Genetic variation in genes expressed in the germinal center and risk of B cell lymphoma among Caucasians

The germinal center reaction is integral to B-cell maturation, where class switch recombination (CSR) and somatic hypermutation (SHM) are targeted to the immunoglobulin (Ig) locus to facilitate antibody diversity.[1] Selection against B cells with auto-reactive or low affinity antigen receptors promotes the generation of highly effective B cells.

SHM is initiated following deamination of cytidine to uracil, resulting in U:G mismatches.[2] Direct removal of uracil by uracil-DNA glycosylase (UNG) can lead to mutation if DNA is replicated across these non-informative abasic sites. Uracil-containing mispairs can also be recognized by mismatch repair (MMR) and base excision repair (BER) machinery, key components of which include MLH1, MSH2, PMS2 and XRCC1.[2] Excision of a repair patch and DNA re-synthesis using error-prone DNA polymerases promotes mutation.[2] MMR also functions in CSR where mismatches are recognized by the MSH2/MSH6 heterodimer, ultimately resulting in the DNA double strand breaks integral to class switching.[2]

Reciprocal translocations involving the Ig locus arising during VDJ recombination or CSR are a hallmark of B-cell lymphomas, and can lead to the activation of proto-oncogenes such as *MYC, CYCLIN D1, BCL6* and *BCL2*. Point mutations occur in genes outside the immunoglobulin locus, including *BCL6* and *FAS*.[3,4] These data suggest that translocations and mutations in B-cell lymphomas can arise via mis-targeting of the CSR and SHM machinery specifically during the germinal center reaction, and may allow cells to bypass the normal processes regulating cell proliferation, differentiation and apoptosis.

We hypothesized that genetic variation in genes expressed in B cells during the germinal center reaction, and which are components of CSR, SHM or B-cell selection, may affect the risk of developing lymphoma. We, therefore, examined the frequency of nine common polymorphisms with putative functionality (allele frequency >0.02) in six genes (*PMS2, UNG, XRCC1, MSH2, MLH1* and *FAS*) in 884 patients with lymphoma and 1,019 population controls. Six hundred and forty-nine Caucasian cases of B-cell non-Hodgkin's lymphoma (NHL), 235 Caucasian cases of Hodgkin's lymphoma (HL) and 1,019 Caucasian controls were recruited to a study conducted in the north and southwest of England between January 1998 and July 2003.[5] The study was approved by the UK Multicentre Research Ethics Committee and all participants gave informed consent according to the Declaration of Helsinki.

DNA was genotyped using allelic discrimination single nucleotide polymorphism (SNP) assays (TaqMan, Applied Biosystems (ABI), Warrington, UK). Genotype clusters were ascertained independently by two researchers and genotypes designated only when there was consensus. Assay accuracy was verified in 30 randomly selected patient samples by direct DNA sequencing (100% concordance). Among the controls, all geno-

Table 1. Number of cases and controls, adjusted odds ratios, and 95% confidence intervals by B-cell non-Hodgkin's lymphoma and Hodgkin's lymphoma for polymorphisms in genes expressed in the germinal center amongst Caucasians.

