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Ala394Thr polymorphism in the clock gene *NPAS2*: A circadian modifier for the risk of non-Hodgkin's lymphoma

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Abstract

Circadian disruption is theorized to cause immune dysregulation, which is the only established risk factor for non-Hodgkin's lymphoma (NHL). Genes responsible for circadian rhythm are also involved in cancer-related biological pathways as potential tumor suppressors. However, no previous studies have examined associations between circadian genes and NHL risk. In this population-based case control study ($n = 455$ cases; 527 controls), we examined the only identified nonsynonymous polymorphism (Ala394Thr; rs2305160) in the largest circadian gene, neuronal PAS domain protein 2 (*NPAS2*), in order to examine its impact on NHL risk. Our results demonstrate a robust association of the variant Thr genotypes (Ala/Thr and Thr/Thr) with reduced risk of NHL (OR = 0.66, 95% CI: 0.51–0.85, $p = 0.001$), especially B-cell lymphoma (OR = 0.61, 95% CI: 0.47–0.80, $p \leq 0.0001$). These findings provide the first molecular epidemiologic evidence supporting a role of circadian genes in lymphomagenesis, which suggests that genetic variations in circadian genes might be a novel panel of promising biomarkers for NHL and warrants further investigation.

Keywords

NPAS2; NHL; circadian gene

The universal 24 hr circadian rhythm influences many fundamental biological processes, including immune activity. Circadian rhythms have been observed in Natural Killer (NK) cells, which are an essential component of the innate immune system against infections and cancerous growth.¹ The expression of NK cells oscillates throughout a 24 hr period, with the peak expression occurring in the early morning.² A similar 24 hr oscillation to that of the NK cells has also been seen in numerous other components of the immune system, such as T-helper, T-suppressor and splenic B-cells.³ These findings suggest that the circadian cycle may play a role in regulating immune responses.

Disruption of circadian rhythms may cause disordered immune responses such as aberrant immune cell trafficking and abnormal cell proliferation cycles.^{4–6} For example, sleep disorders have been shown to affect the expression of the hypothalamic-pituitary-adrenal (HPA) axis, which is a major component of the neuroendocrine system that specifically controls the body's reaction to stress and plays an important role in regulating the immune system.⁴ A

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change in the HPA axis's expression consequently affects the expression of the pro-inflammatory cytokine IL-6, which is secreted by T-cells and macrophages of the immune system, and/or TNF- α , which is involved in the immune system's acute response. Moreover, disruption of the circadian rhythm in NK cells and phagocytic activity has been observed in malignant melanoma cells, leading to a discoordination between the 2 immune system components, which was not observed in healthy individuals.⁷

Given the established association between immune dysregulation and non-Hodgkin's lymphoma (NHL),⁸ we speculate that circadian rhythm might play a role in lymphomagenesis by affecting immune activity. Furthermore, we hypothesize that genetic variation in genes responsible for maintaining circadian rhythm might act as a novel panel of biomarkers associated with an individual's susceptibility to NHL.

Neuronal PAS domain protein 2 (*NPAS2*), the largest of the circadian genes, is expressed primarily in the mammalian forebrain. *NPAS2* encodes for a member of the basic helix-loop-helix-PAS domain class of transcription factors. BMAL1/*NPAS2* heterodimers can also transcriptionally regulate expression of 2 other circadian genes, *per* and *cry*, which are required for maintaining biological rhythms in mammals.^{9,10} Previous evidence has suggested that deficiency in *NPAS2* leads to decreased oscillation of other circadian genes, such as *PER1*, suggesting a negative impact on the periodicity present in normally functioning molecular clock mechanisms.¹¹ An animal study has also shown that the loss of normal *NPAS2* may cause defects in several aspects of the circadian system, such as patterns of sleep and behavior.¹² Moreover, *NPAS2* has been suggested to be involved in tumorigenesis by forming heterodimers with BMAL1, which in turn mediate the promoter of the oncogene *c-myc* and suppress its transcription.¹³

A coding polymorphism (A $\rightarrow$ G; dbSNP ID: rs2305160) in *NPAS2* which alters an amino acid at codon 394 (Ala394Thr) was chosen to be genotyped in this study. It is the only nonsynonymous mutation out of 560 single nucleotide polymorphisms (SNPs) identified in the large *NPAS2* gene (~170 kb) according to the public NCBI dbSNP database. The current study screened for this missense mutation among 455 histologically confirmed NHL cases and 527 controls from a large population-based case-control study of Connecticut women, and investigated whether this polymorphism modified an individuals' risk of NHL.

