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Inherited genetic variation and overall survival following follicular lymphoma

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Abstract

Follicular lymphoma (FL) has variable progression and survival, and improved identification of patients at high risk for progression would aid in identifying patients most likely to benefit from alternative therapy. In a sample of 244 FL cases identified during a population-based case-control study of non-Hodgkin lymphoma (NHL), we examined 6,679 tag SNPs in 488 gene regions for associations with overall FL survival. Over a median follow-up of 89 months with 65 deaths in this preliminary study, we identified 5 gene regions (*BMP7*, *GALNT12*, *DUSP2*, *GADD45B*, and *ADAM17*) that were associated with overall survival from FL. Results did not meet the criteria for statistical significance after adjustment for multiple hypothesis testing. These results, which support a role for host factors in determining the variable progression of FL, serve as an initial examination that can inform future studies of genetic variation and FL survival. However, they require replication in independent populations, as well as assessment in rituximab-treated patients.

Keywords

follicular lymphoma; genetic variation; survival; tag SNP; case-control study

Follicular lymphoma (FL) is one of the most common subtypes of NHL, with over 24,000 new cases diagnosed each year in the United States [1, 2]. Prior to the rituximab era, FL had a median survival of approximately 10 years, but progression varied and survival ranged from less than one year to more than 20 years after diagnosis [3]. Deaths from FL follow resistance to treatments or transformation to a more aggressive lymphoma type, usually diffuse large B-cell lymphoma (DLBCL) [4]. The Follicular Lymphoma International Prognostic Index (FLIPI) separates FL patients into three risk groups based on age, stage of disease, concentrations of β 2-microglobulin and hemoglobin, nodal area involvement, serum lactate dehydrogenase levels, and bone marrow involvement [5, 6]. Treatment strategies for FL have included “watchful waiting”, chemotherapy, immunochemotherapy, radiotherapy, radioimmunoconjugates, and stem cell transplantation [1]. Although new treatment strategies have changed the survival of patients with FL, patients continue to die from disease or complications of survival from disease [7]. Improved identification of patients at high risk for progression would aid in developing targeted approaches for those that might benefit from alternative therapeutic interventions.

The heterogeneous survival times among FL patients reflects genetic properties of the tumors, but the immune microenvironment likely plays a significant role as well [8, 9]. We hypothesized that germline genetic variation could also influence FL survival due to differences in repair or clearance of genetic damage, immune response, inflammatory response, or other processes [9]. We previously reported associations between overall survival of FL and common genetic variants in cytokine genes (*IL8*, *IL2*, *IL12B*, and *IL1RN*) and one-carbon metabolism genes (*MTHFR*, *FTHFD* and *GGH*) in studies of approximately 100 candidate single nucleotide polymorphisms (SNPs) from 88 genes [10, 11]. Because numerous pathways and processes have been implicated in histologic transformation and overall prognosis of FL [8, 12], we conducted a broad, exploratory study of the association of 488 candidate genes with overall survival. Genes were selected for their potential to influence lymphoma survival based on a literature review [8, 13–15], and we comprehensively tagged these genes/gene regions with 6,679 SNPs.

The median age at diagnosis of the 244 FL cases was 56 years, and 42% were age 60 or older. Patients were predominantly white (91%) with similar numbers of men and women (49% female). Participants who reported Hispanic ethnicity (n=10) were categorized based on self-reported race; 8 were included in the “white” category and 2 in the “other” category. The majority of FL cases were diagnosed with advanced stage disease (56%), 14% reported B symptoms, and the most common treatments were chemotherapy-based regimens (63%), observation (25%), and radiation only (11%). Over a median follow-up of 89 months (range, 9–112 months), 65 (27%) patients died of any cause, with 75% of these having lymphoma coded as the underlying cause of death. As described previously, the observed survival for patients in our study was similar to that observed in the SEER program [10]. The risk score constructed to capture effects of the above clinical and demographic factors was significantly associated with overall survival (HR = 3.10, 95% CI = 2.08–6.60) in a Cox model.

Among the 488 gene regions examined, we identified five independent gene regions that were associated ($p < 0.01$) with FL overall survival (Table 1) prior to adjustment for multiple hypothesis testing. The two regions with the smallest gene-based p-values were *BMP7* and *GALNT12* ($p = 0.002$), but neither gene region was significantly associated with

survival after FDR adjustment (FDR $p = 0.41$). Other gene regions of interest included *DUSP2*, *GADD45B* and *ADAM17*, but again associations were not significant after FDR adjustment. In analysis of individual SNPs (Table 2), four SNPs were associated with survival at a $p_{trend} < 0.001$. A SNP in the *BMP7* gene (rs6025446, $p_{trend} = 0.00003$; FDR $p = 0.22$) had the lowest observed p-value for association and was significantly associated with improved overall survival after adjustment for clinical and demographic factors ($HR_{AG} = 0.41$, 95% CI = 0.21–0.69; $HR_{GG} = 0.17$, 95% CI = 0.05–0.54). As with the gene-based tests, none of the SNP associations remained significant after adjustment for multiple hypothesis testing. Other SNPs with suggestive associations that may be of interest for follow-up studies are shown in Table 2, and genotype frequencies and associations with overall survival for all 6,679 SNPs examined are available in Supplemental Table S1. The SNP associations reported in Table 2 were similar when the same models were used with outcomes restricted to death from follicular lymphoma (Supplemental Table S2).

