 1.7 to 11.4). Mutations in HR genes were also associated with an increased rate of subsequent sarcoma among those who received alkylating agent doses in the third tertile (RR, 14.9; 95% CI, 4.0 to 38.0). Mutations in nucleotide excision repair genes were associated with subsequent thyroid cancer for those treated with neck RT  $\geq$  30 Gy (RR, 12.9; 95% CI, 1.6 to 46.6) with marginal statistical significance.

**CONCLUSION** Our study provides novel insights regarding the contribution of genetics, in combination with known treatment-related risks, for the development of SNs. These findings have the potential to facilitate identification of high-risk survivors who may benefit from genetic counseling and/or testing of DRGs, which may further inform personalized cancer surveillance and prevention strategies.

*J Clin Oncol* 38:2728-2740. © 2020 by American Society of Clinical Oncology

## INTRODUCTION

The population of childhood cancer survivors is growing and estimated to exceed 500,000 in the United States by 2020.<sup>1</sup> Childhood cancer survivors experience a substantial burden of long-term chronic health conditions, including a high incidence of subsequent neoplasms (SNs).<sup>2</sup> Although the development of SNs among survivors is largely considered therapy related,<sup>3</sup> pathogenic germline mutations in cancer predisposition genes also contribute to this elevated risk.<sup>4</sup>

Using the St Jude Lifetime Cohort (SJLIFE),<sup>5</sup> we previously characterized the prevalence and spectrum of pathogenic/likely pathogenic (P/LP) mutations in 60 genes associated with well-established autosomal dominant cancer predisposition syndromes and found

that carriers of P/LP mutations have a greater risk of SNs.<sup>4</sup> Notably, some cancer predisposition genes are also DNA repair genes (DRGs), suggesting that pathogenic mutations in DNA repair processes may play a role in the development of SNs among childhood cancer survivors.

DNA repair is essential for the maintenance of genome integrity. The various forms of DNA damage caused by genetic (ie, germline mutations) and/or environmental (ie, genotoxic agents) factors<sup>6</sup> can be repaired through 6 major DNA repair pathways: base excision repair (BER), Fanconi anemia (FA), homologous recombination (HR), mismatch repair (MMR), nucleotide excision repair (NER), and nonhomologous end joining (NHEJ). Defects in some DRGs elevate risks for hereditary cancers or syndromes associated with

## ASSOCIATED CONTENT

### Data Supplement

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

Accepted on April 15, 2020 and published at [ascopubs.org/journal/jco](https://ascopubs.org/journal/jco) on June 4, 2020; DOI <https://doi.org/10.1200/JCO.19.02760>

## CONTEXT

### Key Objective

We aimed to characterize germline pathogenic mutations in DNA repair genes and evaluate the risk of developing subsequent neoplasms on the basis of mutation status among childhood cancer survivors in conjunction with genotoxic cancer treatment exposures.

### Knowledge Generated

By analyzing 4,402 survivors from the St Jude Lifetime Cohort, we identified 3 groups with elevated risk: (1) female survivors who had mutations in homologous recombination genes and received high doses of chest irradiation or anthracyclines have 4-fold increased risk of subsequent breast cancer; (2) survivors who had mutations in homologous recombination genes and received high doses of alkylating agents have 15-fold increased risk of subsequent sarcoma; and (3) survivors who had mutations in nucleotide excision repair genes and received high doses of neck irradiation have 13-fold increased risk of subsequent thyroid cancer.

### Relevance

Identifying survivors at the highest risk of subsequent neoplasms and implementing personalized cancer surveillance and prevention strategies may reduce morbidity and mortality.

specific malignancies.<sup>7,8</sup> Most of these syndromes, such as Bloom syndrome, Nijmegen breakage syndrome, and FA, are characterized by autosomal recessive inheritance of DRG defects with low penetrance in the general population and have a limited contribution to the overall cancer burden.<sup>8</sup> Nevertheless, monoallelic pathogenic mutations of these DRGs have been reported to be associated with increased risk of cancers. For instance, monoallelic mutation carriers of *BLM*,<sup>9</sup> an HR gene, and monoallelic mutation carriers of *MUTYH*,<sup>10</sup> a BER gene, have increased risk of developing colorectal cancer; and monoallelic *FANCC* mutations in the FA pathway increase risk of breast cancer.<sup>11</sup> Moreover, specific DNA repair pathways are responsible for repairing DNA damages caused by certain treatment exposures cancer survivors had received.<sup>12-16</sup> For example, DNA double-strand breaks caused by ionizing radiation or anthracyclines can be repaired by HR or NHEJ pathway.<sup>12</sup> Germline deficiencies in these DNA repair pathways may be exacerbated by these treatment exposures and lead to oncogenic genomic instability and hence increase SN risk. Therefore, we hypothesized that pathogenic mutations in DRGs of specific pathways, regardless of the mode of inheritance of the associated syndromes, may lead to suboptimal DNA repair proficiency resulting in the accumulation of specific DNA errors and contribute to the risk of SNs among childhood cancer survivors, especially those treated with high doses of certain radiotherapy (RT) and/or chemotherapeutic agents.

## METHODS

### Study Participants

The SJLIFE study is a retrospective cohort with prospective clinical follow-up and ongoing enrollment of survivors of

childhood cancer treated at St Jude Children's Research Hospital.<sup>5</sup> For the current analysis, 4,402 survivors completed SJLIFE clinical assessment, provided a blood sample for DNA sequencing, and were followed until December 31, 2016. Procedures of SJLIFE have been approved by the St Jude Institutional Review Board, and participants provided written informed consent. Genetic findings from this study are strictly used for research purpose only, as indicated in the signed consent form.

### Treatment Exposures and Characterization of Subsequent Neoplasms

Region-specific RT exposures used radiation oncology treatment records.<sup>17</sup> Chemotherapy exposures, including cumulative doses of anthracyclines, alkylating agents (cyclophosphamide equivalent dose<sup>18</sup>), and epipodophyllotoxins, were abstracted from medical records. Reported SNs were verified pathologically and/or radiologically, through clinical notes, or from National Death Index reports. The locations of SNs were reviewed in conjunction with RT records and categorized as in or out of the radiation field. The occurrence of any SN and that of each common specific SN diagnosis (nonmelanoma skin cancer [NMSC], meningioma, thyroid cancer, female breast cancer, and sarcoma) were studied.

