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Genetic instability and development of second primary tumors after Hodgkin lymphoma

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ABSTRACT

Hodgkin lymphoma (HL) is a highly curable disease owing to remarkable advances in treatment. However, potential long-term complications of the disease, such as the development of second primary tumors (SPTs), are a major concern for survivors of HL. In the present study, we used cytogenetic biomarkers to identify HL patients at risk of SPTs. Our study cohort consisted of 251 HL patients; the mean follow-up time was 17.3 years. The mean number of chromatid breaks in patients who developed SPTs (3.52 ± 0.43) was significantly higher than that in patients who did not develop SPTs (2.43 ± 0.13; $P<0.01$). Chromosome aberration level and patient age at diagnosis were predictors of SPT development. G-banding revealed that patients who developed SPTs had significantly more structural abnormalities involving chromosomes 2, 4, 11, 14, and X than did patients who did not develop SPTs. Structural changes in chromosome 5 occurred exclusively in HL patients with no SPTs, whereas chromosome 9 and 16 involvement were present exclusively in HL patients with SPTs. Although patients with and patients without SPTs had t(8;14) and t(11;14) chromosomal translocations, the frequency of chromosomal translocations was significantly higher in patients with SPTs than in patients without SPTs. This novel finding suggests that structural chromosome changes in HL patients who develop SPTs are not randomly distributed. Therefore, chromosome aberration analysis may have a role in identifying HL patients at high risk of SPTs and thus, the potential to improve early detection and prevention strategies.

KEYWORDS: Hodgkin's lymphoma, genetic instability, second cancers

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INTRODUCTION

Hodgkin lymphoma (HL) is a neoplasm of the lymphoid tissue. The American Cancer Society estimated that about 8,510 new patients were diagnosed with HL and 1,290 people died from HL in the United States in 2010 [1]. Although research in past decades has yielded many new insights into its biology, HL remains a cancer of unknown etiology.

Integrated chemotherapy and radiotherapy has made HL a largely curable cancer [2]. The current standard of care in patients with early-stage HL has resulted in a 10-year overall survival rate of approximately 90% [3]. Several studies have demonstrated that the cumulative incidence of mortality from HL diminishes with further long-term follow-up; however, mortality from other causes continues to be significantly higher than expected even 15-20 years after the initial HL diagnosis [4; 5]. This is mainly due to late effects such as second primary tumors (SPTs) [6] and cardiovascular events [7], which account for more overall mortality than from failure of HL therapy.

HL survivors have a 7- to 18-fold higher risk of developing SPTs than survivors of other cancers [8; 9]. The estimated 30-year cumulative risk of second malignancy is approximately 18% in men and 26% in women with HL but only 7% in men and 9% in women in the general population [10]. A better understanding of the underlying factors contributing to the development of SPTs could improve early detection strategies and prevention interventions. The most frequently reported SPTs in HL patients are acute myeloid leukemia, non-Hodgkin lymphoma (NHL), and solid tumors such as breast, thyroid, cervical, and lung cancers [11]. Multiple studies have reported that HL patients treated primarily with chemotherapy or radiotherapy develop SPTs [12]. Patients treated with radiotherapy have a higher risk of developing SPTs than patients treated with chemotherapy; the use of large radiation fields and/or combined-modality therapy may further increase this risk. SPTs are sometimes considered a consequence of primary tumor treatment; however the risk of SPT development in HL survivors seems to exceed the effect of therapy alone. In addition to radiation and chemotherapy, a number of potential factors can either contribute to or modify the risk of development of SPTs such as genetic predisposition, hormonal factors, immune function and environmental exposures [13]. Several studies have described the development of SPTs in HL patients treated with chemotherapeutic regimens that do not contain alkylating agents, which have been implicated in increasing the risk for SPTs. These findings suggest that some HL patients may be genetically predisposed to developing SPTs regardless of HL treatment [9; 14]. Such a predisposition may be due in part to an inadequate constitutional capacity to respond to mutagenic or clastogenic insults that drive the cell to chromosome instability, thus contributing to the carcinogenic process [15]. This instability may manifest in the form of cytogenetically distinct clones and aneuploid subclones that are higher in patients predisposed to SPTs than that in patients who are not predisposed to SPTs.

