Polymorphisms of DNA repair and oxidative stress genes in B-cell lymphoma patients

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Abstract. The purpose of the study was to investigate the possible association between *ERCC2* rs28365048, *ERCC5* rs17655, *XRCC3* rs861539 and *NOS2A* rs2297518 polymorphisms with B-cell lymphoma. The study was conducted on 189 patients with CD20$^+$ B-cell lymphoma and 193 controls. The genotype frequencies were compared in the patient and control groups using quantitative polymerase chain reaction, based on allelic discrimination analysis. Our results indicated that variation in *NOS2A* may be significant in B-cell lymphoma in a population ≥50 years old (OR=2.15; 95% CI, 1.17-3.92; P=0.013). No association was observed between variations in *ERCC2*, *ERCC5*, *XRCC3* and B-cell lymphoma in the studied population. Our finding of an association between age and *NOS2A* polymorphisms in lymphoma is unique and requires additional studies. The results concerning *ERCC2*, *ERCC5* and *XRCC3* variations add additional data to studies on genetic polymorphisms in the DNA repair pathway.

Introduction

Exposure to DNA damaging agents and genetic susceptibility have been suggested as potential factors contributing to the etiology of lymphoma (1-3). Previous studies showed that an increased risk of non-Hodgkin's lymphoma (NHL) is associated with certain hereditary syndromes, such as ataxia telangiectasia, Bloom syndrome and Nijmegen breakage syndrome, diseases characterized by a defective DNA repair (4-7). The increased risk of skin cancer in patients with earlier diagnosed NHL has also been proven clinically (8,9). These observations suggest involvement of DNA repair in the pathogenesis of NHL. Genetic variations in DNA repair genes, such as single nucleotide polymorphisms (SNPs) may alter the repair capacity, result in genetic instability and increase the risk of cancer. SNPs in *ERCC2*, *XRCC1* and *XRCC3* have been demonstrated to modulate the repair of ultraviolet (UV)-damaged DNA (10). In their study, Smedby *et al* (11) demonstrated that polymorphic variation in the *XRCC3* gene, but not in the *ERCC2* and *XRCC1* genes, may be important for susceptibility to follicular lymphoma (FL). Additional studies have also shown that genes mediating oxidative stress are associated with the B-cell NHL subtype. Specifically, Wang *et al* (12) observed that the rs2297518 Leu/Leu variation in nitric oxide synthase (NOS) *NOS2A* results in a 3.4-fold increased risk of diffuse large B-cell lymphoma (DLBCL) and a 2.6-fold increased risk of FL. By contrast, Lan *et al* (13), who evaluated SNPs in oxidative stress genes including *NOS2A* in another population, did not detect any statistically significant associations between analyzed SNPs and risk of NHL, with the exception of SNPs in *AKR1A1* and *CYBA* (13). Recently, a pooled analysis of SNPs in 27 gene regions involved in DNA repair based on three studies of almost 2,000 cases and 1,800 controls was published by Shen *et al* (14). Findings of that study showed that only 5 SNPs (in *BLM*, *RAD50*, *FAM82A2*, *ERCC3* and *XRCC4* genes) of the 319 analyzed SNPs were significantly associated with NHL and supported the hypothesis of influencing NHL risk by polymorphisms in DNA repair genes. Since the results of the evaluation of genetic variants in DNA repair and oxidative stress genes were inconsistent, we focused on well-described SNPs in DNA repair genes in patients treated at our institution. These were SNPs in genes involved in two pathways of DNA repair-nucleotide excision repair (*ERCC2* rs28365048 and *ERCC5* rs17655) and homologous recombination (*XRCC3* rs861539). SNP was also analyzed in the oxidative stress gene *NOS2A* rs2297518, and the distribution of analyzed genotypes in patients with common types of CD20$^+$ B-cell lymphoma, i.e., FL and DLBCL as well as in healthy controls was compared.

