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ORIGINAL INVESTIGATION

Genetic polymorphisms in the oxidative stress pathway and susceptibility to non-Hodgkin lymphoma

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Abstract Oxidative damage caused by reactive oxygen species (ROS) and other free radicals is involved in a number of pathological conditions including cancer. In a population-based case-control study of non-Hodgkin lymphoma (NHL) ($n = 518$ cases, 597 controls) among women in Connecticut, we analyzed one or more single nucleotide polymorphisms (SNPs) in ten candidate genes (*AKR1A1*, *AKR1C1*, *AKR1C3*, *CYBA*, *GPX1*, *MPO*, *NOS2A*, *NOS3*, *OGG1*, and *SOD2*) that mediate oxidative stress directly or indirectly in the NADPH oxidase-dependent respiratory burst. Odds ratios (OR) and 95% confidence intervals (CI) were adjusted for age and race. Polymorphisms in *AKR1A1* and *CYBA* were significantly associated with increased risk of NHL. There was a 1.7-fold (95% CI = 1.2–2.4, $P = 0.0047$) increased risk of NHL for individuals who were variant homozygous for the *AKR1A1* (IVS5 + 282T > C) SNP. The effect was most pronounced for risk of diffuse large B-cell lymphoma, but risk estimates were non-significantly elevated for other common B-cell histologies and T-cell lymphomas as well. In addition, individuals variant homozygous for the *CYBA* (Ex4 + 11C > T) SNP had a 1.6-fold (95% CI = 1.1–2.4, $P = 0.019$) increased risk of NHL that was particularly pronounced for T-cell lymphoma (OR = 3.5, 95% CI = 1.3–9.6, $P = 0.013$), but was also associated with non-significant increased risks for each of the common B-cell histologies. These results suggest that SNPs in genes related to the oxidative stress pathway may be associated with increased risk of NHL.

Keywords Non-Hodgkin lymphoma · Oxidative stress · Genetic polymorphism

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Introduction

Oxidative damage caused by reactive oxygen species (ROS) and other free radicals is involved in a number of pathological conditions including ageing and senescence, rheumatoid arthritis, diabetes, atherosclerosis, and cancer (Droge 2002; Poulsen 2005). ROS, such as oxygen ions, free radicals, and hydrogen peroxides, and other reduced nicotinamide adenine dinucleotide phosphate (NADPH) oxidase-dependent oxygen metabolites are toxic agents as they can oxidize macro-molecules, including lipids, proteins, and DNA (Lawson et al. 1999; Heller et al. 2000; Chaudhary et al. 1994). NADPH oxidase is

one of the most important sources of ROS. Due to its bactericidal activity, the NADPH enzyme system plays an important role in immune protection (Cross and Segal 2004). It is known that ROS related oxidative stress plays an important role in the pathogenesis of inflammation (Kawanishi et al. 2006; Halliday 2005). Chronic inflammation and immune-related conditions such as arthritis, Sjogren's syndrome, systemic lumpus erythematosus have been associated with risk of non-Hodgkin lymphoma (NHL) (Baecklund et al. 2006; Smedby et al. 2006; Engels et al. 2005; Xu and Wiernik 2001).

AKR genes (*AKR1A1*, *AKR1C1*, and *AKR1C3*) are members of the aldo-keto reductase (AKR) superfamily, encode NADPH-dependent oxidoreductase and are involved in catalyzing the reduction of aldehydes (Jez and Penning 2001). *AKR* genes can also oxidize polycyclic aromatic hydrocarbon metabolites that in turn produce reactive and redox-active *o*-quinones which can form DNA adducts or ROS leading to oxidative DNA damage. *CYBA* encodes the small sub-unit, p22-phox protein, of the cytochrome b_{558} of the NADPH oxidase that plays a crucial role in NADPH-dependent ROS production. Endogenous defenses against ROS formation and genomic damage includes superoxide dismutase enzymes, glutathione peroxidase, as well as myeloperoxidase (Bewick 1987). Superoxide dismutase converts superoxides into hydrogen peroxide, and glutathione peroxidase converts hydrogen peroxide into water. Multiple DNA repair mechanisms exist to avoid the effects of ROS and maintain genomic integrity (Caporaso 2003). The most important DNA repair pathway of oxidative damage is base-excision repair (Slupphaug et al. 2003) which includes the enzyme OGG1 (Shinmura and Yokota 2001).

We have evaluated the influence of SNPs in NADPH oxidation related genes, *AKR* and *CYBA* genes on NHL risk, as well as SNPs in other genes in related oxidative stress pathways (*GPX1*, *MPO*, *NOS2A*, *NOS3*, *OGG1*, and *SOD2*) (Table 1), in a population-based case-control study among women in Connecticut. The SNPs analyzed in this study were either chosen on the basis of prior functional data in previous association studies, or to help characterize the haplotype structure of the gene of interest (Danesh et al. 2003; Inoue et al. 1998; Lan et al. 2004; Lightfoot et al. 2006; Shimo-Nakanishi et al. 2004; Wang et al. 2006).