Chromosome instability, as measured by chromosome aberrations, has long been suggested to be a biomarker for cancer susceptibility [16]. Chromosome aberrations likely reflect a composite surrogate measure of the end product of many players such as gene polymorphisms involved in carcinogen metabolism, DNA repair, or cell cycle pathways [17]. An increased frequency of chromosome aberrations in peripheral blood lymphocytes has been validated as a biomarker for cancer risk in humans, reflecting both the early biological effects of exposure to genotoxic carcinogens as well as individual patient's susceptibility to cancer [16]. In a previous study in a subset of 105 HL patients, we found that patients who developed SPTs had a significantly higher level of baseline sister chromatid exchanges [as a consequence of chromosomal fragility in response to genetic or environmental factors] than did patients who did not develop SPTs [9]. In the present study, which enrolled a larger cohort of previously untreated adult HL patients, we expanded the panel of cytogenetic assays to include chromosome aberration and G-banding assays in order to confirm our previous findings and to identify specific chromosomes associated with the development of SPTs. Our results suggest that chromosome aberrations are promising biomarkers that could be used to identify HL patients at high risk of developing SPTs. The ability to identify subgroups of HL patients at high risk of SPTs not only would facilitate the early detection of SPTs in HL survivors but also would have substantial impact on the early detection and prevention of other cancers.

MATERIALS AND METHODS

Study population and follow-up interview

The study population consisted of 251 newly diagnosed, previously untreated adult HL patients seen at The University of Texas MD Anderson Cancer Center between January 1987 and December 1992. During that time, these patients were enrolled in a study in which cytogenetic methods were used to investigate the genotoxic effects of alkylating and non-alkylating chemotherapies [18]. Baseline cytogenetic preparations obtained prior to any chemotherapy or radiation treatment were available for all patients. Patients' medical records were reviewed for clinical characteristics at initial presentation, treatment regimen, and complications after treatment. Telephone interviews

with the surviving study participants or, in the cases of deceased study participants, the family member identified as the best available proxy were used to obtain follow-up information including vital status, date and cause of death, occurrence of relapse, and SPT between December 2002 and December 2006. Medical information was verified by reviewing patients' medical records. The Social Security Death Index was searched to ascertain the vital status of all patients as of December 2006 and to confirm deceased patients' dates of death. The study was approved by MD Anderson's Institutional Research Board.

Cytogenetic analysis

At the time of patient enrolment into the study, baseline (pre-treatment) cytogenetic cultures were obtained as described previously [19]. Briefly, 1 ml of fresh whole blood was added to 9 ml of RPMI-I640 medium (Gibco, Grand Island, NY) supplemented with 20% bovine serum (Gibco), 0.18 mg/ml of phytohemagglutinin (Murex Biotech, Dartford, England, UK), 2 mM L-glutamine (Gibco), and 100 U/ml each of penicillin and streptomycin (Gibco). Cells were arrested at metaphase using 0.4 μg/ml Colcemid (Gibco) followed by hypotonic treatment (0.075 M KCI) and fixation in Carnoy's fixative (3:1 methanol to acetic acid). The cytogenetic cultures were stored at -20°C until the time of analysis. Because of the prolonged storage of the fixed cultures, in the present study minor modifications of the standard method were performed including washing the cell pellets twice in freshly prepared fixatives (one wash in fixative with a 3:1 methanol to acetic acid ratio followed by one wash in a fixative with a 5:2 methanol: acetic acid ratio) to relax the chromosome packing and improve the spreads of the metaphases. Cells were then dropped onto clean slides, air-dried, and processed for conventional chromosomal aberrations or G-banding analysis.

Conventional chromosomal aberration

For conventional chromosomal aberrations, the slides were stained with 5% Giemsa solution (Gibco), coded, and scored. One hundred metaphase cells per study subject were scored blindly. Chromatid and chromosome breaks were recorded. In the final computation to generate the total number of breaks, each chromatid break was recorded as one break, and each chromosome-type break was recorded as two chromatid breaks. The criteria for a frank chromatid break were a discontinuity of a single chromatid in which the distance of discontinuity region was wider than the diameter of the chromatid or a clear misalignment of one of the chromatids. The criteria for the chromosome-type breaks were similar to that of chromatid breaks, except both chromatids were involved. The average number of breaks cell was then calculated. Gaps or attenuated regions were recorded but not included in the computation of aberration frequencies.