Patients and methods

Patients. The study comprised 189 patients with histologically confirmed CD20$^+$ B-cell lymphoma (FL-71, DLBCL-118), who were treated at our institution between 2004 and 2007. There were 113 females and 76 males (age, ranging from 22

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to 83; median 52 years). The control group comprised 193 healthy blood donors. One hundred and six cases (55%) and 96 controls (50%) were >50 years old. The study was approved by the Ethics Committee of the Maria Sklodowska-Curie Memorial Institute and Oncology Centre (Warszawa, Poland) and a written informed consent was obtained from each participant.

Genotyping. Characteristics of studied SNPs are shown in Table I. Peripheral blood samples from patients and the controls were collected in tubes with ethylenediaminetetraacetic acid (EDTA) and were frozen at -70˚C until DNA isolation. Genomic DNA was extracted from blood using the NucleoSpin® Blood L kit (Macherey-Nagel, Easton, PA, USA). Genotyping was carried out by TaqMan-based Allelic Discrimination (AD) assay on ABI Prism® 7000 (Applied Biosystems, Carlsbad, CA, USA). For each AD assay, a unique pair of fluorescent dye detectors was used in two TaqMan® MGB probes that target an SNP site. TaqMan® MGB Probes were selected based on the Cancer Genome Anatomy Project SNP500Cancer Database (http://www.snp500cancer.nci.nih.gov). The 20 μl polymerase chain reaction (PCR) mixture contained 200 ng DNA template, 0.2 μM of each primer, 0.9 μM of each probe and 10 μl 2X Taq Man® Master Mix. According to the AD assay PCR reaction conditions were: initial denaturation 10 min at 94˚C, 49 cycles of amplification (30 sec at 92˚C, 1 min at 60˚C). Post-amplification allelic discrimination was performed at 60˚C for 1 min. To confirm the results of the AD assay, in certain cases, gene fragments covering analyzed SNP were sequenced with the use of 3130xl Genetic Analyzer (Applied Biosystems).

Statistical analysis. The Hardy-Weinberg equilibrium was assessed in the control group for genotype frequencies using the standard χ^2 test. The effects of the examined genotypes in $CD20^+$ B-cell lymphoma was estimated by odds ratios (OR) and 95% confidence intervals (CI) using unconditional logistic regression. P<0.05 (two-sided) was considered to indicate a statistically significant difference. The most common haplotype was used as the reference. The Mantel-Haenszel test of homogeneity was performed to estimate the effect of age on OR for the studied polymorphism. The analyses were carried out using STATA 8.2 software (Stata Corp., College Station, TX, USA).

Table I. Patient characteristics.

Characteristics	Value, n (%)
Total no. of patients	189 (100)
Median age (range, years)	52 (22-83)
DLBCL	118 (62)
FL	71 (38)
Gender (female/male)	113/76 (60/40)
Site of initial involvement	
Nodal	134 (71)
Extranodal	54 (29)
Ann Arbor stage	
I	3 (2)
II	52 (27)
III	36 (19)
IV	74 (39)
NA[a]	24 (13)
B symptoms	42/178[b] (24)
Bone marrow involvement	49 (26)

[a]NA, not available. [b]Data available for 178 patients. DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma.

Results

Patient characteristics. Patient characteristics are shown in Table II and genotype distributions for patients and the controls with respective ORs are shown in Table III. The distribution of the genotypes in the control group was in the Hardy-Weinberg equilibrium and was similar to those previously reported in Caucasians (11,15).

ERCC2 rs28365048 and XRCC3 rs861539. No statistically significant differences were observed in patients and the controls regarding the distribution of *ERCC2* and *XRCC3* polymorphisms.

ERCC5 rs17655. In the *ERCC5* gene heterozygous phenotype the GC genotype was identified more often in B-cell lymphoma patients (34%) compared to the controls (26%), but that difference was not statistically significant (OR=1.44; 95% CI, 0.92-2.26; P=0.109). CC genotype was extremely rare, and comparable in patients (3%) and the controls (4%).