Materials and methods

Study population

The study population has previously been described in detail elsewhere (Morton et al. 2003; Zhang et al. 2004; Zheng et al. 2004). Briefly, from 1996 to 2000, all histologically confirmed (Revised European-American Lymphoma (REAL) system) incident female NHL cases aged 21–84 years old in Connecticut, alive at the time of interview and without a previous diagnosis of cancer except for non-melanoma skin cancer, were identified through the Yale Cancer Center's Rapid Case Ascertainment Shared Resource (RCA). Controls were frequency matched to cases on age (±5 years) by adjusting the number of controls randomly selected in each age stratum once every several months during the period of recruitment. The study was approved by the Institutional Review Board at Yale University, the Connecticut Department of Public Health, and the

Table 1 Oxidative stress genes and single nucleotide polymorphisms evaluated in the case-control study for non-Hodgkin lymphoma

Gene	Name	Chromosomal location	SNP rs #	Nucleotide change
AKR1A1	Aldo-keto reductase family 1, member A1	1p33-p32	rs2088102	IVS5 + 282T > C[a]
AKR1C1	Aldo-keto reductase family 1, member C1	10p15-p14	rs8483	Ex9 + 68A > G[a]
AKR1C3	Aldo-keto reductase family 1, member C3	10p15-p14	rs12529	Ex1-70C > G[a]
CYBA	Cytochrome *b*-245, alpha polypeptide	16q24	rs4673	Ex4 + 11T > C[b]
			rs1049255	Ex6-41C > T[a]
			rs7195830	Ex6-16C > T[a]
GPX1	Glutathione peroxidase 1	3p21.3	rs1050450	Ex1-226C > T[a]
MPO	Myeloperoxidase	17q23.1	rs2333227	−642G > A[b]
NOS2A	Nitric oxide synthase 2A	17cen-q11.2	rs2297518	Ex16 + 14C > T[a]
			rs944722	IVS20 + 524G > A[a]
			rs9282801	IVS16 + 88T > G[a]
NOS3	Nitric oxide synthase 3	7q36	rs1799983	Ex8-63G > T[a]
OGG1	8-Oxoguanine DNA glycosylase	3p26.2	rs1052133	Ex6-315C > G[b]
SOD2	Superoxide dismutase 2, mitochondrial	6q25.3	rs4880	Ex2 + 24T > C[b]

[a] Genotyped in blood-based samples only

[b] Genotyped in both blood- and buccal-based samples

National Cancer Institute. Written, informed consent was obtained from each subject and participation was voluntary. About 75% of the interviewed subjects provided blood samples (76.7% of cases and 74.6% of controls), and about 10% of the study subjects (11.0% of cases and 10.4% of controls) provided buccal cell samples (Table 2). In total, 72.2% of the eligible cases completed in-person interviews, 76.7% (461/601) of interviewed cases provided a blood sample, and 11.0% (57) of cases provided a buccal cell sample. Between the two sources of controls, the participation rate for the in-person interview was 69% for the random digit dialing controls and 47% for the Centers for Medicare and Medicaid Services. Blood samples were provided by 74.6% (535/717) of the interviewed controls, where 10.4% (62) of the controls provided buccal cell samples. DNA was extracted from blood or buccal cell samples using phenol–chloroform extraction. Genotyping was carried out by real-time PCR on an ABI 7900HT sequence detection system as described on the SNP500 website (http://www.snp500cancer.nci.nih.gov) (Packer et al. 2006). For subjects who provided only buccal cells, the amount of DNA was limited. Therefore, we first genotyped DNA from subjects who provided a blood sample. If the results were suggestive or if there was a relatively high prior probability that a given SNP was associated with risk of NHL, we subsequently genotyped those subjects who provided buccal cell samples. Among the 14 SNPs, 10 were genotyped only in subjects who provided a blood sample (Table 1). Duplicate samples from 100 study subjects and 40 replicate samples from each of two blood donors were interspersed throughout the plates used for genotype analysis. The concordance rates for quality control samples were 99–100% for all assays.

The chi-square test was used to test for departures from Hardy–Weinberg equilibrium among non-Hispanic Caucasian controls. All of the SNP genotype frequencies in the non-Hispanic Caucasian control subjects were in Hardy–Weinberg equilibrium. The ORs were estimated using unconditional logistic regression, adjusting for age (<50 years, 50–70 years, >70 years) and race (white, African-American, other). Further analyses adjusting for family history (first- and second-degree relatives) or limited to non-Hispanic Caucasians (representing 93.2 and 91.6% of all cases and controls, respectively) resulted in similar results. In this report, we present results of individual SNP analyses that include all study subjects in all models adjusting for age and race. Gene dosage effects were evaluated by assigning the ordinal values 1, 2, or 3 based on the genotype, i.e., homozygote wild-type, heterozygote, and homozygote variant. To maximize statistical power, risks for NHL subtypes were carried out using all controls as the comparison group.

