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Polymorphisms in DNA repair genes, hair dye use, and the risk of non-Hodgkin lymphoma

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Abstract

Purpose—Genetic polymorphisms in DNA repair genes and hair dye use may both have a role in the development of non-Hodgkin lymphoma (NHL). We aimed to examine the interaction between variants in DNA repair genes and hair dye use with risk of NHL in a population-based case–control study of Connecticut women.

Methods—We examined 24 single nucleotide polymorphisms in 16 DNA repair genes among 518 NHL cases and 597 controls and evaluated the associations between hair dye use and risk of overall NHL and common NHL subtypes, stratified by genotype, using unconditional logistic regression.

Results—Women who used hair dye before 1980 had a significantly increased risk of NHL, particularly for the follicular lymphoma (FL) subtype, but not for diffuse large B-cell lymphoma. The following genotypes in combination with hair dye use before 1980 were associated with FL risk: *BRCA2* rs144848 AC+CC [odds ratio (OR) (95 % confidence interval (CI)) 3.28(1.27–8.50)], *WRN* rs1346044 TT [OR(95 % CI) 2.70(1.30–5.65)], *XRCC3* rs861539 CT+TT [OR(95 % CI) 2.76(1.32–5.77)], *XRCC4* rs1805377 GG [OR(95 % CI) 2.07(1.10–3.90)] and rs1056503 TT [OR(95 % CI) 2.17(1.16–4.07)], *ERCC1* rs3212961 CC [OR(95 % CI) 1.93(1.00–3.72)], *RAD23B* rs1805329 CC [OR(95 % CI) 2.28(1.12–4.64)], and *MGMT* rs12917 CC, rs2308321 AA, and rs2308327 AA genotypes [OR(95 % CI) 1.96(1.06–3.63), 2.02(1.09–3.75), and 2.23(1.16–4.29), respectively]. In addition, a significant interaction with risk of overall NHL was observed between *WRN* rs1346044 and hair dye use before 1980 ($p_{\text{interaction}} = 0.032$).

Conclusions—Our results indicated that genetic variation in DNA repair genes modifies susceptibility to NHL in relation to hair dye use, particularly for the FL subtype and in women who began using hair dye before 1980. Further studies are needed to confirm these observations.

Keywords

Non-Hodgkin lymphoma; Hair dye use; DNA repair; Genetic polymorphism; Interaction

Introduction

The incidence of non-Hodgkin lymphoma (NHL) has steadily increased worldwide and in the USA in particular during the past several decades [1]. The factors responsible for the

increase in incidence have been widely investigated, but the etiology of this disease remains largely unexplained. Nevertheless, several putative risk factors for NHL including certain medical conditions affecting the immune system, exposure to some chemicals, and genetic susceptibility have emerged from epidemiological studies conducted over the last two decades and more recently from large consortia studies [2, 3].

Personal use of hair dyes has been suggested to be a risk factor for hematopoietic cancers, especially for NHL [4–6]. Many studies have reported significantly elevated risks of NHL associated with increasing duration of hair dye use and use of dark-colored dyes [7, 8]. One important mechanism underlying this association is that a number of hair dye ingredients, such as phenylenediamines and paraphenylenediamine, are suspected carcinogens that can cause cytogenetic alterations and DNA damage [9, 10]. Of particular note, a variety of hair dye ingredients used in formulations before 1980 were reported to be mutagenic, and in vivo experimental studies carried out in rats and mice also confirmed the carcinogenic effects of some hair dye intermediates [11, 12]. These results provide biologic plausibility for the epidemiological observations that to date have suggested an increased risk of overall NHL and some NHL subtypes in women who began using hair dye products before the year 1980, whereas the association was not observed for those women who started using the products in 1980 or later [6, 7].

We have previously shown that variation in xenobiotic metabolic pathway genes modify the association between hair dye use and NHL risk, particularly for women who started use before 1980 and for the follicular lymphoma (FL) subtype [13]. DNA repair mechanisms are important in maintaining genomic stability, and defects in DNA repair pathways may increase chromosomal aberrations induced by hair dye use [14]. Our previous studies have suggested that polymorphisms in DNA repair genes may affect DNA repair capacities and consequently alter risk of NHL [15]; however, no prior analyses have evaluated the effects of gene–environment interactions between DNA repair genes, hair dye use, and NHL risk. Thus, in order to test the hypothesis that variation in DNA repair genes modifies the association between hair dye use and NHL risk, we conducted a population-based case–control study of women living in Connecticut.