NOS2A rs2297518. The polymorphism analysis of *NOS2A* gene showed slight differences in the distribution of heterozygous genotype CT and homozygous genotype TT between patients and the controls. CT genotype was found in 33% of the patients and in 27% of the controls, but this result was statistically insignificant (OR=1.38; 95% CI, 0.89-2.15; P=0.149). TT genotype was present in 5 of 193 and 10 of 189 cases, and this difference was significant in terms of OR=2.33; 95% CI, 0.81-6.69, yet insignificant statistically (P=0.123).

Distribution of genotypes based on patient age. *ERCC2*, *XRCC3*, *ERCC5* and *NOS2A* polymorphisms were evaluated by baseline patient characteristics, including age. The cut-off age for analysis was 50, and the median age of patients was 52. No differences between patients and the controls were found for *ERCC2*, *XRCC3* and *ERCC5*. However, analysis for *NOS2A* showed that in patients aged ≥50, CT or TT genotype occurred two times more often compared to the control group (OR=2.15; 95% CI, 1.17-3.92; P=0.013) (Table IV). In older patients, homozygous genotype TT bearing two mutated alleles was rare, only 6 patients and no controls had this genotype and the difference between patients and the controls was statistically significant (P=0.016). Moreover, the Mantel-Haenszel test confirmed a statistically significant difference between OR for the CT genotype in the two age groups (OR=1.42; 95% CI, 0.91-2.23; P=0.043).

Table II. Characteristics of studied SNPs.

Gene (locus)	SNP rs no.	Location	Nucleotide change	Amino acid change
ERCC2 (19q13.3)	rs28365048	Ex23+61A>C	A→C	Lys751Gln
ERCC5 (13q22)	rs17655	Ex15-344G>C	G→C	Asp1104His
XRCC3 (14q32.3)	rs861539	Ex8-53C>T	C→T	Thr241Met
NOS2A (17cen-q11.2)	rs2297518	Ex16+14C>T	C→T	Ser608Leu

SNP, single nucleotide polymorphism.

Table III. Distribution of genotypes in DNA repair and oxidative stress genes polymorphism in patients with $CD20^+$ B-cell lymphoma and the controls.

Gene	Genotype	Controls n (%)	Cases n (%)	OR	95% CI	P-value
ERCC2						
rs28365048	All	193	181			
Lys751Gln	AA	54 (28)	57 (31)	1.00 Ref.		
	AC	107 (55)	94 (52)	0.83	0.52-1.32	0.438
	CC	32 (17)	30 (17)	0.89	0.48-1.65	0.709
	CC/AC vs AA	139	124	0.85	0.54-1.32	0.457
ERCC5						
rs17655	All	193	182			
Asp1104His	GG	136 (70)	115 (63)	1.00 Ref.		
	GC	50 (26)	61 (34)	1.44	0.92-2.26	0.109
	CC	7 (4)	6 (3)	1.01	0.35-2.96	0.981
	CC/GC vs GG	57	67	1.39	0.90-2.14	0.134
XRCC3						
rs861539	All	189	189			
Thr241Met	CC	86 (46)	83 (44)	1.00 Ref.		
	CT	85 (45)	85 (45)	1.04	0.68-1.58	0.870
	TT	18 (9)	21 (11)	1.21	0.61-2.41	0.594
	CC/CT vs TT	103	106	1.07	0.71-1.60	0.756
NOS2A						
rs 2297518	All	193	189			
Ser608Leu	CC	135 (69)	116 (61)	1.00 Ref.		
	CT	53 (27)	63 (33)	1.38	0.89-2.15	0.149
	TT	5 (3)	10 (5)	2.33	0.81-6.69	0.123
	TT/CT vs CC	58	73	1.46	0.96-2.23	0.078

Ref, reference group; OR, odds ratio; CI, confidence interval.

Discussion

In this study, we evaluated four selected SNPs for the genes that are involved in DNA repair and oxidative stress. The aim of the study was to evaluate these SNPs in patients with $CD20^+$ B-cell lymphoma in comparison to healthy blood donors. Our findings suggest that variation in oxidative stress gene *NOS2A* may be significant in B-cell lymphoma in patients of ≥50 years of age but not in younger ones. To the best of our knowledge, the association between age and oxidative stress gene polymorphisms in lymphoma is a unique finding that requires additional investigation. Previous studies have shown that SNPs in *MDM2* gene were associated with an earlier age of diagnosis of DLBCL in Ashkenazi Jewish female patients (16), however, this is the only report concerning the association of SNP with the age of onset of DLBCL. Our observation regarding the effect of SNP in *NOS2A* in a population of ≥50 years old on B-cell lymphoma suggest that the pathogenesis of lymphoma may act in a different manner in older individuals. This may be the result of various ways that

Table IV. Distribution of genotypes in *NOS2A* polymorphism based on patient age.