In a sample of 244 FL cases identified during a population-based case-control study of NHL, we identified 5 gene regions (*BMP7*, *GALNT12*, *DUSP2*, *GADD45B*, and *ADAM17*) that were associated with overall survival from FL after accounting for clinical and demographic factors. In addition to SNPs located within the 5 associated gene regions, a SNP in the *IRF2* gene was also associated with FL overall survival. In this exploratory investigation of a large number of genetic variants (>450 genes) and survival after FL, results did not meet the criteria for significance after adjustment for multiple hypothesis testing, thus replication in other populations is critical. Nevertheless, our results suggest a small number of gene regions that may be of particular interest in future studies of survival after FL.

The gene regions identified in our study can all be linked to regulation of growth factors and cell signaling pathways, and expression levels have been associated with FL survival or control of B-cell activity [8, 13]. The bone morphogenetic protein encoded by *BMP7* is known to induce apoptosis, inhibit proliferation, and inhibit metastasis of a number of cancer cell types through antagonism of TGF- β pathways [16]. *ADAM17* gene products have proteinase activity and are responsible for membrane-shedding of TGF- β and other growth-regulators [17]. *GADD45B* has been identified as a pro-survival factor for tumors that mediates activation of the mitogen-activated protein kinase (MAPK) p38/JNK pathway [18]. *DUSP2* encodes a dual-specificity phosphatase that can inhibit MAPK activity via dephosphorylation and is involved in regulation of immune and inflammatory responses [19]. *GALNT12* mutations are associated with aberrant glycosylation, which is a feature of many cancers and can affect cell growth and differentiation [20, 21]. The associations observed in this study may suggest that inherited differences in growth-regulatory pathways in immune cells can impact the phenotype and progression of FL, but FDR-adjusted p-values were not significant and replication in further studies is required. The variants identified are tag SNPs, and none of the identified SNPs are located within coding exons. The most significant SNP in *DUSP2* is located in the 3' UTR, and while rs4806857 tags variation in *GADD45B*, it is located almost 10-kb 3' of the coding region. If additional studies find associations between these genes and survival after FL, then further work to evaluate functional consequences of specific variants may be warranted.

This study examines associations with FL survival for more genetic variants than any reported to date, and our tag SNP approach provides a more comprehensive assessment of genetic variation in these genes and surrounding regions. The genes included in the study were selected *a priori* based on existing literature and knowledge of lymphoma mechanisms. Rigorous quality control procedures ensured high quality genotype data, and our statistical analyses accounted for linkage among SNPs and the large number of genes/SNPs examined. The population-based ascertainment and rapid-reporting mechanisms employed to identify

new FL cases provided a study population likely to be generalizable, as suggested by similarities to the mortality observed in SEER [10].

An important limitation of our study is the lack of detailed treatment data and limited information on prognostic factors, although our clinical risk score predicted at a similar level to FLIPI [10]. Rituximab was not yet widely used as an initial FL therapy at the time of the case-control study, so treatment of patients in our study was different from current practice [22]. Despite the use of rapid reporting mechanisms, some of the most aggressive cases of FL were not included in our study. An analysis of FL etiology in the same case-control study did not find associations for the SNPs reported here [23], which may suggest involvement of different factors in FL etiology and prognosis, although replication is needed.

The gene variants reported here have not previously been linked to FL, and we have made numerous comparisons. These results therefore serve as an important initial examination that can inform future studies, but require replication in independent populations, as well as assessment in rituximab-treated patients. Further investigation is warranted, as identification of inherited genetic variants that affect FL prognosis could yield new insights into clinical outcomes and treatment options.

Materials and Method

Patients included 244 FL cases identified in a population-based case-control study of NHL in the United States, details of which have been reported previously [10, 23]. Briefly, 20–74 year-old patients with histologically-confirmed NHL and without known HIV infection were identified from four Surveillance, Epidemiology, and End Results (SEER) cancer registries (Detroit, MI; Seattle/Puget Sound, Washington; Los Angeles, CA; Iowa) between July 1998 and June 2000. Each of the recruitment centers obtained local IRB approval for the study, the overall study was approved by the National Institutes of Health, and all participants provided written informed consent. Patients with FL were identified using the International Classification of Diseases - Oncology (ICD-O), 3rd edition [24] codes: 9690–9693, 9695–9698. Patients provided data on age, sex, race, Hispanic ethnicity, and education level via interviews conducted for the case-control study. Linkage to routine follow-up from SEER registry databases at each study site in the spring of 2008 provided information on histology, stage, presence of B-symptoms, date of last follow-up, vital status, and first course of therapy (use of single or multiagent chemotherapy, radiation, other therapies exclusive of chemotherapy and/or radiation, and no therapy or observation); information on individual agents or doses was not available.

