Clinical and Genetic Risk Prediction of Subsequent CNS Tumors in Survivors of Childhood Cancer: A Report From the COG ALTE03N1 Study

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ABSTRACT

Purpose

Survivors of childhood cancer treated with cranial radiation therapy are at risk for subsequent CNS tumors. However, significant interindividual variability in risk suggests a role for genetic susceptibility and provides an opportunity to identify survivors of childhood cancer at increased risk for these tumors.

Methods

We curated candidate genetic variants from previously published studies in adult-onset primary CNS tumors and replicated these in survivors of childhood cancer with and without subsequent CNS tumors (82 participants and 228 matched controls). We developed prediction models to identify survivors at high or low risk for subsequent CNS tumors and validated these models in an independent matched case-control sample (25 participants and 54 controls).

Results

We demonstrated an association between six previously published single nucleotide polymorphisms (rs15869 [*BRCA2*], rs1805389 [*LIG4*], rs8079544 [*TP53*], rs25489 [*XRCC1*], rs1673041 [*POLD1*], and rs11615 [*ERCC1*]) and subsequent CNS tumors in survivors of childhood cancer. Including genetic variants in a Final Model containing age at primary cancer, sex, and cranial radiation therapy dose yielded an area under the curve of 0.81 (95% CI, 0.76 to 0.86), which was superior ($P = .002$) to the Clinical Model (area under the curve, 0.73; 95% CI, 0.66 to 0.80). The prediction model was successfully validated. The sensitivity and specificity of predicting survivors of childhood cancer at highest or lowest risk of subsequent CNS tumors was 87.5% and 83.5%, respectively.

Conclusion

It is possible to identify survivors of childhood cancer at high or low risk for subsequent CNS tumors on the basis of genetic and clinical information. This information can be used to inform surveillance for early detection of subsequent CNS tumors.

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INTRODUCTION

Survivors of childhood cancer are at increased risk for developing subsequent histologically distinct CNS tumors.¹ High-grade glioma and meningioma are the most common subsequent CNS tumors and are associated with significant morbidity and mortality.² Exposure to cranial radiation therapy (CRT) is a major risk factor; the risk of subsequent CNS tumors demonstrates a linear relationship with CRT dose,³ especially after CRT exposure at a young age.^{1,3}

Although CRT is an established risk factor, there is significant interindividual variability in

the risk of subsequent CNS tumors,^{3,4} suggesting a role for genetic susceptibility.^{5,6} Inherited predisposition to glioma and meningioma has been studied extensively in adults with a first occurrence of a CNS tumor, using both genome-wide and candidate gene approaches (Data Supplement). The majority of genes implicated in these studies are involved in DNA damage response and repair, telomere homeostasis, or drug metabolism. We hypothesized that single nucleotide polymorphisms (SNPs) in candidate genes involved in the previously mentioned pathways and implicated in the etiology of adult-onset primary CNS tumors could play a role in the

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development of subsequent CNS tumors in survivors of childhood cancer. We further hypothesized that these genetic variants could be used to create a prediction model that identifies survivors of childhood cancer at high or low risk of developing subsequent CNS tumors.

METHODS

Published SNPs: Search Strategy

We searched the Medline and Embase databases for studies published from January 2005 through April 2014. The search was restricted to primary reports in humans published in English. The Medical Subject Heading terms “brain neoplasm,” “brain cancer,” “brain tumor,” “CNS neoplasm,” “meningioma,” “glioma,” “second neoplasm/cancer,” AND “radiotherapy,” and “gene,” “genetic association,” and “SNP” were used in separate searches, yielding 42 publications (Data Supplement) with 129 representative SNPs on 46 genes (Data Supplement) for our replication analysis.

Study Participants and Design

Using a matched case-control study design, we enrolled survivors of childhood cancer with subsequent CNS tumors to a Children's Oncology Group (COG) study (ALTE03N1). One hundred twenty-one COG member institutions contributed participants to the study after obtaining approval from local institutional review boards (Data Supplement). Written informed consent or assent was obtained from all participants and/or their parents or legal guardians. Eligible participants were individuals diagnosed with a primary cancer at age 21 years or younger who subsequently developed a histologically distinct CNS tumor. For each participant, we randomly selected one to four controls from a pool of survivors of childhood cancer with no evidence of subsequent neoplasms using the following matching criteria: primary cancer diagnosis; year of diagnosis; race or ethnicity; and duration of follow-up for controls to exceed time from primary cancer diagnosis to subsequent CNS tumor for

the index participant. Participants provided blood or saliva for germline DNA. A detailed therapeutic summary and validation of subsequent CNS tumors with pathology reports were provided by participating sites.

The study was conducted in the following three phases: phase I, association between previously published SNPs (in adult-onset primary CNS tumors) and subsequent CNS tumors in survivors of childhood cancer; phase II, development of a risk prediction model; and phase III, external validation of the risk prediction model developed in phase II.