Methods and materials

Study subjects

The study population of this case–control study has been described in detail previously [15, 16]. Briefly, female NHL patients were identified from the Yale Comprehensive Cancer Center’s Rapid Case Ascertainment Shared Resource, a component of the Connecticut Tumor Registry, between 1996 and 2000. The recruited eligible patients included female residents of Connecticut diagnosed with NHL (ICD-O, M-9590-9642, 9690-9701, 9740-9750), who were between 21 and 84 years of age at the time of diagnosis, had no previous diagnosis of cancer (except for non-melanoma skin cancer) and were alive at the time of the interview. A total of 601 NHL patients (72 % of all eligible cases) agreed to participate in this study. All NHL patients were histologically confirmed by pathologists and were classified according to the 2001 World Health Organization classification [17].

The population-based control subjects were randomly selected from the Centers for Medicare and Medicaid Services (CMMS) for women 65 years or older or were recruited via random digit dialing (RDD) methods for women younger than 65 years in Connecticut. Forty-seven percent of CMMS controls and 69 % RDD controls agreed to participate in this study. The controls were frequency matched to cases by age (± 5 years). A total of 717 eligible controls completed in-person interviews.

To collect information on the use of hair coloring products, all subjects completed an in-person interview using a standardized, structured questionnaire that has been described previously [7]. Respondents were asked whether they had used hair dye products at any time during their lifetime. For each period of hair dye use, subjects were asked to provide the type and color of the hair coloring product used, age at first use, age when use stopped, the number of years of use, and the frequency of use per year during those years of reported use.

The study subjects provided their informed consent after a clear explanation of study objectives. The study procedures were performed in accordance with a protocol approved by the human investigation committees at Yale University, the Connecticut Department of Public Health, and the National Cancer Institute.

Genotyping

Among the 601 cases and 717 controls enrolled in the study, 518 cases and 597 controls with sufficient blood or buccal cell samples were available for DNA extraction and the subsequent genotyping analysis. The phenol–chloroform extraction method was used to isolate DNA from the blood or buccal cell samples [18]. The genotyping of all selected single nucleotide polymorphisms (SNPs) was carried out using a real-time polymerase chain reaction on an Applied Biosystems 7900HT sequence detection system (Life Technologies Corporation, Carlsbad, California, USA) and was conducted at the National Cancer Institute's Core Genotyping Facility [19]. Sixteen DNA repair genes were chosen for analysis in this study, including seven key processor genes (*BRCA1*, *BRCA2*, *NBS1*, *WRN*, *XRCC2*, *XRCC3*, and *XRCC4*) in the double-strand break repair pathway, six key processor genes (*ERCC1*, *ERCC2*, *ERCC4*, *ERCC5*, *RAD23B*, and *XPC*) in the nucleotide excision repair pathway, four genes (*ADPRT*, *APEX1*, *OGG1*, and *XRCC1*) in the base excision repair pathway, and the alkyltransferase gene *MGMT*, which can repair alkylated bases in DNA.

Single nucleotide polymorphisms (SNPs) were selected for genotyping based on having a minimum allele frequency (MAF) >0.05 , and evidence of association in previous epidemiologic studies, evidence of function, or to extend genomic coverage for a given gene, as described previously [15]. A total of 24 SNPs with a MAF >0.10 and a Hardy–Weinberg equilibrium (HWE) $p > 0.05$ were evaluated in the present analysis (Table 1). The remaining SNPs with MAF <0.10 or HWE $p < 0.05$ were not included in further analyses (Supplemental Table 1). The concordance rates for quality control samples were over 98 % for all SNPs.

Statistical analysis

The results of previous studies that comprehensively reported the relationship between hair dye use and risk of NHL found that women who started using hair dye before 1980 had an increased risk of NHL [odds ratio (OR) 1.3, 95 % confidence interval (CI) 1.0–1.8] [7], but this association was not observed for those women who started using the products in 1980 or later. For this reason, we mainly focused on evaluating the associations between hair dye use before 1980 and the risk of common NHL subtypes [i.e., diffuse large B-cell lymphoma (DLBCL) and FL] within the different genotype strata. While associations with other NHL subtypes could not be evaluated individually in the present study due to limited statistical power, previous evidence has suggested that other subtypes such as chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) may plausibly be linked to hair dye use [6]. Therefore, we further analyzed the associations of hair dye use before 1980 with risk of overall NHL, when stratified by the genotypes of the 24 SNPs. The ORs and 95 % CIs for these associations were calculated using unconditional logistic regression models, and all models were adjusted for age, race (white vs. non-white), and smoking status (smoking vs. never smoking). To increase statistical power, heterozygous and homozygous variant genotypes were combined for all SNPs. The interaction between hair dye use and genotypes was assessed by including an interaction term in the logistic models. The false discovery rate (FDR) method was applied to control for multiple comparisons [20], and a FDR-adjusted $p < 0.2$ was considered noteworthy. All data analyses were carried out with SAS v9.3.

