Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2010 November ; 19(11): 2847–2858. doi: 10.1158/1055-9965.EPI-10-0581.

Germline Variation in Apoptosis Pathway Genes and Risk of non-Hodgkin Lymphoma

Jennifer L. Kelly[1], **Anne J. Novak**[2], **Zachary S. Fredericksen**[3], **Mark Liebow**[4], **Stephen M. Ansell**[2], **Ahmet Dogan**[5], **Alice H. Wang**[3], **Thomas E. Witzig**[2], **Timothy G. Call**[2], **Neil E. Kay**[2], **Thomas M. Habermann**[2], **Susan L. Slager**[3], and **James R. Cerhan**[6]

[1]University of Rochester, School of Medicine and Dentistry, Rochester, New York

[2]Division of Hematology, Department of Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota.

[3]Division of Biomedical Statistics and Informatics, Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, Minnesota.

[4]Division of General Internal Medicine, Department of Medicine, Mayo Clinic College of Medicine, Rochester, Minnesota.

[5]Division of Anatomic Pathology, Department of Laboratory Medicine and Pathology, Mayo Clinic College of Medicine, Rochester, Minnesota.

[6]Division of Epidemiology, Department of Health Sciences Research, Mayo Clinic College of Medicine, Rochester, Minnesota.

Abstract

Background—The t(14;18)(q32;q21) is the most commonly observed chromosomal translocation in non-Hodgkin lymphoma (NHL), resulting in constitutive Bcl-2 expression and apoptosis inhibition. In addition, germline variation in both *BCL2L11* (*BIM*) and *CASP9*, known regulators of apoptosis, have recently been linked to NHL risk. We conducted a comprehensive evaluation of 36 apoptosis pathway genes with risk of NHL.

Methods—We genotyped 226 single nucleotide polymorphisms (SNPs) from 36 candidate genes in a clinic-based study of 441 newly diagnosed NHL cases and 475 frequency matched controls. We used principal components analysis to assess gene-level associations, and logistic regression to assess SNP-level associations. MACH was used for imputation of SNPs in *BCL2L11* and *CASP9*.

Results—In gene level analyses, *BCL2L11* (p=0.0019), *BCLAF1* (p=0.0097), *BAG5* (p=0.026) and *CASP9* (p=0.0022) were associated with NHL risk after accounting for multiple testing (tail strength 0.38; 95% CI 0.05, 0.70). Two of the 5 *BCL2L11* tagSNPs (rs6746608 and rs12613243), both genotyped *BCLAF1* tagSNPs (rs797558 and rs703193), the single genotyped *BAG5* tagSNP (rs7693), and 3 of the 7 genotyped *CASP9* tagSNPs (rs6685648, rs2020902, rs2042370) were significant at p<0.05. We successfully imputed *BCL2L11* and *CASP9* SNPs previously linked to NHL, and replicated all 4 *BCL2L11* and 2 of 3 *CASP9* SNPs.

Corresponding Author: James R. Cerhan, MD, PhD, Department of Health Sciences Research, 200 First St SW, Rochester, MN 55905; Phone: 507-538-0499; Fax: 507-266-2478; cerhan.james@mayo.edu.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Conclusion—We replicated the association of *BCL2L11* and *CASP9* with NHL risk at the gene and SNP-level, and identified novel associations with *BCLAF1* and *BAG5*.

Impact—Closer evaluation of germline variation of genes in the apoptosis pathway with risk of NHL and its subtypes is warranted.

Keywords

Bcl-2 pathways; caspases; molecular epidemiology; non-Hodgkin lymphoma

Introduction

Non-Hodgkin lymphoma (NHL) is the 5th most common cancer overall in the United States, and the lifetime odds of developing NHL is 1 in 45 for men and 1 in 53 for women (1). The remarkable rise in incidence of NHL over the last 50 years suggests a major role for environmental factors in the etiology of this cancer. However, established risk factors to date account for only a relatively small fraction of the cases (2).

The (t14;18)(q32;q21) is a hallmark translocation in follicular lymphoma (3), one of the most common lymphoma subtypes (2). With this translocation event, *BCL2* becomes fused to the immunoglobulin heavy chain (IgH) locus, leading to constitutive Bcl-2 expression and apoptosis inhibition under the control of the IgH enhancer (4,5). Under normal conditions, lymphocytes must strictly regulate growth and apoptosis to provide adequate immunologic defenses against infections while not overwhelming the organism with inappropriate cell numbers. With the (t14;18)(q32;q21) and other less commonly observed translocations of genes in the apoptosis pathway observed among lymphoma cases (6,7), it is clear that dysregulation of the balance between cell proliferation and programmed cell death is a central feature in lymphomagenesis (8). Furthermore, evidence that the (t14;18)(q32;q21) translocation is also present in approximately 30% of diffuse large B-cell lymphomas (DLBCL) and 1-2% of chronic lymphocytic leukemias / small lymphocytic lymphomas (CLL/SLL) (6), and the deletion/down-regulation of Bcl-2 inhibiting micro-RNA species (mir-15 and mir-16) in CLL (9), suggests a broad role for bcl-2 and apoptosis in lymphoma.

Bcl-2 is a member of a large family of pro- and anti-apoptotic proteins which coordinate both extrinsic and intrinsic cell signals to activate caspases, the effector enzymes necessary for apoptosis execution (4,5,10). The high prevalence of the t(14;18) translocation among healthy individuals, estimated as high at 66% at age 50, would indicate that perhaps over-expression of the Bcl-2 protein as a result of this transformation may not sufficient for malignant transformation (11-13). There is accumulating evidence that other Bcl-2 family proteins, caspase family proteases, and genes that encode and regulate their transcription, are important in lymphomagenesis. Somatic mutations in many caspase genes, including *CASP3*, *CASP7*, *CASP8*, and *CASP10*, have been documented in a wide variety of human cancers including NHL (14,15). Furthermore, there is evidence of differential expression of both caspase genes and Bcl-2 family member genes among the NHL subtypes (16-18).

The above mentioned studies have primarily focused on genetic events that effect expression or function of apoptotic proteins within the tumor. However, accumulating epidemiologic evidence suggests that germline genetic variation also plays a role in NHL etiology (19-21). Common variation related to B-cell growth and survival (22), inflammation and immune function (23-28), and DNA repair (29) has been linked to NHL risk. Furthermore, recent genome wide association (GWA) studies have identified novel SNPs that are associated with risk of developing CLL and FL (30,31). To our knowledge, only one other group has comprehensively evaluated the role of germline genetic variation in the apoptosis pathway with regard to NHL etiology, including 8 *BCL2* family members and 12 caspase family members

in a pooled analysis of three independent case-control studies with a total of 1946 cases and 1808 controls (32,33). Statistically significant gene-level associations of *BCL2L11, CASP1, CASP8,* and *CASP9* with NHL risk were identified.

Here, we independently evaluated the hypothesis that germline genetic variation in genes from the apoptosis pathway is associated with risk of developing NHL, and compare these results to those reported in the pooled study in an attempt to validate the relevance of this pathway in NHL etiology. The 36 candidate genes evaluated (Table 1 and Supplemental Table 1) are known to be pro- or anti-apoptotic, and are represented in both the intrinsic and extrinsic apoptotic pathways.