### DNA Sequencing

DNA was isolated from blood samples. Whole-genome sequencing (WGS) and whole-exome sequencing (WES) were performed on the initial 3,006 survivors using the Illumina (San Diego, CA, USA) HiSeq X Ten and HiSeq 4000, respectively.<sup>4</sup> The second set of 1,396 survivors was sequenced with Illumina NovaSeq (WGS, average genome-wide coverage of 38.7×; and WES, average coverage of coding exons of 234.1×).

## Selection of DNA Repair Pathway Genes

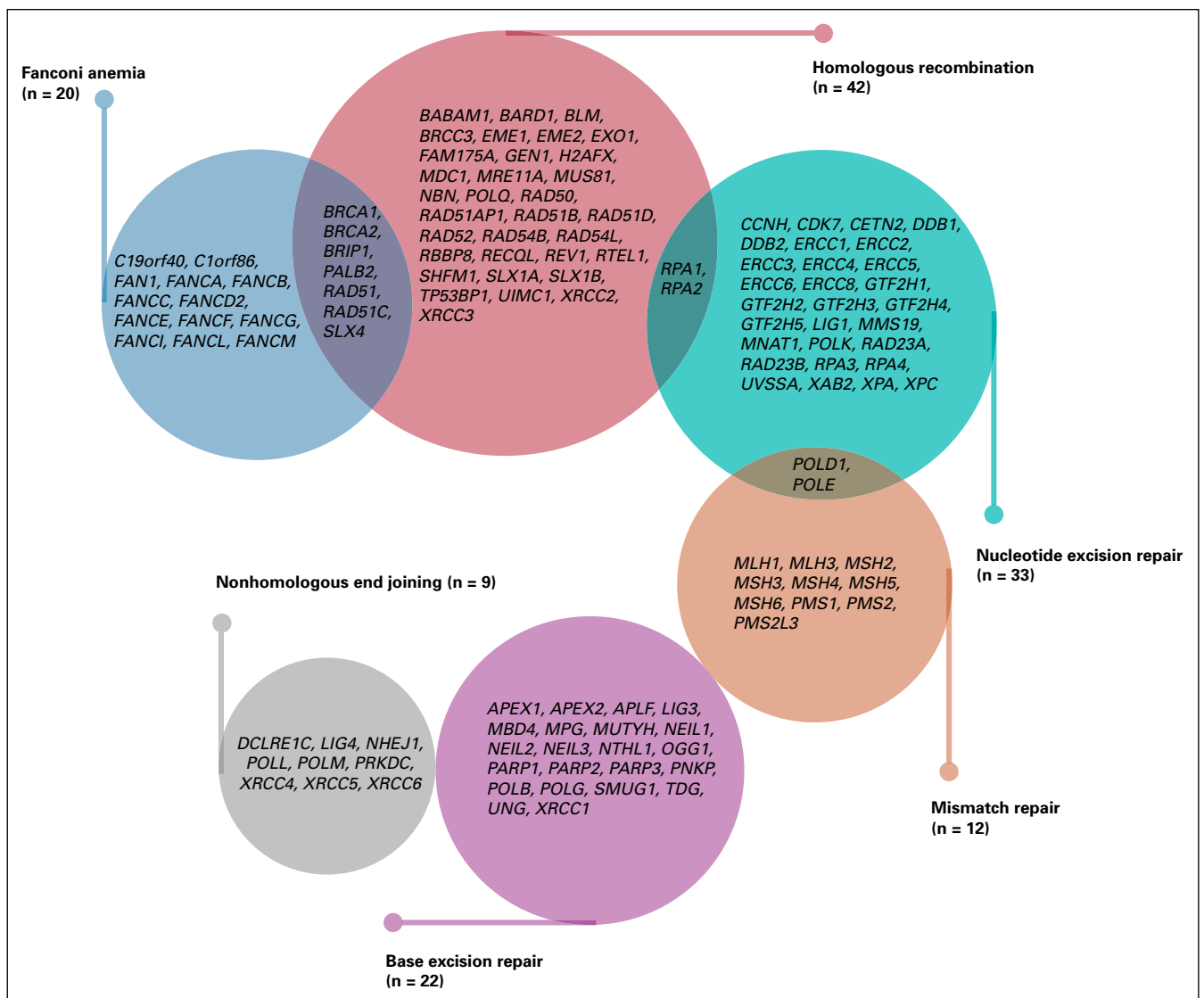
We selected 127 genes in 6 major DNA repair pathways on the basis of information from the MD Anderson DNA repair gene website<sup>19-23</sup> and a literature review. The gene list included 42 HR genes (40 core genes described previously,<sup>24</sup> *POLQ* as a radiosensitivity HR gene,<sup>25</sup> and *RECQL* as an HR gene implicated in breast cancer risk<sup>26</sup>), 12 MMR genes, 33 NER genes, 20 FA genes, 9 NHEJ genes, and 22 BER genes (Fig 1).

## Variant Detection and Classification

Germline single-nucleotide variants, small insertions and deletions, and copy number variations (CNVs) were detected as previously described.<sup>27</sup> Details for the classification of “pathogenic mutations” are provided in the Data Supplement.

## Statistical Analyses

Multivariable piecewise-exponential models,<sup>28</sup> defined as generalized linear models with a logarithmic link function and Poisson error distribution, were used to assess the effect of carrying pathogenic mutations in each DNA repair pathway on the rate of each SN outcome (overall and specific SN types) among survivors of childhood cancer, from which relative rates (RRs) and 95% CIs were estimated. The at-risk follow-up started from the fifth anniversary from childhood cancer diagnosis and ended at the earliest of the occurrence of a specific SN type, last date of contact, or at death. Each survivor’s follow-up time was divided into age-specific segments, where each segment’s age-specific rate of SN was modeled by explanatory variables. Additional details of the modeling approach are provided in the Data Supplement. When estimating the



**FIG 1.** Summary information for 127 genes from 6 major DNA repair pathways selected for germline mutation analysis.

cumulative incidence of each SN by age, death was considered a competing risk event. Gray's method<sup>29</sup> was used to evaluate statistical significance of the differences in cumulative incidence curves between each pair of groups and across all groups. Analyses were performed using SAS 9.4 (SAS Institute, Cary, NC), and figures were generated using R 3.5.1.<sup>30</sup> All tests were 2-sided. Benjamini-Hochberg's method was used to control the false discovery rate (FDR).