Table 2 Characteristics of study participants (n = 1,115)

Characteristics	Cases (n = 518) n (%)	Controls (n = 597) n (%)	P value
Age (years)			0.60
<40	43 (8.3%)	51 (8.5%)	
40–49	59 (11.4%)	66 (11.1%)	
50–59	109 (21.0%)	109 (18.3%)	
60–69	132 (25.5%)	144 (24.1%)	
70+	175 (33.8%)	227 (38.0%)	
Race			0.14
Caucasian	497 (96.0%)	561 (94.0%)	
Non-Hispanic	483 (93.2%)	547 (91.6%)	
Hispanic	12 (2.3%)	14 (2.3%)	
Unknown	2 (0.4)	0 (0%)	
African-American	16 (3.1%)	17 (2.9%)	
Other	5 (1.0%)	19 (3.2%)	
Family history[a]			0.06[b]
No	110 (21.2%)	147 (24.6%)	
NHL	9 (1.7%)	3 (0.5%)	
Other cancer	399 (77.0%)	447 (74.9%)	
DNA source			0.74
Blood	461 (89.0%)	535 (89.6%)	
Buccal cells	57 (11.0%)	62 (10.4%)	
Case pathology			NA
All B cell	411 (79.3%)	NA	
Diffuse large B-cell	161 (31.1%)	NA	
Follicular	119 (23.0%)	NA	
SLL/CLL	59 (11.4%)	NA	
Marginal zone	35 (6.8%)	NA	
Other	37 (7.1%)	NA	
All T cell	39 (7.5%)	NA	
NOS	68 (13.1%)	NA	

NHL non-Hodgkin lymphoma, *DLBCL* diffuse large B-cell lymphoma, *CLL/SLL* B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma, *MZBL* marginal zone B-cell lymphoma, *NA* not applicable, and *NOS* not otherwise specified

[a] Family history of cancer in first- and second-degree relatives

[b] Exact test

For all genes in which more than one SNP was genotyped, haplotype frequencies and analyses were conducted within non-Hispanic Caucasians. HaploView program (http://www.broad.mit.edu/personal/jcbarret/haploview/) was used to evaluate the haplotype block structure (Barrett et al. 2005) the Expectation-Maximization algorithm (Excoffier and Slatkin 1995) was used to estimate the haplotype frequencies, and the omnibus test that was implemented in SAS Genetics was used to test the overall differences in haplotype frequencies between non-Hispanic Caucasian cases and controls, adjusting for age. An unconditional logistic regression model was used to estimate the effect of individual haplotypes while assuming the most likely

haplotype pairs for each person. Unless otherwise specified, all analyses were conducted using SAS 8.2 (SAS Institute Inc., Cary, NC).

The global test of the association between SNPs in the oxidative stress pathway and NHL risk was examined using likelihood ratio Chi-square statistics which compared the logistic regression models that included all SNPs as the main effects with the null model that included none of the SNPs. To test the robustness of our findings, the false discovery rate (FDR) (Benjamini–Hochberg adjustment) method (Benjamini and Hochberg 1995) was applied, which provides the expected ratio of erroneous rejections of the null hypothesis to the total number of rejected hypotheses. The FDR was calculated using results from the additive model (i.e., *P*-trend), as this permits for minimal degrees of freedom across genotypes. FDR values were calculated from the results of 14 tests (i.e., total number of SNPs studied) evaluating the association between each SNP and the risk of NHL, from the 28 tests of association for risk of B- and T-cell lymphoma, and from the 56 tests of association for four B-cell subtypes.

Results

A greater proportion of NHL cases than controls reported having a family history of NHL and other cancers among relatives (Table 2). In terms of age and ethnicity, the NHL cases were comparable to controls. Further analyses which adjusted for family history resulted in similar estimates (data not shown).

Overall, the global test pathway, which incorporates results from all SNPs tested, was borderline significant ($P = 0.06$), suggesting that genetic variation in the oxidative stress pathway may be important for lymphomagenesis. More specifically, individuals homozygous for the *AKR1A1* (IVS5 + 282T > C) SNP had a 1.7-fold (95% CI = 1.2–2.4, $P = 0.0047$) increased risk of NHL (Table 3). The effect was most pronounced for diffuse large B-cell lymphoma (DLBCL) (TT is referent; $OR_{CT} = 1.5$, 95% CI = 0.9–2.3, $P = 0.11$; $OR_{CC} = 1.8$, 95% CI = 1.1–3.1, $P = 0.028$; $P_{trend} = 0.027$) (Table 4), but risks were non-significantly elevated for other common B-cell histologies and T-cell lymphomas. There was some evidence of association for the *AKR1C1* (Ex9 + 68G > A) SNP, particularly for B-cell lymphomas (Table 3). We also found that individuals homozygous for the nonsynonomous *CYBA* (Ex4 + 11C > T) SNP had a 1.6-fold (95% CI = 1.1–2.4, $P = 0.019$) increased risk of NHL that was particularly pronounced for T-cell lymphoma (OR = 3.5, 95% CI = 1.3–9.6, $P = 0.013$) (Table 3), and also associated with non-statistically significant increased risks for each of the common B-cell histologies (Table 4). The FDR value for the *AKR1A1* (IVS5 + 282T > C) SNP and risk of all NHL was 0.06, which is a relatively low