Methods

Study population and data collection

This study was reviewed and approved by the Human Subjects Institutional Review Board at the Mayo Clinic, and all participants provided written informed consent. Full details of this on-going, clinic-based case-control study conducted at the Mayo Clinic in Rochester, Minnesota have been previously reported (27). This analysis is based on Phase 1 of the study, which includes participants enrolled from September 1, 2002 through September 30, 2005. Briefly, eligible patients were within 9 months of their first NHL diagnosis, aged 20 years or older, and were residents of Minnesota, Iowa or Wisconsin at the time of diagnosis. All cases were reviewed and histologically-confirmed by a hematopathologist, and classified according to the WHO criteria (34). Of the 956 eligible cases, 626 (65%) participated in the study. Clinic-based controls were randomly selected from Mayo Clinic Rochester patients aged 20 years or older, who were residents of Minnesota, Iowa or Wisconsin, and were being seen for a prescheduled medical examination in the general medicine divisions of the Department of Medicine. Patients were not eligible if they had a history of lymphoma, leukemia, or HIV infection. Controls were frequency matched to cases by 5-year age group, gender, and geographic region (county groupings based on distance from Rochester, MN and urban/rural status). Of the 818 eligible controls, 572 (70%) participated in the study. All participating subjects were asked to complete a self-administered risk-factor questionnaire and to provide a peripheral blood sample for genetic studies. DNA was extracted from blood samples using a standard procedure (Gentra Inc., Minneapolis, MN).

Genotyping

Genotyping reported here was part of a larger genotyping project to assess the role of immune and other candidate genes in the etiology and prognosis of NHL (27). Most of the genes and SNPs reported here were from the ParAllele (now Affymetrix) Immune and Inflammation SNP panel that included 1253 genes and 9412 SNPs (35). The Immune and Inflammation panel was supplemented by a second round of genotyping using a custom Illumina Goldengate (36) OPA that included 384 SNPs from 100 candidate genes. Full genotyping details and quality control measures for both of these genotyping platforms have been previously described (27,28). Briefly, tagging SNPs were selected using CEPH (European-American) and Yoruba (African) samples from release 16 (Immune and Inflammation panel) and 21 (Illumina panel) of the HapMap Consortium (37). Tagging SNPs covered 5 kb up and downstream of each gene with minor allele frequency (MAF) ≥0.05 and pairwise r^2 threshold of 0.8. Across both platforms, the overall sample success rate was >98%, the assay call rate was >93% (99.1% for ParAllelle and 93.5% for Illumina), and the concordance rate of sample duplicates was >98%; the concordance rate among the 71 SNPs that were duplicated across the two platforms was 99.7%. A total of 916 people (441 cases and 475 controls) were genotyped in both assays and passed all quality control measures (28). This combined master dataset was restricted to subjects who reported their race as Caucasian. After the duplicate SNPs with the lower platform-specific

SNP call rates were dropped and SNPs that had a minor allele frequency less than 1% (N=935) were excluded, 8034 SNPs remained in the dataset. For this analysis, we evaluated 226 SNPs (Supplemental Table 1) from 22 *BCL2* and 14 caspase family genes (Table 1).

Statistical analysis

Allele frequencies from cases and controls were estimated using observed genotype frequencies. The frequencies in the controls were compared to genotype frequencies expected under Hardy-Weinberg Equilibrium (HWE) using a Pearson goodness-offit test or Fisher's exact test (MAF<0.05). In this analysis, 14 of the 226 evaluated SNPs had a HWE $p<0.05$ (Supplemental Table 2); since no genotype calling errors were identified, these SNPs were not excluded from analysis. We previously found no evidence of population stratification in our data (27).

Two methods were used when analyzing the association between each gene and case-control status. The first approach used a principal components analysis to create orthogonal (e.g., uncorrelated) linear combinations of the SNP minor allele count variables that provide an alternate, and equivalent, representation of the SNP genotype count variables. These component linear combinations were then ranked according to the amount of the total SNP variance explained. The resulting smallest subset that accounted for at least 90% of the variability amongst the SNPs was included in a multivariable logistic regression model. A gene-specific global test using the resultant principal components was then carried out using a multiple degree-of-freedom likelihood ratio test. This method decreases the dimensionality of the data when SNPs are correlated by reducing the number of independent degrees of freedom that comprise the statistical test. The second method in which the gene-level association was tested used the global score test of Schaid et al (38) as implemented in the S-plus program *Haplo.stats.* Since the haplotype results were similar to the principal components analysis, we only report these results. Gene level tests with $p<0.05$ were declared of interest.

Individual SNPs were examined using unconditional logistic regression to estimate odds ratios (ORs) and corresponding 95% confidence intervals (CIs) separately for heterozygotes and minor allele homozygotes, using homozygotes for the major allele as the reference. ORs and corresponding 95% CIs were also estimated per copy of variant allele for each SNP, and p-trend was calculated assuming an ordinal (log-additive) genotypic relationship. SNPs with a $p_{trend}<0.05$ in the setting of a global gene test of $p<0.05$ were declared of interest. We also evaluated the association between SNPs in genes of interest with NHL risk by major NHL subtype (DLBCL, diffuse large B cell lymphoma; follicular lymphoma; and CLL/SLL, chronic lymphocytic leukemia / small lymphocytic lymphoma). We used polytomous logistic regression to simultaneously calculate ORs and 95% CIs for each subtype relative to controls, and to formally test for heterogeneity of the estimated association between each SNP of interest and lymphoma subtype (39).

To assess the robustness of our results in the setting of multiple hypothesis testing, we used the tail strength methods of Taylor and Tibshirani at both the gene- and SNP-level (40). This method tests the global null hypothesis that the distribution of p-values from a large set of univariate tests is uniformly distributed. As such, positive tail strength values significantly greater than 0 indicate that the observed number of small p-values is greater than would be expected by chance alone. In addition, we have also estimated q-values at the SNP level to estimate the strength of the association with respect to the positive false discovery rate (pFDR) (41).

In order to allow for SNP-level comparison with previously published associations between *BCL2* (33) and caspase (32) family genes from a pooled analysis of three case-control studies, we used the MACH 1.0.14 to impute genotypes for SNPs not directly observed in our study

population (42). The 60 unrelated HapMap CEU samples (from release 23a / phase II Mar08, NCBI build 36, dbSNP build 12) were used to obtain the phased chromosomes, and the expected genotype dosage was computed based on the posterior probability. SNPs with imputation r^2 larger than 0.30 were deemed of sufficient quality and were examined for their association with NHL case/control status using the SNP dosage estimated from MACH.

Analyses were implemented using SAS (SAS Institute, Cary, NC, Version 8, 1999) and S-Plus (Insightful Corp, Seattle, WA, Version 7.05, 2005) software systems. All analyses were adjusted for age and gender.

Results

Participant characteristics

There were 441 cases and 475 controls available for analysis. For cases, the mean age was 60.1 years and 58% were male, while for controls the mean age was 61.7 years and 55% were male. Additional patient characteristics have been previously published (28). The most common NHL subtypes were SLL/CLL (N=123), FL (N=113), and DLBCL (N=69).

Gene-level analysis

We first evaluated gene-level associations between the 36 candidate apoptosis pathway genes and NHL, all subtypes combined. Using principal components analysis, we observed 4 genes to be significantly associated with NHL risk at $p<0.05$ (Table 1): *BAG5* (p=0.026), *BCL2L11*, also known as *BIM*, (p=0.0019), *BCLAF1* (p=0.0097), and *CASP9* (p=0.0022). In addition, *BCL2*, *BCL2L13*, *BCL2L14*, *BID*, *APAF1*, *CASP7*, *CASP10*, and *DFFB* each had 1 or more SNPs at $p<0.05$ but in the setting of a gene level test of $p\geq0.05$, and thus were not considered further. All SNP-level associations from non-significant genes are available in Supplemental Table 2.

