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A functional TNFRSF5 polymorphism and risk of non-Hodgkin lymphoma, a pooled analysis

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Abstract

Interaction between CD40 and its ligand, CD154, has a key function in immune regulation. Recent experimental data support a role of deregulated CD40 signalling in lymphomagenesis. Data from earlier studies that are part of this pooling study implicate a functional polymorphism (−1C>T, rs1883832) in the *TNFRSF5* gene encoding CD40 in the etiology of follicular lymphoma. Here, the association of this variant with non-Hodgkin lymphoma (NHL) risk was replicated in a European multicenter study of 855 NHL cases and 1,206 controls. In the combined analysis of 2,617 cases and 3,605 controls, carrying the TT genotype was associated with an increased risk for all NHL (OR = 1.4; *p* for linear trend = 0.00009), diffuse large B-cell lymphoma (OR = 1.6; *p* for linear trend = 0.002) and follicular lymphoma (OR = 1.6; *p* for linear trend = 0.001). These data suggest a possible role of this functional polymorphism in lymphomas originating within the germinal center.

Keywords

lymphoma; TNFRSF5; CD40; polymorphism; epidemiology

Deregulation of immune responses and infectious agents play a role in the etiology of lymphoma.[1,2] There is increasing evidence that variants in a number of immune regulatory genes drive interindividual differences in lymphoma susceptibility.[3] Recently, Skibola et al. reported that the homozygous variant −1TT genotype of a functional single nucleotide polymorphism (SNP) in the *TNFRSF5* gene (−1C>T, rs1883832), located in the Kozak

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sequence of the 5′UTR,[4] was associated with an increased risk of follicular lymphoma (FL).[5] TNFRSF5 encodes CD40, a member of the tumor necrosis factor (TNF) receptor family that interacts with its ligand, CD154 encoded by *TNFSF5*, and orchestrates a multitude of processes that influence B-cell and T-cell biology. The *TNFRSF5* −1T risk allele has been associated with reduced CD40 translational efficiency on B cells,[6] lower CD40 expression on dendritic cells and reduced circulating levels of soluble CD40 in humans.[5] Deregulated CD40 signaling was recently implicated in the initiation of B-cell transformation in mice.[7]

The objective of our study was to replicate within Epi-Lymph, a European multicenter study, the results of an association between the *TNFRSF5* −1TT genotype and risk of FL found in the previous study[5] and to conduct a pooled analysis with increased power for subtype-specific analyses.

Materials and Methods

Study populations in Europe and the United States

EpiLymph—The EpiLymph multicenter case–control study consisting of 2,302 lymphoma cases (including non-Hodgkin lymphoma [NHL] and Hodgkin lymphoma) and 2,417 controls was conducted in six countries (Germany, Italy, Spain, Ireland, France and Czech Republic) from 1998 to 2004. Details of the study design have been provided elsewhere.[8] Cases were categorized according to the WHO classification.[8] Controls were drawn randomly from population registers of the study regions (Germany and Italy) or were recruited from the same hospital as cases (remaining countries). All controls were frequency matched to the cases by age (±5 years), sex and study center. In the hospital-based studies, controls were excluded if the main reason for the hospitalization was cancer, organ transplant and/or systemic infection.

San Francisco Bay Area NHL1 and NHL2 studies—Detailed methods for these two large population-based case–control studies (NHL1: cases = 1,591, controls = 2,515; NHL2: cases = 2,055, controls = 2,081) have been published previously (NHL1[9,10]; NHL2[5]). In brief, rapid case ascertainment and cancer registry data were used to identify incident NHL adult cases diagnosed from 1988 to 1993 (NHL1) and from 2001 to 2006 (NHL2) in six San Francisco Bay Area counties. Controls were identified using random digit dial and frequency matched to cases by 5-year age group, sex and county. Diagnostic materials were rereviewed, and NHL subtypes were classified using the Revised European-American Lymphoma (NHL1) and the WHO classification (NHL2). Biospecimens were collected from eligible participants (NHL1: 63% cases, 66% controls; NHL2: 87% cases, 89% controls).

Informed consent was obtained from all participants before enrolment. The institutional review boards of participating centers approved each study.

Genotyping

Genotyping of *TNFRSF5* −1C>T SNP (rs1883832) was performed by Pyrosequencing™, Qiagen, Hilden, Germany and TaqMan®, Applied Biosystems™, Darmstadt, Germany.[5]

Statistical analysis

Data for the *TNFRSF5* −1C>T SNP (rs1883832) for five Epi-Lymph study centers (Spain, France, Italy, Ireland and the Czech Republic) were used to replicate results from an earlier analysis including the German component of the same study and the San Francisco Bay Area NHL1 and 2 studies[5] and to conduct a pooled analysis of the combined data to increase

power for analyses by NHL subtype. Statistical analyses were conducted using SAS version 9 (SAS Institute, Cary, NC) and the rmeta package in R version 2.14.