## RESULTS

### Study Participants

Of 4,402 survivors (Table 1), 52.5% were males, and 81.4% were white. The primary diagnoses included leukemia (32.0%), CNS malignancies (14.7%), lymphoma (16.9%), and other solid tumors (36.4%). Approximately half (49.3%) were treated with RT, 55.8% with anthracyclines, 56.3% with alkylating agents, and 34.0% with epipodophyllotoxins. The median ages at childhood cancer diagnosis and at last follow-up were 6.3 (interquartile range [IQR], 2.8-12.5) years and 30.6 (IQR, 21.9-39.7) years, respectively, with a median follow-up of 22.3 (IQR, 14.2-32.1) years.

### SNs

A total of 1,269 SNs were diagnosed among 495 (11.2%) survivors. Median time from childhood cancer diagnosis to the first SN was 26.5 (IQR, 19.1-33.9) years, with the median age at first SN of 35.9 (IQR, 29.0-43.2) years. The most common SNs were NMSC (4.3%; 686 in 191 survivors), meningioma (2.7%; 272 in 120 survivors), female breast cancer (2.7%; 64 in 57 survivors), thyroid cancer (1.6%; 73 in 72 survivors), and sarcoma (0.7%; 30 in 30 survivors).

### Prevalence and Spectrum of Mutations

A total of 538 pathogenic mutations in 98 DRGs were identified in 508 survivors (prevalence, 11.5%; 95% CI, 10.6% to 12.5%), including 192 HR gene mutations in 185 survivors (prevalence, 4.2%; 95% CI, 3.6% to 4.8%), 36 MMR gene mutations in 36 survivors (prevalence, 0.8%; 95% CI, 0.6% to 1.1%), 98 NER gene mutations in 97 survivors (prevalence, 2.2%; 95% CI, 1.8% to 2.7%), 143 FA gene mutations in 140 survivors (prevalence, 3.2%; 2.7% to 3.7%), 26 NHEJ gene mutations in 26 survivors (prevalence, 0.6%; 95% CI, 0.4% to 0.9%), and 112 BER gene mutations in 110 survivors (prevalence, 2.5%; 95% CI, 2.1% to 3.0%). The proportions (with 95% CIs) of mutation carriers in each DNA repair pathway were reported among survivors without SN, with any SN, or with a specific SN (Data Supplement). Of the 538 pathogenic mutations, 70 were missense mutations, 175 were nonsense mutations, 196 were frameshift mutations, one was an in-frame protein deletion, 78 were splicing mutations, and 18 were CNVs. Of the 508 mutation carriers, no one had > 1 pathogenic mutation in the same gene, 11 carried

≥ 2 pathogenic mutations in the same DNA repair pathway, and 16 had > 1 pathogenic mutation in different pathways (Data Supplement).

The most frequently altered DRGs were *POLG* (n = 30), *MUTYH* (n = 27), *ERCC2* (n = 25), *BRCA2* (n = 20), *FANCA* (n = 16), *FAN1* (n = 15), *FANCC* (n = 15), *BRCA1* (n = 14), *BLM* (n = 13), *ERCC3* (n = 13), *PALB2* (n = 13), *POLQ* (n = 13), *LIG4* (n = 12), *RECQL* (n = 10), and *ERCC6* (n = 10; Data Supplement). Thirty-three mutations in 27 DRGs (eg, *BRCA1*, *BRCA2*, *ERCC2*, *FAN1*) with a low variant allele frequency (range, 0.13-0.30) were considered as germline mosaicism (Data Supplement).

### Associations Between Mutation Status and Subsequent Neoplasms

Any SN and SN-specific baseline clinical models are shown in Table 2. We observed significantly increased rates of any SN among carriers of mutations in HR (RR, 1.5; 95% CI, 1.1 to 2.2), NER (RR, 1.6; 95% CI, 1.1 to 2.5), or MMR genes (RR, 2.1; 95% CI, 1.2 to 3.7; Table 3). The subsequent breast cancer rate was significantly increased among female survivors carrying pathogenic mutations in HR (RR, 3.7; 95% CI, 1.8 to 7.7) or FA genes (RR, 3.7; 95% CI, 1.3 to 10.6; Table 3). We also observed a higher rate of subsequent sarcoma (RR, 5.0; 95% CI, 1.4 to 12.8) among survivors carrying pathogenic mutations in HR genes, and higher rates of subsequent thyroid cancer (RR, 2.7; 95% CI, 1.1 to 6.9) and NMSC (RR, 2.9; 95% CI, 1.1 to 7.9) among survivors carrying pathogenic mutations in NER genes, but these associations were not statistically significant after controlling FDR (Table 3). We found no significant association for subsequent meningioma (Table 3). For HR, FA, and MMR pathways, we performed additional analyses by excluding 61 mutation carriers of 7 well-established cancer predisposition genes (14 in *BRCA1*, 20 in *BRCA2*, 13 in *PALB2*, 8 in *MSH6*, and 6 in *PMS2*; no carrier of mutations in *MLH1* or *MSH2*) and found that the estimated RRs ranged from 1.35-3.15, although they were not statistically significant (Data Supplement).