SNP-level analysis

Next, we formally evaluated SNP-level associations within the 4 genes with a $p<0.05$ from the gene-level analysis (Table 2). The single genotyped *BAG5* tagSNP (rs7693) was significantly associated with NHL risk at $p<0.05$: OR=1.24 per T allele copy (95% CI 1.02, 1.50). For *BCL2L11*, 2 of the 5 tagSNPs were significant at $p<0.05$, and variant alleles were associated with decreased NHL risk for both: rs6746608, OR=0.82 per A allele copy (95% CI 0.68, 1.00); and rs12613243, OR=0.58 per C allele copy (95% CI 0.38, 0.87). There was little evidence of correlation between these two *BCL2L11* SNPs ($r^2=0.046$), and both genotyped SNPs remained statistically significant when modeled in a single logistic regression model, confirming that these two SNPs represent separate NHL risk signals (p=0.011 and p=0.0015, respectively). Both genotyped *BCLAF1* tagSNPs were significantly associated with NHL risk: rs797558, OR=1.38 per G allele copy (95% CI 1.07, 1.80); and rs703193, OR=1.42 per T allele copy (95% CI 1.10, 1.84). These two SNPs were in strong linkage disequilibrium with each other in our population ($r^2=0.96$). Neither SNP reached statistical significance when both were modeled in the same logistic regression model, indicating that they represent the same signal related to NHL risk. Finally, three of the 7 genotyped *CASP9* tagSNPs were significant: rs6685648, OR=1.41 per C allele copy (95% CI 1.14, 1.73); rs2020902, OR=0.74 per C allele copy (95% CI 0.57, 0.95); and rs2042370, OR=0.82 per C allele copy (95% CI 0.68, 1.00). The pairwise correlation of these SNPs is fairly low (r^2 between 0.079 and 0.34); however, they appear to represent the same signal when jointly modeled. That is, when we added either rs2020902 or rs2042370 to the logistic regression model with the most significant SNP, rs6685648, the added SNP was non-significant based on the likehood ratio test ($p> 0.1$). Of note, all of the SNPs that were significantly associated with NHL risk were intronic, with the

exception of the *BAG5* SNP rs7693, which is in the non-coding region interval of an mRNA transcript.

Multiple testing

To evaluate the effect of multiple testing, we estimated both the tail strength of the p-values generated in the gene-level and SNP-level analyses and the q-values with respect to the pFDR for each SNP. The tail strength for the 36 gene-level p-values was 0.38 (95% CI 0.05, 0.70), while the tail strength estimate for the 226 SNP-level p-values was 0.20 (95% CI 0.07, 0.33). As the tail strength estimates and 95% CIs exclude the null in both the gene-level and SNP-level analyses, we can conclude that the distribution of p-values are more extreme than we would have expected by chance alone, and thus observed significant associations at both the gene and SNP level remain noteworthy. However, individual SNP q-values within the genes of interest (Table 2) ranged from 0.22 to 0.38 in the SNPs we have considered significant for the purpose of this analysis.

Subtype analysis

In exploratory analyses, similar associations (as assessed by direction and magnitude of ordinal odds ratios and p-heterogeneity obtained from polytomous logistic regression) were observed for significant SNPs from *BCLAF1* and *BAG5* for the subtypes of CLL/SLL, follicular lymphoma, and DLBCL. In contrast, there is some evidence that the associations between individual tagSNPs within *BCL2L11 and CASP9* differ among the three NHL subtypes (Table 3). For *BCL2L11*, the decreased risk of NHL with copies of the variant A allele at rs6746608 appears similar across NHL subtype ($p_{heterogeneity}$ =0.57), but the decreased NHL risk with copies of the variant C allele at rs12613243 appears limited to CLL/SLL and follicular lymphoma ($p_{heterogeneity}$=0.047). The pattern for *CASP9* SNPs appeared most differential by subtype. The decreased NHL risk with copies of the A allele at both rs4646077 and rs2020902 was limited to DLBCL ($p_{heterogeneity}$=0.049 and 0.17, respectively); the decreased NHL risk with copies of the A and C variant alleles at rs4646018 and rs2042370, respectively, was limited to CLL/SLL (rs4646018 $p_{heterogeneity}$=0.0098; rs2042370 $p_{heterogeneity}$=0.0046); the remaining CASP9 SNPs had $p_{heterogeneity}$>0.25. However, caution is warranted in interpreting the subtype results due to small sample sizes, especially SNPs with lower minor allele frequencies.

Comparison to published estimates

There were 17 genes that were evaluated in both this study and the pooled analysis of three case-control studies from the United States and Australia (32,33): 13 genes that were not associated with NHL risk in either study (*BAX*, *BCL2*, *BCL2A1*, *BCL2L1*, *BCL2L2*, *BCL2L10*, and *CASP2*, *3*, *4*, *5*, *6*, *7*, *10*), 2 genes (*CASP1* and *CASP8)* that were associated with NHL risk in the pooled study but were not associated with NHL risk in our study, and 2 genes (*BCL2L11* and *CASP9)* that were significantly associated at the gene-level with NHL risk in both studies. For these two genes (*BCL2L11* and *CASP9)*, we compared individual SNP-level significance (based on ordinal ORs) between these two studies. The results for the overlapping observed and imputed SNPs are presented in Table 4. Figure 1 illustrates the relative position and linkage disequilibrium (based on HapMap CEPH population genotypes) of each *BCL2L11* (Panel A) and *CASP9* (Panel B) genotyped SNPs.

For *BCL2L11*, there was no overlap in the SNPs genotyped in the two studies. For the Mayo case-control study, we imputed genotypes for the 4 SNPs observed to be significantly associated with NHL risk in the pooled study (rs7567444, rs3789068, rs686952, and rs6760053). The estimated NHL risk with variant allele copies was similar in direction and magnitude across all four SNPs, and reached significance for rs3789038 (p_{trend}=0.0018) and rs6760053 (p_{trend}=0.0031). In exploratory analyses of these four SNPs by NHL subtype, we observed that the association between the imputed SNP genotypes in the Mayo population

were strongest in the CLL/SLL subtype but were also observed in follicular lymphoma at a magnitude consistent with the pooled study, which observed an association specific to the follicular lymphoma subtype (Table 5).

The *CASP9* SNP rs2020902 was genotyped in both studies, although the results were inconsistent. The observed association between copies of the variant G allele and NHL risk was OR=0.74 (95% CI 0.57, 0.95; p_{trend}=0.019) in the Mayo case-control study and OR=1.02 (95% CI 0.89, 1.16; p_{trend}=0.82) in the pooled study. Alternatively, two *CASP9* SNPS were observed to be significantly associated with NHL risk in the pooled study (rs4661636 and rs4646047), and the magnitude and direction of the estimate associated with Mayo imputed genotypes was consistent for both. An inverse association with the number of variant T alleles at both rs4661636 and rs4646047 was observed in both studies, although associations did not reach significance in the Mayo case-control study.

The pooled study did not genotype *BCLAF* or *BAG5*, so we were not able to compare our findings for these two genes. Further, we did not have the original genotyping data from the pooled analysis, so we were not able to impute genotypes in their population for comparison to SNPs significant in the Mayo case-control study.