Phase I: Association Between Candidate SNPs and Subsequent CNS Tumors

Genomic DNA was isolated from peripheral blood (QIAamp/Qiagen kits; Qiagen, Hilden Germany) or saliva (Oragene kits; DNA Genotek, Ottawa, Ontario, Canada). Genotyping was performed on the Illumina (San Diego, CA) semicustom HumanExome+vl.1 array specifically designed for COG-ALTE03N1. Quality control for genotype data was performed with PLINK (<http://zzz.bwh.harvard.edu/plink/>).⁷ No individuals were disqualified on the basis of discordant sex or sample contamination. One individual was removed as a result of low genotyping (missing fraction > 0.025). Thirty-four SNPs with minor allele frequency < 0.05 were excluded, leaving 95 SNPs for the final analysis. No SNP with $P < .001$ (0.05 of 95) for Hardy-Weinberg equilibrium was identified. The final analysis included 310 individuals (82 participants and 228 controls) with a total genotyping rate exceeding 99.8%. Analyses were conducted for all subsequent CNS tumors taken together. Exploratory analyses were also conducted for glioma and meningioma. SAS 9.4 software (SAS Institute, Cary, NC) was used for all analyses.

To test the main effect of each SNP that passed quality control, we used multivariable conditional logistic regression to calculate odds ratios (ORs) and 95% CIs, as shown in Fig 1A (Model 1). Variables in Model 1 included age at diagnosis of primary cancer (continuous variable), sex, race and ethnicity (categorical variable: non-Hispanic white, other), and CRT (binary variable using prescribed CRT dose as $< v \geq 24$ Gy). In separate models, CRT dose as a continuous variable and CRT as a binary variable (exposed v not exposed) were also examined.

A

$$\text{logit}(p) = \text{matched set} + \text{agedx} + \text{sex} + \text{race} + \text{CRT} + \text{SNP} \quad (\text{Model 1})$$

p , probability of disease in a subject conditional on matched set; SNP, genotype for each SNP in additive coding; agedx, age at diagnosis of primary cancer (continuous variable); sex, sex of participants (male or female); race, race or ethnicity (categorical variable: non-Hispanic white or other); CRT, cranial radiation therapy (dose of cranial radiation $< v \geq 24$ Gy)

B

$$\text{logit}(p) = \text{matched set} + \text{agedx} + \text{sex} + \text{race} \quad (\text{Base Model – Model 2})$$

$$\text{logit}(p) = \text{matched set} + \text{agedx} + \text{sex} + \text{race} + \text{CRT} \quad (\text{Clinical Model – Model 3})$$

$$\text{logit}(p) = \text{matched set} + \text{agedx} + \text{sex} + \text{race} + \text{SNP}_1 + \dots + \text{SNP}_k \quad (\text{Genetic Model – Model 4})$$

$$\text{logit}(p) = \text{matched set} + \text{agedx} + \text{sex} + \text{race} + \text{CRT} + \text{SNP}_1 + \dots + \text{SNP}_k \quad (\text{Final Model – Model 5})$$

p , probability of disease in a subject conditional on matched set; SNP, genotype for each SNP in additive coding; we used forward stepwise selection to add genetic predictors with $P < .1$; agedx, age at diagnosis of primary cancer (continuous variable); sex, sex of participants (male or female); race, race or ethnicity (categorical variable: non-Hispanic white or other); CRT, cranial radiation (dose of cranial radiation $< v \geq 24$ Gy)

Fig 1. (A) Multivariable conditional logistic regression model used to test the main effect of each single nucleotide polymorphism (SNP) that passed quality control, including variables outlined in Model 1. (B) Risk prediction models using the following four sequential conditional logistic regression models (Models 2 to 5): Base Model (Model 2), Clinical Model (Model 3), Genetic Model (Model 4), and Final Model (Model 5), including the variables outlined in each model.

Phase II: Building Risk Prediction Models

We derived the following risk prediction models sequentially (Fig 1B): Base Model (Model 2), Clinical Model (Model 3), Genetic Model (Model 4), and Final Model (Model 5). SNPs with main effect $P < .1$ (from phase I) were considered in building the Genetic Model, using stepwise forward selection. The Final Model included all variables in the Clinical Model plus the SNPs retained in the Genetic Model. Models were developed for all subsequent CNS tumors taken together and individually for glioma and meningioma.

Receiver operating characteristic analysis was performed to evaluate the predictive use of clinical or genetic variables alone or clinical variables combined with genetic variables for assessing the risk of subsequent CNS tumors overall and individually for glioma and meningioma. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated. We used the Final Model to infer predictive probability, which is the predictive value for a subject to develop subsequent CNS tumors when age at diagnosis of primary cancer, sex, race and ethnicity, CRT dose, and retained SNPs are given. We defined three risk groups using predictive values obtained from the Final Model (high risk: predictive value > 0.5 ; low risk: predictive value < 0.1 ; and intermediate risk: predictive value 0.1 to 0.5).

Phase III: External Validation of Risk Prediction Models

For validation, we used an independent sample of survivors of childhood cancer (25 participants and 54 controls) derived from COG-ALTE03N1 after enrollment for phase I was complete. SNPs retained in Model 4 of phase II were genotyped in the validation set (Data Supplement). Clinical and Final Models (using SNPs retained in Model 4 of phase II) were used to estimate areas under the curve (AUCs) for validation.