Results

Demographic and other characteristics of the study population are shown in Table 2. The mean age was 62 years old for both NHL cases and control subjects, while 497 (95.9 %) of the cases and 561 (94.0 %) of the controls were Caucasian. There were 288 (55.6 %) and 315 (52.8 %) ever-smokers in the case and control groups, respectively. No significant differences were apparent for the distributions of age, race, and smoking status between the case and control groups (all $p > 0.05$). One hundred and twenty-five NHL patients (24.1 %) and 173 (29.0 %) control subjects had never used hair dye during their lifetime; 261 NHL patients (50.4 %) and 247 controls (41.4 %) started to use hair dye before 1980, while 132 NHL patients (25.5 %) and 177 controls (29.7 %) started using hair dye in 1980 or later. NHL cases were significantly more likely to start using hair dye before 1980 compared with controls ($p = 0.011$). Among the NHL cases, 161 (31.1 %) were diagnosed as DLBCL and 119 (23.0 %) were diagnosed as FL, and these histological subtypes were the two most common among cases in the study population (Table 2).

The significant associations between DNA repair gene polymorphisms, use of hair dye before 1980, and risk of DLBCL and FL are shown in Table 3, while associations with all evaluated SNPs, including those that did not achieve statistical significance after accounting for multiple comparisons, are shown in Supplemental Table 2. Compared to women who never used hair dye during their lifetime, women who started using hair dye before 1980 had a significantly increased risk of FL if they carried the following genotypes: *BRCA2* rs144848 AC+CC [OR(95 % CI) 3.28(1.27–8.50)], *WRN* rs1346044 TT [OR(95 % CI) 2.70(1.30–5.65)], *XRCC3* rs861539 CT+TT [OR(95 % CI) 2.76(1.32–5.77)], *XRCC4*

rs1805377 GG [OR(95 % CI) 2.07(1.10–3.90)] and rs1056503 TT [OR(95 % CI) 2.17(1.16–4.07)], *ERCC1* rs3212961 CC [OR(95 % CI) 1.93(1.00–3.72)], *RAD23B* rs1805329 CC [OR(95 % CI) 2.28(1.12–4.64)], and *MGMT* rs12917 CC, rs2308321 AA, and rs2308327 AA genotypes [OR(95 % CI) 1.96(1.06–3.63), 2.02(1.09–3.75), and 2.23(1.16–4.29), respectively] (Table 3). The associations with these SNPs remained noteworthy after adjustment for multiple testing (all FDR-adjusted $p < 0.2$). No significant associations for starting hair dye use before 1980 and risk of FL were observed for the other 14 SNPs (Supplementary Table 2), and there were no associations for hair dye use before 1980 with risk of DLBCL when stratifying by the genotypes for any of the 24 SNPs (Table 3 and Supplementary Table 2) after FDR adjustment.

The significant associations for overall NHL are shown in Table 4, while associations with all evaluated SNPs are shown in Supplemental Table 3. Compared to never users of hair dye, women who started to use hair dye before 1980 had a significantly higher risk of developing NHL if they carried the *WRN* rs1346044 TT genotype (OR 1.88, 95 % CI 1.26–2.80, $p = 0.002$), but this effect was not significant for the *WRN* rs1346044 TC+CC genotype (OR 0.95, 95 % CI 0.58–1.54) ($p = 0.820$). A significant interaction was also observed between hair dye use before 1980 and *WRN* rs1346044 for risk of NHL ($p_{\text{interaction}} = 0.032$). However, in women with either rs1346044 TT or TC+CC genotypes, there were no significant associations with risk of NHL for women who began using hair dye in 1980 and later (both $p > 0.05$) (Table 3). After accounting for multiple comparisons, the associations with overall NHL for the *WRN* rs1346044 TT genotype (FDR $p = 0.122$) and the interaction between this SNP and starting hair dye use before 1980 (FDR $p = 0.164$) remained noteworthy (data not shown).