G-banding technique

G-banding was performed to identify specific chromosome regions associated with SPTs. The analysis was done on all HL patients who developed SPTs and on gender-, race-, and age-matched subset of an equal number of HL patients who did not develop SPTs. Patients with non-melanoma skin cancer were not included in this analysis. Banding was performed as described previously [20]. Briefly, the slides were aged at 60°C overnight and treated with a 0.06% trypsin solution. Slides were then stained with 4% Giemsa solution, rinsed, and air-dried. One hundred well-spread metaphase cells per patient were analyzed, and karyotypes were constructed. Metaphase imaging and karyotype production were done using the CytoVision® Genus software program (Applied Imaging, Inc., San Jose, CA).

Statistical analyses

All statistical analyses were carried out using SAS software (version 9, SAS Institute, Cary, NC). Baseline levels of chromatid and chromosome breaks were expressed as the mean ± the standard error of the mean (SEM). The two-sided Mann-Whitney test was used to compare differences between two subgroups of patients, and the Kruskal-Wallis test was used to compare differences among three groups of patients. P values <0.05 were considered statistically significant. Time to SPT development was measured from the date of HL diagnosis to the date of SPT diagnosis. The Cox proportional hazards model was used to ascertain possible risk factors for the development of SPTs, including age at diagnosis, gender, ethnicity, disease stage, histology, B symptoms, and chromosome aberrations. For the current analysis, we used the

75th percentile as a cut-off point to categorize chromosomal aberrations as either low- or high-risk. Variables that were significant at the $P = 0.20$ level in univariate analyses were included in the final multivariate Cox analysis. Pearson's χ^2 test was used to assess differences in structural abnormalities by chromosome between patients with SPT and matched patients without SPT. In addition, the nonparametric Wilcoxon rank-sum test was used to compare the distribution of numerical changes such as aneuploidy and polyploidy between patients with and without SPT.

RESULTS

Study population

Patients' demographic and clinical characteristics are summarized in Table 1. Of the 251 patients included in the study, 138 (55.0%) were men and 113 (45.0%) were women. The patients' mean age ± the standard deviation (SD) at diagnosis was 31.65 ± 12.52 years; 148 patients (59.0%) were younger than 32 years. The majority of patients were Caucasian (71.3%), had stage I or II HL (61.0%), and had nodular sclerosis HL (77.7%). Approximately 28% of the patients had B-symptoms, and about 20% of the patients had a relapse during the follow-up period. Thirty-three patients (13.0%) received radiotherapy, 35 patients (14%) received chemotherapy, and 179 patients (73.0%) received both chemotherapy and radiotherapy. The mean survival time was 15.8 years, and the overall survival rate was 76.3%. The mean follow-up time was 17.3 years, during which 29 patients (12.0%) developed SPTs, of which approximately 66% were solid tumors and 35% were hematological malignancies. The follow-up interview response rate was 75%; 25% of the patients were lost to follow-up.

Frequency of chromosome aberrations in HL patients

The frequency distribution of baseline pretreatment chromosome breaks, chromatid breaks, and total breaks for the selected demographics and clinical characteristics among the study participants are summarized in Table 2. Patients older than 32 years had non-significant higher levels of total breaks than younger patients 32 years or younger did (4.44 ± 0.30 and 3.95 ± 0.27, respectively). Women had slightly more total breaks than men did (4.29 ± 0.30 and 4.03 ± 0.28, respectively); however, this difference was not statistically

Table 1. Demographic and clinical characteristics of the study cohort.