RESULTS

Demographic and Clinical Characteristics

The demographic and clinical characteristics of the study participants for phases I, II, and III are listed in Table 1.

Phases I and II. The median age at primary cancer diagnosis was 3.5 years (range, 0 to 21 years) for the participants and 5 years (range, 0 to 20 years) for the controls. Median interval between diagnosis of primary cancer and subsequent CNS tumor (participants, 13.2 years) or study participation (controls, 13.2 years) was comparable. The proportion of participants exposed to CRT was significantly greater (91.5%) compared with controls (39.5%; $P < .001$). Table 1 also lists the demographic and clinical characteristics of participants with glioma and meningioma and matched controls.

Phase III. As shown in Table 1, the median age at primary cancer diagnosis was 6.5 years (range, 0.2 to 15.1 years) for participants and 6.9 years (range, 0.2 to 19 years) for controls. The median interval between diagnosis of the primary cancer and subsequent CNS tumor (participants, 14.7 years) or study participation (controls, 15.2 years) was comparable. Participants were more likely to be exposed to CRT compared with controls (72% v 55.6%, respectively); however, the difference did not reach statistical significance ($P = .16$).

Phase I: Association Between Candidate Genes and Subsequent CNS Tumors

All subsequent CNS tumors. We found associations between six SNPs (rs15869 [BRCA2], rs1805389 [LIG4], rs8079544 [TP53], rs25489 [XRCC1], rs1673041 [POLDD1], and rs11615 [ERCC1])

and subsequent CNS tumors (Table 2). These SNPs were associated with increased risk for subsequent CNS tumors, conferring ORs ranging from 1.6 (95% CI, 1.0 to 2.5) to 4.0 (95% CI, 1.1 to 15.2).

Subsequent glioma. We found associations between three SNPs (rs2909430 [TP53], rs1805389 [LIG4], and rs15869 [BRCA2]) and risk of subsequent glioma (Table 2). The ORs ranged from 3.7 (95% CI, 1.3 to 10.2) to 19.7 (95% CI, 2.5 to 156.4).

Subsequent meningioma. We found associations between four SNPs (rs15869 [BRCA2], rs25489 [XRCC1], rs1801270 [CDKN1A], and rs1673041 [POLDD1]) and risk of subsequent meningioma (Table 2). The ORs ranged from 2.0 (95% CI, 1.0 to 3.8) to 9.6 (95% CI, 1.1 to 85.4).

Phase II: Developing Risk Prediction Models for Subsequent CNS Tumors

All subsequent CNS tumors. The Clinical Model (AUC, 0.73; 95% CI, 0.66 to 0.80) performed significantly better than the Base Model (AUC, 0.59; 95% CI, 0.52 to 0.66; $P < .001$). The following seven SNPs were included in the Genetic Model: rs15869 (BRCA2), rs8079544 (TP53), rs498872 (PHLBI), rs1673041 (POLDD1), rs25489 (XRCC1), rs11615 (ERCC1), and rs828699 (XRCC5). When these seven SNPs were included in the Final Model, the AUC was 0.81 (95% CI, 0.76 to 0.86; Fig 2; Data Supplement) and was superior to the Clinical Model ($P = .0017$). Replacing CRT dose as a binary variable ($< v \geq 24$ Gy) with CRT dose as a continuous variable or treating CRT as a different binary variable (exposed v unexposed) did not alter the findings (Data Supplement).

Subsequent glioma. The Clinical Model (AUC, 0.76; 95% CI, 0.66 to 0.87) performed significantly better than the Base Model (AUC, 0.61; 95% CI, 0.49 to 0.73; $P = .02$). Using stepwise forward selection to build the Genetic Model, we were able to retain the following three SNPs: rs15869 (BRCA2), rs2909430 (TP53), and rs2518471 (TPMT). When these three SNPs were included in the Final Model, the AUC was 0.85 (95% CI, 0.77 to 0.93; Fig 3A and Data Supplement) and was superior to the Clinical Model ($P = .03$). Replacing CRT dose as a binary variable ($< v \geq 24$ Gy) with CRT dose as a continuous variable or treating CRT as a different binary variable (exposed v unexposed) did not alter the findings (Data Supplement).

Subsequent meningioma. The Clinical Model (AUC, 0.70; 95% CI, 0.60 to 0.79) performed better than the Base Model (AUC, 0.60; 95% CI, 0.50 to 0.70; $P = .07$). Using stepwise forward selection, we were able to retain the following five SNPs in the Genetic Model: rs15869 (BRCA2), rs1673041 (POLDD1), rs25489 (XRCC1), rs1801270 (CDKN1A), and rs828699 (XRCC5). The AUC for the Genetic Model was 0.82 (95% CI, 0.74 to 0.89; Fig 3B and Data Supplement) and was superior to the Clinical Model ($P = .01$). Replacing CRT dose as a binary variable ($< v \geq 24$ Gy) with CRT dose as a continuous variable or treating CRT as a different binary variable (exposed v unexposed) did not alter the findings (Data Supplement).