Discussion

In this case-control study, we demonstrate gene- and SNP-level association of *BCL2L11* (*BIM), BCLAF1*, *BAG5*, and *CASP9* with NHL risk that remained noteworthy after accounting for multiple testing. While the pFDR q-values estimated for *BCL2L11* and *CASP9* SNPs do indicate that there is a moderate chance that any of these associations individually may be false positives, the tail strength estimates indicated that the distribution of p-values for the group of SNPs from these genes is more extreme than we would have expected by chance alone. Moreover, the significant gene-level associations for *BCL2L11* and *CASP9* were consistent with previously published data from a pooled analysis of three studies (NCI-SEER, Connecticut, and New South Wales) that included 1946 cases and 1808 controls (32,33). While there was minimal overlap in the individual tagSNPs genotyped across our study and the pooled study, we were able to impute genotypes for the SNPs in our study that were not directly observed, and we found associations that were largely consistent in magnitude and direction across all four significant *BCL2L11* SNPs and 2 of 3 *CASP9* SNPs from the pooled study, making this the first independent replication of these results. The associations from our study with *BCLAF1* and *BAG5* have not been tested in an independent dataset and thus require replication.

Strengths of our study include a carefully designed case-control study, central pathology review, and high quality genotyping. Although this study was not population-based, both case and control participation was restricted to those residing in the region surrounding Mayo Clinic (Minnesota, Iowa, and Wisconsin), thus minimizing the effect of referral bias, and increasing the internal validity of using frequency-matched general medicine controls from the same region. Common HapMap SNPs were used to tag genes of interest, and through other genotyping projects we have ruled out the presence of significant population stratification (27). The major limitations are the use of an exclusively Caucasian population, which limits generalizability, and the relatively small sample size, which in particular precludes robust estimation of NHL subtype associations. There are several apoptosis genes evaluated in this study for which the genotyped tagSNPs provided <70% gene coverage, and thus an association between these genes and NHL risk cannot yet be ruled out. In addition, there are greater than 100 human genes more broadly identified with apoptosis pathway involvement in which germline variants may play a role in NHL risk, including genes with caspase-recruitment

domains, death domains, and death effector domains (43). The genes in the current analysis represent only the core of a larger set of genes involved in apoptosis-related pathways.

The two genes that replicated have strong biologic plausibility in NHL pathogenesis. *BCL2L11* balances the anti-apoptotic influence of *BCL2* and coordinates pro-apoptotic signaling through the intrinsic apoptosis pathway (4). In addition, *BCL2L11* is required for negative selection of autoreactive lymphocytes (44). Functional silencing of *BCL2L11* through methylation has been observed in Burkitt lymphoma cell lines and primary tumor biopsies, and reduced *BCL2L11* mRNA and protein expression has also been documented in other tumors including renal cell carcinoma, melanoma, and colon cancer (45). Of note, we did not identify any association between *BCL2* and NHL risk at the gene level, and only one of the 53 genotyped SNPs was significant at the SNP-level. While these 53 SNPs comprised only 74% gene coverage, it does suggest that *BCL2* germline variation may not play a role in NHL risk. This would be consistent with the hypothesis that most of the *BCL2* variation in lymphoma tumors is a result of hypermutation following the t(14;18) translocation event (4). This hypothesis should be further explored by comparing somatic and germline mutation among patients stratified by the t(14;18) translocation.

CASP9, the other gene to be replicated, is a pro-apoptotic protease integral to the intrinsic apoptotic pathway, and is responsible for effector caspase activation and apoptosis execution following activation by Apaf-1 bound to cytochrome *c* released from mitochondria (46). Hyperphosphorylation of caspase-9 may lead to aberrant apoptosis inhibition, and the relevance of this process has been demonstrated in a number of other cancer types (46). Also of note, upon mitochondrial release of cytochrome *c* via both the intrinsic and extrinsic apoptosis pathways, the cytoplasmic protein Apaf-1 binds caspase-9 to form the apoptosome, in turn activating the caspase cascade (4). *APAF1* did not reach gene-level statistical significance in our population, although 6 of the 13 genotyped tagSNPs in this gene were significant at $p<0.05$. Given the gene-level significance of *CASP9*, there may be some clinical relevance of the individual tagSNPs significance in *APAF1*, and further follow-up on this gene is warranted.

To our knowledge, this is the first report of an association between germline variation in *BAG5* and *BCLAF1* with regard to lymphoma risk. *BCLAF1* and *BAG5* are both Bcl-2 family members that suppress *BAX* (pro-apoptotic) gene expression, in turn suppressing the *APAF1* gene and inhibiting apoptosis (4,47). These associations should be confirmed in follow-up with an independent study population.

While underpowered to assess NHL subtypes, our data provide some evidence that there may be subtype-specific associations in the apoptosis pathway, particularly *BCL2L11 and CASP9*. Evidence of differential association of germline variants in the *BCL2* and *CASP* families was observed in the pooled analysis of 3 case-control studies (32,33), although the *BCL2L11* association was largely limited to follicular lymphoma (33), a pattern we observed for both follicular and CLL/SLL in our study.

In conclusion, our results support an association of four genes from the apoptosis pathway, with NHL risk, and these associations may vary by NHL subtype. In light of the importance of the apoptosis pathway to human lymphomagenesis, further characterization of the key players within this pathway is warranted.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank Sondra Buehler for her editorial assistance.

Grant Support: This work was supported by awards from the National Institutes of Health National, Cancer Institute [R01 CA92153]. Dr. Kelly was supported by the National Institutes of Health, National Heart Lung and Blood Institute [HL007152].

References

1. Jemal A, Siegel R, Ward E, Hao Y, Xu J, Thun MJ. Cancer statistics, 2009. CA Cancer J Clin 2009;59:225–49. [PubMed: 19474385]
2. Alexander DD, Mink PJ, Adami HO, et al. The non-Hodgkin lymphomas: a review of the epidemiologic literature. Int J Cancer 2007;120(Suppl 12):1–39. [PubMed: 17405121]
3. Masir N, Campbell LJ, Goff LK, et al. BCL2 protein expression in follicular lymphomas with t(14;18) chromosomal translocations. Br J Haematol 2009;144:716–25. [PubMed: 19120369]
4. Reed JC. Bcl-2-family proteins and hematologic malignancies: history and future prospects. Blood 2008;111:3322–30. [PubMed: 18362212]
5. Rossi D, Gaidano G. Messengers of cell death: apoptotic signaling in health and disease. Haematologica 2003;88:212–8. [PubMed: 12604411]
6. Dyer MJ. The pathogenetic role of oncogenes deregulated by chromosomal translocation in B-cell malignancies. Int J Hematol 2003;77:315–20. [PubMed: 12774917]
7. Lin P, Jetly R, Lennon PA, Abruzzo LV, Prajapati S, Medeiros LJ. Translocation (18;22)(q21;q11) in B-cell lymphomas: a report of 4 cases and review of the literature. Hum Pathol 2008;39:1664–72. [PubMed: 18656237]
8. Cory S, Adams JM. The Bcl2 family: regulators of the cellular life-or-death switch. Nat Rev Cancer 2002;2:647–56. [PubMed: 12209154]
9. Buggins AG, Pepper CJ. The role of Bcl-2 family proteins in chronic lymphocytic leukaemia. Leuk Res 34:837–842. [PubMed: 20359747]
10. Lamkanfi M, Festjens N, Declercq W, Vanden Berghe T, Vandenabeele P. Caspases in cell survival, proliferation and differentiation. Cell Death Differ 2007;14:44–55. [PubMed: 17053807]
11. Basecke J, Griesinger F, Trumper L, Brittinger G. Leukemia- and lymphoma-associated genetic aberrations in healthy individuals. Ann Hematol 2002;81:64–75. [PubMed: 11907785]
12. Hui D, Satkunam N, Al Kaptan M, Reiman T, Lai R. Pathway-specific apoptotic gene expression profiling in chronic lymphocytic leukemia and follicular lymphoma. Mod Pathol 2006;19:1192–202. [PubMed: 16763612]
13. Schuler F, Dolken L, Hirt C, et al. Prevalence and frequency of circulating t(14;18)-MBR translocation carrying cells in healthy individuals. Int J Cancer 2009;124:958–63. [PubMed: 19030176]
14. Soung YH, Lee JW, Kim SY, et al. Somatic mutations of CASP3 gene in human cancers. Hum Genet 2004;115:112–5. [PubMed: 15127291]
15. Shin MS, Kim HS, Kang CS, et al. Inactivating mutations of CASP10 gene in non-Hodgkin lymphomas. Blood 2002;99:4094–9. [PubMed: 12010812]
16. Yamanaka K, Clark R, Dowgiert R, et al. Expression of interleukin-18 and caspase-1 in cutaneous T-cell lymphoma. Clin Cancer Res 2006;12:376–82. [PubMed: 16428475]
17. Rummel MJ, de Vos S, Hoelzer D, Koeffler HP, Hofmann WK. Altered apoptosis pathways in mantle cell lymphoma. Leuk Lymphoma 2004;45:49–54. [PubMed: 15061196]
18. Alizadeh AA, Eisen MB, Davis RE, et al. Distinct types of diffuse large B-cell lymphoma identified by gene expression profiling. Nature 2000;403:503–11. [PubMed: 10676951]
19. Wang SS, Slager SL, Brennan P, et al. Family history of hematopoietic malignancies and risk of non-Hodgkin lymphoma (NHL): a pooled analysis of 10 211 cases and 11 905 controls from the International Lymphoma Epidemiology Consortium (InterLymph). Blood 2007;109:3479–88. [PubMed: 17185468]