Table 3 Association between *AKR1A1*, *AKR1C1*, *CYBA* polymorphisms and non-Hodgkin lymphoma (NHL) among all NHL, B- and T-cell lymphoma cases and controls and odds ratios adjusted for age and race

Genotype	Controls (%)	All NHL			B-cell NHL			T-cell NHL		
		Cases (%)	OR (95% CI)	*P* value	Cases (%)	OR (95% CI)	*P* value	Cases (%)	OR (95% CI)	*P* value
AKR1A1 rs2088102 IVS5 + 282T > C										
TT	160 (30)	103 (22)	1.0 (ref)		160 (30)	1.0 (ref)		10 (30)	1.0 (ref)	
CT	262 (49)	236 (52)	1.4 (1.0–1.9)	0.037	192 (52)	1.4 (1.0–1.9)	0.066	12 (36)	0.8 (0.3–1.8)	0.51
CC	110 (21)	119 (26)	1.7 (1.2–2.4)	0.0047	90 (25)	1.5 (1.0–2.3)	0.029	11 (33)	1.7 (0.7–4.1)	0.26
CT or CC	372 (70)	355 (78)	1.5 (1.1–2.0)	0.0087	282 (77)	1.4 (1.0–1.9)	0.029	23 (70)	1.0 (0.5–2.2)	0.97
Trend[a]				0.0044			0.026			0.29
AKR1C1 rs8483 Ex9 + 68G > A										
GG	211 (40)	158 (35)	1.0 (ref)		123 (34)	1.0 (ref)		10 (30)	1.0 (ref)	
AG	237 (45)	219 (48)	1.3 (0.9–1.7)	0.12	185 (51)	1.4 (1.0–1.8)	0.042	15 (45)	1.4 (0.6–3.1)	0.46
AA	81 (15)	76 (17)	1.3 (0.9–1.9)	0.23	55 (15)	1.2 (0.8–1.8)	0.41	8 (24)	2.0 (0.8–5.3)	0.17
AG or AA	318 (60)	295 (65)	1.3 (1.0–1.6)	0.094	240 (66)	1.3 (1.0–1.7)	0.054	23 (70)	1.5 (0.7–3.3)	0.28
Trend				0.14			0.18			0.17
CYBA rs4673 (Tyr/His) Ex4 + 11C > T										
CC	245 (44)	187 (40)	1.0 (ref)		154 (41)	1.0 (ref)		9 (27)	1.0 (ref)	
CT	253 (45)	209 (44)	1.1 (0.8–1.4)	0.61	165 (44)	1.0 (0.8–1.4)	0.87	16 (48)	1.7 (0.8–4.0)	0.20
TT	62 (11)	74 (16)	1.6 (1.1–2.4)	0.019	57 (15)	1.5 (1.0–2.2)	0.065	8 (24)	3.5 (1.3–9.6)	0.013
CT or TT	315 (56)	283 (60)	1.2 (0.9–1.5)	0.21	222 (59)	1.1 (0.9–1.5)	0.43	24 (73)	2.1 (1.0–4.6)	0.065
Trend				0.040			0.13			0.015

[a] For trend tests, the homozygote wild-type, heterozygote, and homozygote variant genotypes were assigned values of 0, 1, and 2, respectively, with the genotype treated as a continuous variable

Table 4 Association between *AKR1A1*, *AKR1C1*, *CYBA* polymorphisms and non-Hodgkin lymphoma (NHL) among diffuse, follicular, marginal zone and B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma cases and controls and odds ratios adjusted for age and race