In addition, compared to women who never used hair dye, women who started using hair dye before 1980 had a significantly increased risk of NHL if they carried the following genotypes: *BRCA2* rs144848 AC+CC [OR(95 % CI) 1.71(1.07–2.71)], *XRCC3* rs861539 CT+TT (OR 1.65, 95 % CI 1.11–2.45), *XRCC4* rs1805377 GG (OR 1.54, 95 % CI 1.08–2.19) and rs1056503 TT (OR 1.57, 95 % CI 1.11–2.23), *ERCC1* rs3212961 CC [OR(95 % CI) 1.50(1.05–2.17)], *ERCC2* rs1799793 GA+AA (OR = 1.74, 95 % CI 1.15–2.65), *RAD23B* rs1805329 CC (OR 1.57, 95 % CI 1.09–2.25), *APEX1* rs1130409 TG+GG (OR 2.51, 95 % CI 1.81–5.33), *XRCC1* rs25487 GA+AA [OR(95 % CI) 1.62(1.08–2.42)], and *MGMT* rs12917 CC (OR = 1.59, 95 % CI 1.12–2.26), rs2308321 AA (OR 1.57, 95 % CI 1.12–2.19), and rs2308327 AA (OR 1.61, 95 % CI 1.14–2.26) (all FDR-adjusted $p < 0.2$) (Table 4). No significant associations for starting hair dye use before 1980 and risk of NHL were observed for the other 12 SNPs (Supplementary Table 3).

Discussion

To our knowledge, this is the first study to evaluate the interactions between hair dye use and genetic variation in DNA repair genes in relation to risk of NHL. We investigated 24 SNPs in 16 DNA repair genes and found that women who started using hair dye before 1980 had a significantly increased risk of NHL if they carried the following genotypes: *BRCA2* rs144848 AC+CC, *WRN* rs1346044 TT, *XRCC3* rs861539 CT+TT, *XRCC4* rs1805377 GG and rs1056503 TT, *ERCC1* rs3212961 CC, *RAD23B* rs1805329 CC, *XRCC1* rs25487 GA

+AA, and *MGMT* rs12917 CC, rs2308321 AA, and rs2308327 AA. Each of these associations remained noteworthy after adjustment for multiple comparisons and were apparent for the FL subtype but not for DLBCL. In addition, a significant interaction was also observed between *WRN* rs1346044 and hair dye use before 1980 for risk of NHL.

Our study provided evidence that the risk of NHL in relation to hair dye use is modified by genetic polymorphisms in key DNA repair genes. Our previous study and the large sample-sized InterLymph study both investigated the associations between detailed characteristics of hair dye use and risk of all NHL and NHL subtypes. These studies both showed that an increased risk of NHL was observed among women who began using hair dye before 1980 (both OR 1.3) [6, 7]. While our study is the first to our knowledge to evaluate hair dye use and NHL risk in relation to DNA repair polymorphisms, two previous studies have reported that genetic variation in xenobiotic metabolic pathway genes and in *NAT1* and *NAT2* genes modify the risk of NHL with hair dye use and that these associations are also limited to hair dye use before 1980 [13, 21]. One possible explanation for these differences in risk by time period observed in our study may relate to higher levels of mutagenic chemicals in hair dye formulations before 1980, as reformulation of hair dye products beginning in the early 1980s involved the replacement or elimination of some of the dyes that had been reported to produce tumors in NCI bioassays [22]. Alternatively, the lack of an association in women who began using products after 1980 may be a result of an insufficient latency period, considering that the strongest associations with NHL have generally been observed in those with the longest durations of hair dye exposure [7]. This hypothesis is plausible, given that putative carcinogens still exist in contemporary hair dye products, but will require further study. In particular, further studies are warranted to show whether the observed association by year of first use reflects the change in hair dye formula contents during the past two decades or indicates that more recent users are still in their induction and latent periods.

We observed that women who used hair dye before 1980 had a significant increased risk of FL and overall NHL if they carried the *WRN* rs1346044 TT genotype, and a significant interaction was also shown for *WRN* rs1346044 and use of hair dye before 1980 in increasing susceptibility to overall NHL. *WRN* is a RecQ helicase with an associated 3'-5' exonuclease activity involved in many aspects of DNA metabolism, including DNA transcription, replication, recombination, and repair of double-strand DNA breaks [23, 24]. Following exposure to chemical agents that result in DNA damage, WRN protein migrates from the nucleoli to form nuclear foci and interacts with a number of DNA metabolic pathway proteins such as RPA and Rad51 to facilitate the repair of DNA double-strand breaks [25]. Mutations in the *WRN* gene are believed to result in a deleterious change in normal WRN functions as a DNA helicase and exonuclease, which could result in a breakdown in genome integrity [26]. The present study showed that women who used hair dye before 1980 and carried the *WRN* rs1346044 TT (1367Cys/Cys) genotype had a 2.70-fold increased risk of FL and 1.88-fold increased risk of overall NHL, but this effect was not seen in women carrying the *WRN* rs1346044 TC or CC (1367Cys/Arg + Arg/Arg) genotypes (OR 0.95). It had been revealed by our previous study that, compared with women carrying the *WRN* rs1346044 TT genotype, women with TC or CC genotypes had a significantly decreased risk of NHL (OR 0.71) [15]. The *WRN* rs1346044 SNP results in a missense change of Cysteine to Arginine at the 1367 code site of *WRN*, and the observed

association with this SNP for FL and overall NHL in our study provides some evidence that variation in this region may modify the repair of hair dye-induced DNA damage.