Risk groups. We defined the following three risk groups on the basis of the predictive values of the Final Model. For all subsequent tumors, 46 individuals (15%) were classified as high risk, 169 individuals (55%) as intermediate risk, and 95 individuals (30%) as low risk (Table 3). When restricting the risk prediction to only

Table 1. Characteristics of the Discovery Study Population

Variable	All Study Participants				Phases I and II				Phase III			
	Participants (n = 82)*		Controls (n = 228)		P		Glioma		Meningioma		Study Participants	
	Participants (n = 82)*	Controls (n = 228)	Participants (n = 28)	Controls (n = 93)	P	Matched	Participants (n = 46)	Controls (n = 104)	P	Matched	Participants (n = 25)	Controls (n = 54)
Race/ethnicity, No. (%)†												
Non-Hispanic white	65 (79.3)	191 (83.8)	20 (71.4)	73 (78.5)		Matched	38 (82.6)	88 (84.6)		Matched	19 (76)	48 (88.9)
Hispanic	8 (9.7)	19 (8.3)	4 (14.3)	11 (11.8)			4 (8.7)	7 (6.7)			2 (8)	2 (3.7)
Other	9 (11.0)	18 (7.9)	4 (14.3)	9 (9.7)			4 (8.7)	9 (8.7)			4 (16)	4 (7.4)
Median age at primary cancer diagnosis, years (range)	3.5 (0-21)	5 (0-20)	4.5 (0-15)	6 (0-20)	.05	.4	4 (0-21)	5 (0-16)	.1	.1	6.5 (0.2-15.1)	6.9 (0.2-19)
Median age at study participation, years (range)	22.5 (5-51)	19 (4-55)	16.5 (7-27)	16 (4-37)	.2	.2	28.5 (12-51)	25 (9-55)	.9	.9	18.7 (6-54)	22.9 (10-49)
Female sex, No. (%)	36 (43.9)	98 (42.9)	11 (39.3)	35 (37.6)	.8	.7	25 (54.4)	59 (56.7)	.9	.9	15 (60)	35 (64.8)
Primary diagnosis, No. (%)†												
Hodgkin lymphoma	1 (1.2)	2 (0.9)	0 (0)	0 (0)		Matched	1 (2.2)	2 (1.9)		Matched	1 (4)	2 (3.7)
Non-Hodgkin lymphoma	3 (3.7)	3 (1.3)	0 (0)	0 (0)			3 (6.5)	3 (2.9)			2 (8)	4 (7.4)
Acute lymphocytic leukemia	39 (47.6)	109 (47.8)	10 (35.7)	37 (39.8)			25 (54.4)	52 (50)			4 (16)	10 (18.5)
Acute myeloid leukemia	1 (1.2)	1 (0.4)	0 (0)	0 (0)			1 (2.2)	1 (0.96)			0 (0)	0 (0)
Brain cancer	24 (29.3)	76 (33.3)	12 (42.9)	37 (39.8)			11 (23.9)	36 (34.6)			14 (56)	30 (55.5)
Sarcoma	7 (8.5)	19 (8.3)	3 (10.7)	9 (9.7)			3 (6.5)	7 (6.7)			1 (4)	1 (1.9)
Other	7 (8.5)	18 (7.9)	3 (10.7)	10 (10.8)			2 (4.4)	3 (2.9)			3 (12)	7 (12.9)
Year of primary cancer diagnosis, No. (%)†												
≤ 1990	45 (54.9)	77 (33.8)	4 (14.3)	7 (7.5)		Matched	38 (82.6)	64 (61.5)		Matched	9 (36)	20 (37.0)
1991-2000	27 (32.9)	122 (53.5)	15 (53.6)	62 (66.7)			7 (15.2)	37 (35.6)			7 (28)	25 (46.3)
2001-2008	10 (12.2)	29 (12.7)	9 (32.1)	24 (25.8)			1 (2.2)	3 (2.9)			6 (24)	9 (16.7)
≥ 2009	0 (0)	0 (0)	0 (0)	0 (0)			0 (0)	0 (0)			3 (12)	0 (0)
Median length of follow-up, years (range)†	13.2 (1.9-36.9)	13.2 (2.4-40.3)	9.9 (2.2-26)	10.5 (2.4-27.2)			20.7 (4.2-6.9)	19.5 (4.2-40.3)			14.7 (2.9-50.5)	15.2 (5.04-42.1)
Received cranial radiation, No. (%)	75 (91.5)	90 (39.5)	23 (82.1)	34 (36.6)	< .001	< .001	45 (97.8)	50 (48.1)	< .001	< .001	18 (72)	30 (55.6)

NOTE. P values were obtained from conditional logistic regression or a generalized linear model taking into consideration the matched set. Study phases were as follows: phase I, association between candidate single nucleotide polymorphism (previously published in association with primary CNS tumors in adults) and subsequent CNS tumors; phase II, development of a risk prediction model; and phase III, external validation of the risk prediction model.

*The 82 participants with subsequent CNS tumors included 28 participants with subsequent glioma, 46 participants with subsequent meningioma, five participants with subsequent primitive neuroectodermal tumor, and three participants with subsequent schwannoma/neurosarcoma. †As a result of variation in the number of controls per participant, the percentages of controls and participants in each category of a specific matching variable may not be identical.