### Associations Between Mutation Status and Subsequent Neoplasms by Treatment Exposures

When stratified by treatment exposures, the associations of mutations in HR or FA genes with increased rates of subsequent breast cancer were slightly stronger among female survivors treated with chest RT ≥ 20 Gy (HR genes: RR, 4.4; 95% CI, 1.6 to 12.4;  $P_{fdr} = 0.02$ ; FA genes: RR, 5.5; 95% CI, 1.5 to 14.2;  $P_{fdr} = 0.03$ ) and those who received anthracycline doses in the second or third tertile (≥ 98 mg/m<sup>2</sup>; HR genes: RR, 4.4; 95% CI, 1.7 to 11.4;  $P_{fdr} = 0.01$ ; FA genes: RR, 4.2; 95% CI, 1.4 to 12.3;  $P_{fdr} = 0.03$ ; Fig 2A; Data Supplement). The association of HR gene mutations with subsequent sarcoma was substantially stronger among survivors treated with alkylating agent doses in the third tertile (≥ 10,451 mg/m<sup>2</sup>; RR, 14.9;

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

Characteristics	All Survivors	
	No.	%
Total	4,402	100.0
Sex		
Male	2,312	52.5
Female	2,090	47.5
Race <sup>a</sup>		
White	3,585	81.4
Black	708	16.1
Other	109	2.5
Ethnicity <sup>a</sup>		
Hispanic	138	3.1
Non-Hispanic	4,264	96.9
Diagnosis		
Leukemia		
Acute lymphoblastic leukemia	1,260	28.6
Acute myeloid leukemia	140	3.2
Other leukemia	7	0.2
CNS tumors		
Astrocytoma or glioma	278	6.3
Medulloblastoma or PNET	162	3.7
Ependymoma	66	1.5
Other CNS tumor	142	3.2
Lymphoma		
Hodgkin lymphoma	467	10.6
Non-Hodgkin lymphoma	279	6.3
Sarcoma		
Ewing sarcoma	118	2.7
Osteosarcoma	157	3.6
Rhabdomyosarcoma	149	3.4
Nonrhabdomyosarcoma	125	2.8
Embryonal		
Wilms tumor	277	6.3
Neuroblastoma	218	5.0
Germ cell tumor	96	2.2
Retinoblastoma	259	5.9
Hepatoblastoma	26	0.6
Other		
Melanoma	41	0.9
Carcinomas	61	1.4
Others	74	1.7

(continued in next column)

**TABLE 1.** Characteristics of Participants in the St Jude Lifetime Cohort Study (continued)

Characteristics	All Survivors	
	No.	%
Radiotherapy		
Any	2,169	49.3
Cranial	858	19.5
Neck	601	13.7
Chest	628	14.3
Abdomen	567	12.9
Pelvis	506	11.5
Chemotherapy		
Alkylating agent	2,480	56.3
Anthracyclines	2,455	55.8
Epipodophyllotoxins	1,496	34.0
Median age at diagnosis, years (IQR)	6.3 (2.8-12.5)	
Median age at follow-up, years (IQR)	30.6 (21.9-39.7)	
Median length of follow-up, years (IQR)	22.3 (14.2-32.1)	

Abbreviations: IQR, interquartile range; PNET, primitive neuroectodermal tumor.

<sup>a</sup>Race/ethnicity was based on self-report.

95% CI, 4.0 to 38.0;  $P_{fdr} = 0.001$ ; Fig 2B). NER gene mutations were associated with a higher rate of subsequent thyroid cancer among those treated with neck RT  $\geq 30$  Gy (RR, 12.9; 95% CI, 1.6 to 46.6;  $P_{fdr} = 0.06$ ; Fig 2C) and an increased rate of subsequent NMSC occurring in the RT field (RR, 3.2; 95% CI, 1.1 to 9.2;  $P_{fdr} = 0.06$ ; Data Supplement), both with marginal statistical significance after accounting for multiple comparisons.

### Associations Between Mutation Status and Subsequent Neoplasms by Age

When stratified by age during follow-up (Data Supplement), the associations of HR or FA gene mutations and increased rates of specific SNs appeared stronger during the follow-up period corresponding to age  $\leq 35$  years, including any SN (HR genes: RR, 2.1; 95% CI, 1.3 to 3.3; FA genes: RR, 1.9; 95% CI, 1.0 to 3.5), subsequent female breast cancer (HR genes: RR, 6.4; 95% CI, 2.3 to 18.1; FA genes: RR, 8.7; 95% CI, 2.4 to 22.2), and subsequent sarcoma (HR genes: RR, 9.2; 95% CI, 2.5 to 23.5). In contrast, the associations of NER gene mutations with the rates of any SN (RR, 2.0; 95% CI, 1.2 to 3.4), or subsequent NMSC (RR, 3.5; 95% CI, 1.4 to 8.5) and the association of MMR gene mutations with the rate of any SN (RR, 2.3; 95% CI, 1.0 to 5.1) appeared stronger during the follow-up period corresponding to age  $> 35$  years.

### Cumulative Incidence of Second Neoplasms by Mutation Status and Treatment Exposures

Cumulative incidence curves of subsequent female breast cancer at age 20-55 years and that of subsequent sarcoma

**TABLE 2.** Baseline Nongenetic Multivariable Model, Including Diagnostic and Treatment Variables, of Relative Rates of Any and Specific Subsequent Neoplasms

