Vitamin D receptor gene polymorphisms and risk of non-Hodgkin's lymphoma

We studied three vitamin D receptor gene (*VDR*) polymorphisms (*BsmI, TaqI, FokI*) in a case-control study of non-Hodgkin's lymphoma. The *BsmI B* and *TaqI t* alleles were associated with an increased risk of diffuse large B-cell lymphoma. These findings suggest that variants in *VDR* may influence lymphomagenesis.

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Recent epidemiologic findings suggesting an inverse association between sunlight exposure and risk of non-Hodgkin lymphoma (NHL) have generated interest in whether vitamin D, a photosynthesized hormone with anti-neoplastic properties, inhibits NHL development.[1-3] Vitamin D has been reported to inhibit proliferation and induce differentiation in lymphocytes[4] and lymphoma cell lines.[5] In an early-phase trial of a vitamin D analog, 24% of 34 treated patients with progressive low-grade lymphoma experienced regression.[6]

Investigations of single-nucleotide polymorphisms (SNPs) in the vitamin D receptor gene (*VDR*) may offer an insight into the etiologic relevance of vitamin D. Three SNPs, located in *FokI, BsmI* and *TaqI* restriction sites, have been extensively studied as possible susceptibility factors for different diseases. The *FokI* (rs10735810) polymorphism, located at the 5' end of *VDR*, alters the transcription initiation site, resulting in a protein three amino acids shorter in length with higher activity as a transcription factor.[7] The *BsmI* (rs1544410) and *TaqI* (rs731236) polymorphisms are located close to one another at the 3' end. While these SNPs are unlikely to have functional relevance, they may be in linkage disequilibrium with other functionally relevant variants.[7,8] We investigated the relationship between *FokI, BsmI* and *TaqI* and NHL risk within a population-based case-control study conducted in New South Wales, Australia. This study was approved by the human research ethics committee at each participating institution. Detailed descriptions of the study design and methods have been published previously.[9] Briefly, 704 cases of NHL (86% of 819 eligible and contactable individuals) and 694 controls (61% of 1,136 eligible and contactable individuals) were interviewed. Of the 597 cases and 525 controls who provided blood, genotyping was successfully performed for 584 cases (83% of participants, 69% of eligible contactable individuals) and 518 controls (75% and 46%). Genotyping was carried out at the National Cancer Institute Core

Table 1. Associations between *FokI, BsmI* and *TaqI VDR* polymorphisms and risk of all NHL.

			Controls		Cases			
Gene	SNP	Genotype*	n	%	n	%	OR (95%)	p
VDR	FokI	FF	196	40	216	39	1.00	
		Ff	234	47	255	46	1.00 (0.77-1.31)	0.99
		ff	65	13	83	15	1.13 (0.77-1.66)	0.53
		Ff+ff	299	60	338	61	1.03 (0.80-1.32)	0.82
		Trend						0.61
	BsmI	bb	196	40	189	35	1.00	
		Bb	215	44	267	49	1.31 (1.00-1.72)	0.05
		BB	83	17	92	17	1.14 (0.80-1.64)	0.47
		Bb + BB	298	61	359	66	1.26 (0.98-1.63)	0.08
		Trend						0.24
	TaqI	TT	197	41	192	36	1.00	
		Tt	207	43	269	50	1.37 (1.04-1.79)	0.02
		tt	79	16	80	15	1.05 (0.73-1.53)	0.78
		Tt+tt	286	59	349	65	1.28 (0.99-1.65)	0.06
		Trend						0.34

*n: number of individuals; OR: odds ratio; CI: confidence interval. *Genotypes expressed in RFLP nomenclature: upper-case letters (F,B,T) indicate the presence of a restriction sequence (FokI, BsmI, TaqI respectively); lower case letters denote the absence of the sequence.*

Table 2. Associations between *FokI, BsmI* and *TaqI VDR* polymorphisms and NHL subtypes (B-cell- and T-cell lymphoma; follicular- and diffuse large B-cell lyphoma).

