Variants at 6q21 implicate \( \text{PRDM1} \) 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 \( \text{PRDM1} \) (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–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 \( \text{ATG5} \) (encoding autophagy protein 5) and \( \text{PRDM1} \). The strongest evidence for association in this region was for rs4946728 (\( P = 1.09 \times 10^{-8} \), OR\(_{\text{allelic}} = 4.22 \) (95% confidence interval (CI) = 2.53–7.05)). rs8083533 mapped to 18q11.2, intronic to \( \text{TAF4B} \) (encoding transcription initiation factor TFIIId subunit 4B) (\( P = 4.98 \times 10^{-8} \), OR\(_{\text{allelic}} = 3.78 \) (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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combined set, the odds of an SMN were increased over threefold per copy of the major allele for rs4946728 (OR\textsubscript{allelic} = 3.32 (95% CI: 2.25–4.90), combined P = 5.99 × 10\textsuperscript{-10}) and over twofold for rs1040411 (OR\textsubscript{allelic} = 2.39 (95% CI: 1.73–3.30), combined P = 1.18 × 10\textsuperscript{-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\textsuperscript{2} = 0.4) are noncoding SNPs located between PRDM1 and ATG5 on chromosome 6q21 (Fig. 1a). Imputation of the locus with the 100 Genomes reference panel\textsuperscript{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\textsuperscript{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 expression of 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\textsuperscript{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\textsuperscript{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.

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

<table>
<thead>
<tr>
<th>SNP</th>
<th>Risk allele</th>
<th>Genotype count\textsuperscript{a}</th>
<th>OR\textsuperscript{b}</th>
<th>P value\textsuperscript{b}</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs4946728</td>
<td>C</td>
<td>Cases: 2/37, Controls: 12/43/27</td>
<td>Discovery\textsuperscript{d}</td>
<td>4.22 (2.53–7.05)</td>
</tr>
<tr>
<td>rs1040411</td>
<td>T</td>
<td>Discovery: 7/47/42, Replication\textsuperscript{b}</td>
<td>3.27 (2.11–5.06)</td>
<td>6.43 × 10\textsuperscript{-8}</td>
</tr>
<tr>
<td>rs4946728</td>
<td>T</td>
<td>Combined: 3/40/114, 17/85/60</td>
<td>Combined: 3.32 (2.25–4.90)</td>
<td>5.99 × 10\textsuperscript{-10}</td>
</tr>
<tr>
<td>rs4946728</td>
<td>T</td>
<td>Replication: 16/77/64, 46/78/28</td>
<td>Combined: 3.29 (1.73–3.30)</td>
<td>1.18 × 10\textsuperscript{-7}</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Number of individuals genotyped as homozygous for the protective allele/heterozygous/homozygous for the risk allele.

\textsuperscript{b}OR for homozygous carriage of risk allele compared to combined set, the odds of an SMN were increased over threefold per copy of the major allele for rs4946728 (OR\textsubscript{allelic} = 3.32 (95% CI: 2.25–4.90), combined P = 5.99 × 10\textsuperscript{-10}) and over twofold for rs1040411 (OR\textsubscript{allelic} = 2.39 (95% CI: 1.73–3.30), combined P = 1.18 × 10\textsuperscript{-7}).
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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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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