Variable	Any SN <sup>a</sup>			Meningioma <sup>a,b,c</sup>			Sarcoma			Breast <sup>a,d</sup>			Thyroid			NMSC <sup>a,c</sup>		
	RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P	
Age at diagnosis, years																		
0-4	Ref			Ref			Ref			Ref			Ref			Ref		
5-9	0.7 (0.6 to 1.0)	.018		0.5 (0.3 to 0.8)	.0069		0.7 (0.2 to 3.2)	.64		0.2 (0.1 to 0.6)	.0043							
10-14	1.0 (0.8 to 1.2)	.77		0.4 (0.2 to 0.8)	.0072		3.3 (1.1 to 9.9)	.033		1.1 (0.6 to 2.0)	.75							
≥ 15	0.8 (0.7 to 1.1)	.16		0.1 (0.1 to 0.4)	< .0001		3.3 (1.9 to 10.1)	.036		0.5 (0.2 to 1.0)	.042							
Sex																		
Female v male	1.5 (1.3 to 1.8)	< .0001		1.9 (1.2 to 3.1)	.0056		1.1 (0.5 to 2.4)	.90		1.7 (1.0 to 2.7)	.040		1.0 (0.6 to 1.6)	.93				
RT regions																		
Cranial RT, Gy																		
None	Ref			Ref			Ref			Ref			Ref					
> 0 to < 18	1.9 (0.9 to 4.1)	.10		10.1 (1.1 to 95.9)	.045					2.3 (0.4 to 12.3)	.34							
18-29	1.9 (1.6 to 2.3)	< .0001		80.0 (28.2 to 226.8)	< .0001					3.6 (2.0 to 6.4)	< .0001							
≥ 30	1.9 (1.4 to 2.5)	< .0001		102.3 (32.2 to 325.4)	< .0001					3.2 (1.7 to 6.0)	< .0001							
Unknown	3.0 (1.9 to 4.7)	< .0001		112.4 (33.2 to 380.5)	< .0001					3.6 (1.2 to 10.8)	.021							
Abdomen RT, Gy																		
None	Ref			Ref			Ref			Ref			Ref					
> 0 to < 30	1.5 (1.1 to 1.9)	.011								5.5 (2.4 to 12.8)	< .0001							
≥ 30	1.5 (1.1 to 2.1)	.0060								5.6 (2.3 to 13.5)	.0016							
Unknown	1.8 (0.6 to 5.4)	.33								0.3 (0.1 to 0.8)	.022							
Pelvis RT, Gy																		
None	Ref			Ref			Ref			Ref			Ref					
> 0 to < 20										0.2 (0.0 to 0.7)	.011							
≥ 20										0.4 (0.2 to 0.9)	.018							
Unknown										9.7 (3.5 to 27.2)	< .0001							
Chest RT, Gy																		
None	Ref			Ref			Ref			Ref			Ref					
> 0 to < 20	1.1 (0.7 to 1.8)	.80		4.3 (1.4 to 13.1)	.012													
≥ 20	1.8 (1.4 to 2.3)	< .0001		4.0 (2.3 to 7.0)	< .0001													
Unknown	1.4 (0.5 to 3.6)	.50		3.4 (0.5 to 24.6)	.23													

(continued on following page)



**TABLE 2.** Baseline Nongenetic Multivariable Model, Including Diagnostic and Treatment Variables, of Relative Rates of Any and Specific Subsequent Neoplasms (continued)

Variable	Any SN <sup>a</sup>			Meningioma <sup>a,b,c</sup>			Sarcoma			Breast <sup>a,d</sup>			Thyroid			NMSC <sup>a,e</sup>		
	RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P		RR (95% CI)	P	
Neck RT, Gy																		
None																		
> 0 to 10																		
11-19																		
20-29																		
≥ 30																		
Unknown																		
Alkylating agents																		
None																		
1st tertile																		
2nd tertile																		
3rd tertile																		
Anthracyclines																		
None																		
1st tertile																		
2nd tertile																		
3rd tertile																		
Epipodophylotoxins																		
None																		
1st tertile																		
2nd tertile																		
3rd tertile																		

Abbreviations: NMSC, nonmelanoma skin cancer; Ref, reference; RR, relative rate; RT, radiotherapy; SN, subsequent neoplasm.

<sup>a</sup>Generalized estimating equation was used to account for repeated events.

<sup>b</sup>All regression models were adjusted for attained age as cubic splines and eigenvectors when applicable (ie, any SN, breast cancer, and NMSC).

<sup>c</sup>Meningioma and NMSC data are from multiple-events analyses.

<sup>d</sup>Only female survivors were included in these analyses.

**TABLE 3.** Adjusted Associations Between Pathogenic Mutations in DNA Repair Genes and Rates of Subsequent Neoplasms Among Childhood Cancer Survivors

DNA Repair Pathway	Any SN			Breast			Sarcoma <sup>a</sup>			Thyroid			Meningioma			NMSC		
	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>	RR (95% CI)	P	<i>P</i> <sub>HR</sub> <sup>b</sup>
HR	1.5 (1.1 to 2.2)	.017	.051	3.7 (1.8 to 7.7)	.00041	.0016	5.0 (1.4 to 12.8)	.018	.090	1.5 (0.6 to 3.7)	.42	.86	0.9 (0.4 to 2.1)	.82	.82	1.3 (0.4 to 4.1)	.70	.70
FA	1.3 (0.7 to 2.1)	.40	.48	3.7 (1.3 to 10.6)	.017	.034	3.1 (0.4 to 11.3)	.27	.45	1.0 (0.1 to 3.6)	.99	.99	0.3 (0.1 to 1.3)	.10	.48	0.7 (0.3 to 1.6)	.39	.65
MMR	2.1 (1.2 to 3.7)	.014	.051	NA	NA	NA	4.9 (0.1 to 27.0)	.37	.46	3.0 (0.1 to 16.5)	.57	.86	1.5 (0.5 to 5.1)	.50	.75	0.7 (0.2 to 2.2)	.53	.66
BER	0.7 (0.4 to 1.3)	.24	.36	1.3 (0.2 to 4.7)	.91	.91	NA	NA	NA	0.5 (0.0 to 2.9)	.87	.99	0.4 (0.1 to 1.8)	.24	.48	0.6 (0.2 to 1.5)	.25	.63
NER	1.6 (1.1 to 2.5)	.027	.054	1.7 (0.5 to 6.2)	.65	.87	2.0 (0.1 to 11.2)	.79	.79	2.7 (1.1 to 6.9)	.037	.22	2.1 (0.8 to 5.6)	.16	.48	2.9 (1.1 to 7.9)	.036	.18
NHEJ	1.1 (0.3 to 2.9)	.96	.96	NA	NA	NA	7.6 (0.2 to 42.3)	.25	.45	3.7 (0.1 to 20.4)	.48	.86	1.4 (0.2 to 9.9)	.77	.82	NA	NA	NA