20. Zhang Y, Wang R, Holford TR, et al. Family history of hematopoietic and non-hematopoietic malignancies and risk of non-Hodgkin lymphoma. Cancer Causes Control 2007;18:351–9. [PubMed: 17206533]
21. Skibola CF, Curry JD, Nieters A. Genetic susceptibility to lymphoma. Haematologica 2007;92:960–9. [PubMed: 17606447]
22. Novak AJ, Slager SL, Fredericksen ZS, et al. Genetic variation in B-cell-activating factor is associated with an increased risk of developing B-cell non-Hodgkin lymphoma. Cancer Res 2009;69:4217–24. [PubMed: 19383901]
23. Purdue MP, Lan Q, Kricker A, et al. Polymorphisms in immune function genes and risk of non-Hodgkin lymphoma: findings from the New South Wales non-Hodgkin Lymphoma Study. Carcinogenesis 2007;28:704–12. [PubMed: 17056605]
24. Rothman N, Skibola CF, Wang SS, et al. Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. Lancet Oncol 2006;7:27–38. [PubMed: 16389181]
25. Purdue MP, Lan Q, Wang SS, et al. A pooled investigation of Toll-like receptor gene variants and risk of non-Hodgkin lymphoma. Carcinogenesis 2009;30:275–81. [PubMed: 19029192]
26. Wang SS, Purdue MP, Cerhan JR, et al. Common gene variants in the tumor necrosis factor (TNF) and TNF receptor superfamilies and NF-kB transcription factors and non-Hodgkin lymphoma risk. PLoS ONE 2009;4:e5360. [PubMed: 19390683]
27. Cerhan JR, Ansell SM, Fredericksen ZS, et al. Genetic variation in 1253 immune and inflammation genes and risk of non-Hodgkin lymphoma. Blood 2007;110:4455–63. [PubMed: 17827388]
28. Cerhan JR, Liu-Mares W, Fredericksen ZS, et al. Genetic variation in tumor necrosis factor and the nuclear factor-kappaB canonical pathway and risk of non-Hodgkin's lymphoma. Cancer Epidemiol Biomarkers Prev 2008;17:3161–9. [PubMed: 18990758]
29. Schuetz JM, MaCarthur AC, Leach S, et al. Genetic variation in the NBS1, MRE11, RAD50 and BLM genes and susceptibility to non-Hodgkin lymphoma. BMC Med Genet 2009;10:117. [PubMed: 19917125]
30. Skibola CF, Bracci PM, Halperin E, et al. Genetic variants at 6p21.33 are associated with susceptibility to follicular lymphoma. Nat Genet 2009;41:873–5. [PubMed: 19620980]
31. Slager SL, Goldin LR, Strom SS, et al. Genetic susceptibility variants for chronic lymphocytic leukemia. Cancer Epidemiol Biomarkers Prev 2010;19:1098–102. [PubMed: 20332261]
32. Lan Q, Morton LM, Armstrong B, et al. Genetic variation in caspase genes and risk of non-Hodgkin lymphoma: a pooled analysis of three population-based case-control studies. Blood 2009;114:264–7. [PubMed: 19414860]
33. Morton LM, Purdue MP, Zheng T, et al. Risk of non-Hodgkin lymphoma associated with germline variation in genes that regulate the cell cycle, apoptosis, and lymphocyte development. Cancer Epidemiol Biomarkers Prev 2009;18:1259–70. [PubMed: 19336552]
34. Jaffe, ES.; Harris, N.; Stein, H.; Vardiman, J. World Health Organization Classification of Tumours Pathology and Genetics, Tumors of Hematopoietic and Lymphoid Tissues. IARC Press; Lyon: 2001.
35. Hardenbol P, Yu F, Belmont J, et al. Highly multiplexed molecular inversion probe genotyping: over 10,000 targeted SNPs genotyped in a single tube assay. Genome Res 2005;15:269–75. [PubMed: 15687290]
36. Oliphant A, Barker DL, Stuelpnagel JR, Chee MS. BeadArray technology: enabling an accurate, cost-effective approach to high-throughput genotyping. Biotechniques 2002;(Suppl):56–8. 60–1. [PubMed: 12083399]
37. The International HapMap Consortium. The International HapMap Project. Nature 2003;426:789–96. [PubMed: 14685227]
38. Schaid DJ, Rowland CM, Tines DE, Jacobson RM, Poland GA. Score tests for association between traits and haplotypes when linkage phase is ambiguous. Am J Hum Genet 2002;70:425–34. [PubMed: 11791212]
39. Hosmer, DW.; Lemeshow, S. Applied Logistic Regression. John Wiley & Sons, Inc.; New York: 2000.
40. Taylor J, Tibshirani R. A tail strength measure for assessing the overall univariate significance in a dataset. Biostatistics 2006;7:167–81. [PubMed: 16332926]

41. Storey JD. A direct approach to false discovery rates. J R Statist Soc B 2002;64:479–98.
42. Nothnagel M, Ellinghaus D, Schreiber S, Krawczak M, Franke A. A comprehensive evaluation of SNP genotype imputation. Hum Genet 2009;125:163–71. [PubMed: 19089453]
43. Kitada S, Pedersen IM, Schimmer AD, Reed JC. Dysregulation of apoptosis genes in hematopoietic malignancies. Oncogene 2002;21:3459–74. [PubMed: 12032782]
44. Bouillet P, Purton JF, Godfrey DI, et al. BH3-only Bcl-2 family member Bim is required for apoptosis of autoreactive thymocytes. Nature 2002;415:922–6. [PubMed: 11859372]
45. Pinon JD, Labi V, Egle A, Villunger A. Bim and Bmf in tissue homeostasis and malignant disease. Oncogene 2009;27(Suppl 1):S41–52. [PubMed: 19641506]
46. Allan LA, Clarke PR. Apoptosis and autophagy: Regulation of caspase-9 by phosphorylation. FEBS J 2009;276:6063–73. [PubMed: 19788417]
47. Achiron A, Feldman A, Mandel M, Gurevich M. Impaired expression of peripheral blood apoptotic-related gene transcripts in acute multiple sclerosis relapse. Ann N Y Acad Sci 2007;1107:155–67. [PubMed: 17804543]