Genotype	Controls (%)	DLBCL			FL			CLL/SLL			MZBL		
		Cases (%)	OR (95% CI)	*P* value	Cases (%)	OR (95% CI)	*P* value	Cases (%)	OR (95% CI)	*P* value	Cases (%)	OR (95% CI)	*P* value
AKR1A1 rs2088102 IVS5 + 282T > C													
TT	160 (30)	31 (21)	1.0 (ref)		26 (25)	1.0 (ref)		12 (23)	1.0 (ref)		5 (16)	1.0 (ref)	
CT	262 (49)	77 (52)	1.5 (0.9–2.3)	0.11	51 (49)	1.2 (0.7–1.9)	0.60	28 (53)	1.5 (0.7–2.9)	0.30	21 (68)	2.9 (0.9–6.7)	0.080
CC	110 (21)	39 (27)	1.8 (1.1–3.1)	0.028	28 (27)	1.6 (0.9–2.8)	0.14	13 (25)	1.6 (0.7–3.7)	0.26	5 (16)	1.4 (0.4–5.0)	0.59
CT or CC	372 (70)	116 (79)	1.6 (1.0–2.4)	0.045	79 (75)	1.3 (0.8–2.1)	0.34	41 (77)	1.5 (0.76–2.9)	0.24	26 (84)	2.2 (0.8–5.8)	0.12
Trend				0.027			0.15			0.25			0.50
AKR1C1 rs8483 Ex9 + 68G > A													
GG	211 (40)	50 (35)	1.0 (ref)		33 (31)	1.0 (ref)		21 (39)	1.0 (ref)		10 (33)	1.0 (ref)	
AG	237 (45)	73 (51)	1.3 (0.9–2.0)	0.21	55 (52)	1.6 (1.0–2.5)	0.062	27 (50)	1.2 (0.6–2.1)	0.64	14 (47)	1.3 (0.6–3.0)	0.57
AA	81 (15)	21 (15)	1.1 (0.6–1.9)	0.77	18 (17)	1.5 (0.8–2.8)	0.25	6 (11)	0.7 (0.3–1.9)	0.54	6 (20)	1.7 (0.6–4.7)	0.35
AG or AA	318 (60)	94 (65)	1.3 (0.9–1.8)	0.27	73 (69)	1.5 (1.0–2.4)	0.060	33 (61)	1.1 (0.6–1.9)	0.88	20 (67)	1.4 (0.6–3.0)	0.43
Trend				0.51			0.13			0.75			0.34
CYBA rs4673 (Tyr/His) Ex4 + 11C > T													
CC	245 (44)	59 (40)	1.0 (ref)		45 (42)	1.0 (ref)		29 (51)	1.0 (ref)		11 (35)	1.0 (ref)	
CT	253 (45)	69 (47)	1.1 (0.8–1.6)	0.62	49 (45)	1.0 (0.7–1.6)	0.92	16 (28)	0.5 (0.3–1.0)	0.06	16 (52)	1.4 (0.6–3.1)	0.41
TT	62 (11)	20 (14)	1.4 (0.8–2.5)	0.29	14 (13)	1.3 (0.7–2.5)	0.46	12 (21)	1.6 (0.8–3.4)	0.19	4 (13)	1.4 (0.4–4.6)	0.56
CT or TT	315 (56)	89 (60)	1.2 (0.8–1.7)	0.45	63 (58)	1.1 (0.7–1.6)	0.74	28 (49)	0.8 (0.4–1.3)	0.32	20 (65)	1.4 (0.7–3.0)	0.39
Trend				0.31			0.55			0.75			0.43

For trend tests, the homozygote wild-type, heterozygote, and homozygote variant genotypes were assigned values of 0, 1, and 2, respectively, with the genotype treated as a continuous variable

NHL non-Hodgkin lymphoma, *DLBCL* diffuse large B-cell lymphoma, *FL* follicular lymphoma, *CLL/SLL* B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma, *MZBL* marginal zone B-cell lymphoma

value and indicates that the association may be particularly robust. The FDR value for the *CYBA* (Ex4 + 11C > T) SNP and risk of all NHL was 0.28. FDR values for B and T cell-specific analysis shown in Table 3, and for the B-cell subtype analyses shown in Table 4, were above 0.28.

Haplotype analysis of all genes with two or more SNPs in linkage disequilibrium showed the same general pattern as observed in Table 3 (Online Supplemental Table 3). Although additional SNPs were significantly associated with one or more NHL subtypes [i.e., *NOS3* IVS1-762C > T and T-cell lymphoma (Online Supplemental Table 1)]; *AKR1C3* (Ex1-70C > G) and DLBCL; *MPO* (642G > A) and follicular lymphoma; and *GPX1* (Ex1-226C > T) and marginal zone B-cell lymphoma (Online Supplemental Table 2), the number of subjects with the homozygous genotype was either relatively small or there was no indication of a gene dosage effect. We did not find significant associations between the remaining eleven SNPs and NHL or any subtype (Online Supplemental Tables 1 and 2).

Discussion

We evaluated 14 SNPs that were drawn from ten key genes that play a direct or indirect role in mediation of oxidative stress and risk of NHL. Overall, we observed that common genetic variants in genes related to the NADPH oxidase, *AKR1A1* and *CYBA* were significantly associated with an increased overall risk of NHL.

There is increasing evidence that ROS generated by the NADPH oxidase plays an important role in signaling molecules for proliferation of lymphocytes and tumor cells. Inflammatory stimuli or infection can trigger a NADPH respiratory burst, which activates the NADPH oxidase system and produces ROS (Almeida et al. 2005). ROS are also important secondary messengers generated in response to environmental stress and can regulate many cellular actions, including apoptosis, cell differentiation and proliferation, often via activation of transcription factors (including NF-κB) (Almeida et al. 2005; Sauer et al. 2001). In response to stress conditions such as infection and inflammation, an inactive form of NF-κB present in the cytoplasm can be activated. Activated NF-κB transcription factors have been associated with several features of tumorigenesis, including the proliferation, differentiation, and survival of lymphocytes (Piva et al. 2006). In addition, there is evidence that NF-κB activity plays an important role in the pathogenesis of activated B-cell-like DLBCL cell lines (Davis et al. 2001; Alizadeh et al. 2000), which is critical in lymphomagenesis.

