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Variants at 6q21 implicate *PRDM1* in the etiology of therapy-induced second malignancies after Hodgkin's lymphoma

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Survivors of pediatric Hodgkin's lymphoma are at risk for radiation therapy–induced second malignant neoplasms (SMNs). We identified two variants at chromosome 6q21 associated with SMNs in survivors of Hodgkin's lymphoma treated with radiation therapy as children but not as adults. The variants comprise a risk locus associated with decreased basal expression of *PRDM1* (encoding PR domain containing 1, with ZNF domain) and impaired induction of the PRDM1 protein after radiation exposure. These data suggest a new gene-exposure interaction that may implicate PRDM1 in the etiology of radiation therapy-induced SMNs.

Patients treated successfully for Hodgkin's lymphoma in childhood develop SMNs, with a cumulative incidence of 18.4% by 30 years after treatment and an absolute excess risk of 6.9 per 1,000 person-years of follow-up[1]. This high prevalence makes SMNs the second leading cause of mortality in Hodgkin's lymphoma survivors. SMNs primarily affect organs in the involved mediastinal radiation therapy field, including the thyroid, skin, gastrointestinal tract and female breast[2,3]. Risk is positively associated with cumulative radiation dose and inversely correlated with age at treatment[4,5].

Despite the clinical importance of this devastating late consequence of radiation therapy exposure, little is known about predisposing risk factors. We performed a genome-wide association study (GWAS) to identify variants associated with radiation therapy–induced SMNs in Hodgkin's lymphoma survivors. In studies of sporadic cancers, nongenetic heterogeneity can obscure genetic associations[6], but here radiation therapy exposure is common to patients with Hodgkin's lymphoma who did and did not develop SMNs. Thus, we hypothesized that limiting our study to radiation therapy–treated survivors would improve our power to detect the genetic contribution to SMN risk.

The discovery set consisted of 100 SMN cases and 89 SMN-free controls (**Supplementary Table 1a** and **Supplementary Table 2**). All cases and controls were diagnosed with Hodgkin's lymphoma as children (median age: 15.6, range: 8–20) and treated with 25–44 Gy radiation therapy with or without alkylating chemotherapy[7]. Cases developed SMNs with a mean latency of 20.0 years (s.d. = 5.8 years, range: 6–34). Controls were followed for at least 27 years (median: 32 years, range: 27–38) to ensure that the maximal contamination of controls by future cases was <2%. The **Supplementary Methods** contain a detailed description of the study populations and experimental protocols.

After genotype quality control, we successfully genotyped 665,313 single nucleotide polymorphisms (SNPs) in 96 cases and 82 controls. We compared allele frequencies between cases and controls using a Chi-square test of homogeneity. A quantile-quantile plot of the expected and observed distribution of *P* values revealed no evidence for systematic genotype calling error or hidden population substructure (genomic control $\lambda = 1.007$) (**Supplementary Fig. 1**)[8]. Principal component analysis using Eigenstrat indicated cases and controls were of European descent (**Supplementary Fig. 2**)[9].

We empirically determined the threshold for a genome-wide false positive rate of 0.05 by permutation ($P < 1.0 \times 10^{-7}$). At this threshold, our study had 80% power to detect a SNP with a frequency of 35% and an odds ratio (OR) of 3.5 (**Supplementary Fig. 3**). Three SNPs (rs4946728, rs1040411 and rs8083533) achieved genome-wide significance (**Supplementary Fig. 4** and **Table 1**). rs4946728 and rs1040411 mapped to chromosome 6q21, intergenic between *ATG5* (encoding autophagy protein 5) and *PRDM1*. The strongest evidence for association in this region was for rs4946728 ($P = 1.09 \times 10^{-8}$, $OR_{allelic} = 4.22$ (95% confidence interval (CI) = 2.53–7.05)). rs8083533 mapped to 18q11.2, intronic to *TAF4B* (encoding transcription initiation factor TFIID subunit 4B) ($P = 4.98 \times 10^{-8}$, $OR_{allelic} = 3.78$ (95% CI = 2.31–6.18)). Logistic regression, adjusting for gender, age at diagnosis, year of Hodgkin's lymphoma diagnosis, gonadal radiation (in females) and alkylating chemotherapy exposure, indicated that these risk variables had no effect on the observed associations (**Supplementary Table 3**).