Material and methods

Study subjects

A detailed description of this study population has been reported previously.¹⁴ Briefly, histologically-confirmed female incident cases of NHL (ICD-O, M-9590–9642, 9690–9701, 9740–9750) from Connecticut were identified through the Yale Cancer Center's Rapid Case Ascertainment (RCA) between 1996 and 2000. Female population-based controls from Connecticut were recruited by: (1) random digit dialing methods for those younger than 65 years of age; or (2) random selection from Health Care Financing Administration files for those aged 65 years or more. Cases and controls were frequency matched 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 participation rate was 72% for cases, 69% for controls contacted by random digit dialing and 47% for controls contacted through health care records. A total of 601 cases and 717 controls were enrolled and completed in-person interviews. Pathology slides from all patients were obtained from the original pathology departments and specimens were classified using the Revised European–American Lymphoma (REAL) system. Blood samples for genotyping were available for 75.7% (461/601) of cases and 73.5% (535/717) of controls.

Genotyping the Ala394Thr variation

Genomic DNA was isolated from peripheral blood lymphocytes. TaqMan Assays-on-Demand primers and probes (Cat no.: C_15976652_10; Applied Biosystems, Foster City, CA) were used for detecting this variant, according to the instructions provided by the manufacturer. The TaqMan reactions were performed on a Stratagene MX3000P instrument with PCR conditions of 95°C for 5 min, followed by 40 cycles of 95°C for 15 sec and 60°C for 60 sec, with an indefinite hold at 20°C. The plates were then read by the instrument and data were analyzed by the allelic discrimination function of the MX3000P software (Stratagene Corp.). Duplicate samples from 100 study subjects were interspersed throughout the plates used for genotype analysis, and a 99% concordance rate was achieved.

Statistical analysis

All statistical analyses were performed using the STATA statistical software (StataCorp.; College Station, TX). The Hardy–Weinberg equilibrium (HWE) assumption was assessed by comparing the observed number of individuals in the different genotype categories with those expected under HWE for the estimated allele frequency. Adjusted odds ratios (ORs) with 95% confidence intervals (CI) were calculated by unconditional logistic regression to estimate the relative risk associated with different genotypes. Analyses were adjusted for age (continuous), race (Caucasian, African–American, other) and family history. Analyses limited to Caucasians (representing 96.26% and 94.12% of all cases and controls, respectively) yielded very similar results to analyses including all study subjects, so only results from all study subjects were reported (Table II).

The common homozygous genotype was used as the reference group. Tests for trend were conducted by assigning the ordinal values 1, 2 and 3 to the common homozygous, heterozygous and variant homozygous genotypes, respectively. Risks for NHL subtypes were carried out using all controls as the comparison group, to maximize statistical power.

Prediction of NPAS2 domains by a bioinformatics tool

In order to determine whether the Ala394Thr substitution falls in a domain critical for protein function, a search of the Protein Domain Database (ProDom; <http://prodes.toulouse.inra.fr/prodom/current/html/form.php>) was carried out using the NPAS2 protein accession number Q99743. The methodology of this bioinformatics tools has been previously described.¹⁵ Briefly, the ProDom database is constructed from a nonredundant set created from SWISS-PROT, TrEMBL and the complete proteomes available on the ExPASy server and the Proteome Analysis pages by employing a method whereby homologous domains are clustered into families which can then be matched to the queried protein based on sequence similarity.

Results

Compared to controls, NHL cases reported a higher proportion of having a family history of NHL and other cancers in their first-degree relatives (Table I). There were no significant differences in age and race between cases and controls. The genotypic frequencies were 41.4% homozygous Ala/Ala, 46.1% heterozygous Ala/Thr and 12.5% homozygous Thr/Thr in the control population (Table II). The Hardy–Weinberg equilibrium of genotype frequencies was met in both controls ($\chi^2 = 0.066$, $p = 0.849$) and NHL patients ($\chi^2 = 0.271$, $p = 0.647$).