Table 2. Marginal Genetic Susceptibility in the Development of Subsequent CNS Tumors

SNP	Chromosome	Position	Effect Allele	MAF (participants)	MAF (controls)	Other Allele	All Subjects		Gene	Function	Study
							OR (95% CI)	P			
All subsequent CNS tumors											
rs15869	13	32398875	C*	0.32	0.19	A	2.5 (1.5 to 4.2)	< .001	BRCA2	UTR	Zhao et al ⁸
rs1805389	13	108211261	A*	0.09	0.04	G	3.7 (1.4 to 9.9)	.008	LIG4	Exon	Zhao et al ⁸
rs8079544	17	7676734	G	0.02	0.07	A*	4.0 (1.1 to 15.2)	.04	TP53	Intron	Bethke et al ^{9,10}
rs498872	11	118606652	G	0.25	0.31	A*	1.8 (1.0 to 3.3)	.06	PHLDB1	Exon	Walsh et al ¹¹
rs4295627	8	129673211	A	0.13	0.18	C*	1.7 (0.9 to 3.2)	.06	CCDC26	Intron	Wang et al ¹²
rs25489	19	43552260	G	0.02	0.07	A*	3.6 (1.1 to 11.5)	.03	XRCC1	Exon	Jacobs et al ¹³
rs1673041	19	50406132	A*	0.29	0.24	C	1.9 (1.1 to 3.1)	.01	POLD1	Intron	Bethke et al ^{9,10}
rs11615	19	45420395	A*	0.35	0.41	G	1.6 (1.0 to 2.5)	.04	ERCC1	Synonymous	Rodriguez-Hernandez, et al ¹⁴
rs828699	2	216123238	A*	0.53	0.44	C	1.5 (1.0 to 2.2)	.06	XRCC5	Intron	He et al ¹⁵
Subsequent glioma											
rs2909430	17	7675327	G*	0.15	0.05	A	7.5 (1.5 to 36.9)	.01	TP53	Intron	Bethke et al ^{9,10}
rs1805389	13	108211261	A*	0.13	0.04	G	19.7 (2.5 to 156.4)	.005	LIG4	Exon	Zhao et al ⁸
rs15869	13	32398875	C*	0.33	0.17	A	3.7 (1.3 to 10.2)	.01	BRCA2	UTR	Zhao et al ⁸
rs2518471	6	18148282	G*	0.1	0.04	A	3.9 (0.9 to 16.7)	.07	TPMT	Intron	Relling et al ¹⁶
Subsequent meningioma											
rs15869	13	32398875	C*	0.32	0.19	A	2.4 (1.2 to 4.9)	.01	BRCA2	UTR	Zhao et al ⁸
rs828699	2	216123238	A	0.59	0.45	C	1.7 (0.9 to 3.1)	.07	XRCC5	Intron	Rajaraman et al ¹⁷
rs25489	19	43552260	G	0.04	0.10	A*	6.9 (1.1 to 43.6)	.04	XRCC1	Exon	Kiuru et al ¹⁸
rs1801270	6	36684194	C	0.03	0.10	A*	9.6 (1.1 to 85.4)	.04	CDKN1A	Exon	Jin et al ¹⁹
rs1673041	19	50406132	A*	0.17	0.26	C	2.0 (1.0 to 3.8)	.04	POLD1	Intron	Bethke et al ^{9,10}

NOTE. P values were obtained using conditional logistical regression by adjusting for age at diagnosis, sex, and cranial radiotherapy. The genotypes of SNPs were coded in additive.

Abbreviations: MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphism; UTR, untranslated region.

*Minor allele in non-Hispanic whites.

those classified as high risk or low risk, the sensitivity of the prediction model was 87.5% and specificity was 83.5%; the PPV was 60.9% and the NPV was 95.8%. For subsequent glioma, the PPV was 68.8%, the NPV was 96%, and the sensitivity and specificity of the prediction model were 84.6% and 90.6%, respectively. For subsequent meningioma, the PPV was 68.6%, the NPV was 86.5%, and the sensitivity and specificity of the prediction model were 82.8% and 74.4%, respectively.

Phase III: Risk Prediction in Validation Cohort

We replicated five of the seven SNPs from the phase II Final Model (rs15869 [*BRCA2*], rs8079544 [*TP53*], rs498872 [*PHLDB1*], rs1673041 [*POLD1*], and rs828699 [*XRCC5*]). When these five SNPs were included in the Replication Final Model, the AUC was 0.89 and was superior to the Clinical Model (AUC, 0.73; $P = .07$; Data Supplement).