We sought to replicate these findings in an independent set of 62 cases with SMNs and 71 SMN-free controls, all treated for Hodgkin's lymphoma in childhood with 25–44 Gy mediastinal radiation therapy (**Supplementary Table 1b**). We observed significant associations with SMNs for both SNPs on chromosome 6q21, rs4946728 ($P = 0.002$) and rs1040411 ($P = 0.03$), but not for rs8083533 ($P = 0.82$) (**Table 1**). In the

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Table 1 Association of SNPs with SMNs after Hodgkin's lymphoma treatment

SNP	Risk allele	Genotype count[a]			OR[b]			*P* value[e]
		Cases	Controls	Stage	Per allele	Heterozygote[c]	Homozygote[d]	
rs4946728[f]	C	2/23/71	12/43/27	Discovery[g]	4.22 (2.53–7.05)	3.21 (0.66–15.59)	15.78 (3.31–75.18)	1.09×10^{-8}
		1/17/43	6/32/33	Replication[h]	2.43 (1.33–4.46)	3.19 (0.35–28.69)	7.82 (0.90–68.14)	0.002
		3/40/114	18/75/60	Combined[i]	3.32 (2.25–4.90)	3.20 (0.89–11.52)	11.4 (3.23–40.25)	5.99×10^{-10}
rs1040411[f]	T	7/47/42	27/45/10	Discovery	3.27 (2.11–5.06)	4.03 (1.60–10.17)	16.2 (5.50–47.71)	6.43×10^{-8}
		9/30/22	19/33/18	Replication	1.59 (0.97–2.59)	1.92 (0.75–4.89)	2.58 (0.94–7.07)	0.03
		16/77/64	46/78/28	Combined	2.39 (1.73–3.30)	2.84 (1.48–5.44)	6.57 (3.19–13.52)	1.18×10^{-7}
rs8083533	T	21/44/31	4/21/57	Discovery	3.78 (2.31–6.18)	2.51 (0.76–8.23)	9.65 (3.04–30.65)	4.98×10^{-8}
		2/31/26	8/33/29	Replication	0.89 (0.52–1.53)	0.27 (0.05–1.35)	0.28 (0.05–1.43)	0.82
		23/75/57	12/54/86	Combined	1.86 (1.32–2.62)	1.38 (0.63–3.01)	2.89 (1.33–6.27)	4.00×10^{-4}

[a]Number of individuals genotyped as homozygous for the protective allele/heterozygous/homozygous for the risk allele. [b]OR (95% CI). [c]OR for heterozygous carriage of risk allele compared to homozygous carriage of protective allele. [d]OR for homozygous carriage of risk allele compared to homozygous carriage of protective allele. [e]Two-sided X^2 *P* value for discovery and combined sets; one-sided for replication. [f]rs4946728 and rs1040411 are in linkage disequilibrium ($r^2 = 0.4$). [g]Discovery set: 96 cases and 82 controls. [h]Replication set: 62 cases and 71 controls. [i]Combined set: 158 cases and 153 controls.

combined set, the odds of an SMN were increased over threefold per copy of the major allele for rs4946728 ($OR_{allelic} = 3.32$ (95% CI = 2.25–4.90), combined $P = 5.99 \times 10^{-10}$) and over twofold for rs1040411 ($OR_{allelic} = 2.39$ (95% CI = 1.73–3.30), combined $P = 1.18 \times 10^{-7}$).

We found no evidence that the association of rs4946728 and rs1040411 differed between breast cancer and other SMNs (Breslow-Day test, $P = 0.41$ for rs4946728 and $P = 0.58$ for rs1040411) or between males and females ($P = 0.83$ for rs4846728 and $P = 0.29$ for rs1040411) (**Supplementary Table 4a**,**b**). To determine whether rs4946728 or rs1040411 were associated with SMNs after adult Hodgkin's lymphoma, we genotyped both SNPs in 57 SMN cases and 37 controls who were treated with radiation therapy as adults (median age: 24.0, range: 21–43) (**Supplementary Table 1b**). We did not observe an association for either rs4946728 ($P = 0.87$) or rs1040411 ($P = 0.65$) (**Supplementary Table 5**), suggesting that age of radiation therapy–exposure modifies the association between these variants and SMN risk. However, these results should be interpreted with caution, given the small number of individuals genotyped.