	Age <50 (years)				Age ≥50 (years)			
Genotype	No. of cases/controls	OR	95% CI	P-value	No. of cases/controls	OR	95% CI	P-value
CC	51/62	Ref.			65/73	Ref.		
CT/TT	29/35	1.00	0.55-1.86	0.982	44/23	2.15	1.17-3.92	0.013

Ref, reference group; OR, odds ratio; CI, confidence interval.

damage caused by oxidative stress is efficiently repaired, or the damage induced by the oxidative stress with an increase in age. *NOS2A* is one of the oxidative stress genes coding inducible nitric oxide synthase (iNOS). High levels of iNOS and nitric oxide were identified in B-cell NHL and T-cell leukemia (17), which increased the production of pro-inflammatory cytokines including TNF (18) and resulted in chronic inflammation. Wang *et al* (12) suggested that a prolonged oxidative stress leading to chronic inflammation might contribute to lymphomagenesis. They evaluated the difference in the distribution of *NOS2A* rs2297518 in 1321 newly diagnosed NHL and 1057 controls. Statistically significant differences in DLBCL and FL were shown for T/T homozygotes only. By contrast, Lan *et al* (13) found no difference between patients and healthy blood donors regarding this polymorphism in a study of 161 DLBCL and 119 FL patients.

Smedby *et al* (11) suggested that polymorphic variation in the *XRCC3* gene, but not in the *ERCC2* and *XRCC1* genes, may be essential in the pathogenesis of FL. By contrast, we have demonstrated no difference between patients and the controls regarding the incidence of polymorphisms in *XRCC3* gene as well as in the *ERCC2* and *ERCC5* genes. In their study, Shen *et al* (15) showed that the distribution of SNPs in *ERCC5* and *ERCC2* varies between patients and the controls with DLBCL, but not with FL. No difference regarding the incidence of polymorphisms for *XRCC3* and *ERCC5* SNPs was reported in the study by Hill *et al* (19). Moreover, in their recent study, Shen *et al* (14) have demonstrated no involvement of the abovementioned SNPs in NHL in the pooled three studies conducted on 1,946 cases and 1,808 controls from the US and Australia. In that study, tag SNP analysis was performed to provide broad coverage of variation within each examined gene, demonstrating that five additional SNPs within the DNA repair pathways were associated with NHL and subtypes of NHL. Of those, *XRCC4* rs13178127 was associated with NHL and *BLM* rs441399, whereas *FAM82A2* rs2304583 was associated with FL.

A limitation of our study is the relatively small size of the FL patient group. Consequently, we analyzed the FL and DLBCL groups together as one $CD20^+$ B-cell lymphoma group. The size of the patient group in this study is comparable to other published reports (15,19,20). However, our results need to be confirmed on a larger group of patients from the Polish population.

The role of polymorphisms in certain DNA repair genes in the pathogenesis of lymphoma was implicated in several studies (11,21,22). However, even in a large multicenter study, e.g., by Shen *et al* (14), of 319 studied polymorphisms only a small number (i.e. five) were identified to be associated with NHL. The results of our study are consistent with these findings. Several SNPs have to be analyzed in order to find detectable differences in the distribution of polymorphisms in NHL. A number of them may be contributing to the pathogenesis of B-cell lymphoma. The results of our study are believed to contribute to a consecutive meta-analysis of the pooled data from various studies allowing for the clarification of the role of DNA repair in lymphoma. However, we postulate to perform such meta-analysis in two age groups, since as suggested by our findings the pathogenesis of lymphoma in older individuals may be different compared to younger individuals.

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