XRCC3 (X-ray cross-complementing group 3) belongs to the RecA/Rad51-related protein family that can participate in homologous recombination, while *XRCC4* is an important component of non-homologous end-joining to maintain chromosome stability and repair double-strand breaks in DNA caused by DNA-damaging chemicals [27, 28]. A recent meta-analysis of *XRCC3* rs861539 (Thr241Met) polymorphism suggested that the rs861539 T allele was associated with increased risks of breast cancer and bladder cancer in Caucasians [29]. The *XRCC4* rs1805377 and rs1056503 SNPs were in complete linkage disequilibrium ($r = 0.99$) in this study population. Although rs1056503 is a synonymous SNP, a functional analysis demonstrated that this polymorphism might play a role in alternative splicing of *XRCC4* mRNA [30].

RAD23B proteins are involved in the nucleotide excision repair (NER) pathways, and rs1805329 in *RAD23B*, which was associated with risk of FL and overall NHL in our study, is a common non-synonymous coding region variant (Ala249Val) that may affect the normal protein functions of RAD23B and contribute to unrepaired DNA damage in the human genome, resulting in susceptibility to tumorigenesis.

The *MGMT* gene encodes O6-alkylguanine-DNA alkyltransferase, which is a critical defense against alkylation-induced mutagenesis and potential cancer development [31]. Our previous studies have demonstrated effect modification of the *MGMT* rs12917 SNP on the association between exposure to chlorinated solvents and the risk of NHL [32]. When compared with women who had no occupational exposure to chlorinated solvents, exposed women had an increased risk of NHL if they carried the rs12917 CT/TT genotypes [32]. In this study, we found that women who were first exposed to hair dye before 1980 and who carried the rs12917 CC genotype but not the CT/TT genotype had an elevated risk of FL and overall NHL. The three SNPs of the *MGMT* gene analyzed in this study were in high LD (pair-wise $r > 0.85$, data not shown). Because of the low penetration of these SNPs and the moderate sample size in this study, these results need further investigation into functional studies and validation in larger case-control studies.

Given increasing evidence of etiologic heterogeneity for specific NHL subtypes [33], we investigated the associations between DNA repair gene polymorphisms and the two most common NHL subtypes, DLBCL and FL, separately in our study population. A previous large pooled epidemiology study incorporating 4,461 NHL cases and 5,799 controls from InterLymph indicated that personal use of hair dyes may play a role in risk of NHL mainly for women who started using these products before 1980, particularly for FL and CLL/SLL, but not for DLBCL [6], which is consistent with the results of our study. Because of the small sample size for the other NHL subtypes in our study, such as marginal zone B-cell lymphoma (MZBCL) and T-cell lymphoma, it is not clear whether there are associations of hair dye use with these NHL subtypes. It was therefore important and of interest to explore associations for both overall NHL and for specific subtypes that had enough cases to evaluate individually, particularly exploring the relationship for FL.

Studies investigating the etiologic heterogeneity among NHL subtypes also pointed out that use of permanent hair dyes before 1980 appeared to increase risk of FL more than other NHL subtypes [33]. Therefore, FL would be the main subtype to demonstrate an association with hair dye use among women with predisposing genotypes. However, the results of the InterLymph study also suggested a significant association between hair dye use before 1980 and risk of CLL/SLL. In our study, the sample size for CLL/SLL was relatively small (only 59 cases), while fewer other studies have investigated CLL/SLL risk in relation to hair dye exposure. Given that direct DNA damage from environmental carcinogens is a possible factor associated with the development of CLL/SLL [33], larger epidemiologic studies are needed to determine whether CLL/SLL is another subtype associated with hair dye use before 1980 among women with predisposing genotypes of DNA repair genes.

Several strengths should be considered when interpreting our findings. First, a total of 18 key genes in DNA repair pathways were evaluated in this study, which enabled a broad evaluation of potential DNA repair mechanisms involved in the pathogenesis of hair dye-induced NHL. In addition, our study used a population-based study design and enrolled histologically confirmed NHL cases, which minimized the potential for disease misclassification. Interpretation of our results should also take into account several limitations. First, due to small subtype-specific sample sizes and to some degree of misclassification of the hair dye use information, we did not stratify the subjects by other aspects of hair dye, such as type and color of the hair coloring product used, age at first use, duration of use, the total number of applications, and years since first use. Thus, while the results of our study are consistent with previous evidence suggesting that the year of first hair dye use is an important factor in influencing risk of NHL, larger studies in the future will be needed to examine the potential interaction between genetic variation in DNA repair genes and these other metrics of hair dye use in relation to NHL risk. Further, future studies will be useful in determining the mechanism for the observed associations by year of first use; that is, whether the absence of an association in women who began using hair dye more recently (i.e., C1980) is due to an insufficient latency period, the result of more recent changes to hair dye formulations, and/or the relationship of this variable with other metrics of hair dye exposure. Second, we were unable to evaluate associations with other NHL subtypes in the stratified analyses, including CLL/SLL and MZBCL, because we did not have sufficient cases for these subtypes. Finally, due to the modest sample size, the statistical power of this study was limited, especially for analyzing the interactions of genetic polymorphisms and hair dye use in relation to risk of NHL subtypes. As a consequence, the results of this study require replication in additional epidemiologic studies with larger sample sizes, as well as in male subjects to evaluate whether the observed associations are generalizable.