Both rs4946728 and rs1040411 ($r^2 = 0.4$) are noncoding SNPs located between *PRDM1* and *ATG5* on chromosome 6q21 (**Fig. 1a**). Imputation of the locus with the 1000 Genomes reference panel[10] did not reveal any variant with a stronger association than either genotyped SNP (**Supplementary Table 6**). Logistic regression conditioning on rs4946728 revealed a modest residual association for rs1040411 ($P = 0.05$) (**Supplementary Table 7**), suggesting that an unobserved causal variant may be correlated with a haplotype harboring both SNPs.

rs4946728 and rs1040411 form three common haplotypes in individuals of European descent that represent 99.9% of the haplotypes at this locus (**Supplementary Table 8**). As noncoding risk variants frequently regulate gene expression[11], we performed expression quantitative trait locus analysis to determine whether these haplotypes were associated with expression of *PRDM1*, *ATG5* or other genes within five megabases. We found that increasing dosage of the risk haplotype (comprised of the risk alleles for both rs4946728 and rs1040411) was significantly associated with lower *PRDM1* mRNA expression ($P = 0.03$) (**Supplementary Fig. 5**). In contrast, we observed no association with expression for any other gene, including *ATG5* ($P = 0.39$).

Because SMNs after Hodgkin's lymphoma are caused by radiation exposure, we investigated the relationship between ionizing radiation exposure and PRDM1 protein abundance in cell lines homozygous for either the risk haplotype ($n = 4$) or the protective haplotype (comprised of the protective alleles for both rs4946728 and rs1040411, $n = 4$). In untreated cells, PRDM1 was more abundant in cells homozygous for the protective haplotype than in cells homozygous for the risk haplotype ($P = 0.048$) and was significantly induced within 2 h of ionizing radiation exposure ($P = 0.020$) (**Fig. 1b**). Strikingly, PRDM1 was not induced by ionizing radiation in cells homozygous for the risk haplotype ($P = 0.19$).

PRDM1 (also known as *BLIMP1*; Online Mendelian Inheritance in Man 603423), encodes a zinc finger transcriptional repressor involved in a variety of cellular processes including proliferation, differentiation and apoptosis[12]. It was recently shown to be a tumor suppressor in activated B cell–like diffuse large B cell lymphoma[13,14] and is frequently lost in many cancer types, including solid tumors[15]. Of note, loss of heterozygosity at chromosome 6q was found to be significantly more common in breast cancers after radiation therapy for Hodgkin's lymphoma than in sporadic breast cancers (42% versus 10%), suggesting this region may be targeted for loss in these radiation therapy–induced cancers[16].

PRDM1 negatively regulates proproliferative genes such as *MYC*[17]. Therefore, we investigated whether the 6q21 variants were associated with repression of *MYC* by radiation concomitant with PRDM1 induction. Although basal MYC protein levels did not correlate with carriage of the 6q21 risk haplotype ($P = 0.19$), MYC was significantly more repressed after ionizing radiation exposure in cells homozygous for the protective haplotype than in cells homozygous for the risk haplotype ($P = 0.02$) (**Supplementary Fig. 6**).

In summary, these data indicate that variants at 6q21 are strongly associated with risk for SMNs after radiation therapy treatment for Hodgkin's lymphoma in childhood and suggest that common variants can have large effect sizes in the context of specific exposures. The SNPs we identified are associated with basal and radiation-induced PRDM1 expression, as well as radiation-induced MYC repression. Taken together, our findings support a previously unknown role for PRDM1 as a radiation-responsive tumor suppressor. However, we cannot rule out either long-range effects of these variants on other genes or tissue-specific differences in PRDM1 function. Additionally, the observation that SNPs intergenic between *PRDM1* and *ATG5* are associated with autoimmune disease[18,19] raises the possibility that altered immune function or inflammation may be associated with SMN risk. Although the radiation therapy doses used currently to treat Hodgkin's lymphoma are considerably lower than the radiation therapy doses used to treat the children analyzed in this study, recent data indicate that children treated with lower-dose radiation therapy for Hodgkin's lymphoma remain at risk for SMNs[20]. Thus, our findings may be crucial for understanding the etiology of SMNs in these pediatric Hodgkin's lymphoma survivors, as well as in other cancer patients treated with radiation therapy.