DISCUSSION

Approximately 30% of survivors of childhood cancer carry a history of exposure to CRT,¹ a major risk factor for subsequent CNS tumors. Interindividual variability in risk makes it important to determine the genetic factors that likely moderate the risk of radiation-related subsequent CNS tumors. Furthermore, the high burden of morbidity and mortality associated with CNS tumors makes it important to identify those at highest risk such that

intervention strategies can be personalized on the basis of individual risk. We curated a list of genetic variants previously identified to be associated with an increased risk of adult-onset primary CNS tumors and examined their association with subsequent CNS tumors in survivors of childhood cancer. We identified several intriguing SNPs, including rs15869 (*BRCA2*), rs1805389 (*LIG4*), rs25489 (*XRCC1*), rs1673041 (*POLD1*), rs8079544 (*TP53*), and rs11615 (*ERCC1*) associated with all subsequent CNS tumors; rs2909430 (*TP53*), rs1805389 (*LIG4*), and rs15869 (*BRCA2*) associated with subsequent gliomas; and rs15869 (*BRCA2*), rs25489 (*XRCC1*), rs1801270 (*CDKN1A*), and rs1673041 (*POLD1*) associated with subsequent meningioma. We found that the prediction model that included clinical and genetic variables (Final Model) performed better than either the Clinical Model or the Genetic Model, with AUCs consistently exceeding 80%. Importantly, we were able to validate the prediction model in an independent set of survivors of cancer.

SNP rs15869 on *BRCA2* was associated with an increased risk of both glioma and meningioma. *BRCA2* interacts with *RAD51*, a central player in homologous recombination by targeting *RAD51* to single-stranded DNA while inhibiting binding to double-stranded DNA. These reinforcing activities of *BRCA2* culminate in the correct positioning of *RAD51* onto a processed DNA double-strand break to initiate its faithful repair by homologous recombination.²⁰ The association between *BRCA2* and subsequent CNS tumors is likely a result of exposure to DNA-damaging agents (radiation therapy) in the presence of impaired DNA repair mechanism.

Fig 2. Receiver operating characteristic curves from the risk prediction models for subsequent CNS tumors.

We also found an association between rs1805389 on gene *LIG4* and subsequent CNS tumors and glioma. DNA ligase IV, or *LIG4*, works with the x-ray repair cross-complementing group 4 (*XRCC4*) in the nonhomologous end-joining DNA repair pathway.²¹ Upon recognition of a double-strand break, the *LIG4/XRCC4* complex is recruited to perform the end-joining reaction. Mouse embryonic cells with disruption of *LIG4* or *XRCC4* show reduced proliferation, radiation hypersensitivity, chromosomal instability, and severely impaired V(D)J recombination.²²

We found an association between rs25489 on gene *XRCC1* and risk of subsequent CNS tumors and of meningioma. Base excision repair is the predominant DNA repair pathway for small base lesions resulting from oxidation and alkylation damage.²³ *XRCC1* is an important component of base excision repair; the *XRCC1* gene encodes a scaffolding protein that coordinates numerous protein-protein interactions with DNA ligase III and DNA polymerase at the site of damage.²⁴

We also identified an association between SNP rs2909430 on the *TP53* gene and glioma risk. The *TP53* gene is at the crossroads of cellular pathways including cell cycle checkpoints, DNA repair, and apoptosis. These pathways maintain stability of the genome during cellular stress from DNA damage. *TP53* mutation is one of the most frequent genetic alterations in primary glioma,²⁵ and several studies have reported associations between *TP53* polymorphisms and primary glioma risk.^{26,27}

A previous study found a higher incidence of subsequent CNS tumors in patients with childhood acute lymphoblastic leukemia with defective *TPMT* (42.9% at 8 years) compared with wild-type *TPMT* (8.3% at 8 years; $P = .008$).¹⁶ In our study, we found that *TPMT* polymorphism (rs2518471) was retained as one of the three SNPs in the risk prediction model for subsequent glioma. Children with neurofibromatosis type 1 are at risk for both primary and subsequent CNS tumors^{28,29}; however, we found no associations between 22 published SNPs on the *NF1* gene and risk of subsequent CNS tumors.

Identifying survivors at highest risk may provide a targeted approach to intensified screening and early detection of subsequent CNS tumors, thus potentially reducing morbidity and mortality. For prediction of any CNS tumor, glioma, or meningioma after childhood cancer, the Final Model (AUC, 0.81, 0.85, and 0.82, respectively) performed significantly better than the Clinical Model (AUC, 0.73, 0.76, and 0.70, respectively). We found that inclusion of CRT as a binary variable (CRT dose $< v \geq 24$ Gy or CRT as yes v no) or as a continuous variable did not materially alter our findings. In fact, the Clinical Model had the highest AUCs with CRT as a continuous variable, likely a reflection of the small sample. When restricting the risk prediction to only those classified as high risk or low risk, the sensitivity of the prediction model was 87.5%, specificity was 83.5%, PPV was 60.9%, and NPV was 95.8%. Currently, we recommend annual history and physical examination for survivors of childhood cancer exposed to CRT.³⁰ However, among survivors identified to be at highest risk of subsequent CNS tumors, screening could be intensified (eg, with periodic imaging), thus reducing morbidity and mortality associated with delayed diagnosis of subsequent CNS tumors. Whether

Fig 3. (A) Receiver operating characteristic curves from the risk prediction models for subsequent glioma. (B) Receiver operating characteristic curves from the risk prediction models for subsequent meningioma.