Logistic regression analysis showed that the NHL risk was significantly reduced among people with the heterozygous Ala/Thr genotype (OR = 0.69, 95% CI: 0.53–0.90; $p = 0.006$), the homozygous variant Thr/Thr genotype (OR = 0.55, 95% CI: 0.36–0.85; $p = 0.007$) and the variant Thr genotypes (Ala/Thr & Thr/Thr) (OR = 0.66, 95% CI: 0.51–0.85; $p = 0.001$) when

compared to those with the homozygous Ala/Ala genotype. Similar reduced risks were detected for B-cell lymphoma among individuals with the heterozygous Ala/Thr genotype (OR = 0.64, 95% CI: 0.48–0.85; $p = 0.002$), the homozygous variant Thr/Thr genotype (OR = 0.51, 95% CI: 0.32–0.82; $p = 0.005$) and the variant Thr genotypes (OR = 0.61, 95% CI: 0.47–0.80; $p < 0.0001$). However, no significant associations were detected among patients with T-cell lymphoma. There were also statistically significant trends for the variant genotypes (genotypes Ala/Thr and Thr/Thr) and the risk of NHL overall and B-cell lymphoma ($p = 0.0024$ and 0.0007 , respectively).

After stratification by subtypes of B-cell lymphoma, significantly decreased risks were observed between the variant Thr genotypes and 2 major subtypes: B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma (CLL/PLL/SLL) (OR = 0.63, 95% CI: 0.43–0.93; $p = 0.019$) and follicular lymphoma (FL) (OR = 0.45, 95% CI: 0.29–0.69; $p < 0.0001$). A significant trend was detected for the variant genotypes associated with FL ($p = 0.0028$). Further analyses restricted to the Caucasian subjects produced results similar to those reported above (data not shown).

The ProDom search returned 8 predicted domain families including a “good quality” (NorMD value = 1.122) 99 amino acid alignment beginning at residue 307 and thus including the polymorphic site located at residue 394. The Ala394Thr substitution is predicted to be included near the end of this domain which belongs to the PAS family of sensory domains. Therefore, the polymorphism may fall in the PAS fold region of NPAS2 and may lead to changes in protein sensory capacity thereby altering its ability to effectively mediate signal transduction. The Ala394Thr polymorphism may also interfere with NPAS2/BMAL1 heterodimerization, which is essential for normal sensory function.

Discussion

Our results demonstrate for the first time that a functional polymorphism in the circadian gene *NPAS2* modifies an individual's susceptibility to NHL. Observed associations of the *NPAS2* Ala394Thr variation with multiple histological subtypes of B-cell lymphoma also suggest that this circadian biomarker might be a common etiologic factor shared by these subtypes. Our findings are in accordance with previous observations which suggest a role of circadian rhythm in NHL prognosis. For example, circadian disruptions of interleukin-2 receptors, serum thymidine kinase and β -2-microglobulin in blood samples were detected in NHL patients and might be related to therapeutic outcome.¹⁶ At the cellular level, significant circadian cell cycle variations have been found in pathological lymph nodes from NHL patients.¹⁷ Recent findings have demonstrated that expression levels of the circadian gene *PER2* were reduced in lymphoma cell lines and in acute myeloid leukemia (AML) patient samples.¹⁸ It has also been shown that a significant number of *PER2* mutant mice die from spontaneous lymphomas before the age of 16 months.¹³ These observations together with our findings suggest that circadian biomarkers might not only be related to NHL prognosis, but also contribute to the etiology of lymphomagenesis.

The possible causal association of *NPAS2* with NHL detected in our study supports the proposed role of circadian genes as tumor suppressors.¹⁹ Circadian genes have been shown to affect expression of 2–10% of mammalian genes.²⁰ Emerging data from animal models have further demonstrated a substantial impact of circadian genes on tumor-related biological pathways such as cell proliferation, cell cycle control and apoptosis.¹⁹ For example, mice with mutations in the circadian gene *PER2* have deficiencies in DNA damage responses and are more prone to tumorigenesis.¹³ Also, heterodimers of the circadian proteins BMAL1/NPAS2 may regulate expression of the major oncogene *c-myc*.¹³ In human tumor cells, studies have shown that the expression patterns of the period (*PER*) circadian gene fail to maintain daily

rhythms.^{21,22} Our results are also congruent with findings from a recent molecular epidemiologic study that reported an association between a structural genetic variation in another circadian gene, *PERIOD3*, and breast cancer.²³ Therefore, although explicit mechanisms are currently unknown, *NPAS2*, as well as other circadian genes, might serve as biomarkers for an individual's susceptibility to NHL and possibly other cancer types.