AKRs are NADPH-dependent oxido-reductases involved in both catalytic reactions and in the conversion of aldehydes and ketones into their respective alcohols through the oxidizing of an NADPH cofactor (Jez and Penning 2001). These enzymes are ubiquitous in nature among mammals, amphibians and plants as well as in lower life forms such as in yeast, protozoa and bacteria. AKRs have a broad substrate spectrum including aliphatic and aromatic aldehydes, monosaccharides, steroids, prostaglandins, and polycyclic aromatic hydrocarbons, which suggests that they may be involved in various biological processes in a variety of tissues. Few published studies have reported associations between *AKR* genes and cancer, although associations between *AKRs* and lung cancer and diabetes have been reported (Lan et al. 2004; Danesh et al. 2003). It is noteworthy that the only association we report here with an FDR value less than 0.20 was the *AKR1A1* (IVS5 + 282T > C) SNP (FDR = 0.06, for risk of all NHL), which provides additional support that this is an important locus for risk of NHL. Additional work is needed to extensively evaluate genetic variants in this locus with respect to risk for NHL, particularly because the functional significance of this intronic SNP is unknown. The *AKR1C1* (Ex9 + 68G > A) variant was found to be associated with NHL risk in our study, and is located in the 3′ untranslated region, which could change gene translation through ribosome binding, initiation, or elongation, and therefore potentially affect mRNA stability (Kuersten and Goodwin 2003). Alternatively, the two *AKR* polymorphisms could be in linkage disequilibrium with other variants in a region associated with increased risk that we did not study.

The SNP (C242T) (His72Tyr) in the *CYBA* gene located in exon 4, which is found in putative heme-binding sites, results in the replacement of histidine with tyrosine. This SNP has been suggested to have functional relevance for the p22-phox protein because of the structural change it causes in the enzyme structure (Inoue et al. 1998). One study has shown that compared with the C allele, the T allele conferred an increase in NADPH oxidase activity both in human probands and in cells transfected with *CYBA* expression constructs (Shimo-Nakanishi et al. 2004). However, Guzik reported that the T allele was associated with reduced basal and NADPH-stimulated superoxide production (Guzik et al. 2000). Similarly, there are inconsistent reported associations between the T allele and diabetes and coronary heart disease (Matsunaga-Irie et al. 2004; Zalba et al. 2005; Santos et al. 2005; Doi et al. 2004). Our finding that genetic variation in *CYBA* was associated with risk of NHL,

particularly T-cell lymphoma, is consistent with one report that found some evidence that *CYBA* polymorphisms were associated with risk of T-cell NHL (Wang et al. 2006). However, the number of cases with T-cell lymphoma in both studies was small and the finding requires replication in larger, pooled analyses.

We also found additional SNPs were significantly associated with one or more NHL subtypes. However, the sample size is relatively small, and these could be false positive results (Wacholder et al. 2004). It is worth noting that the *GPX1* Ex1-226C > T was significantly associated with marginal zone B-cell lymphoma, which has been suspected to be associated with chronic inflammation. Lightfoot et al. (2006) recently found that the same variant is associated with an increased NHL risk in both DLBCL and follicular lymphoma. Interestingly, a recent report of microarray findings from analysis of tumor samples found that decreased expression of glutathione peroxidase was associated with the worst prognosis for DLBCL patients (Tome et al. 2005).

Our study has several strengths. It is a population-based, case-control study with both incident cases that are histologically confirmed, and highly accurate genotyping data. The primary limitation of our study is that the sample size is modest and the number of cases in several histologic subgroups was small. This resulted in reduced power to detect associations for SNPs with low allele frequencies. Further, the response rate in our study is moderate and could potentially result in biased risk estimates if willingness to participate in our study was associated with genotype frequency. However, a recent report observed that subject participation status was unrelated to genotype frequencies for a wide spectrum of genes (Bhatti et al. 2005). The positive findings in our report require replication in larger studies with greater power, which will be particularly valuable if tagged SNPs with full genomic coverage of the most promising candidate genes are used.

In summary, we report that common genetic variants in the oxidative stress pathway may be associated with the risk of NHL. A detailed, extensive genomic analysis of genes that play a role in the oxidative stress pathway is warranted. Further, these findings require replication in larger studies and ultimately in pooled analyses.

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References

Alizadeh AA, Eisen MB, Davis RE, Ma C, Lossos IS, Rosenwald A, Boldrick JC, Sabet H, Tran T, Yu X, Powell JI, Yang L, Marti GE, Moore T, Hudson J Jr, Lu L, Lewis DB, Tibshirani R, Sherlock G, Chan WC, Greiner TC, Weisenburger DD, Armitage JO, Warnke R, Levy R, Wilson W, Grever MR, Byrd JC, Botstein D, Brown PO, Staudt LM (2000) Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 403:503–511

Almeida AC, Rehder J, Severino SD, Martins-Filho J, Newburger PE, Condino-Neto A (2005) The effect of IFN-gamma and TNF-alpha on the NADPH oxidase system of human colostrum macrophages, blood monocytes, and THP-1 cells. J Interferon Cytokine Res 25:540–546

Baecklund E, Iliadou A, Askling J, Ekbom A, Backlin C, Granath F, Catrina AI, Rosenquist R, Feltelius N, Sundstrom C, Klareskog L (2006) Association of chronic inflammation, not its treatment, with increased lymphoma risk in rheumatoid arthritis. Arthritis Rheum 54:692–701

Barrett JC, Fry B, Maller J, Daly MJ (2005) Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics 21:263–265.