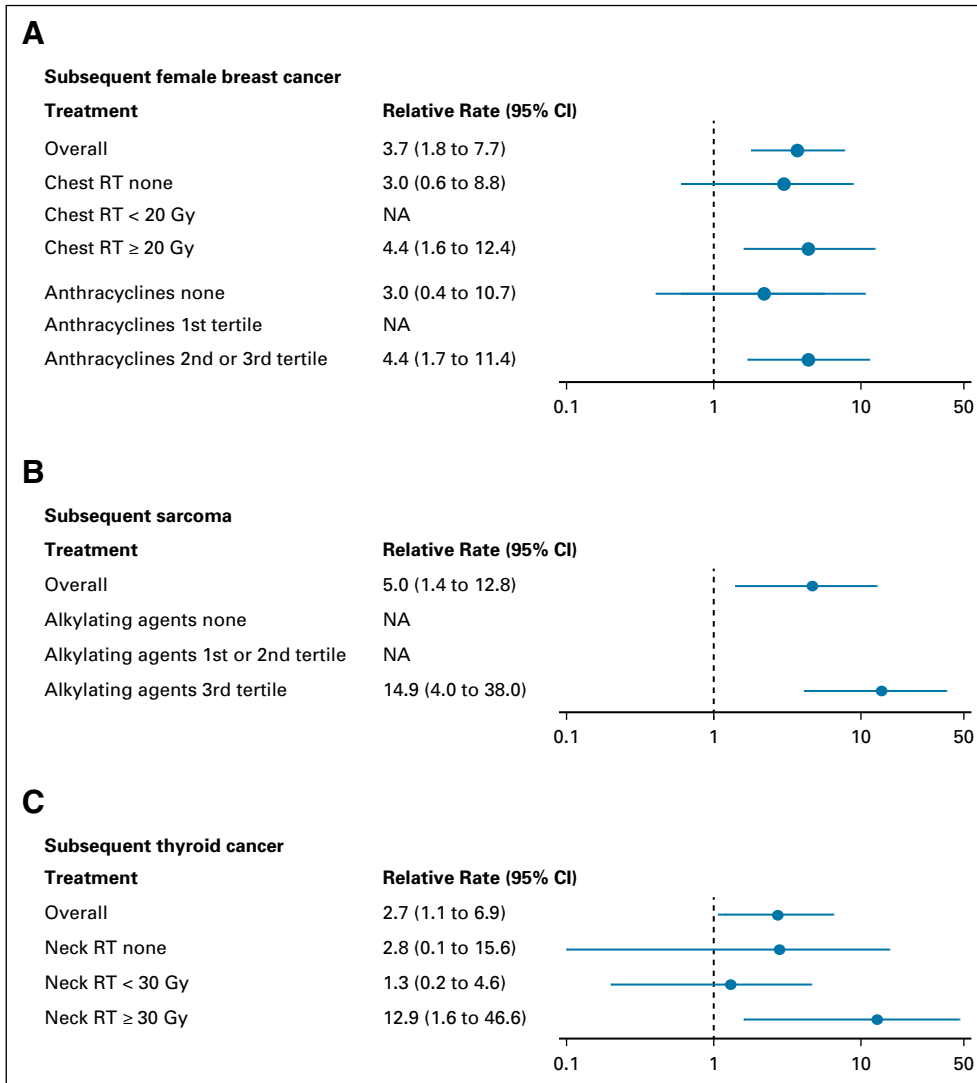
NOTE. Associations were adjusted for sex, age at diagnosis of the primary cancer, ancestry principal components, and the treatment exposures relevant to the specific SN type.

Abbreviations: BER, base excision repair; FA, Fanconi anemia; HR, homologous recombination; MMR, mismatch repair; NA, not applicable (for regression models that did not converge); NER, nucleotide excision repair; NHEJ, nonhomologous end joining; NMSC, nonmelanoma skin cancer; RR, relative rate; SN, subsequent neoplasm.

<sup>a</sup>In modeling rate of subsequent sarcoma, 6 survivors with *RBI* mutations who developed leiomyosarcoma during follow-up were not counted as events.

<sup>b</sup>Benjamini-Hochberg adjusted *P* values.





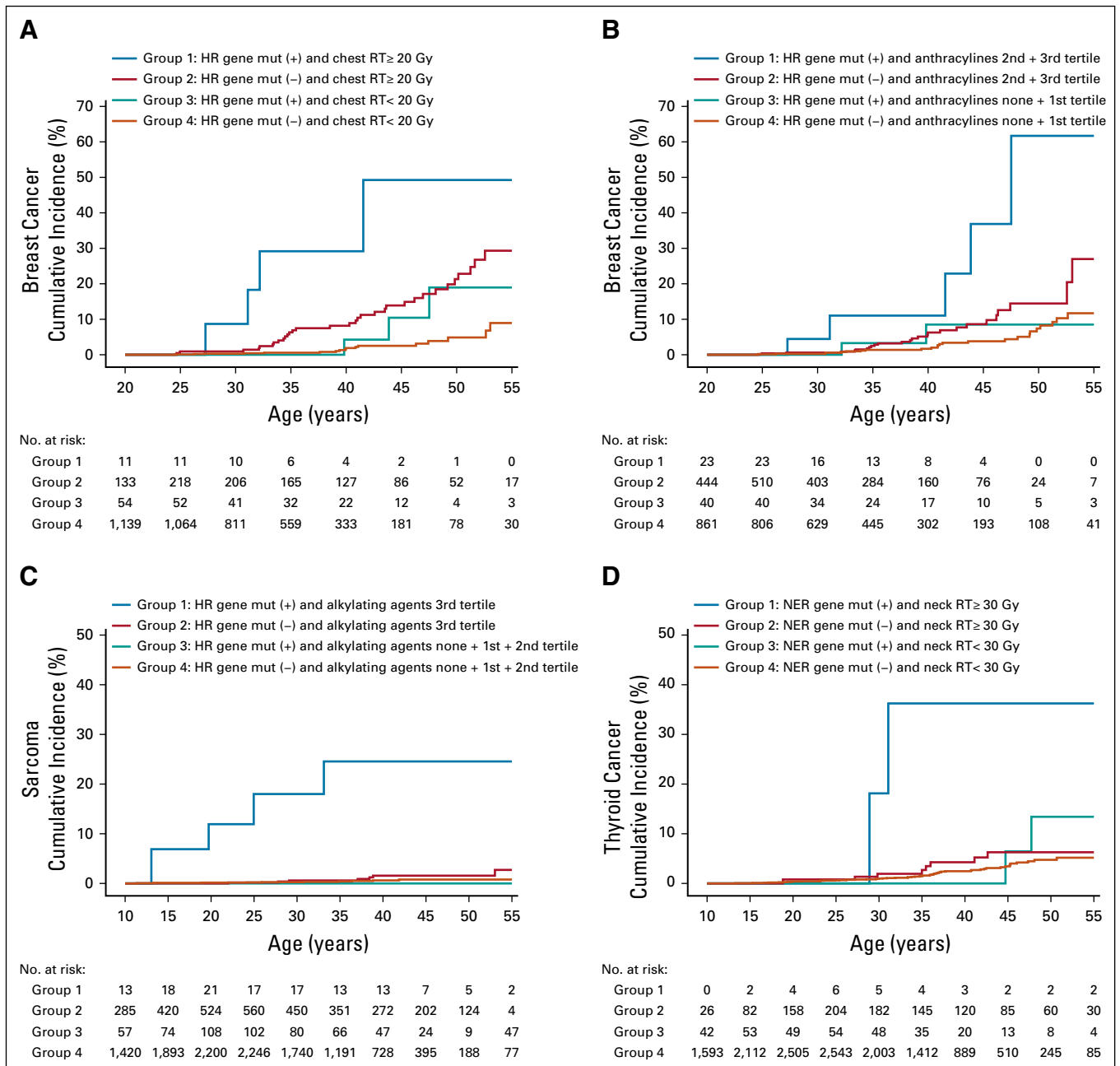
**FIG 2.** Adjusted associations of pathogenic mutations in DNA repair genes with rates of subsequent neoplasms by treatment exposures. (A) The associations of mutations in homologous recombination (HR) genes and the rate of subsequent female breast cancer. (B) The association of mutations in HR genes and the rate of subsequent sarcoma. (C) The association of mutations in nucleotide excision repair (NER) genes and the rate of subsequent thyroid cancer. The relative rate is denoted as not applicable (NA) if the model did not converge. RT, radiotherapy.