This nonsynonymous mutation (rs2305160) may have 2 potential impacts on *NPAS2* functionality. The amino acid change (Ala → Thr) caused by this polymorphism may alter the *NPAS2* protein structure. Our bioinformatics search predicts that the *NPAS2* Ala394Thr polymorphism is located in the PAS sensory domain. Therefore, it may affect protein sensory capacity directly or interfere with *NPAS2*/*BMAL1* heterodimerization, which is essential for normal sensory function. Additionally, the mutation affects DNA sequences of a putative exonic splicing enhancer (ESE) responsive to the human SR protein SRp55 based on our search using the ESEfinder.²⁴ ESEs are common in both alternative and constitutive exons where they act as binding sites for splicing factors. The ESE, SRp55, that the *NPAS2* mutation potentially affects, may regulate tissue-specific alternative splicing of the calcitonin/CGRP pre-mRNA.²⁵ Changes in this binding site could lead to inaccurate recognition of exon/intron boundaries or differential binding of transcription factors.

Changes in *NPAS2* functionality and *NPAS2*-mediated gene expression could interfere with *CLOCK* regulated-pathways because *NPAS2* is a paralog of *CLOCK*, another member of the basic Helix-Loop-Helix PAS domain family of transcription factors.²⁶ Both proteins are major regulators of the molecular clock, and act by forming heterodimers with *BMAL1* and binding to E box sequences in the promoter regions of target genes.²⁷ However, while *CLOCK* is mainly expressed in the “central pacemaker” of the circadian system, the suprachiasmatic nucleus of the hypothalamus, *NPAS2* is expressed mainly in the forebrain.¹¹ This suggests that while these 2 genes are functionally analogous, they might be involved in separate circadian-controlled processes. Although the amino acid sequences of *NPAS2* and *CLOCK* are highly similar, especially in the regions corresponding to functional domains, the *NPAS2* Ala394Thr polymorphism examined in the study is not shared by *CLOCK*.

In summary, our study reports a robust association between a functional polymorphism in the clock gene *NPAS2* and risk of NHL, suggesting for the first time that genetic variations in circadian genes may confer inherited susceptibility to NHL. Given that NHL incidence is increasing and its etiology is largely unknown, our findings provide a novel panel of promising biomarkers for NHL risk and prognosis, which warrants further investigation.

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References

1. Arjona A, Sarkar DK. Evidence supporting a circadian control of natural killer cell function. *Brain Behav Immun* 2006;20:469–76. [PubMed: 16309885]
2. Arjona A, Sarkar DK. Circadian oscillations of clock genes, cytolytic factors, and cytokines in rat NK cells. *J Immunol* 2005;174:7618–24. [PubMed: 15944262]
3. Esquifino AI, Selgas L, Arce A, Maggiore VD, Cardinali DP. Twenty-four-hour rhythms in immune responses in rat submaxillary lymph nodes and spleen: effect of cyclosporine. *Brain Behav Immun* 1996;10:92–102. [PubMed: 8811933]
4. Vgontzas AN, Chrousos GP. Sleep, the hypothalamic-pituitary-adrenal axis, and cytokines: multiple interactions and disturbances in sleep disorders. *Endocrinol Metab Clin N Am* 2002;31:15–36.