Benjamini Y, Hochberg Y (1995) Controlling the false discovery rate: a practical and powerful approach to multiple testing. J R Stat Soc B 57:289–300

Bewick M, Coutie W, Tudhope GR (1987) Superoxide dismutase, glutathione peroxidase and catalase in the red cells of patients with malignant lymphoma. Br J Haematol 65:347–350

Bhatti P, Sigurdson AJ, Wang SS, Chen J, Rothman N, Hartge P, Bergen AW, Landi MT (2005) Genetic variation and willingness to participate in epidemiologic research: data from three studies. Cancer Epidemiol Biomarkers Prev 14:2449–2453

Caporaso N (2003) The molecular epidemiology of oxidative damage to DNA and cancer. J Natl Cancer Inst 95:1263–1265

Chaudhary AK, Nokubo M, Reddy GR, Yeola SN, Morrow JD, Blair IA, Marnett LJ (1994) Detection of endogenous malondialdehyde-deoxyguanosine adducts in human liver. Science 265:1580–1582

Cross AR, Segal AW (2004) The NADPH oxidase of professional phagocytes-prototype of the NOX electron transport chain systems. Biochim Biophys Acta 1657:1–22

Danesh FR, Wada J, Wallner EI, Sahai A, Srivastava SK, Kanwar YS (2003) Gene regulation of aldose-, aldehyde- and a renal specific oxido reductase (RSOR) in the pathobiology of diabetes mellitus. Curr Med Chem 10:1399–1406

Davis RE, Brown KD, Siebenlist U, Staudt LM (2001) Constitutive nuclear factor kappaB activity is required for survival of activated B cell-like diffuse large B cell lymphoma cells. J Exp Med 194:1861–1874

Doi K, Noiri E, Tokunaga K (2004) The association of NAD(P)H oxidase p22phox with diabetic nephropathy is still uncertain: response to Hodgkinson, Millward, and Demaine. Diabetes Care 27:1518–1519

Droge W (2002) Free radicals in the physiological control of cell function. Physiol Rev 82:47–95

Engels EA, Cerhan JR, Linet MS, Cozen W, Colt JS, Davis S, Gridley G, Severson RK, Hartge P (2005) Immune-related conditions and immune-modulating medications as risk factors for non-Hodgkin's lymphoma: a case-control study. Am J Epidemiol 162:1153–1161

Excoffier L, Slatkin M (1995) Maximum-likelihood estimation of molecular haplotype frequencies in a diploid population. Mol Biol Evol 12:921–927

Guzik TJ, West NE, Black E, McDonald D, Ratnatunga C, Pillai R, Channon KM (2000) Functional effect of the C242T polymorphism in the NAD(P)H oxidase p22phox gene on vascular superoxide production in atherosclerosis. Circulation 102:1744–1747

Halliday GM (2005) Inflammation, gene mutation and photoimmunosuppression in response to UVR-induced oxidative damage contributes to photocarcinogenesis. Mutat Res 571:107–120

Heller JI, Crowley JR, Hazen SL, Salvay DM, Wagner P, Pennathur S, Heinecke JW (2000) *p*-Hydroxyphenylacetaldehyde, an aldehyde generated by myeloperoxidase, modifies phospholipid amino groups of low density lipoprotein in human atherosclerotic intima. J Biol Chem 275:9957–9962

Inoue N, Kawashima S, Kanazawa K, Yamada S, Akita H, Yokoyama M (1998) Polymorphism of the NADH/NADPH oxidase p22 phox gene in patients with coronary artery disease. Circulation 97:135–137

Jez JM, Penning TM (2001) The aldo-keto reductase (AKR) superfamily: an update. Chem Biol Interact 130–132:499–525

Kawanishi S, Hiraku Y, Pinlaor S, Ma N (2006) Oxidative and nitrative DNA damage in animals and patients with inflammatory diseases in relation to inflammation-related carcinogenesis. Biol Chem 387:365–372

Kuersten S, Goodwin EB (2003) The power of the 3′ UTR: translational control and development. Nat Rev Genet 4:626–637

Lan Q, Mumford JL, Shen M, Demarini DM, Bonner MR, He X, Yeager M, Welch R, Chanock S, Tian L, Chapman RS, Zheng T, Keohavong P, Caporaso N, Rothman N (2004) Oxidative damage-related genes AKR1C3 and OGG1 modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. Carcinogenesis 25:2177–2181