SNP	*Controls (N=1019)*	*B-cell NHL*[a] *Cases (N=649)*	*OR*[b]	*95% CI*	*HL*[a] *Cases (N=235)*	*OR*[b]	*95% CI*
UNG 1082 T>A (rs1018783)[c]							
TT	655 (67.3)	422 (68.1)	1	(ref)	143 (66.2)	1	(ref)
TA	287 (29.5)	189 (30.5)	1.01	0.80-1.26	67 (31.0)	1.15	0.81-1.63
AA	31 (3.2)	9 (1.4)	0.43	0.20-0.92	6 (2.8)	1.08	0.42-2.81
TA+AA	318 (32.7)	198 (31.9)	0.95	0.76-1.18	73 (33.8)	1.14	0.81-1.60
XRCC1 399 G>A (rs25487)[c]							
GG	366 (38.4)	277 (44.2)	1	(ref)	67 (34.2)	1	(ref)
GA	456 (47.9)	266 (42.4)	0.77	0.62-0.96	94 (48.0)	1.05	0.73-1.51
AA	131 (13.7)	84 (13.4)	0.84	0.61-1.16	35 (17.8)	1.41	0.87-2.30
GA+AA	587 (61.6)	350 (55.8)	0.78	0.64-0.96	129 (65.8)	1.12	0.79-1.59
PMS2 622 G>A (rs1805324)[c]							
GG	924 (95.8)	604 (95.3)	1	(ref)	216 (96.4)	1	(ref)
GA	41 (4.2)	30 (4.7)	1.11	0.68-1.81	8 (3.6)	0.91	0.40-2.07
AA	0	0		-	0		
GA+AA	41 (4.2)	30 (4.7)	1.11	0.68-1.81	8 (3.6)	0.90	0.40-2.05
PMS2 511 A>G (rs2228007)[c]							
AA	868 (94.9)	594 (94.4)	1	(ref)	153 (96.8)	1	(ref)
AG	47 (5.1)	35 (5.6)	1.05	0.67-1.65	5 (3.2)	0.73	0.28-1.93
GG	0	0		-	0		
AG+GG	47 (5.1)	35 (5.6)	1.05	0.67-1.66	5 (3.2)	0.75	0.28-1.99
MSH2 IVS1 +8 C>G (rs2303426)[c]							
GG	351 (37.0)	217 (35.0)	1	(ref)	73 (33.3)	1	(ref)
GC	455 (48.0)	312 (50.3)	1.11	0.88-1.39	118 (53.9)	1.26	0.89-1.79
CC	143 (15.0)	91 (14.7)	1.00	0.73-1.37	28 (12.8)	1.03	0.62-1.72
GC+CC	598 (63.0)	403 (65.0)	1.08	0.87-1.34	146 (66.7)	1.21	0.87-1.70
MSH2 IVS12 -6 T>C (rs2303428)[c]							
TT	758 (80.3)	491 (80.9)	1	(ref)	166 (81.0)	1	(ref)
TC	173 (18.3)	111 (18.3)	0.98	0.75-1.29	39 (19.0)	1.08	0.71-1.64
CC	13 (1.4)	5 (0.8)	0.58	0.20-1.67	0		
TC+CC	186 (19.7)	116 (19.1)	0.96	0.74-1.24	39 (19.0)	1.00	0.66-1.51
MLH1 -93 G>A (rs1800734)[c]							
GG	610 (64.8)	375 (62.4)	1	(ref)	137 (65.9)	1	(ref)
GA	310 (32.9)	205 (34.1)	1.06	0.85-1.32	65 (31.2)	1.01	0.71-1.43
AA	22 (2.3)	21 (3.5)	1.52	0.81-2.84	6 (2.9)	1.26	0.43-3.67
GA+AA	332 (35.2)	226 (37.6)	1.09	0.88-1.35	71 (35.3)	1.02	0.72-1.43
FAS -1377 G>A (rs2234767)[c]							
GG	701 (78.5)	454 (79.3)	1	(ref)	171 (80.7)	1	(ref)
GA	181 (20.2)	112 (19.6)	0.98	0.75-1.28	38 (17.9)	0.85	0.56-1.29
AA	12 (1.3)	6 (1.1)	0.66	0.24-1.80	3 (1.4)	1.72	0.40-7.35
GA+AA	193 (21.5)	118 (20.7)	0.96	0.74-1.24	41 (19.3)	0.89	0.59-1.33
FAS -670 G>A (rs1800682)[c]							
AA	277 (29.2)	183 (29.4)	1	(ref)	57 (26.5)	1	(ref)
AG	477 (50.2)	297 (47.8)	0.96	0.76-1.23	108 (50.2)	1.04	0.71-1.53
GG	196 (20.6)	142 (22.8)	1.14	0.85-1.52	50 (23.3)	1.16	0.73-1.83
AG+GG	673 (70.8)	439 (70.6)	1.01	0.81-1.27	158 (73.5)	1.08	0.75-1.54

[a]*NHL: non-Hodgkin's lymphoma; HL: Hodgkin's lymphoma.* [b]*Odds ratios adjusted for age, sex and geographical region were estimated using unconditional logistic regression.* [c]*Test for Hardy-Weinberg equilibrium among controls: UNG 1082 T>A χ^2=0.004, p=0.95; XRCC1 399 G>A χ^2=0.34, p=0.56; PMS2 622 G>A χ^2=0.46, p=0.50; PMS 511 A>G χ^2=0.64, p=0.43; MSH2 IVS1 +8 C>G χ^2=0.05, p=0.82; MSH2 IVS12 -6 T>C χ^2=0.75, p=0.39; MLH1 -93 G>A χ^2=5.78, p=0.02; FAS -1377 G>A χ^2=0.01, p=0.93; FAS -670 G>A χ^2=0.09, p=0.77. Samples did not amplify for 8 cases and 46 controls for UNG 1082 T>A; 61 cases and 66 controls for XRCC1 399 G>A; 26 cases and 54 controls for PMS 622 G>A; 97 cases and 104 controls for PMS 511 A>G; 45 cases and 70 controls for MSH2 IVS1 +8 C>G; 70 cases and 73 controls for MSH2 IVS12 -6 T>C; 73 cases and 75 controls for MLH1 -93 G>A; 98 cases and 123 controls for FAS -1377 G>A; and 47 cases and 69 controls for FAS -670 G>A.*