In conclusion, our results indicate that genetic variation in DNA repair genes modifies susceptibility to NHL in relation to hair dye use, particularly for the FL subtype and in women who began using hair dye before 1980. Although our preliminary results will require additional replication in future studies, *WRN* rs1346044 in particular may modify the effect of hair dye use on risk of NHL.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NHL	Non-Hodgkin lymphoma
DLBCL	Diffuse large B-cell lymphoma
FL	Follicular lymphoma
MAF	Minor allele frequency
CI	Confidence interval

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Table 1

Selected DNA repair genes and single nucleotide polymorphisms in this study

Pathway	Gene	SNP	Position	Alleles	Function	MAF	HWE
Double-strand break repair							
<i>BRCA1</i>		rs16940	Exon-12	T/C	Leu730Leu	0.316	0.289
		rs799917	Exon-12	C/T	Pro830Leu	0.353	0.151
		rs16942	Exon-12	A/G	Lys1142Arg	0.325	0.528
<i>BRCA2</i>		rs144848	Exon-10	A/C	Asn372His	0.259	0.458
		rs543304	Exon-11	T/C	Val1269Val	0.183	0.801
		rs1805794	Exon-5	G/C	Glu185Gln	0.334	0.365
<i>NBS1</i>		rs1801195	Exon-26	G/T	Leu1074Phe	0.422	0.055
<i>WRN</i>		rs1346044	Exon-34	T/C	Cys1367Arg	0.265	0.298
<i>XRCC3</i>		rs861539	Exon-8	C/T	Thr241Met	0.379	0.051
<i>XRCC4</i>		rs1805377	IVS-7	G/A		0.127	0.085
		rs1056503	Exon-8	T/G	Ser307Ser	0.128	0.095
Nucleotide excision repair							
<i>ERCC1</i>		rs3212961	IVS-5	C/A		0.142	0.884
<i>ERCC2</i>		rs1799793	Exon-10	G/A	Asp312Asn	0.354	0.557
		rs13181	Exon-23	A/C	Lys751Gln	0.368	0.375
<i>ERCC5</i>		rs17655	Exon-15	G/C	Asp1104His	0.206	0.146
<i>RAD23B</i>		rs1805329	Exon-7	C/T	Ala249Val	0.180	0.093
<i>XPC</i>		rs2228001	Exon-16	A/C	Lys939Gln	0.427	0.554
Base excision repair							
<i>ADPRT</i>		rs1136410	Exon-17	T/C	Val762Ala	0.172	0.246
<i>APEX1</i>		rs1130409	Exon-5	T/G	Asp148Glu	0.394	0.053
<i>OGG1</i>		rs1052133	Exon-7	C/G	Ser326Cys	0.225	0.539
<i>XRCC1</i>		rs25487	Exon-10	G/A	Arg399Gln	0.351	0.086
Direct reversal of damage							
<i>MGMT</i>		rs12917	Exon-2	C/T	Leu84Phe	0.127	0.985
		rs2308321	Exon-4	A/G	Ile143Val	0.108	0.495
		rs2308327	Exon-4	A/G	Lys178Arg	0.100	0.071

Table 2

Characteristics of the study population

Characteristic	Case (n, %) (n = 518)	Control (n, %) (n = 597)	<i>p</i> [*]
Age (years)			0.193
<55	156 (30.1)	183 (30.7)	
55–69	187 (36.1)	187 (31.3)	
70	175 (33.8)	227 (38.0)	
Race			0.135
Caucasian	497 (95.9)	561 (94.0)	
African–American	16 (3.1)	17 (2.8)	
Others	5 (1.0)	19 (3.2)	
Cigarette smoking			0.344
Non-smokers	230 (44.4)	282 (47.2)	
Ever-smokers	288 (55.6)	315 (52.8)	
Hair dye use			0.011
Never use	125 (24.1)	173 (29.0)	
Start before 1980	261 (50.4)	247 (41.4)	
Start 1980 or later	132 (25.5)	177 (29.7)	
Common NHL subtypes			
DLCBC	161 (31.1)		
FL	119 (23.0)		
CLL/SLL	59 (11.4)		
MZBCL	35 (6.8)		
T-cell lymphoma	39 (7.5)		
Others	105 (20.3)		

