bjh short report

Polymorphisms in cytochrome P450 17A1 and risk of non-Hodgkin lymphoma

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Summary

Broad cross-talk exists between the endocrine and immune systems. Estrogen receptor expression in lymphocytes suggests that hormonal modulation may influence lymphoma risk. Analysis of genetic polymorphisms that affect oestrogen production, such as cytochrome P450 17A1 (*CYP17A1*) −34T>C, may provide insight into oestrogen's role in lymphomagenesis. *CYP17A1* −34T>C and *CYP17A1* IVS2 105A>C polymorphisms were analyzed in a non-Hodgkin lymphoma (NHL) population-based case–control study. The *CYP17A1* −34CC genotype was positively associated with NHL [odds ratio (OR) = 1·44, 95% confidence interval (CI) 1·02–2·03], particularly diffuse large B-cell lymphoma (OR = 1·76, CI 1·14–2·71). Associations of *CYP17A1* polymorphisms with increased risk of NHL suggest a role for oestrogen in lymphomagenesis.

Keywords: lymphoma, single nucleotide polymorphism, genetic associations, oestrogen, CYP17A1.

Immune function, believed to be important in lymphomagenesis, is influenced by cross-talk between the endocrine and immune systems via hormones, such as oestrogen (reviewed in McMurray, 2001). Oestrogen has multiple effects on lymphopoietic cells including the modulation of lymphocyte growth, antigen presentation, and production of antibodies and cytokines (Olsen & Kovacs, 1996). Furthermore, oestrogen receptors are expressed in various hematopoietic cell types including lymphocytes, bone marrow and lymphoma cell lines. Therefore, the risk of lymphoproliferative diseases such as non-Hodgkin lymphoma (NHL) may be influenced by hormonal modulation. Few studies have examined the relationship between oestrogen exposure, reproductive factors and lymphoma and published findings are contradictory (Nelson *et al*, 2001; Cerhan *et al*, 2002; Beiderbeck *et al*, 2003).

Levels of circulating hormone are affected by genetic variability, and analysis of genetic polymorphisms that influence oestrogen production may provide insight into its potential role in lymphomagenesis. Cytochrome P450 17A1 (*CYP17A1*) is a key enzyme involved in sex hormone and glucocorticoid production (Fig 1). A single nucleotide polymorphism (SNP) in the 5′ untranslated region of the *CYP17A1* gene (−34T>C, rs743572), 34 bp upstream of the initiation site of translation (−34T>C), creates an additional Sp1-type promoter site hypothesized to enhance CYP17 transcriptional efficiency and enzyme activity (Carey *et al*, 1994). This SNP has been associated with elevated serum dehydroepiandrosterone sulphate (DHEAS) and oestradiol levels in premenopausal women (reviewed in Sharp *et al*, 2004). The effects on androgen levels are inconsistent (reviewed in Ntais *et al*, 2003). To investigate the role of *CYP17A1* SNPs in lymphoma risk, we examined the *CYP17A1* −34T>C polymorphism and an additional SNP located in intron 2 (IVS2 105A>C, rs743575) in a lymphoma case–control study conducted in the UK. While no known functional data exists on the IVS2 105A>C polymorphism, it was chosen as a tagging SNP to estimate *CYP17A1* haplotypes using data from the Hapmap provided by the International HapMap Project (http://www.hapmap.org/index.html) and HAPLOVIEW software (http://www.broad.mit.edu/mpg/haploview/index.php).

Methods

Study population

Full details of the study are described elsewhere (Willett *et al*, 2004). The study was in compliance with the principles of the Declaration of Helsinki. Briefly, participants recruited

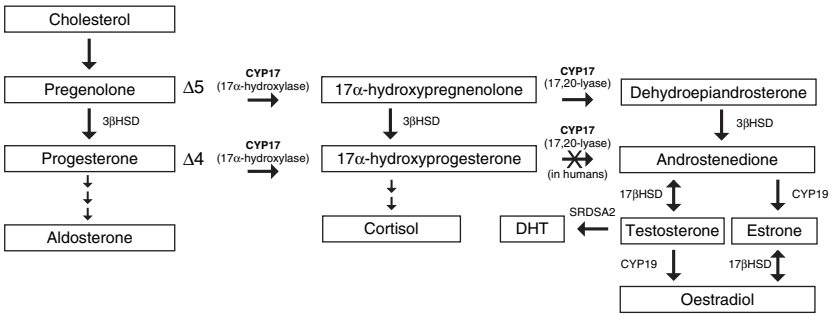

Fig 1. Schematic of the synthesis of oestradiol. CYP17A1, cytochrome P450 17A1; CYP19, cytochrome P450 19, SRD5A2, 5α-reductase type 2, 3β-HSD, 3β-hydroxysteroid dehydrogenase, 17β-HSD, 17β-hydroxysteroid dehydrogenase. Through the delta 5 pathway, CYP17A1 converts pregnenolone to dehydroepiandrosterone (DHEA), the precursor for oestrogen and testosterone.

into the population-based case–control study were aged 18–64 years and diagnosed with NHL between 1998 and 2001. For each case, one control was randomly selected from the same general practice list as the case, and matched on sex, ethnicity, and date of birth. Participating subjects gave consent to an interview, allowed access to their medical records, and provided mouthwash and blood samples. The present analysis includes 700 Caucasian cases with a confirmed diagnosis of NHL and 915 Caucasian controls. Of these subjects, DNA was available for 620 cases and 762 controls. There were no differences by age, sex or diagnostic subtype between the subjects who did and did not have DNA available for genotyping.