and thyroid cancer at age 10-55 years by mutation status and treatment modality are shown in Figure 3 and the Data Supplement. Among the 4 groups in Figure 3A, cumulative incidence of subsequent breast cancer varied greatly, being highest among female survivors who carried HR gene mutations and received chest RT  $\geq$  20 Gy: at age 45 years, it was 49.2%, 13.9%, 10.4%, and 2.5%, for the 4 subgroups. Among the 4 subgroups in Figure 3B, cumulative incidence of subsequent breast cancer was the highest among female survivors who carried HR gene mutations and received moderate to high cumulative doses of anthracyclines (the second or third tertile); at age 45 years, it was 36.9%, 8.6%, 8.5%, and 3.8%, for the 4 subgroups. Cumulative incidence of subsequent sarcoma was the highest among survivors who carried HR gene mutations and received high doses of alkylating agents (the third tertile); at age 45 years it was 24.6%, 1.6%, 0.8%, and 0.0%, for the 4 subgroups (Fig 3C). Cumulative incidence of subsequent thyroid cancer was the highest among survivors who carried NER-gene mutations and

received high-dose neck RT ( $\geq$  30 Gy); at age 45 years it was 36.2%, 6.4%, 6.3%, and 3.4%, for the 4 subgroups (Fig 3D).

#### Proportions of Mutation Carriers Among High-Risk Groups

For female survivors who developed subsequent breast cancer and were exposed to high-dose chest RT ( $\geq$  20 Gy) or moderate to high cumulative doses of anthracyclines (the second or third tertile), the proportions of HR gene mutation carriers were 11.1% (95% CI, 4.4% to 25.3%) and 16.1% (95% CI, 7.1% to 32.6%), which were 2- to 3-fold higher than that observed in all survivors. Among survivors exposed to high cumulative doses of alkylating agents (the third tertile) who developed subsequent sarcoma, the prevalence of HR gene mutation carriers was 30.8% (95% CI, 12.7% to 57.6%), more than 7-fold higher than that observed in all survivors. Finally, for survivors who developed subsequent thyroid cancer after high-dose neck RT ( $\geq$  30 Gy) exposure, the prevalence of NER gene mutation carriers was 20.0% (95% CI, 5.7% to 51.0%),



**FIG 3.** Cumulative incidence of subsequent neoplasms by mutation (mut) status of DNA repair genes and treatment exposures. (A) Subsequent female breast cancer by mutation status of homologous recombination (HR) genes and dose of chest radiotherapy (RT). (B) Subsequent female breast cancer by mutation status of HR genes and dose of anthracyclines. (C) Subsequent sarcoma by mutation status of HR genes and dose of alkylating agents. (D) Subsequent thyroid cancer by mutation status of nucleotide excision repair (NER) genes and dose of neck RT. The cumulative incidence curves are statistically different ( $P < .001$ ) in all 4 panels. Additional statistical tests between group pairs and across the 3 groups except Group 1 (blue line) are included in the Data Supplement. A similar set of cumulative incidence curves with years since diagnosis as the x-axis is provided in the Data Supplement.

more than 9-fold higher than that in all survivors (Data Supplement).

#### Genetic Heterogeneity of DNA repair Gene Mutations in Subsequent Neoplasms

We observed substantial genetic heterogeneity and pleiotropic effects (Data Supplement). Although survivors who

developed the same type of SN carry mutations in different DRGs (genetic heterogeneity), carriers of mutations in the same DRG developed different types of SNs (pleiotropy). For example, subsequent breast cancers developed in female survivors who carried mutations in HR genes (*BRCA1*, *BRCA2*, *NBN*, *BRIP1*, *EME2*, *RECQL*, and *XRCC3*), FA genes (*BRCA1*, *BRCA2*, and *BRIP1*), and NER genes

(*ERCC3* and *GTF2H4*), as well as 1 BER gene (*POLG*); subsequent sarcomas developed in those who carried mutations in HR genes (*BRCA2*, *PALB2*, *BRIP1*, *POLQ*, and *RAD54L*) and FA genes (*BRCA2*, *PALB2*, and *BRIP1*), as well as 1 MMR gene (*PMS1*), NER gene (*XPC*), and NHEJ gene (*LIG4*); subsequent thyroid cancer occurred in survivors who carried mutations in HR genes (*BRCA2*, *EME1*, *RAD50*, *RAD54L*, and *UIMC1*), FA genes (*BRCA2* and *FAN1*), and NER genes (*ERCC2*, *ERCC3*, *POLK*, *CDK7*, and *RPA3*), as well as 1 MMR gene (*MSH5*), BER gene (*POLG*), and NHEJ gene (*POLM*).