DLBCL diffuse large B-cell lymphoma, *FL* follicular lymphoma, *CLL/SLL* chronic lymphocytic leukemia/small lymphocytic lymphoma, *MZBCL* marginal zone B-cell lymphoma

^{*} Chi-square test

Table 3

Associations between DNA repair gene polymorphisms, starting hair dye use before 1980, and risk of DLBCL and FL

Gene	SNP	Controls		Diffuse large B-cell lymphoma (DLBCL)				Follicular lymphoma (FL)					
		Never use	Hair dye use before 1980	Never use		Hair dye use before 1980		Never use		Hair dye use before 1980			
				Cases	OR	Cases	OR(95% CI)	p*	Cases	OR	Cases	OR(95% CI)	p*
BRCA2	rs144848												
	AA	91	129	26	1.00	31	0.82(0.45–1.49)	0.507	16	1.00	27	1.22(0.61–2.42)	0.576
	AC?CC	70	100	16	1.00	36	1.78(0.88–3.60)	0.111	7	1.00	30	3.28(1.27–8.50)	0.014
WRN	rs1346044												
	TT	100	108	29	1.00	43	1.34(0.77–2.33)	0.3	12	1.00	36	2.70(1.30–5.65)	0.008
	TC?CC	58	111	13	1.00	23	1.01(0.46–2.19)	0.99	10	1.00	19	1.05(0.44–2.49)	0.917
XRCC3	rs861539												
	CC	66	89	18	1.00	27	1.16(0.58–2.32)	0.682	10	1.00	12	0.93(0.37–2.34)	0.872
	CT?TT	92	129	24	1.00	39	1.15(0.64–2.07)	0.65	12	1.00	43	2.76(1.32–5.77)	0.007
XRCC4	rs1805377												
	GG	124	164	31	1.00	46	1.20(0.71–2.03)	0.496	17	1.00	44	2.07(1.10–3.90)	0.024
	GA?AA	31	53	9	1.00	18	0.88(0.32–2.40)	0.798	5	1.00	10	1.25(0.35–4.47)	0.736
MG	rs1056503												
	TT	127	164	32	1.00	48	1.24(0.74–2.08)	0.412	17	1.00	45	2.17(1.16–4.07)	0.016
	TG?GG	30	54	10	1.00	17	0.75(0.28–2.01)	0.561	4	1.00	10	1.53(0.39–6.05)	0.544
ERCC1	rs3212961												
	CC	110	162	28	1.00	47	1.11(0.65–1.90)	0.698	15	1.00	42	1.93(1.00–3.72)	0.048
	CA?AA	44	53	13	1.00	17	1.15(0.49–2.72)	0.744	7	1.00	13	1.49(0.50–4.47)	0.476
RAD23B	rs1805329												
	CC	113	147	29	1.00	49	1.28(0.75–2.19)	0.375	13	1.00	38	2.28(1.12–4.64)	0.024
	CT?TT	50	83	12	1.00	8	0.93(0.40–2.14)	0.857	10	1.00	19	1.09(0.47–2.57)	0.836
MGMT	rs2308321												
	AA	133	178	36	1.00	60	1.30(0.80–2.10)	0.29	17	1.00	45	1.96(1.06–3.63)	0.031
	AG?GG	31	52	6	1.00	6	0.52(0.15–1.85)	0.316	5	1.00	11	1.28(0.34–4.86)	0.721
MG	rs2308327												
	AA	130	168	35	1.00	58	1.35(0.83–2.21)	0.23	17	1.00	44	2.02(1.09–3.75)	0.026

Gene	SNP	Controls		Diffuse large B-cell lymphoma (DLBCL)				Follicular lymphoma (FL)			
		Never use		Hair dye use before 1980		Never use		Never use		Hair dye use before 1980	
						Cases	OR	Cases	OR	Cases	OR(95 % CI) <i>p</i> *
	AG?GG	27	43			6	1.00	8	1.00	10	1.71(0.40–7.38) 0.47
	rs12917										
	CC	126	168			30	1.00	43	1.00	43	2.23(1.16–4.29) 0.017
	CT?TT	37	61			12	1.00	24	1.00	14	1.37(0.41–3.15) 0.806

* Multiple unconditional logistic regression models, with adjustment for age, race, and smoking status

Table 4

Associations between DNA repair gene polymorphisms, use of hair dye before 1980, and risk of non-Hodgkin's lymphoma