Isolation of DNA and genotyping

DNA was isolated from peripheral blood mononuclear cells using a phenol–chloroform extraction and quantified using PicoGreen® dsDNA Quantification kits (Molecular Probes, Eugene, OR, USA). Genotyping was performed using Taqman®-based assays [Applied Biosystems (ABI), Foster City, CA, USA] with the following protocol on a 7700 ABI Sequence Detection System: 95°C for 10 min, then 40 cycles of 95°C for 15 s and 60°C for 1 min. Primers and probes used for rs743572 were 5′-CCACGAGCTCCCACATGGT-3′ (forward primer), 5′-GCCTCCTTGTGCCCTAGAGTT-3′ (reverse primer), 5′-VIC-TCCACTGCTGTCTAT-3′ (T-allele specific probe) and 5′-6FAM-CTCCACCGCTGTC-3′ (C-allele specific probe). For rs743575, genotyping was performed using ABI Assays on Demand™, SNP reference sequence no. hCV3284579. Allele specific probes used were 5′-VIC-ATGGCAGCAGTAGCCAAGAAAAGGCGGCATTGCGCTCTAGTCCTAACCCTT -3′ (C-allele specific probe) and 5′ -6FAM-ATGGCAGCAGTAGCCAAGAAAAGGCTGCATTGCGCTCTAGTCCTAACCCTT-3′ (A-allele specific probe).

Statistical analysis

All analyses were restricted to Caucasian cases and controls. Odds ratios (OR) and 95% confidence intervals (CI), adjusted for age and sex, were calculated by unconditional logistic regression. The likelihood ratio test was used to test for trend and for interaction between the two SNPs. All analyses including assignment of haplotypes and calculation of haplotype ORs were conducted using STATA v.8 (College Station, TX, USA).

Results and discussion

Control genotype frequencies were in accordance with Hardy–Weinberg equilibrium. The *CYP17A1* −34CC genotype was associated with an increased risk for NHL (OR = 1·44, 95% CI 1·02–2·03) and diffuse large B-cell lymphoma (DLBCL) (OR = 1·76, 95% CI 1·14–2·71), but not for follicular lymphoma (FL) (OR = 1·03, 95% CI 0·63–1·68) (Table I). Stratification by sex revealed no gender differences for total NHL, although the *CYP17A1* 105CC genotype was positively associated with FL in males (OR = 2·12, 95% CI 1·01–4·43). There was no evidence of statistical interaction ($\chi^2 = 7{\cdot}4$, $P = 0{\cdot}29$), but the SNPs were in linkage disequilibrium $D' = 0{\cdot}92$). Control frequencies of the four possible haplotypes were Hap1 −34T/105A, 63%; Hap2 −34T/105C, 1%; Hap3 −34C/105A, 8%; and Hap4 −34C/105C, 28%. Hap3, which contained the high-risk −34C allele, was associated with an increased risk of NHL (OR = 1·3, 95% CI 0·99–1·79) and DLBCL (OR = 1·5, 95% CI 1·04–2·17), being present in 10% and 11% of NHL and DLBCL cases, respectively. Further, an increased risk of FL was observed with the low frequency Hap2 haplotype, present in 3% of FL cases (OR = 2·8, 95% CI 1·24–6·21).

The association of the *CYP17A1* −34CC genotype and Hap3 haplotype with increased risk of DLBCL in both men and women suggests a role for oestrogen and possibly other cholesterol metabolites formed via CYP17A1 in the pathogenesis of DLBCL. Oestrogen exerts pleiotropic effects on lymphocyte activation, proliferation, cell cycle progression and apoptosis, factors that may influence lymphoma risk (Grimaldi *et al*, 2002). In B-cells, oestrogen mediates its anti-apoptotic effects through upregulation of BCL-2. BCL-2 overexpression promotes survival of both autoreactive B-cells arising in bone marrow that would normally be deleted and B-cells that acquire autoreactivity following somatic mutation in germinal centres. Furthermore, oestrogen upregulates

Table 1. Number of cases & controls, adjusted odds ratios* (OR) and 95% confidence intervals (CI) by subtype of NHL for *CYP17A1*−34T < C and *CYP17A1* IVS2 105A > C stratified by sex.