DNA was extracted from blood clots, buffy coats, or buccal cell samples as described previously [23], and genotype frequencies were equivalent for individuals who provided blood or buccal cell samples [25]. Genotyping of 7,943 tag SNPs from 488 gene regions putatively involved in lymphoma survival was conducted at the NCI Core Genotyping Facility as previously described [23]. Tag SNPs were selected within the region spanning 20-kb 5' of the transcription start site to 10-kb 3' of the end of the last exon and were grouped using $r^2 > 0.80$ to define a gene region. Genotyping was successfully completed for 6,830 tag SNPs. Of these, SNPs with a low completion rate (<90% of samples) or with a concordance less than 95% in QC samples were also excluded, resulting in a final analytic study population of 244 FL cases with data for 6,679 SNPs. Of the 1,321 cases in the case-control study, 1,286 had valid registry data at the time of linkage, of which 328 were FL. Of the 328 FL cases, 50 were excluded because they did not provide a blood or buccal sample and 34 were excluded based on genotyping QC. This left a total of 244 FL cases for analysis.

We examined the association between individual SNPs and overall survival in FL patients by time-to-event analysis using multivariable Cox proportional hazards regression models to estimate hazard ratios (HRs) and 95% confidence intervals (95% CI). Models were adjusted for a prognostic risk score generated by the linear combination of the following clinical and demographic variables multiplied by their Cox regression coefficients: stage (local, regional, distant, missing), presence of B-symptoms (yes, no, missing), type of initial therapy (chemotherapy + radiation, chemotherapy + other therapy, radiation only, no therapy), age (<60 years, 60+ years, as categorized in FLIPI), sex, race (white, all other), study center (Detroit, Iowa, Los Angeles, Seattle), and years of education (<12, 12–15, 16+ years) [10]. We examined associations at the gene level using principal components analysis as described previously [26]. Briefly, linear combinations of ordinal-scaled SNPs were used to identify principal components that explained 90% of the SNP variance within the gene region. The principal components were then included in multivariable Cox proportional hazards regression models [26]. To address the potential for type I error due to multiple comparisons, we calculated the false discovery rate (FDR) for individual SNPs and gene-level tests. The FDR is defined as the expected proportion of falsely rejected hypotheses among all rejected hypotheses [27]. A secondary analysis restricted to lymphoma-specific death was conducted for genes and SNPs associated with overall survival.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary of gene-level and SNP-level association tests for genes associated with overall survival¹ in follicular lymphoma patients at $p<0.01$

Table 1

Gene	No. tag SNPs	No. tag SNPs $p_{trend} < 0.01$	Gene-based p -value ²	FDR	Tag SNP with smallest p_{trend} ⁴	
					dbSNP ID	p_{trend}
<i>GALNT12</i>	11	2	0.002	0.41	rs10987898	0.00067
<i>BMP7</i>	26	8	0.002	0.41	rs6025446	0.00003
<i>DUSP2</i>	2	0	0.003	0.41	rs1724120	0.09953
<i>GADD45B</i>	6	1	0.007	0.76	rs4806857	0.00112
<i>ADAM17</i>	8	0	0.009	0.76	rs7580419	0.04081

¹ Dichotomous survival: yes/no

² Determined using principal components analysis

³ Adjusted for multiple hypothesis testing using the False Discovery Rate

⁴ Cox proportional hazards models

Table 2

Individual SNPs most strongly associated with overall survival¹ in follicular lymphoma patients ($p_{\text{trend}} < 0.001$)

Gene	Tag SNP with p_{trend}	Heterozygous for minor allele		Homozygous for minor allele		FDR	
		Minor allele/MAF	N	HR (95% CI) ²	N	HR (95% CI) ²	p_{trend} ³
<i>BMP7</i>	rs6025446	G / 0.36	117	0.41 (0.21–0.69)	30	0.17 (0.05–0.54)	0.00003
<i>IRF2</i>	rs3775567	T / 0.05	24	3.18 (1.72–5.87)	0	--	0.00022
<i>GALNT12</i>	rs10987898	G / 0.29	90	0.48 (0.27–0.83)	25	0.18 (0.04–0.74)	0.00067
<i>GALNT12</i>	rs10819377	T / 0.44	127	0.62 (0.36–1.03)	43	0.24 (0.09–0.59)	0.00095

¹ Dichotomous survival: yes/no

² Adjusted for prognostic risk score; HR based on Cox proportional hazards models with “homozygous for major allele” as the reference group

³ Adjusted for multiple hypothesis testing using the False Discovery Rate