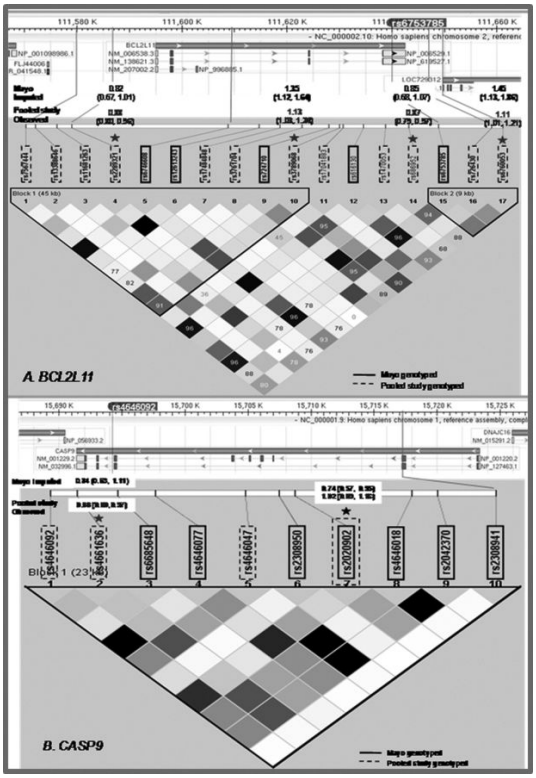

Figure 1.

Linkage disequilibrium plot of SNPs genotyped in *BCL2L11* (Panel A) and *CASP9* (Panel B), Mayo Clinic Case-control study of NHL, 2002-2005 and SEER/CT/NSW pooled case-control study of NHL. The numbers indicate D' values; the darker shading indicates higher r^2 values of correlation between SNPs. ORs and 95% CIs for significant SNPs genotyped in the SEER/CT/NSW pooled study and either genotyped or imputed in the current Mayo NHL case-control study are indicated above each SNP rs number.

Table 1

Gene-level results, Mayo Case-Control Study of NHL, 2002-2005.

Gene* (alias)	Function^	HapMap† SNPs, N	SNPs genotyped, N	Gene coverage‡	SNPs p≤0.05, N	Principal Components Analysis DF	Principal Components Analysis p-value
BCL2 family members							
BAD (BCL2L8)	pro	7	2	71.4%	0	2	0.71
BAG1	anti	10	2	80.0%	0	2	0.19
BAG3	anti	42	8	57.1%	0	4	0.36
BAG4	anti	10	2	100.0%	0	1	0.78
BAG5	anti	**10**	**1**	**50.0%**	**1**	**1**	**0.026**
BAK1 (BCL2L7)	pro	19	2	52.6%	0	2	0.20
BAX	pro	10	2	40.0%	0	1	0.44
BCL2	anti	179	53	74.3%	1	21	0.28
BCL2A1	anti	13	4	76.9%	0	1	0.27
BCL2L1 (BCL-XL)	anti/pro	41	4	82.9%	0	2	0.94
BCL2L10 (Diva)	anti	9	1	55.6%	0	1	0.65
BCL2L11(BIM)	pro	**31**	**5**	**45.2%**	**2**	**2**	**0.0019**
BCL2L12	pro	4	2	75.0%	0	2	0.49
BCL2L13	pro	83	13	91.6%	1	4	0.15
BCL2L14 (BCL-G)	pro	82	26	54.9%	4	9	0.26
BCL2L2 (BCL-W)	anti	6	2	66.7%	0	2	0.13
BCLAF1 (BTF)	anti	**8**	**2**	**87.5%**	**2**	**1**	**0.0097**
BID	pro	44	10	43.2%	1	6	0.46
BIK	pro	31	6	77.4%	0	3	0.71
BNIP2	pro	55	3	70.9%	0	2	0.24
BNIP3	pro	16	2	75.0%	0	2	0.19
HRK	pro	12	1	50.0%	0	1	0.94
Caspase family members							
AIF1	anti	12	3	75.0%	0	3	0.47
APAF1 (CED4)	pro	50	13	82.0%	6	4	0.065
BIRC3 (AIP1)	anti	9	2	88.9%	0	2	0.35
CASP1 (IL1BC)	pro	30	1	76.7%	0	1	0.36

						Principal Components Analysis	
Gene* (alias)	**Function^**	**HapMap† SNPs, N**	**SNPs genotyped, N**	**Gene coverage‡**	**SNPs p≤0.05, N**	**DF**	**p-value**
CASP10	pro	11	9	100.0%	1	4	0.12
CASP2	pro	10	3	90.0%	0	3	0.29
CASP3	pro	24	1	29.2%	0	1	0.90
CASP4	pro	22	6	72.7%	0	3	0.45
CASP5	pro	22	2	36.4%	0	2	0.15
CASP6	pro	15	2	60.0%	0	2	0.29
CASP7	pro	59	10	81.4%	2	4	0.31
CASP8 (MACH)	pro	34	12	67.6%	0	5	0.88
CASP9 (APAF3)	pro	**58**	**7**	**96.6%**	**3**	**4**	**0.0022**
DFFB	pro	19	2	10.5%	1	2	0.097

*As defined in Entrez Gene

^anti, anti-apoptotic; pro, pro-apoptotic

†Total number of SNPs (MAF>0.05) from HapMap version Build 36 dbSNP 126

‡Gene coverage is defined as =(total hapmap snps – number of SNPs not tagged) / total hapmap snps

Cancer Epidemiol Biomarkers Prev. Author manuscript; available in PMC 2011 November 1.

Table 2

SNP level associations from genes with p≤0.05 from the gene level test , Mayo Case-Control Study of NHL, 2002-2005.