Variable	No. of patients [%] N = 251
Age at diagnosis	
<32 years	148 [59.0]
≥32 years	103 [41.0]
Gender	
Female	113 [45.0]
Male	138 [55.0]
Ethnicity	
Caucasian	179 [71.3]
Other	72 [28.7]
Smoking status	
Ever	103 [41.0]
Never	99 [39.4]
Unknown	49 [19.5]
Disease stage	
I or II	153 [61.0]
III or IV	97 [38.6]
Histology[a]	
NS	195 [77.7]
LP, MC, or LD	55 [21.9]
B symptoms	
No	181 [72.1]
Yes	69 [27.5]
Relapses	
No	200 [79.7]
Yes	51 [20.3]
Treatment	
Radiotherapy	33 [13.4]
Chemotherapy	35 [14.2]
Chemotherapy and radiotherapy	179 [72.5]
Second primary cancer	
No	222 [88.4]
Yes	29 [11.6]

[a] NS, nodular sclerosis; LP, lymphocytic predominance; MC, mixed cellularity; LD, lymphocytic depletion.

Table 2. Distribution frequency of chromosomal aberrations in untreated Hodgkin lymphoma patients.

Characteristic	No. of patients	Chromosome breaks, mean ± SEM	*P*[a]	Chromatid breaks, mean ± SEM	*P*[a]	Total breaks, mean ± SEM	*P*[a]
Overall	251	0.79±0.06		2.55±0.12		4.15±0.20	
Age at diagnosis							
<32 years	148	0.70±0.08	0.03	2.51±0.16	0.55	3.95±0.27	0.09
≥32 years	103	0.91±0.10		2.61±0.19		4.44±0.30	
Sex							
Female	113	0.86±0.10	0.44	2.55±0.18	0.83	4.29±0.30	0.34
Male	138	0.73±0.08		2.56±0.17		4.03±0.28	
Disease stage							
I or II	153	0.73±0.08	0.25	2.50±0.15	0.87	3.97±0.24	0.63
III or IV	97	0.89±0.11		2.65±0.22		4.44±0.36	
Histology							
NS	195	0.81±0.08	0.87	2.61±0.14	0.40	4.24±0.24	0.62
LP, MC, or LD	55	0.75±0.11		2.38±0.26		3.86±0.38	
Relapses							
No	200	0.79±0.07	0.92	2.61±0.14	0.27	4.21±0.23	0.69
Yes	51	0.78±0.14		2.35±0.28		3.92±0.41	
Treatment							
Radiotherapy	33	0.67±0.16	0.25	2.48±0.34	0.81	3.82±0.48	0.50
Chemotherapy	35	0.57±0.14		2.60±0.40		3.77±0.55	
Chemotherapy and radiotherapy	179	0.85±0.08		2.54±0.14		4.27±0.25	
Secondary primary tumor							
No	222	0.78±0.07	0.97	2.43±0.13	<0.01	4.01±0.21	0.09
Yes	29	0.83±0.22		3.52±0.43		5.21±0.73	
Cancer type							
Solid	19	0.79±0.29	0.55	3.47±0.53	0.89	5.16±0.97	0.8
Hematologic	10	0.9±0.31		3.6±0.75		5.3±1.11	

[a]*P* values from two-sided nonparametric Mann-Whitney [two groups] or Kruskal-Wallis [three groups] tests.
Note: SEM, standard error of the mean; NS, nodular sclerosis; LP, lymphocytic predominance; MC, mixed cellularity; LD, lymphocytic depletion.

significant. The frequency of total breaks was slightly higher in Caucasian patients (4.21 ± 0.25) than in other patients (3.99 ± 0.33). The difference in the mean frequency of any of the chromosome aberrations between never smokers and ever smokers was not significant. Patients with B symptoms had a non-significantly higher frequency of breaks (4.49 ± 0.43) than patients with no

B symptoms (4.03 ± 0.23). Patients with stage I or II HL had a slightly lower frequency of breaks than patients with stage III or IV HL did. With regard to histology, patients with nodular sclerosis HL had a non-significantly higher level of total breaks than patients with other HL histologies did (4.24 ± 0.24 and 3.86 ± 0.38, respectively).