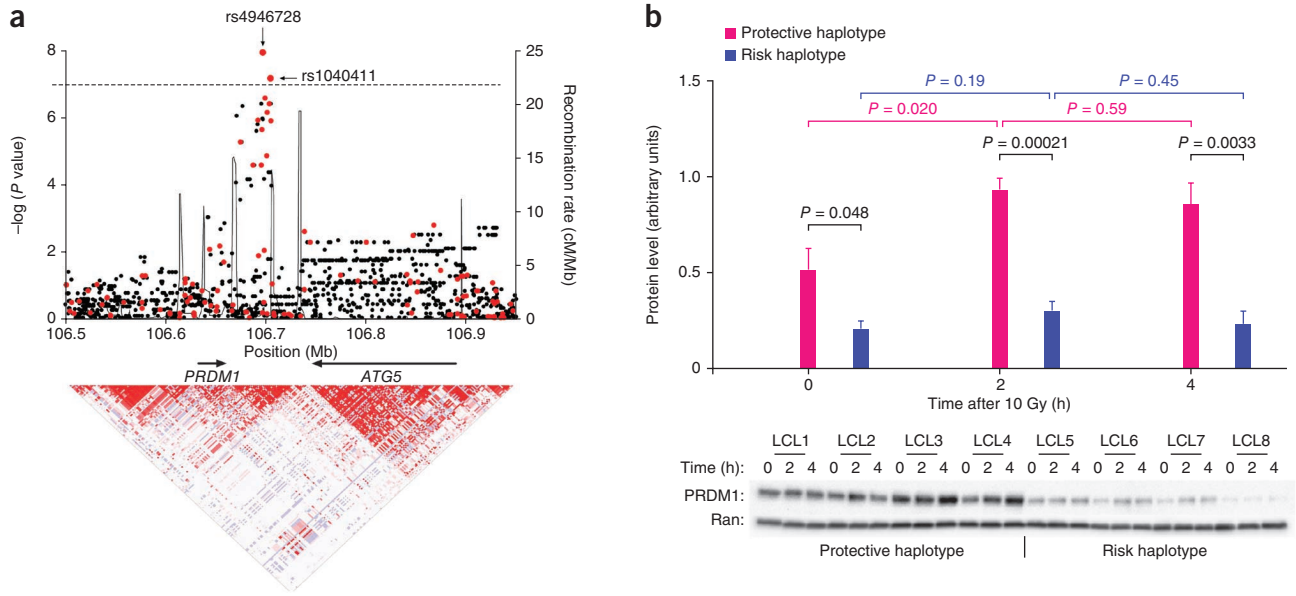

Figure 1 Variants at 6q21 are associated with both radiation therapy–induced SMNs and PRDM1 protein abundance before and after radiation exposure. (**a**) Regional association plot of the 6q21 locus. The –log (*P* value) for SNPs in this region is shown with respect to genomic position. Genotyped SNPs are in red; imputed SNPs are in black. The line at –log (*P*) = 7 denotes the threshold for genome-wide significance. Recombination rates (centimorgans per megabase, taken from HapMap) and genes within this region are also shown (top). Linkage disequilibrium structure based on D′ values for the GWAS data are shown (bottom). National Center for Biotechnology Information build 36 was used for all map locations. (**b**) Western blot analysis over time for PRDM1 in eight lymphoblastoid cell lines (LCLs), four homozygous for the chromosome 6q21 protective haplotype and four homozygous for the risk haplotype, treated with 10 Gy gamma irradiation. PRDM1 protein levels were quantified relative to Ran loading control.

Note: Supplementary information is available on the Nature Medicine website.

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AUTHOR CONTRIBUTIONS

T.B. and K. Onel designed the study and wrote the manuscript with substantial contributions from A.D.S., Y.Y., S.B., L.C.S., R.S.H., T.M.M., D.V.C., K. Offit, W.C. and L.L.R.; T.B. performed the experiments and undertook the analysis; D.L., A.D.S., T.K., and Y.Y. performed data analysis; S.A.J., S.M.D., K.L.N., O.I.O., W.C. and L.L.R. provided clinical samples and performed analysis of subject data; K. Onel directed the project. All authors contributed to the final manuscript.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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