Table 3. Risk Group Comparisons for Subsequent CNS Tumors

Predicted Probability Group	Development of Subsequent CNS Tumor (No.)		OR (95% CI)	P
	Yes (participants)	No (controls)		
Phase II: All subsequent CNS tumors				
High-risk group (n = 46); predicted probability > .5)	28	18	23.2 (5.29 to 101.4)	< .001
CRT ≥ 24 Gy	26	16		
CRT < 24 Gy	2	2		
Intermediate-risk group (n = 169; .1 ≤ predicted probability ≤ .5)	50	119	8.6 (2.5 to 29.6)	< .001
CRT ≥ 24 Gy	29	51		
CRT < 24 Gy	21	68		
Low-risk group (n = 95; predicted probability < .1)	4	91	1.0	—
CRT ≥ 24 Gy	0	7		
CRT < 24 Gy	3	84		
Phase II: Subsequent gliomas				
High-risk group (n = 16; predicted probability > .5)	11	5	18.3 (2.0 to 163.6)	.009
CRT ≥ 24 Gy	11	5		
CRT < 24 Gy	0	0		
Intermediate-risk group (n = 55; .1 ≤ predicted probability ≤ .5)	15	40	5.5 (1.1 to 28.1)	.04
CRT ≥ 24 Gy	9	20		
CRT < 24 Gy	6	20		
Low-risk group (n = 50; predicted probability < .1)	2	48	1.0	—
CRT ≥ 24 Gy	0	3		
CRT < 24 Gy	2	45		
Phase II: Subsequent meningioma				
High-risk group (n = 35; predicted probability > .5)	24	11	18.7 (3.3 to 105.1)	< .001
CRT ≥ 24 Gy	19	9		
CRT < 24 Gy	5	2		
Intermediate-risk group (n = 78; .1 ≤ predicted probability ≤ .5)	17	61	1.99 (0.5 to 7.6)	.3
CRT ≥ 24 Gy	11	25		
CRT < 24 Gy	6	36		
Low-risk group (n = 37; predicted probability < .1)	5	32	1.0	—
CRT ≥ 24 Gy	1	7		
CRT < 24 Gy	3	25		

Abbreviations: CRT, cranial radiotherapy; OR, odds ratio.

patients identified as low risk could receive higher radiation doses without incurring the risk of subsequent CNS tumors requires additional investigation.

This study has to be placed in the context of its limitations. First, we used a prevalent case-control study design, which excludes fatal end points from the participant set. Presence of survival bias has the potential for underascertainment of genotypes associated with high lethality, with consequent underestimation of the effect size for genotypes associated with both increased disease risk and disease-associated lethality.³¹ However, because it is logistically impossible to prove this within the context of a large multi-institutional study, we do acknowledge the possibility that participants with a high-risk allele in the candidate genes may have been more likely to have developed brain tumors and died, thus becoming lost from the sampling frame and eroding the true association between high-risk variants and subsequent CNS tumors. The relatively small sample size when examining individual tumor type (meningioma and glioma) also likely limited the ability to identify additional associations. The validation case-control set was largely comparable to the discovery case-control set, but there were subtle differences. In the discovery set, participants were significantly more likely to have received CRT when compared with controls. In the validation set, although patients were more likely to have received CRT than controls, the difference did not reach statistical significance, in part because of

a smaller validation sample and also because the difference in proportion exposed to CRT was smaller. Nonetheless, after adjusting for CRT, we were able to replicate the findings. These limitations notwithstanding, this study represents, to our knowledge, the first attempt to understand the role of genetic variants in moderating the risk of radiation-related subsequent CNS tumors and to incorporate the genetic profile in identifying those at high risk for this outcome.

Using a carefully curated list of genetic variants previously investigated in determining primary, adult-onset glioma or meningioma risk, we demonstrated that several candidate SNPs involved with DNA damage response modify the risk of radiation-related subsequent CNS tumors. Our findings also demonstrate that it is possible to discriminate between individuals at high and low risk for subsequent CNS tumors on the basis of genetic information in combination with clinical factors. This information can be used to inform post-treatment surveillance.