		Cases n(%)					
	Controls n (%)	Total NHL†	OR (95% CI)	DLBCL	OR (95% CI)	FL	OR (95% CI)
Total	n = 762 (100)	n = 620 (100)		n = 283 (100)		n = 214 (100)	
CYP17A1−34T < C							
−34TT	308 (40·7)	237 (39·2)	1	95 (34·8)	1	97 (46·0)	1
−34CT	366 (48·3)	274 (45·4)	0·95 (0·75–1·19)	133 (48·7)	1·17 (0·86–1·58)	86 (40·8)	0·71 (0·51–0·99)
−34CC	83 (11·0)	93 (15·4)	1·44 (1·02–2·03)	45 (16·5)	1·76 (1·14–2·71) ‡	28 (13·3)	1·03 (0·63–1·68)
sna	5	16	–	10		3	
Males							
−34TT	173 (42·4)	126 (40·3)	1	55 (37·7)	1	43 (43·4)	1
−34CT	197 (48·3)	137 (43·8)	0·94 (0·68–1·29)	70 (47·9)	1·11 (0·74–1·67)	41 (41·4)	0·81 (0·50–1·30)
−34CC	38 (9·3)	50 (16·0)	1·80 (1·11–2·92)	21 (14·4)	1·74 (0·94–3·21)	15 (15·2)	1·57 (0·79–3·13)
sna	1	6		3		1	
Females							
−34TT	135 (38·7)	111 (38·1)	1	40 (31·5)	1	54 (48·2)	1
−34CT	169 (48·4)	137 (47·1)	0·96 (0·68–1·35)	63 (49·6)	1·25 (0·79–1·97)	45 (40·2)	0·63 (0·40–1·00)
−34CC	45 (12·9)	43 (14·8)	1·15 (0·70–1·88)	24 (18·9)	1·80 (0·98–3·31) §	13 (11·6)	0·70 (0·35–1·40)
sna	4	10	–	7	–	2	
CYP17A1 IVS2 105A>C							
AA	373 (50·3)	288 (49·8)	1	125 (47·5)	1	103 (51·2)	1
AC	315 (42·5)	233 (40·3)	0·94 (0·74–1·18)	113 (43·0)	1·06 (0·79–1·43)	74 (36·8)	0·82 (0·58–1·15)
CC	53 (7·2)	57 (9·9)	1·37 (0·91–2·05)	25 (9·5)	1·40 (0·83–2·34)	24 (11·9)	1·61 (0·95–2·75)
sna	21	42		20		13	
Males							
AA	203 (51·0)	158 (53·2)	1	73 (52·9)	1	48 (51·6)	1
AC	169 (42·5)	112 (37·7)	0·83 (0·60–1·14)	55 (39·9)	0·89 (0·60–1·34)	32 (34·4)	0·76 (0·46–1·25)
CC	26 (6·5)	27 (9·1)	1·32 (0·74–2·36)	10 (7·2)	1·06 (0·49–2·30)	13 (14·0)	2·12 (1·01–4·43)
sna	11	22		11		7	
Females							
AA	170 (49·6)	130 (46·3)	1	52 (41·6)	1	55 (50·9)	1
AC	146 (42·6)	121 (43·1)	1·07 (0·77–1·50)	58 (46·4)	1·30 (0·84–2·00)	42 (38·9)	0·87 (0·55–1·38)
CC	27 (7·9)	30 (10·7)	1·43 (0·81–2·53)	15 (12·0)	1·81 (0·90–3·67)	11 (10·2)	1·23 (0·57–2·64)
sna	10	20		9		6	

*Odds ratios (OR) and 95% confidence intervals (CI) were estimated using unconditional logistic regression adjusted for age and sex.
†NHL, non-Hodgkin lymphoma; DLBCL, diffuse large B-cell lymphoma; FL, follicular lymphoma.
Likelihood ratio test used to test for trend: ‡$\chi^2 = 5{\cdot}8$, $P = 0{\cdot}02$; §$\chi^2 = 3{\cdot}48$, $P = 0{\cdot}06$.
sna, sample not amplified.

expression of several angiogenic factors in endothelial cells via activation of the nuclear factor (NF)-κB pathway (Seo *et al*, 2004) and it is known that NF-κB is an important regulator of programmed cell death, proliferation and growth arrest. Through the delta 4 pathway, CYP17A1 also converts progesterone to 17α-hydroxyprogesterone, a substrate in the production of cortisol (Conley & Bird, 1997) (Fig 1). Depending on levels, cortisol can either suppress or stimulate immune function, so modulation of its production could potentially influence NHL risk. To date, no functional studies have reported whether the *CYP17A1* −34T>C SNP or CYP17A1 haplotypes affect glucocorticoid production, and examination of this pathway in future studies may be warranted. In conclusion, our data suggest that oestrogen and/or other cholesterol metabolites involving CYP17A1 may promote lymphomagenesis, although these findings will need to be replicated in other independent studies.

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