Lawson JA, Rokach J, FitzGerald GA (1999) Isoprostanes: formation, analysis and use as indices of lipid peroxidation in vivo. J Biol Chem 274:24441–24444

Lightfoot TJ, Skibola CF, Smith AG, Forrest MS, Adamson PJ, Morgan GJ, Bracci PM, Roman E, Smith MT, Holly EA (2006) Polymorphisms in the oxidative stress genes, superoxide dismutase, glutathione peroxidase and catalase and risk of non-Hodgkin's lymphoma. Haematologica 91:1222–1227

Matsunaga-Irie S, Maruyama T, Yamamoto Y, Motohashi Y, Hirose H, Shimada A, Murata M, Saruta T (2004) Relation between development of nephropathy and the p22phox C242T and receptor for advanced glycation end product G1704T gene polymorphisms in type 2 diabetic patients. Diabetes Care 27:303–307

Morton LM, Holford TR, Leaderer B, Boyle P, Zahm SH, Zhang Y, Flynn S, Tallini G, Zhang B, Owens PH, Zheng T (2003) Cigarette smoking and risk of non-Hodgkin lymphoma subtypes among women. Br J Cancer 89:2087–2092

Packer BR, Yeager M, Burdett L, Welch R, Beerman M, Qi L, Sicotte H, Staats B, Acharya M, Crenshaw A, Eckert A, Puri V, Gerhard DS, Chanock SJ (2006) SNP500Cancer: a public resource for sequence validation, assay development, and frequency analysis for genetic variation in candidate genes. Nucleic Acids Res 34:D617–D621

Piva R, Belardo G, Santoro MG (2006) NF-kappaB: a stress-regulated switch for cell survival. Antioxid Redox Signal 8:478–486

Poulsen HE (2005) Oxidative DNA modifications. Exp Toxicol Pathol 57(Suppl 1):161–169

Santos KG, Canani LH, Gross JL, Tschiedel B, Souto KE, Roisenberg I (2005) Relationship of p22phox C242T polymorphism with nephropathy in type 2 diabetic patients. J Nephrol 18:733–738

Sauer H, Wartenberg M, Hescheler J (2001) Reactive oxygen species as intracellular messengers during cell growth and differentiation. Cell Physiol Biochem 11:173–186

Shimo-Nakanishi Y, Hasebe T, Suzuki A, Mochizuki H, Nomiyama T, Tanaka Y, Nagaoka I, Mizuno Y, Urabe T (2004) Functional effects of NAD(P)H oxidase p22(phox) C242T mutation in human leukocytes and association with thrombotic cerebral infarction. Atherosclerosis 175:109–115

Shinmura K, Yokota J (2001) The OGG1 gene encodes a repair enzyme for oxidatively damaged DNA and is involved in human carcinogenesis. Antioxid Redox Signal 3:597–609

Slupphaug G, Kavli B, Krokan HE (2003) The interacting pathways for prevention and repair of oxidative DNA damage. Mutat Res 531:231–251

Smedby KE, Hjalgrim H, Askling J, Chang ET, Gregersen H, Porwit-MacDonald A, Sundstrom C, Akerman M, Melbye M, Glimelius B, Adami HO (2006) Autoimmune and chronic inflammatory disorders and risk of non-Hodgkin lymphoma by subtype. J Natl Cancer Inst 98:51–60

Tome ME, Johnson DB, Rimsza LM, Roberts RA, Grogan TM, Miller TP, Oberley LW, Briehl MM (2005) A redox signature score identifies diffuse large B-cell lymphoma patients with a poor prognosis. Blood 106:3594–3601

Wacholder S, Chanock S, Garcia-Closas M, El Ghormli L, Rothman N (2004) Assessing the probability that a positive report is false: an approach for molecular epidemiology studies. J Natl Cancer Inst 96:434–442

Wang SS, Davis S, Cerhan JR, Hartge P, Severson RK, Cozen W, Lan Q, Welch R, Chanock SJ, Rothman N (2006) Polymorphisms in oxidative stress genes and risk for non-Hodgkin lymphoma. Carcinogenesis 27:1828–1834

Xu Y, Wiernik PH (2001) Systemic lupus erythematosus and B-cell hematologic neoplasm. Lupus 10:841–850

Zalba G, San Jose G, Moreno MU, Fortuno A, Diez J (2005) NADPH oxidase-mediated oxidative stress: genetic studies of the p22(phox) gene in hypertension. Antioxid Redox Signal 7:1327–1336

Zhang Y, Holford TR, Leaderer B, Boyle P, Zahm SH, Flynn S, Tallini G, Owens PH, Zheng T (2004) Hair-coloring product use and risk of non-Hodgkin's lymphoma: a population-based case-control study in Connecticut. Am J Epidemiol 159:148–154

Zheng T, Holford TR, Leaderer B, Zhang Y, Zahm SH, Flynn S, Tallini G, Zhang B, Zhou K, Owens PH, Lan Q, Rothman N, Boyle P (2004) Diet and nutrient intakes and risk of non-Hodgkin's lymphoma in Connecticut women. Am J Epidemiol 159:454–466