Gene	SNP ID†	Type*	Major/Minor	MAF		Adjusted OR^ (95% Confidence Interval)			p-trend	q-value
				Case	Cont	Per copy of variant allele	One copy of variant allele	Two copies of variant allele		
BAG5	rs7693	mrna-utr	C/T	0.39	0.34	1.24 (1.02, 1.50)	1.15 (0.87, 1.52)	1.62 (1.07, 2.45)	**0.031**	0.384
BCL2L11	rs6746608	int	G/A	0.39	0.44	0.82 (0.68, 1.00)	0.77 (0.57, 1.03)	0.70 (0.47, 1.04)	**0.049**	0.384
BCL2L11	rs12613243	int	T/C	0.04	0.07	0.58 (0.38, 0.87)	0.60 (0.40, 0.92)	--	**0.0087**	0.332
BCL2L11	rs724710	c-s	C/T	0.28	0.31	0.89 (0.72, 1.09)	0.88 (0.67, 1.15)	0.81 (0.49, 1.33)	0.26	0.622
BCL2L11	rs616130	int	A/C	0.41	0.45	0.87 (0.72, 1.05)	0.84 (0.62, 1.13)	0.76 (0.52, 1.13)	0.15	0.542
BCL2L11	rs6753785	mrna-utr	C/A	0.39	0.44	0.84 (0.69, 1.02)	0.80 (0.60, 1.08)	0.72 (0.48, 1.08)	0.078	0.453
BCLAF1	rs797558	int	C/G	0.18	0.14	1.38 (1.07, 1.80)	1.44 (1.07, 1.93)	1.55 (0.60, 3.99)	**0.015**	0.363
BCLAF1	rs703193	int	C/T	0.18	0.14	1.42 (1.10, 1.84)	1.50 (1.12, 2.00)	1.54 (0.63, 3.77)	**0.0073**	0.332
CASP9	rs6685648	int	T/C	0.33	0.26	1.41 (1.14, 1.73)	1.29 (0.98, 1.69)	2.31 (1.37, 3.91)	**0.0013**	0.219
CASP9	rs4646077	int	G/A	0.23	0.26	0.87 (0.70, 1.08)	0.91 (0.69, 1.20)	0.68 (0.39, 1.21)	0.20	0.561
CASP9	rs2308950	int	G/A	0.02	0.02	0.73 (0.35, 1.49)	0.73 (0.35, 1.49)	--	0.38	0.699
CASP9	rs2020902	int	T/C	0.14	0.18	0.74 (0.57, 0.95)	0.68 (0.51, 0.92)	0.76 (0.33, 1.75)	**0.019**	0.363
CASP9	rs4646018	int	G/A	0.44	0.48	0.84 (0.69, 1.02)	0.91 (0.67, 1.24)	0.69 (0.47, 1.03)	0.078	0.453
CASP9	rs2042370	int	T/C	0.43	0.47	0.82 (0.68, 1.00)	0.88 (0.65, 1.19)	0.67 (0.46, 0.98)	**0.044**	0.384
CASP9	rs2308941	c-ns	C/T	0.02	0.02	0.72 (0.36, 1.45)	0.72 (0.36, 1.45)	--	0.36	0.675

† Reference sequence ID from dbSNP

* SNP function as defined in dbSNP: c-ns, coding- non-synonymous, c-s, coding-synonymous; int, intronic; l-r, variation in region of gene, but not in transcript; mrna-utr, variation in mrna transcript, but not in coding region interval; utr, variation in transcript, but not in coding region interval

^ ORs adjusted for age and sex.

Table 3

Adjusted ORs (95% confidence interval) for selected SNPs by NHL subtype, Mayo Case-Control Study of NHL, 2002-2005.

Gene	SNP ID†	Major/Minor	All NHL (n=441)		CLL/SLL (n=123)		Follicular Lymphoma (n=113)		DLBCL (n=69)		p-heterogeneity^
			Ordinal OR*	p-trend	Ordinal OR*	p-trend	Ordinal OR*	p-trend	Ordinal OR*	p-trend	
BAG5	rs7693	C/T	**1.24 (1.02, 1.50)**	**0.031**	1.25 (0.94, 1.67)	0.13	1.31 (0.97, 1.77)	0.084	**1.67 (1.15, 2.43)**	**0.0074**	0.48
BCL2L11	rs6746608	G/A	**0.82 (0.68, 1.00)**	**0.049**	**0.74 (0.54, 1.00)**	**0.049**	0.87 (0.65, 1.18)	0.38	0.91 (0.62, 1.34)	0.63	0.57
BCL2L11	rs12613243	T/C	**0.58 (0.38, 0.87)**	**0.0087**	**0.41 (0.19, 0.88)**	**0.022**	0.57 (0.28, 1.12)	0.10	1.24 (0.64, 2.39)	0.52	0.047
BCL2L11	rs724710	C/T	0.89 (0.72, 1.09)	0.26	0.78 (0.56, 1.09)	0.14	0.94 (0.68, 1.29)	0.69	1.07 (0.72, 1.59)	0.73	0.39
BCL2L11	rs616130	A/C	0.87 (0.72, 1.05)	0.15	0.79 (0.59, 1.07)	0.13	0.88 (0.66, 1.29)	0.42	0.98 (0.68, 1.43)	0.93	0.65
BCL2L11	rs6753785	C/A	0.84 (0.69, 1.02)	0.078	0.74 (0.55, 1.01)	0.057	0.94 (0.70, 1.28)	0.71	0.87 (0.60, 1.28)	0.49	0.45
BCLAF1	rs797558	C/G	**1.38 (1.07, 1.80)**	**0.015**	1.39 (0.94, 2.05)	0.10	1.24 (0.82, 1.88)	0.30	1.32 (0.81, 2.14)	0.27	0.89
BCLAF1	rs703193	C/T	**1.42 (1.10, 1.84)**	**0.0073**	1.43 (0.98, 2.10)	0.066	1.32 (0.88, 1.97)	0.17	1.40 (0.88, 2.25)	0.16	0.94
CASP9	rs6685648	T/C	**1.41 (1.14, 1.73)**	**0.0013**	**1.60 (1.17, 2.19)**	**0.0036**	1.21 (0.86, 1.69)	0.27	1.26 (0.84, 1.88)	0.27	0.36
CASP9	rs4646077	G/A	0.87 (0.70, 1.08)	0.20	1.07 (0.78, 1.46)	0.68	0.79 (0.55, 1.12)	0.18	**0.55 (0.34, 0.89)**	**0.016**	0.049
CASP9	rs2308950	G/A	0.73 (0.35, 1.49)	0.38	0.21 (0.03, 1.57)	0.13	0.92 (0.31, 2.78)	0.88	0.34 (0.04, 2.60)	0.30	0.34
CASP9	rs2020902	T/C	**0.74 (0.57, 0.95)**	**0.019**	0.85 (0.58, 1.24)	0.40	0.72 (0.47, 1.10)	0.12	**0.43 (0.23, 0.80)**	**0.0076**	0.17
CASP9	rs4646018	G/A	0.84 (0.69, 1.02)	0.078	**0.65 (0.49, 0.88)**	**0.0049**	1.03 (0.76, 1.39)	0.84	1.23 (0.85, 1.79)	0.27	0.0098
CASP9	rs2042370	T/C	**0.82 (0.68, 1.00)**	**0.044**	**0.62 (0.46, 0.83)**	**0.0015**	1.07 (0.80, 1.44)	0.65	1.14 (0.79, 1.64)	0.48	0.0046
CASP9	rs2308941	C/T	0.72 (0.36, 1.45)	0.36	0.83 (0.28, 2.49)	0.74	1.49 (0.61, 3.63)	0.38	0.30 (0.04, 2.31)	0.25	0.27

*ORs adjusted for age and sex

†Reference sequence ID from dbSNP

^p-value for test of heterogeneity between the CLL/SLL, Follicular Lymphoma, and DLBCL NHL subtypes as determined by polytomous logistic regression; CLL/SLL, chronic lymphocytic leukemia / small lymphocytic lymphoma; DLBCL, diffuse large B-cell lymphoma

Table 4

Comparison of observed and imputed Mayo Case-Control Study of NHL (2002-2005) SNP-level results with published observed SNP-level results from the pooled NCI-SEER, Connecticut, and NSW NHL Case-Control Studies