			Cell Lineage						Common B-Cell Subtype					
			B-cell			T-cell			Follicular			Diffuse large B-Cell		
SNP	Genotype*	$N_{Controls}$	n	OR (95%)	p	n	OR (95%)	p	n	OR (95%)	p	n	OR (95%)	p
FokI	FF	196	211	1.00		2	1.00		78	1.00		73	1.00	
	Ff	234	245	0.99 (0.76-1.29)	0.94	10	4.25 (0.90-20.1)	0.07	98	1.02 (0.71-1.47)	0.92	80	0.97 (0.67-1.42)	0.89
	ff	65	79	1.10 (0.75-1.62)	0.62	4	7.09 (1.23-41.0)	0.03	31	1.13 (0.67-1.89)	0.65	26	1.08 (0.63-1.85)	0.77
	Ff+ff	299	324	1.01 (0.79-1.31)	0.92	14	4.82 (1.06-21.8)	0.04	129	1.04 (0.74-1.47)	0.81	106	1.00 (0.70-1.43)	0.99
	Trend				0.72			0.02			0.69			0.86
BsmI	bb	196	178	1.00		10	1.00		75	1.00		54	1.00	
	Bb	215	260	1.36 (1.03-1.79)	0.03	5	0.53 (0.17-1.60)	0.26	104	1.32 (0.92-1.90)	0.14	88	1.55 (1.03-2.31)	0.03
	BB	83	91	1.21 (0.84-1.75)	0.30	1	0.18 (0.02-1.53)	0.12	30	0.97 (0.58-1.61)	0.90	32	1.49 (0.89-2.50)	0.13
	Bb+BB	298	351	1.32 (1.02-1.70)	0.04	6	0.41 (0.14-1.15)	0.09	134	1.22 (0.86-1.72)	0.26	120	1.53 (1.05-2.23)	0.03
	Trend				0.14			0.06			0.71			0.07
TaqI	TT	197	181	1.00		10	1.00		77	1.00		56	1.00	
	Tt	207	262	1.42 (1.08-1.86)	0.01	5	0.53 (0.18-1.63)	0.27	104	1.34 (0.93-1.92)	0.12	90	1.62 (1.09-2.42)	0.02
	tt	79	79	1.12 (0.77-1.62)	0.57	1	0.20 (0.02-1.62)	0.13	23	0.79 (0.46-1.36)	0.40	28	1.34 (0.79-2.29)	0.28
	Tt+tt	286	341	1.33 (1.03-1.73)	0.03	6	0.42 (0.15-1.19)	0.10	127	1.19 (0.84-1.68)	0.33	118	1.55 (1.06-2.26)	0.02
	Trend				0.21			0.07			0.93			0.11

*N: number of individuals; OR: odds ratio; CI, confidence interval. *Genotypes expressed in RFLP nomenclature: upper-case letters (F,B,T) indicate the presence of a restriction sequence (FokI, BsmI, TaqI respectively); lower case letters denote the absence of the sequence.*

Genotyping Facility (*http://cgf.nci.nih.gov*) using TaqMan® assays (Applied Biosystems Inc., Foster City, CA, USA). The concordance rates for 95 duplicate samples were 100% for all assays. All SNPs passed a Hardy-Weinberg equilibrium test. The analysis was restricted to individuals of European or Asian ethnicity (561 cases, 506 controls; 97% of subjects) and was performed using SAS Version 8.2 (SAS Institute, Cary, North Carolina, USA). Analyses restricted to individuals of European ancestry gave almost identical findings and are not presented. To estimate the relative risk of NHL according to SNP genotype, odds ratios (ORs) and 95% confidence intervals (CIs) were calculated using unconditional logistic regression adjusted for sex, age, state and ethnicity. The most prevalent homozygous genotype among controls was selected as the referent group. We also calculated subtype-specific ORs by cell lineage (B-cell, n=542; T-cell, n=16) and for the two most prevalent B-cell subtypes: follicular lymphoma (FL, n=211) and diffuse large B-cell lymphoma (DLBCL, n=180). We express genotypes using restriction-fragment length polymorphism nomenclature. Upper-case letters (i.e., F,B,T) indicate alleles coding for the restriction sequence in question (*FokI, BsmI, TaqI* respectively), while lower case letters denote the absence of the sequence.

The *FokI*, *BsmI* and *TaqI* polymorphisms were not clearly associated with all NHL in our population (Table 1). We did observe an excess risk of DLBCL with carriage of *BsmI* B and *TaqI* t alleles, but no evidence of a dose-response relationship was observed (Table 2). Experimental studies of functional activity and association studies involving these SNPs have not provided consistent findings,[7] although these variants have been fairly consistently linked to shorter adult height.[10] These SNPs, located close to one another at the 3' end of *VDR*, are unlikely to have direct functional effects, although they are in linkage disequilibrium with variants in the nearby 3'-UTR that may affect mRNA stability and *VDR* transcriptional activity. *BsmI* and *TaqI* are also situated within the *block B* linkage disequilibrium block spanning exons 3 to 9,[8] and may be linked to other functional variants residing within this region. Further research is needed to identify possible underlying causal variants.

We also found the *FokI f* allele to be associated with an increased risk of T-cell lymphoma. This is linked to decreased *VDR* transactivation activity *in vitro*[7] and is consistent with the hypothesis that vitamin D inhibits lymphomagenesis. However, given the small number of T-cell lymphomas in our study, we cannot rule out the possibility that this is due to chance.

In conclusion, our findings suggest that the *FokI*, *BsmI* and *TaqI* polymorphisms in *VDR* may be associated with some NHL subtypes. However, these findings must be confirmed in further studies if meaningful conclusions are to be drawn.

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Key words: vitamin D receptor, case-control study, non-Hodgkin's lymphoma, polymorphisms, Australia.

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