Analyses were restricted to HIV-negative, non-Hispanic whites to diminish potential confounding effects because of underlying population structure (2,617 cases, 3,605 controls; 4 cases and 5 controls without genotype information, see Supporting Information Table 2). Unconditional logistic regression models adjusted for age as a continuous variable, sex and individual study centers (six EpiLymph, two San Francisco Bay Area) were used to obtain odds ratios (OR) as estimates of relative risk (hereafter called risk). The contribution of individual studies to the overall effect estimates was assessed in sensitivity analyses. Genotype was coded as an ordinal variable based on the number of rare alleles (0, 1 and 2) and as a binary variable assuming dominant inheritance. The referent group for genotype analyses was the most common homozygous genotype in controls. Adjusted logistic regression was used to determine linear trend in genotype odds ratios when genotype was included in the model as an ordinal variable.

Results and Discussion

A total of 855 NHL cases and 1,206 controls were available for the replication study. The *TNFRSF5* −1TT genotype was positively associated with risk of NHL (OR = 1.6, *p* value for linear trend = 0.001; Table 1). Risk was increased for diffuse large B-cell lymphoma (DLBCL; OR = 2.1, *p* value for linear trend = 0.001) and FL (OR = 1.9, *p* value for linear trend = 0.04; Table 1), but not for chronic lymphocytic leukemia (OR = 0.78, *p* value for linear trend = 0.84).

In the pooled study population of 2,617 NHL cases and 3,605 controls, carriers of the *TNFRSF5* −1TT genotype showed a 1.4-fold increase in risk of NHL (*p* for trend = 0.00009). The excess risk was seen for DLBCL and FL, where the −1TT genotype conferred a 1.6-fold increase in risk (*p* value for linear trend = 0.002 and 0.001, respectively; Table 1). Risk for chronic lymphocytic leukemia was not increased in association with the −1TT genotype. Study-specific and pooled-adjusted risk estimates and 95% confidence intervals from fixed-effect models for *TNFRSF5* −1C>T for all NHL, DLBCL and FL are depicted in Figure 1. In sensitivity analyses, none of the study altered the observed OR estimate by >10% (Fig. 1). Results from a sensitivity analysis excluding hospital controls were similar to results from the analysis including hospital controls (risk for NHL per allele, OR = 1.15, 95% CI = 1.04–1.26; OR = 1.18, 95% CI = 1.08–1.27; respectively).

Skibola *et al.*[5] recently showed that healthy controls and lymphoma cases with the *TNFRSF5* −1TT genotype had lower circulating soluble CD40 levels and reduced cell surface expression of CD40 on dendritic cells. This is consistent with previous studies that have reported lower CD40 expression in B cells of −1T versus −1C carriers in B-cell lines derived from patients with Grave's disease and in transfected Rat-2 fibroblasts.[6,11,12] Mice and humans that lack *TNFRSF5* or *TNFSF5* gene expression have reduced antibody production and Ig class switching and are unable to mount effective responses against infectious agents.[13]

Interestingly, the *TNFRSF5* −1TT genotype was associated with an elevated risk of the two major NHL subtypes, DLBCL and FL, neoplasms that develop mainly in the germinal center (GC). CD40-CD154 ligation plays a pivotal role in centrocyte differentiation in the GC by downregulation of BCL6.[14] Low BCL6 activity permits differentiation of centrocytes into plasma cells or memory cells, allowing B cells to exit the GC. This process may be attenuated by the *TNFRSF5* −1TT genotype where low CD40 expression could hinder B- and T-cell interactions, allowing B cells to linger in GCs and undergo further somatic

hypermutation and proliferation. This may increase the likelihood of B cells with preneoplastic lesions to progress to malignancy.

The strength of our study is its large sample size that allows the investigation of the main effects of *TNFRSF5* −1C>T in relation to the risk of major NHL subtypes. However, small numbers limited the analysis of rarer lymphoma subtypes and subgroups stratified by age and sex.