5. Mormont MC, Levi F. Circadian-system alterations during cancer processes: a review. *Int J Cancer* 1997;70:241–7. [PubMed: 9009166]
6. Matsuo T, Yamaguchi S, Mitsui S, Emi A, Shimoda F, Okamura H. Control mechanism of the circadian clock for timing of cell division in vivo. *Science* 2003;302:255–9. [PubMed: 12934012]
7. Gamaleia NF, Skivka LM, Fedorchuk AG, Shishko ED. Circadian rhythms of cytotoxic activity in peripheral blood mononuclear cells of patients with malignant melanoma. *Exp Oncol* 2006;28:54–60. [PubMed: 16614709]
8. Filipovich AH, Mathur A, Kamat D, Shapiro RS. Primary immunodeficiencies: genetic risk factors for lymphoma. *Cancer Res* 1992;52(Suppl 19):5465s–5467s. [PubMed: 1327508]
9. Vitaterna MH, King DP, Chang AM, Kornhauser JM, Lowrey PL, McDonald JD, Dove WF, Pinto LH, Turek FW, Takahashi JS. Mutagenesis and mapping of a mouse gene, clock, essential for circadian behavior. *Science* 1994;264:719–25. [PubMed: 8171325]
10. Bunker MK, Wilsbacher LD, Moran SM, Clendenin C, Radcliffe LA, Hogenesch JB, Simon MC, Takahashi JS, Bradfield CA. Mop3 is an essential component of the master circadian pacemaker in mammals. *Cell* 2000;103:1009–17. [PubMed: 11163178]
11. Reick M, Garcia JA, Dudley C, McKnight SL. NPAS2: an analog of clock operative in the mammalian forebrain. *Science* 2001;293:506–9. [PubMed: 11441147]
12. Dudley CA, Erbel-Sieler C, Estill SJ, Reick M, Franken P, Pitts S, McKnight SL. Altered patterns of sleep and behavioral adaptability in NPAS2-deficient mice. *Science* 2003;301:379–83. [PubMed: 12843397]
13. Fu L, Pelicano H, Liu J, Huang P, Lee C. The circadian gene Period2 plays an important role in tumor suppression and DNA damage response in vivo. *Cell* 2002;111:41–50. [PubMed: 12372299]
14. Zhang Y, Lan Q, Rothman N, Zhu Y, Zahm SH, Wang SS, Holford TR, Leaderer B, Boyle P, Zhang B, Zou K, Chanock S, et al. A putative exonic splicing polymorphism in the BCL6 gene and the risk of non-Hodgkin lymphoma. *J Natl Cancer Inst* 2005;97:1616–18. [PubMed: 16264183]
15. Servant F, Bru C, Carrere S, Courcelle E, Gouzy J, Peyruc D, Kahn D. ProDom: automated clustering of homologous domains. *Brief Bioinform* 2002;3:246–51. [PubMed: 12230033]
16. Micke O, Schafer U, Wormann B, Hiddemann W, Willich N. Circadian variations of interleukin-2 receptors, serum thymidine kinase and β -2-microglobulin in patients with non-Hodgkin's lymphoma and normal controls. *Anticancer Res* 1997;17:3007–10. [PubMed: 9329587]
17. Smaaland R, Lote K, Sothorn RB, Laerum OD. DNA synthesis and ploidy in non-Hodgkin's lymphomas demonstrate inpatient variation depending on circadian stage of cell sampling. *Cancer Res* 1993;53:3129–38. [PubMed: 8319221]
18. Gery S, Gombart AF, Yi WS, Koeffler C, Hofmann WK, Koeffler HP. Transcription profiling of C/EBP targets identifies Per2 as a gene implicated in myeloid leukemia. *Blood* 2005;106:2827–36. [PubMed: 15985538]
19. Fu L, Lee CC. The circadian clock: pacemaker and tumour suppressor. *Nat Rev Cancer* 2003;3:350–61. [PubMed: 12724733]
20. Storch KF, Lipan O, Leykin I, Viswanathan N, Davis FC, Wong WH, Weitz CJ. Extensive and divergent circadian gene expression in liver and heart. *Nature* 2002;417:78–83. [PubMed: 11967526]
21. You S, Wood PA, Xiong Y, Kobayashi M, Du-Quito J, Hrushesky WJ. Daily coordination of cancer growth and circadian clock gene expression. *Breast Cancer Res Treat* 2005;91:47–60. [PubMed: 15868431]
22. Chen ST, Choo KB, Hou MF, Yeh KT, Kuo SJ, Chang JG. Deregulated expression of the PER1, PER2 and PER3 genes in breast cancers. *Carcinogenesis* 2005;26:1241–6. [PubMed: 15790588]
23. Zhu Y, Brown HN, Zhang Y, Stevens RG, Zheng T. Period3 structural variation: a circadian biomarker associated with breast cancer in young women. *Cancer Epidemiol Biomarkers Prev* 2005;14:268–70. [PubMed: 15668506]
24. Cartegni L, Chew SL, Krainer AR. Listening to silence and understanding nonsense: exonic mutations that affect splicing. *Nat Rev Genet* 2002;3:285–98. [PubMed: 11967553]
25. Tran Q, Roesser JR. SRp55 is a regulator of calcitonin/CGRP alternative RNA splicing. *Biochemistry* 2003;42:951–7. [PubMed: 12549914]

26. King DP, Zhao Y, Sangoram AM, Wilsbacher LD, Tanaka M, Antoch MP, Steeves TD, Vitaterna MH, Kornhauser JM, Lowrey PL, Turek FW, Takahashi JS. Positional cloning of the mouse circadian clock gene. *Cell* 1997;89:641–53. [PubMed: 9160755]
27. Kondratov RV, Kondratova AA, Lee C, Gorbacheva VY, Chernov MV, Antoch MP. Post-translational regulation of circadian transcriptional CLOCK(NPAS2)/BMAL1 complex by cryptochromes. *Cell Cycle* 2006;5:890–5. [PubMed: 16628007]