types were in Hardy-Weinberg equilibrium, with the exception of *MLH1* -93 G>A (p=0.02), although our data are similar to genotype frequencies for Caucasian populations presented on the NCBI dbSNP database (*http://www.ncbi.nlm.nih.gov/SNP/snp_ref.cgi?rs=1800734*; accessed 11/02/08). Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression using all controls in an unmatched analysis adjusting for age, sex and region of residence, and the likelihood ratio test was used to investigate interactions. Homozygosity for the UNG 1082 A variant was significantly associated with a decreased risk of B-cell NHL (OR 0.43, 95% CI 0.20-0.92; Table 1). There was some suggestion that this risk varied by gender (test for interaction: χ^2 = 6.45, p=0.01), with a decreased risk among female (OR (GA/AA *vs.* GG) 0.70, 95% CI 0.50-0.97), but not male carriers of the A allele (OR (GA/AA *vs.* GG) 1.23 95% CI 0.91-1.66). Compared to controls,

Table 2. Number of cases and controls, adjusted odds ratios, and 95% confidence intervals by B-cell non-Hodgkin's lymphoma subtypes for polymorphisms UNG 1082 T>A and XRCC1 399 G>A amongst Caucasians.

SNP	Controls (N=1019)	DLBCL[a] Cases (N=286)	OR[b]	95% CI	FL[a] Cases (N=216)	OR[b]	95% CI	MZL[a] Cases (N=112)	OR[b]	95% CI	MCL[a] Cases (N=35)	OR[b]	95% CI
UNG 1082 T>A (rs1018783)													
TT	655 (67.3)	198 (71.2)	**1**	**(ref)**	133 (65.8)	**1**	**(ref)**	72 (67.3)	**1**	**(ref)**	19 (57.6)	**1**	**(ref)**
TA	287 (29.5)	75 (27.0)	0.84	0.62-1.14	67 (33.2)	1.09	0.78-1.52	33 (30.8)	1.09	0.70-1.71	14 (42.4)	1.81	0.88-3.72
AA	31 (3.2)	5 (1.8)	0.52	0.20-1.36	2 (1.0)	0.31	0.07-1.33	2 (1.9)	0.56	0.13-2.44	0 (0)	0[c]	0-4.34[c]
TA+AA	318 (32.7)	80 (28.8)	0.81	0.60-1.09	69 (34.2)	1.02	0.74-1.41	35 (32.7)	1.05	0.68-1.62	14 (42.4)	1.65	0.80-3.39
XRCC1 399 G>A (rs25487)													
GG	366 (38.4)	125 (44.6)	**1**	**(ref)**	95 (44.2)	**1**	**(ref)**	43 (43.4)	**1**	**(ref)**	14 (42.4)	**1**	**(ref)**
GA	456 (47.9)	114 (40.7)	0.74	0.55-0.99	95 (44.2)	0.79	0.57-1.09	45 (45.5)	0.82	0.52-1.28	12 (36.4)	0.66	0.30-1.46
AA	131 (13.7)	41 (14.6)	0.92	0.61-1.38	25 (11.6)	0.72	0.44-1.18	11 (11.1)	0.74	0.37-1.50	7 (21.2)	1.25	0.48-3.23
GA+AA	587 (61.6)	155 (55.4)	0.78	0.59-1.02	120 (55.8)	0.77	0.57-1.05	56 (56.6)	0.81	0.53-1.23	19 (57.6)	0.80	0.39-1.64

[a]DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; MZL: marginal zone lymphoma; MCL: mantle cell lymphoma. [b]Odds ratios adjusted for age, sex and geographical region were estimated using unconditional logistic regression. [c]Odds ratio and 95% confidence interval estimated using exact methods.

homozygotes for the UNG 1082 A genotype were under-represented in all subtypes of B-cell NHL (Table 2). No association was observed between UNG 1082 T>A and HL in total (Table 1), or when stratified by Epstein-Barr virus (EBV) status (*data not shown*).