Association between chromosome aberrations and adverse long-term late effects

The frequency of chromatid breaks in the HL patients who developed SPTs (3.52 ± 0.43) was significantly higher than that in the HL patients who did not develop SPTs (2.43 ± 0.13; $P<0.01$). The frequency of chromatid breaks and the development of relapses were not associated (Table 2). Of the 29 patients who developed SPTs, 6 developed non-melanoma skin cancer, 5 developed melanoma, 1 developed uterine cancer, 2 developed head and neck cancer, 1 developed lung cancer, 4 developed breast cancer, 4 developed acute myeloid leukemia, and 4 developed NHL. The remaining 2 patients were diagnosed with HL more than 10 years after their initial HL diagnoses. Because HL relapse typically occurs within 4 years after the original diagnosis, and because the cut-off time for late HL relapse is unclear, we were uncertain whether these patients' tumors should be classified as late relapsed HL or SPTs. Therefore, we included and excluded these 2 patients as SPTs in separate data analyses.

Univariate Cox proportional hazards model for ascertaining possible risk factors for SPTs revealed that age at diagnosis, sex, HL histology, level of chromatid breaks, and level of total breaks were significant predictors of SPT development at the $P = 0.20$ level (data not shown). Because chromatid breaks and total breaks are highly correlated, only chromatid breaks were included in the multivariable Cox model to avoid colinearity issues. The multivariate Cox proportional hazard model revealed that the level of chromatid breaks was a significant positive predictor of SPT development (hazard ratio (HR) = 2.13; 95% confidence interval (CI) = 1.02-4.45; $P = 0.04$). Age at diagnosis was also a significant predictor of SPTs (HR = 2.35; 95% CI = 1.12-4.95; $P = 0.025$). Men had a non-significantly lower risk of developing SPTs than women did (HR = 0.63; 95% CI = 0.30-1.32). Patients who had lymphocyte-predominant, mixed cellularity, or lymphocyte-depleted HL were 65% less likely than were patients with nodular sclerosis HL to develop SPTs (HR = 0.35; 95% CI = 0.11-1.18).

Structural chromosomal aberrations

We used G-banding techniques to determine whether the structural chromosomal changes in patients who developed SPTs were different from those of a well-matched subset of HL patients who did not develop SPTs. The demographic characteristics of the HL patients who did and did not develop SPT and who were included in the G-banding analysis are shown in Table 3. G-banding

Table 3. Demographic characteristics of a selected subset of Hodgkin lymphoma patients[a].

	Second primary tumor status		
Variable	No [N = 25]	Yes [N = 25]	P[b]
Mean age ± SEM at diagnosis, years	34.4 ± 0.02	34.38 ± 0.01	0.89
Gender			
Female	12 [48]	14 [56]	0.62
Male	13 [52]	11 [44]	
Ethnicity			
Caucasian	21[84]	20 [80]	0.47
Other	4 [16]	5 [20]	

[a]Data are no. of patients [%] unless otherwise indicated, [b]P values from χ^2 test.
Note: SEM, standard error of the mean.

Table 4. Distribution of structural abnormalities in cells with normal chromosome numbers [46 XX/XY] from Hodgkin lymphoma patients with and without second primary tumors [SPTs].

Patients without SPTs		Patients with SPTs	
Band	**N [%]**	**Band**	**N [%]**
Chromosome 1		**Chromosome 1**	
1q11	2 [8]	1q11	13 [52]
1q25	2 [8]	-	
1q32	2 [8]	-	
1p13	5 [20]	-	
1p21	2 [8]	-	
Chromosome 2		**Chromosome 2**	
-		2p16	6 [24]
-		2p21	5 [20]
-		2p24	6 [24]
Chromosome 4		**Chromosome 4**	
-		4p16	3 [12]
-		4q 25	12 [48]
Chromosome 5		**Chromosome 5**	
5q25	5 [20]	-	
5q31	3 [12]	-	
Chromosome 7		**Chromosome 7**	
7q23	3 [12]	7q31	6 [24]
Chromosome 9		**Chromosome 9**	
-		9q31	8 [32]
Chromosome 11		**Chromosome 11**	
-		11p13	6 [24]
Chromosome 12		**Chromosome 12**	
12q24	6 [24]	12 q22,23	7 [28]
Chromosome 14		**Chromosome 14**	
-		14q11	4 [16]
-		14q32	9 [36]
Chromosome 15		**Chromosome 15**	
15q24	5 [20]	15q22	3 [12]
Chromosome 16		**Chromosome 16**	
-		16q13	9 [36]
-		16q22	7 [28]

Table 4 continued..