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

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REFERENCES

1. Bowers DC, Nathan PC, Constine L, et al: Subsequent neoplasms of the CNS among survivors of childhood cancer: A systematic review. *Lancet Oncol* 14:e321-e328, 2013
2. Morris EB, Gajjar A, Okuma JO, et al: Survival and late mortality in long-term survivors of pediatric CNS tumors. *J Clin Oncol* 25:1532-1538, 2007
3. Neglia JP, Robison LL, Stovall M, et al: New primary neoplasms of the central nervous system in survivors of childhood cancer: A report from the Childhood Cancer Survivor Study. *J Natl Cancer Inst* 98:1528-1537, 2006
4. Taylor AJ, Little MP, Winter DL, et al: Population-based risks of CNS tumors in survivors of childhood cancer: The British Childhood Cancer Survivor Study. *J Clin Oncol* 28:5287-5293, 2010
5. Flint-Richter P, Sadetzki S: Genetic predisposition for the development of radiation-associated meningioma: An epidemiological study. *Lancet Oncol* 8:403-410, 2007
6. Adel Fahmideh M, Schwartzbaum J, Frumentio P, et al: Association between DNA repair gene polymorphisms and risk of glioma: A systematic review and meta-analysis. *Neuro Oncol* 16:807-814, 2014
7. Purcell S, Neale B, Todd-Brown K, et al: PLINK: A tool set for whole-genome association and population-based linkage analyses. *Am J Hum Genet* 81:559-575, 2007
8. Zhao P, Zou P, Zhao L, et al: Genetic polymorphisms of DNA double-strand break repair pathway genes and glioma susceptibility. *BMC Cancer* 13:234, 2013
9. Bethke L, Murray A, Webb E, et al: Comprehensive analysis of DNA repair gene variants and risk of meningioma. *J Natl Cancer Inst* 100:270-276, 2008
10. Bethke L, Webb E, Murray A, et al: Comprehensive analysis of the role of DNA repair gene polymorphisms on risk of glioma. *Hum Mol Genet* 17:800-805, 2008
11. Walsh KM, Rice T, Decker PA, et al: Genetic variants in telomerase-related genes are associated with an older age at diagnosis in glioma patients: Evidence for distinct pathways of gliomagenesis. *Neuro Oncol* 15:1041-1047, 2013
12. Wang SS, Hartge P, Yeager M, et al: Joint associations between genetic variants and reproductive factors in glioma risk among women. *Am J Epidemiol* 174:901-908, 2011
13. Jacobs DI, Bracken MB: Association between XRCC1 polymorphism 399 G->A and glioma among Caucasians: A systematic review and meta-analysis. *BMC Med Genet* 13:97, 2012
14. Rodriguez-Hernandez I, Perdomo S, Santos-Briz A, et al: Analysis of DNA repair gene polymorphisms in glioblastoma. *Gene* 536:79-83, 2014
15. He X, Zhu X, Li L, et al: The relationship between polymorphisms of XRCC5 genes with astrocytoma prognosis in the Han Chinese population. *Oncotarget* 7:85283-85290, 2016
16. Relling MV, Rubnitz JE, Rivera GK, et al: High incidence of secondary brain tumours after radiotherapy and antimetabolites. *Lancet* 354:34-39, 1999
17. Rajaraman P, Melin BS, Wang Z, et al: Genome-wide association study of glioma and meta-analysis. *Hum Genet* 131:1877-1888, 2012
18. Kiuru A, Lindholm C, Heinävaara S, et al: XRCC1 and XRCC3 variants and risk of glioma and meningioma. *J Neurooncol* 88:135-142, 2008
19. Jin TB, Zhang JY, Li G, et al: RTEL1 and TERT polymorphisms are associated with astrocytoma risk in the Chinese Han population. *Tumour Biol* 34:3659-3666, 2013
20. Jensen RB: BRCA2: One small step for DNA repair, one giant protein purified. *Yale J Biol Med* 86:479-489, 2013
21. Lieber MR, Ma Y, Pannicke U, et al: Mechanism and regulation of human non-homologous DNA end-joining. *Nat Rev Mol Cell Biol* 4:712-720, 2003
22. Barnes DE, Stamp G, Rosewell I, et al: Targeted disruption of the gene encoding DNA ligase IV leads to lethality in embryonic mice. *Curr Biol* 8:1395-1398, 1998
23. Almeida KH, Sobol RW: A unified view of base excision repair: Lesion-dependent protein complexes regulated by post-translational modification. *DNA Repair (Amst)* 6:695-711, 2007
24. Vidal AE, Boiteux S, Hickson ID, et al: XRCC1 coordinates the initial and late stages of DNA abasic site repair through protein-protein interactions. *EMBO J* 20:6530-6539, 2001
25. Ohgaki H, Kleihues P: Genetic pathways to primary and secondary glioblastoma. *Am J Pathol* 170:1445-1453, 2007
26. Stacey SN, Sulem P, Jonasdottir A, et al: A germline variant in the TP53 polyadenylation signal confers cancer susceptibility. *Nat Genet* 43:1098-1103, 2011
27. Shi M, Huang R, Pei C, et al: TP53 codon 72 polymorphism and glioma risk: A meta-analysis. *Oncol Lett* 3:599-606, 2012
28. Sharif S, Ferner R, Birch JM, et al: Second primary tumors in neurofibromatosis 1 patients treated for optic glioma: Substantial risks after radiotherapy. *J Clin Oncol* 24:2570-2575, 2006
29. Cnossen MH, Stam EN, Cooman LC, et al: Endocrinologic disorders and optic pathway gliomas in children with neurofibromatosis type 1. *Pediatrics* 100:667-670, 1997
30. Landier W, Bhatia S, Eshelman DA, et al: Development of risk-based guidelines for pediatric cancer survivors: The Children's Oncology Group long-term follow-up guidelines from the Children's Oncology Group Late Effects Committee and Nursing Discipline. *J Clin Oncol* 22:4979-4990, 2004
31. Hernán MA, Hernández-Díaz S, Robins JM: A structural approach to selection bias. *Epidemiology* 15:615-625, 2004

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AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

Clinical and Genetic Risk Prediction of Subsequent CNS Tumors in Survivors of Childhood Cancer: A Report From the COG ALTE03N1 Study

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