UNG is a key component of SHM where it mediates the removal of uracil arising as a consequence of cytidine deamination in DNA.[2] Aberrant targeting of SHM machinery in the germinal center leading to mutation and translocation has been proposed as a mechanism involved in lymphomagenesis.[4] Supporting this theory, mice null for Ung (*Ung -/-*) have a greater than 20 fold increase in the incidence of B-cell lymphoma compared to Ung proficient mice, which is thought to result primarily from inappropriate targeting of SHM in the germinal center and concomitant mutation outside the Ig locus. Humans with a constitutional mutation in UNG also exhibit abnormalities in B-cell development, as illustrated by attenuated CSR manifesting as an accumulation of IgM and low levels of IgA, IgE and IgG.[6] The findings presented here suggest an association between B-cell NHL and a polymorphism in UNG, although at present the functionality of the 1082 T>A SNP is unknown.

Carriers of the *XRCC1* 399 A allele were significantly under-represented in B-cell NHL cases compared to controls (Table 1). Under-representation of heterozygotes and homozygotes for the A allele extended across DLBCL, FL, mantle cell and marginal zone lymphomas but was not statistically significant (Table 2). The significance of these findings is unclear given that previous studies have shown that *XRCC1* 399 was not associated with risk of NHL.[7-9] For *PMS2*, *MSH2, MLH1* and FAS, associations were not observed with B-cell NHL or its subtypes, or HL stratified by EBV status, either when considering each polymorphism alone (Table 1) or with limited constructed haplotypes (*data not shown*). This study used a large collection of lymphoma cases and controls to test for association between genetic variants in genes expressed in B cells during the germinal center reaction and risk of lymphoma, specifically those involved in SHM, CSR and B-cell selection. The polymorphisms investigated here are by no means exhaustive and others expressed in the germinal center may define risk alleles for lymphoma or its subtypes. A number of statistical tests were conducted and so it is possible that the associations may be due to chance. Other studies are required to confirm our findings in particular our decreased risk with UNG 1082 T>A which may be restricted to females. This may be best achieved in the context of multi-center collaborative initiatives, such as the InterLymph consortium,[10] where the accrual of large case numbers will allow risk alleles to be more confidently identified.

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References

1. Küppers R, Dalla-Favera R. Mechanisms of chromosomal translocations in B cell lymphomas. Oncogene 2001;20: 5580-94.
2. Lee GS, Brandt VL, Roth DB. B cell development leads off with a base hit: dU:dG mismatches in class switching and hypermutation. Mol Cell 2004;16:505-8.
3. Muschen M, Rajewsky K, Kronke M, Kuppers R. The origin of CD95-gene mutations in B-cell lymphoma. Trends

Immunol 2002;23:75-80.
4. Pasqualucci L, Neumeister P, Goossens T, Nanjangud G, Chaganti RS, Kuppers R, et al. Hypermutation of multiple proto-oncogenes in B-cell diffuse large-cell lymphomas. Nature 2001;412:341-6.
5. Willett EV, Smith AG, Dovey GJ, Morgan GJ, Parker J, Roman E. Tobacco and alcohol consumption and the risk of non-Hodgkin lymphoma. Cancer Causes Control 2004; 15:771-80.
6. Imai K, Slupphaug G, Lee WI, Revy P, Nonoyama S, Catalan N, et al. Human uracil-DNA glycosylase deficiency associated with profoundly impaired immunoglobulin class-switch recombination. Nat Immunol 2003;4:1023-8.
7. Hill DA, Wang SS, Cerhan JR, Davis S, Cozen W, Severson RK, et al. Risk of Non-Hodgkin lymphoma (NHL) in relation to germline variation in DNA repair and related genes. Blood 2006;108:3161-7.
8. Smedby KE, Lindgren CM, Hjalgrim H, Humphreys K, Schollkopf C, Chang ET, et al. Variation in DNA repair genes ERCC2, XRCC1, and XRCC3 and risk of follicular lymphoma. Cancer Epidemiol Biomarkers Prev 2006;15: 258-65.
9. Matsuo K, Hamajima N, Suzuki R, Andoh M, Nakamura S, Seto M, et al. Lack of association between DNA base excision repair gene XRCC1 Gln399Arg polymorphism and risk of malignant lymphoma in Japan. Cancer Genet Cytogenet 2004;149:77-80.
10. Rothman N, Skibola CF, Wang SS, Morgan G, Lan Q, Smith MT, et al. Genetic variation in TNF and IL10 and risk of non-Hodgkin's lymphoma: a report from the InterLymph Consortium. Lancet Oncol 2006;7:27-38.