Chromosome 20		**Chromosome 20**	
20q11	3 [12]	-	
Chromosome 22		**Chromosome 22**	
-		22q12	2 [8]
Chromosome X		**Chromosome X**	
Xp21	2 [8]	Xp21	3 [12]
-		Xq21	7 [28]

revealed significant differences in the number of structural events in cells with the 46 XX/XY karyotype between the 2 groups of patients. In the patients with SPTs, the most frequent structural changes involved chromosomes 2, 4, 9, 16, and X (P<0.01). Patients with SPTs also had significantly more structural changes involving chromosomes 11 and 14 than patients without SPTs did (P = 0.02 and 0.01), respectively. The frequency of structural changes on chromosome 5 was significantly higher in HL patients with no SPTs than in HL patients without SPTs (P<0.01). In addition, chromosomes 7, 12, 15, and 22 as well as t(8;14) and t(11; 14) translocations were involved in HL patients with and HL patients without SPTs (Table 4). There were no statistically significant differences between the patients with and patients without SPTs for any of the other analyzed chromosomes.

DISCUSSION

The goal of the present study was to identify genetic events that could enable the identification of HL patients at risk for SPTs. Understanding the underlying mechanisms involved in SPTs would facilitate the formulation of more effective treatments that could in turn have a substantial impact on clinical outcomes and healthcare costs. We found that HL patients who developed SPTs had a significantly higher frequency of spontaneous chromosome aberrations, and thus higher levels of genomic instability, than HL patients who did not develop SPTs. This genomic instability may not only contribute to certain patients' predisposition to HL but also play a key role in SPT development after HL.

Numerous studies have found a high incidence of chromosomal instability in different types of cancers [21-23]. Other studies have found higher frequencies of chromosome aberrations in lymphocytes in untreated HL patients than in healthy individuals [24; 25]. M'kacher *et al*., reported that regardless of age, newly diagnosed HL patients had a higher frequency of spontaneous chromosomal abnormalities, higher *in vitro* radiosensitivity, and shorter telomeres than healthy controls [24]. We found that in addition to chromosome aberration level, patient age at the time of diagnosis was the only other strong predictor of SPT development. Because most of the patients in the present study were young adults, our findings support the hypothesis that an individual's inherent genome instability, rather than the damage to the genome accumulated throughout his or her lifetime, is the key factor in SPT development after HL. The level of chromosome aberrations in patients who developed SPTs shortly after their initial HL diagnosis was not significantly different from that of patients who developed SPTs years after their initial HL diagnosis. Given that blood samples were obtained at the time of the patients' initial HL diagnosis, our findings suggest that SPTs were the result of an early onset of disease development rather than a consequence of treatment. Our Cox proportional hazards model revealed that clinical variables such as stage of disease, B symptoms, HL relapse, and treatment type did not strongly influence SPT development. Two patients developed disease relapse more than 10 years after the primary HL diagnosis; however, distinguishing clearly between an SPT and recurrent disease in such instances is biologically difficult. Although few studies have identified risk factors for HL recurrence 7 or more years after its remission, two possible explanations for such late events have been suggested. First, the late reappearance of HL

could actually represent a second HL malignancy, not an HL relapse, in which case the patient's susceptibility to both the development of the primary and second HL may be related to underlying T-cell immunosuppression [26]. Second, the primary HL and the late relapse of HL may be related to viral reactivation in patients who are susceptible to HL [27]. Researchers have proposed several mechanisms by which chromosomal aberrations contribute to the carcinogenic process, including defective mitotic processes, centrosome duplication, or aberrant DNA repair [15]. Another possibility is that recurrent deletions along hotspots located in specific chromosome regions result in structural abnormalities in these regions. Non-random chromosome involvement in different cancers has been reported [28]. In the present study, G-banding revealed that patients who developed SPTs had a significantly higher number of structural events than patients who did not develop SPTs did, which suggests that multiple specific genetic lesions were associated with SPT development. Structural changes on chromosomes 2, 4, 9, 11, 14, 16, and X were significantly associated with SPT. However, structural changes on chromosome 5 were significantly associated with HL patients with no SPT. These results are consistent with previous studies' findings that suggested that the presence of several changes in the 5q region is associated with lymphoma and HL etiology [29].