			Mayo Actual Results				Mayo Imputed Results						Pooled Study Actual Results			
Gene	**SNP ID†**	**position**	**W/V**	**Variant Freq***	**OR Ordinal (95% CI)**	**p-trend**	**W/V**	**Variant Freq***	**OR Ordinal (95% CI)**	**p-trend**	**quality**	**rsq**	**W/V**	**Variant Freq***	**OR Ordinal (95% CI)**	**p-trend**
BCL2L11	rs7567444	111579909			not evaluated	--	C/T	0.44	0.82 (0.67, 1.01)	0.056	0.94	0.87	C/T	0.46	**0.88 (0.80, 0.96)**	**0.0055**
BCL2L11	rs13388646	111581386			not evaluated	--	C/T	0.05	26.0 (5.04, 134.06)	0.00010	0.91	0.09	C/T	0.08	1.12 (0.95, 1.34)	0.18
BCL2L11	rs11681263	111584481			not evaluated	--	C/A	0.27	1.73 (1.20, 2.50)	0.0036	0.64	0.33	C/A	0.23	1.05 (0.94, 1.17)	0.38
BCL2L11	rs2289321	111586691			not evaluated	--	T/C	0.06	**0.59 (0.38, 0.90)**	**0.014**	0.99	0.90	T/C	0.06	1.07 (0.89, 1.28)	0.46
BCL2L11	rs6746608	111609455	G/A	0.42	**0.82 (0.68, 1.00)**	**0.049**			--	--	--	--			not evaluated	
BCL2L11	rs12613243	111613977	T/C	0.06	**0.58 (0.38, 0.87)**	**0.0087**			--	--	--	--			not evaluated	
BCL2L11	rs17484848	111617031			not evaluated	--	T/C	0.11	3.19 (1.33, 7.65)	0.0092	0.79	0.12	T/C	0.10	1.09 (0.94, 1.26)	0.27
BCL2L11	rs3761704	111620169			not evaluated	--	A/G	0.14	3.35 (1.70, 6.58)	0.00046	0.75	0.16	A/G	0.12	1.11 (0.97, 1.28)	0.13
BCL2L11	rs724710	111624162	C/T	0.29	0.89 (0.72, 1.09)	0.27			--	--	--	--			not evaluated	
BCL2L11	rs3789068	111625718			not evaluated	--	A/G	0.52	**1.35 (1.12, 1.64)**	**0.0018**	0.99	0.99	A/G	0.46	**1.13 (1.03, 1.24)**	**0.0093**
BCL2L11	rs17041883	111626213			not evaluated	--	C/A	0.11	5.83 (2.55, 13.37)	0.00003	0.79	0.13	C/A	0.12	1.08 (0.94, 1 24)	0.28
BCL2L11	rs616130	111629152	A/C	0.43	0.87 (0.72, 1.05)	0.15			--	--	--	--			not evaluated	
BCL2L11	rs1470053	111632417			not evaluated	--	G/T	0.21	1.94 (1.22, 3.1)	0.0052	0.66	0.25	G/T	0.17	1.05 (0.93, 1.18)	0.43
BCL2L11	rs686952	111635817			not evaluated	--	C/A	0.28	0.85 (0.68, 1.06)	0.15	0.96	0.90	C/A	0.29	**0.87 (0.79, 0.97)**	**0.010**
BCL2L11	rs6753785	111640101	C/A	0.42	0.84 (0.69, 1.02)	0.078			--	--	--	--			not evaluated	
BCL2L11	rs726430	111647892			not evaluated	--	T/C	0.20	2.41 (1.47, 3.94)	0.00047	0.67	0.23	T/C	0.18	1.11 (0.98, 1.24)	0.096
BCL2L11	rs6760053	111649468			not evaluated	--	C/G	0.42	**1.45 (1.13, 1.86)**	**0.0031**	0.76	0.59	C/G	0.44	**1.11 (1.01, 1.21)**	**0.031**
CASP9	rs4646092	15694260			not evaluated	--	C/T	0.21	0.67 (0.51, 0.89)	0.0057	0.88	0.68	C/T	0.23	1.09 (0.97-1.21)	0.14
CASP9	rs4661636	15695648			not evaluated	--	C/T	0.39	0.84 (0.63, 1.11)	0.23	0.68	0.45	C/T	0.33	**0.88 (0.8-0.97)**	**0.011**
CASP9	rs6685648	15697782	T/C	0.29	**1.41 (1.14, 1.73)**	**0.0013**			--	--	--	--			not evaluated	
CASP9	rs4646077	15699723	G/A	0.21	0.87 (0.70, 1.08)	0.20			--	--	--	--			not evaluated	
CASP9	rs4646047	15704370			not evaluated	--	C/T	0.51	0.88 (0.70, 1.10)	0.26	0.85	0.72	C/T	0.46	**0.90 (0.82-0.99)**	**0.033**
CASP9	rs2308950	15706093	G/A	0.02	0.73 (0.35, 1.49)	0.38			--	--	--	--			not evaluated	
CASP9	rs2020902	15706947	A/G	0.16	**0.74 (0.57, 0.95)**	**0.019**			--	--	--	--	A/G	0.14	1.02 (0.89-1.16)	0.82
CASP9	rs4646018	15712961	G/A	0.49	0.84 (0.69, 1.02)	0.078			--	--	--	--			not evaluated	
CASP9	rs2042370	15714329	T/C	0.49	**0.82 (0.68, 1.00)**	**0.044**			--	--	--	--			not evaluated	

Gene	SNP ID†	position	Mayo Actual Results				Mayo Imputed Results						Pooled Study Actual Results			
			W/V	Variant Freq*	OR Ordinal (95% CI)	p-trend	W/V	Variant Freq*	OR Ordinal (95% CI)	p-trend	quality	rsq	W/V	Variant Freq*	OR Ordinal (95% CI)	p-trend
CASP9	rs2308941	15717305	C/T	0.01	0.72 (0.36, 1.45)	0.36			--	--	--	--			not evaluated	

*Variant allele frequency among the controls.

Table 5

Comparison of imputed and observed SNPs in *BCL2L11* and risk of NHL subtypes, Mayo Case-Control Study compared with published results from the pooled NCI-SEER, Connecticut, and NSW NHL Case-Control Studies

		CLL/SLL		Follicular Lymphoma		DLBCL	
SNP ID[†]	**Major/Minor**	**Ordinal OR**	**p-trend**	**Ordinal OR**	**p-trend**	**Ordinal OR**	**p-trend**
Mayo Study (imputed results)							
rs7567444	C/T	0.73 (0.53, 1.01)	0.059	0.87 (0.64, 1.20)	0.41	0.91 (0.61, 1.36)	0.64
rs3789068	A/G	**1.56 (1.16, 2.10)**	**0.0032**	1.28 (0.95, 1.72)	0.10	1.05 (0.72, 1.52)	0.79
rs686952	C/A	**0.74 (0.52, 1.06)**	**0.097**	0.91 (0.65, 1.29)	0.60	1.01 (0.66, 1.54)	0.98
rs6760053	C/G	**1.68 (1.15, 2.46)**	**0.0077**	1.31 (0.89, 1.92)	0.17	1.10 (0.68, 1.79)	0.69
Pooled Study (observed results)							
rs7567444	C/T	0.93 (0.74, 1.17)	0.54	**0.79 (0.68, 0.91)**	**0.0009**	0.91 (0.79, 1.04)	0.15
rs3789068	A/G	1.03 (0.82, 1.29)	0.80	**1.28 (1.12, 1.47)**	**0.0004**	1.10 (0.97, 1.26)	0.14
rs686952	C/A	0.93 (0.72, 1.20)	0.56	**0.78 (0.67, 0.92)**	**0.0023**	0.87 (0.75, 1.01)	0.078
rs6760053	C/G	1.00 (0.79, 1.26)	0.99	**1.19 (1.03, 1.36)**	**0.015**	1.09 (0.95, 1.24)	0.22