## DISCUSSION

Within this cohort of 4,402 SJLIFE survivors, approximately 11.5% were carriers of a pathogenic mutation in  $\geq 1$  DRG. These mutations were significantly associated with increased rates of subsequent female breast cancer, sarcoma, thyroid cancer, and NMSC among survivors, particularly within treatment- and age-specific subgroups. This finding is of great interest to the research and clinical communities, as it not only provides novel insights into genetic susceptibility underlying SN development but also identifies populations with substantially higher probability of carrying a pathogenic mutation in a DRG and populations at significantly increased SN risk.

Although DNA damage caused by genotoxic agents for cancer therapy cures primary childhood cancers by killing tumor cells, it also produces harmful genetic lesions within normal cells, which may lead to SNs.<sup>31</sup> Thus, survivors with a pathogenic DRG mutation are at elevated risk for therapy-related SNs. The Data Supplement summarizes cancer therapy-associated genotoxicity with well-known clinical presentations.<sup>12-16</sup> Mechanistically, the end products of DNA damage associated with specific anticancer therapies and their correction through specific repair pathways are consistent with and support our findings of increased SN rates among survivors who carry mutations in a specific DNA repair pathway and received specific genotoxic treatment.

Given the increased SN risk experienced by childhood cancer survivors, efforts to define those survivors at highest risk have potential to further inform clinical care recommendations and cancer surveillance practices. On the basis of our previous finding of a prevalence of 5.8% for P/LP mutations in 60 genes involved in autosomal dominant cancer predisposition syndromes with moderate to high penetrance, we recommended that all childhood cancer survivors be referred for genetic counseling.<sup>4</sup> The associations observed within SJLIFE between pathogenic DRG mutations and SN risk add substantial new information regarding genetic contribution along with known treatment-related risks. Within the context of genetic counseling and potential clinical genetic testing of childhood cancer survivors, consideration should be given to assessing DRG mutations, especially among those survivors with treatment exposures known to increase the risk of

female breast cancer, subsequent sarcomas, NMSC, and thyroid cancer. Establishing the presence of a genetic mutation in cancer predisposition and/or DRGs has potential to inform future precision medicine approaches for the treatment of high-risk patients, for example by avoiding or limiting exposure to radiation therapy.<sup>32</sup> Moreover, mutation status may inform precision prevention/intervention strategies designed to reduce risk and/or provide early detection of SNs, beyond what is recommended in current clinical guidelines.<sup>33</sup> Last, survivors' genetic information may provide family members with valuable information about potential cancer risk to consider in decision-making about personal genetic testing and participation in strategies that facilitate early detection/prevention.

The interpretation of our findings should be considered with the following limitations. First, because WGS data were available only for participants who were alive at SJLIFE recruitment, mutations associated with increased mortality, either from a childhood cancer or SN, may be underrepresented. Thus, the mutation prevalence and associations between mutation carrier status and SN risk are likely underestimated in our analysis. Second, the increased SN risk among mutation carriers was associated with mutations in multiple genes. The substantial genetic heterogeneity and limited sample size prevented us from conducting more granular analyses of specific SN-gene associations and differentiating the contributions of mutations in genes associated with autosomal dominant syndromes with relatively higher penetrance versus those with autosomal recessive syndromes with relatively lower penetrance. Although excluding mutation carriers of 7 well-established cancer predisposition genes in HR, FA, and MMR pathways attenuated the associations of these pathways with specific SN rates, the directions of association remained consistent, suggesting that other DRGs also contributed but to a lesser extent. Third, our findings of higher SN rates among survivors with defined dose exposures were not based on a rigorous investigation of dose categories and their interactions with DRG mutations; additional research with larger populations is warranted to better determine the thresholds of treatment doses with strength of these genetic associations. Finally, because SJLIFE survivors are relatively young, with a median age of 36 years, SN risk in DRG mutation carriers may change with increasing age and extended follow-up.

Collectively, our findings provide compelling evidence of increased SN risk among childhood cancer survivors with DRG mutations and prior genotoxic treatment exposures. Thus, the results have the potential for informing future clinical recommendations for the broad range of medical professionals who provide health care for this growing population. Identifying survivors at the highest SN risk and implementing personalized cancer surveillance and prevention strategies may reduce the substantial morbidity and mortality associated with these outcomes.

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## SUPPORT

Supported by funding from the American Lebanese Syrian Associated Charities and by Grants No. CA021765, CA195547, and CA216354 from the National Institutes of Health to St Jude Children's Research Hospital. The funders of the study had no role in the design and conduct of the study: collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; or decision to submit manuscript for publication.

## DATA AVAILABILITY STATEMENT

The entire collection of WGS data for 4402 survivors, including raw sequence reads, aligned BAM files, and joint genotype calls (gVCFs), is accessible through St. Jude Cloud (<https://stjude.cloud>).

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## AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST AND DATA AVAILABILITY STATEMENT

Disclosures provided by the authors and data availability statement (if applicable) are available with this article at DOI <https://doi.org/10.1200/JCO.19.02760>.

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## ACKNOWLEDGMENT

We thank all the individuals who participated in this study; we also thank Dr Angela J. McArthur (St Jude Children's Research Hospital) for scientific editing of this manuscript.

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**AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST**

**Pathogenic Germline Mutations in DNA Repair Genes in Combination With Cancer Treatment Exposures and Risk of Subsequent Neoplasms Among Long-Term Survivors of Childhood Cancer**

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**Research Funding:** Incyte/Novartis, Alpine Immune Sciences

No other potential conflicts of interest were reported.