Interestingly, the specific break points on several of the chromosomes identified by G-banding either map to or are in the vicinity of known common fragile sites, which are highly recombinogenic and are sites of frequent chromosome breakage and rearrangements that may alter and/or inactivate the associated genes and thus contribute to genetic instability [30]. HL patients who did not develop SPTs had breaks at the specific fragile sites in chromosomes 1q25, 1p21, 5q31, 12q24, and Xp21, whereas HL patients who developed SPTs had breaks at fragile sites in chromosomes 2p16, 4p16, 7q31, 9q31, 15q22, 16q22, and Xp21 (Figure 1). An NCBI gene literature search identified several genes that are crucial players in the carcinogenic process. For example, the DNA repair genes *MSH2* and *MSH6* map to the fragile site on chromosome 2p16, and the DNA repair gene *RAD50* maps to the fragile site on chromosome 5q31. *MSH2* and *MSH6* play a role in mismatch repair, in which the encoded proteins combine to form a mismatch recognition complex that functions as a bidirectional molecular switch that exchanges ADP and ATP as DNA mismatches are bound and dissociated [31]. The Rad50 protein plays a role in repairing DNA double strand breaks, activating cell cycle checkpoints, telomere maintenance, and meiotic recombination [32]. The telomere maintenance genes *POT1*, *PINX1*, and *PIF1* map to the fragile sites on chromosomes 7q31, 12q24, and 15q22, respectively, and are essential for chromosome stability [33-35]. Alterations in telomere maintenance affect telomere length and lead to illegitimate recombination, chromosome instability, and abnormal chromosome segregation [36]. Several other aberration sites we detected harbor genes that encode proteins involved in various cellular processes. These genes include *TRADD* (11q22) and *CASP6* (4p16), which play central roles in programmed cell death [37, 38], and *EWSR1* (22q12), which affects gene expression, cell signalling, RNA processing and transport [39].

Common fragile sites, which are found on all chromosomes in all individuals, are expressed as gaps or breaks on metaphase chromosomes under mild DNA replicative stress [40]. The increased frequency of expression of common fragile sites may predict cancer [41]. Traditionally, exposure to a low dose of aphidicolin, a DNA polymerase inhibitor, induces the expression of common fragile sites [42]; however, inherent alterations in the DNA damage response and/or cell cycle control may also lead to the disruption of DNA replication and thus the expression of common fragile sites. Studies have reported that mutations in the checkpoint pathways may allow cell proliferation and tumor progression, leading to more DNA replication stress and additional genomic instability [43]. We previously suggested that genetic variants in DNA repair pathways lead to suboptimal DNA repair and increase patients' susceptibility to HL [44]. Given the characteristics of the genomic regions in which these events take place, the possible roles of repeated DNA