TABLE I
DISTRIBUTION OF SELECTED CHARACTERISTICS BY CASE CONTROL STATUS

Variable	Cases (<i>N</i> = 455) <i>N</i> ^{<i>I</i>}	Controls (<i>N</i> = 527) <i>N</i> ^{<i>I</i>}	<i>p</i> -value
Mean age (years)	61.88	62.34	0.607
Race			
Caucasian	438 (96.26)	496 (94.12)	
African-American	13 (2.86)	14 (2.66)	
Other	4 (0.88)	17 (3.22)	0.108
Family history of cancer in first degree relatives			
No	96 (21.10)	130 (24.67)	
NHL	9 (1.98)	2 (0.38)	
Other cancer	350 (76.92)	395 (74.95)	0.030
Case pathology			
All B cell	365 (80.22)		
Diffuse large B-cell	135 (36.99)		
Follicular	105 (28.77)		
SLL/PLL/CLL	54 (14.79)		
Marginal zone	30 (8.22)		
Other	41 (11.23)		
All T cell	32 (7.03)		
NOS	58 (12.75)		

^{*I*} Figures in parenthesis are percentages.

ASSOCIATIONS BETWEEN NPAS2 ALA394THR GENOTYPES AND NHL RISK

TABLE II

Genotype	All NHL (n = 455)			B-cell lymphoma (n = 365)			T-cell lymphoma (n = 32)		
	Controls (n = 527)			Cases	OR (95% CI) ^J	p value	Cases	OR (95% CI) ^J	p value
Ala/Ala Ala/Thr	218 (41.4) 243 (46.1)			194 (53.1) 140 (38.4)	1 0.64 (0.48– 0.85)		14 (43.8) 14 (43.7)		1 0.765
						0.006			
Thr/Thr	66 (12.5)			31 (8.5)	0.51 (0.32– 0.82)	0.007	4 (12.5)	0.90 (0.28– 2.87)	0.865
<i>P for trend</i> Variant Thr	309 (58.6)			171 (46.9)	0.61 (0.47– 0.80)	0.0007	18 (56.2)	0.89 (0.43– 1.84)	0.504 0.762
						<0.0001			
B-cell lymphoma									
CLL/SLL (n = 54)									
Ala/Ala Ala/Thr	218 (41.4) 243 (46.1)			70 (51.8) 50 (37.0)	1 0.62 (0.41– 0.94)		15 (50.0) 12 (40.0)		
						0.023			
Thr/Thr	66 (12.5)			15 (11.1)	0.67 (0.36– 1.26)	0.217	3 (10.0)	0.67 (0.19– 2.41)	0.543
<i>P for trend</i> Variant Thr	309 (58.6)			65 (48.1)	0.63 (0.43– 0.93)	0.352 0.019	15 (50.0)	0.70 (0.33– 1.47)	0.375 0.346
DLBL (n = 135)									
Ala/Ala Ala/Thr	218 (41.4) 243 (46.1)			70 (51.8) 50 (37.0)	1 0.62 (0.41– 0.94)		15 (50.0) 12 (40.0)		
						0.023			
Thr/Thr	66 (12.5)			15 (11.1)	0.67 (0.36– 1.26)	0.217	3 (10.0)	0.67 (0.19– 2.41)	0.543
<i>P for trend</i> Variant Thr	309 (58.6)			65 (48.1)	0.63 (0.43– 0.93)	0.352 0.019	15 (50.0)	0.70 (0.33– 1.47)	0.375 0.346
FL (n = 105)									
Ala/Ala Ala/Thr	218 (41.4) 243 (46.1)			70 (51.8) 50 (37.0)	1 0.62 (0.41– 0.94)		15 (50.0) 12 (40.0)		
						0.023			
Thr/Thr	66 (12.5)			15 (11.1)	0.67 (0.36– 1.26)	0.217	3 (10.0)	0.67 (0.19– 2.41)	0.543
<i>P for trend</i> Variant Thr	309 (58.6)			65 (48.1)	0.63 (0.43– 0.93)	0.352 0.019	15 (50.0)	0.70 (0.33– 1.47)	0.375 0.346

CLL/SLL, B-cell chronic lymphocytic leukemia/prolymphocytic leukemia/small lymphocytic lymphoma; DLBL, Diffuse large B-cell lymphoma; MZBL, Marginal zone B-cell lymphoma; FL, Follicular lymphoma.

^J Adjusted for age (as continuous), race, and family history of NHL in first-degree relatives.