Gene	SNP	Never use		OR	Started before 1980		OR(95% CI)	* p	* p _{int}
		Controls	Cases		Controls	Cases			
<i>BRCA2</i>	rs144848								0.142
	AA	91(55.8)	72(44.2)	1.00	129(52.4)	117(47.6)	1.16(0.78–1.73)	0.470	
	AC+CC	70(59.3)	48(40.7)	1.00	100(45.1)	122(54.9)	1.71(1.07–2.71)	0.025	
<i>WRN</i>	rs1346044								0.032
	TT	100(58.1)	72(41.9)	1.00	108(42.5)	146(57.5)	1.88(1.26–2.80)	0.002	
	TC+CC	58(56.3)	45(43.7)	1.00	111(57.5)	82(42.5)	0.95(0.58–1.54)	0.820	
<i>XRCC3</i>	rs861539								0.145
	CC	66(57.4)	49(42.6)	1.00	89(55.6)	71(44.4)	1.13(0.69–1.85)	0.631	
	CT+TT	92(57.9)	67(42.1)	1.00	129(45.0)	158(55.0)	1.65(1.11–2.45)	0.013	
<i>XRCC4</i>	rs1805377								0.384
	GG	124(59.1)	86(40.9)	1.00	164(48.8)	172(51.2)	1.54(1.08–2.19)	0.017	
	GA+AA	31(54.4)	26(45.6)	1.00	53(50.5)	52(49.5)	1.08(0.56–2.11)	0.811	
<i>ERCC1</i>	rs1056503								0.222
	TT	127(59.1)	88(40.9)	1.00	164(48.2)	176(51.7)	1.57(1.11–2.23)	0.011	
	TG+GG	30(51.7)	28(48.3)	1.00	54(50.5)	53(49.5)	0.92(0.61–1.39)	0.694	
<i>ERCC2</i>	rs3212961								0.327
	CC	110(58.8)	77(41.2)	1.00	162(48.4)	173(51.6)	1.50(1.05–2.17)	0.028	
	CA+AA	44(53.0)	39(47.0)	1.00	53(50.4)	52(49.5)	1.11(0.31–1.27)	0.721	
<i>RAD23B</i>	rs1799793								0.074
	GG	63(51.6)	59(48.4)	1.00	96(51.1)	92(48.9)	1.00(0.63–1.59)	0.989	
	GA+AA	94(62.7)	56(37.3)	1.00	123(48.6)	130(51.4)	1.74(1.15–2.65)	0.009	
<i>APEX1</i>	rs1805329								0.303
	CC	113(57.4)	84(42.6)	1.00	147(45.8)	174(54.2)	1.57(1.09–2.25)	0.016	
	CT+TT	50(58.8)	35(41.2)	1.00	83(55.7)	66(44.3)	1.06(0.61–1.82)	0.849	
	rs1130409								0.150
	TT	135(57.0)	102(43.0)	1.00	202(49.5)	206(50.5)	1.32(0.96–1.83)	0.092	
	TG+GG	36(66.7)	18(33.3)	1.00	41(47.1)	46(52.9)	2.51(1.18–5.33)	0.017	

Gene	SNP	Never use		Started before 1980		OR(95% CI)	<i>p</i> *	<i>p</i> _{int} *
		Controls	Cases	Controls	Cases			
<i>XRCC1</i>	rs25487							0.372
	GG	64(54.2)	54(45.8)	95(48.7)	100(51.3)	1.17(0.73–1.86)	0.518	
	GA+AA	93(60.0)	62(40.0)	127(48.5)	135(51.5)	1.62(1.08–2.42)	0.020	
<i>MGMT</i>	rs12917							0.198
	CC	126(60.6)	82(39.4)	168(48.7)	177(51.3)	1.59(1.12–2.26)	0.010	
	CT+TT	37(50.7)	36(49.3)	61(50.0)	61(50.0)	1.02(0.57–1.83)	0.954	
	rs2308321							0.167
	AA	133(58.3)	95(41.7)	178(47.1)	200(52.9)	1.57(1.12–2.19)	0.008	
	AG+GG	31(57.4)	23(42.6)	52(59.1)	36(40.9)	0.82(0.40–1.67)	0.586	
	rs2308327							0.391
	AA	130(58.0)	94(42.0)	168(46.4)	194(53.6)	1.61(1.14–2.26)	0.006	
	AG+GG	27(58.7)	19(41.3)	43(55.1)	35(44.9)	1.04(0.49–2.23)	0.914	

*p*_{int} *p* value for interaction of SNP and hair dye use before 1980

* Multiple unconditional logistic regression models, with adjustment for age, race, and smoking status