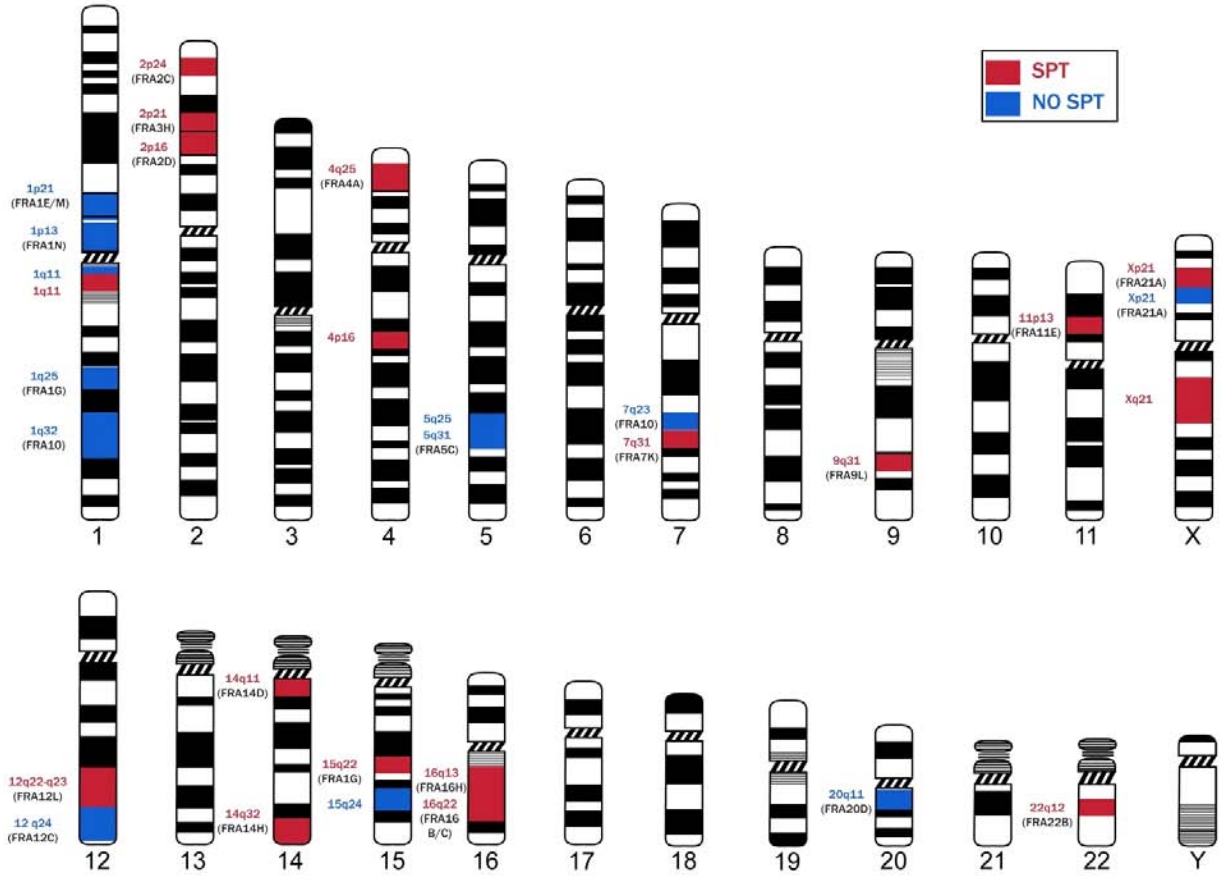

Figure 1. Distribution of structural abnormalities in relation to common fragile sites among Hodgkin lymphoma patients with and without second primary tumors. Blue: Breakpoints in non-SPT patients. Red: Breakpoints in SPTs patients. Black text in parenthesis: indicates the common fragile sites observed in different Studies.

sequences or long interspersed repetitive elements in the development of SPTs warrant further study. Novara *et al.* described events such as ectopic V(D)J recombination, changes in repeated sequences, and non-B DNA structures as mechanisms related to specific breakpoints-such as the 9p21 deletion breakpoint-in acute lymphoblastic leukemia [45]. Similarly, Wang *et al.* proposed a model in which breakpoint hotspots occur as a consequence of non-canonical DNA structures [46].

CONCLUSIONS

The present study suggests that multiple genetic lesions are associated with SPT development in HL patients who are highly susceptible to SPTs and identifies specific genetic lesions associated not only with HL but also with the development of SPTs in HL patients. Because lifestyle choices, environmental or occupational exposures, and primary cancer treatment may contribute to the development of SPTs, identifying patients who are susceptible to SPTs is crucial to improving treatment decisions among newly diagnosed HL patients and early SPT detection among HL survivors. A limitation of our study is the relatively small number of patients that developed SPTs, and therefore our results should be interpreted with caution. Based on these results and given the probable underlying mechanisms involved, future larger studies are warranted to determine the roles of the specific events in the etiology of primary HL and the development of SPTs.

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CONFLICT OF INTEREST

The authors state that there are no conflicts of interest in this manuscript.

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