In conclusion, this pooled analysis highlights the important role of the functional *TNFRSF5* −1C>T variant in the etiology of DLBCL and FL. Future studies with careful molecular characterization of GC lymphomas are needed to assess risk related to *BCL6* and *BCL2* mutation status and further clarify mechanisms that contribute to lymphomagenesis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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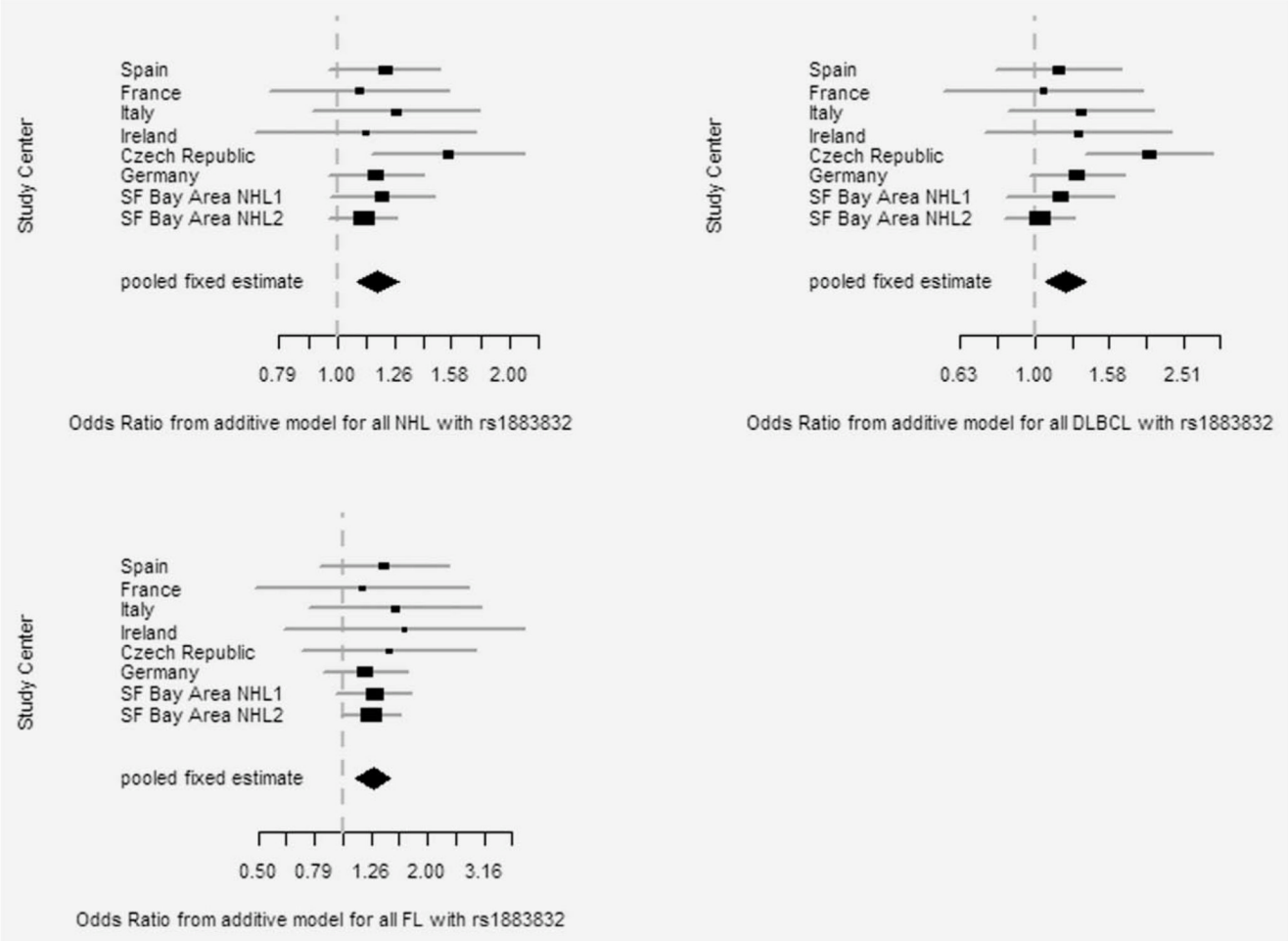

Figure 1.

Odds ratios (ORs) and 95% confidence intervals (CIs) for risk per allele for the rs13883832 polymorphism associated with risk of all non-Hodgkin lymphoma (NHL), diffuse large B-cell (DLBCL) and follicular lymphoma (FL) obtained from age- and sex-adjusted unconditional logistic regression models restricted to HIV-negative non-Hispanic whites by study center. Rectangular shapes represent the study-specific ORs, the diamond shape represents the pooled OR with the size of the rectangles proportional to the weight (sample size) of the individual study in the pooled estimate (diamond). Study centers are identified on the y-axis and ORs and 95% CIs along the x-axis. The broken vertical line represents the OR = 1.0 (null effect).

Table 1

Odds ratios (OR) and 95% confidence intervals (CI) for risk of non-Hodgkin lymphoma (NHL) and NHL by histologic subtype groups associated with the single nucleotide polymorphism (SNP) at position −1 of the Kozak consensus sequence of CD40 gene (rs1883832, C>T), restricted to HIV-negative non-Hispanic white participants

		Replication set[1]		Pooled set[2]	
NHL	**Genotype**	**Cases/controls**	**OR[3] (95%CI)**	**Cases/controls**	**OR[3] (95%CI)**
All NHL	CC	420/661	1.0	1,343/1,992	1.0
	CT	351/460	1.2 (1.0–1.5)	1,041/1,366	1.1 (1.0–1.3)
	TT	84/85	1.6 (1.2–2.3)	229/242	1.4 (1.2–1.8)
	CT/TT	435/545	1.3 (1.1–1.5)	1,270/1,608	1.2 (1.1–1.3)
p trend			0.001		0.00009
DLBCL	CC	125/661	1.0	391/1,992	1.0
	CT	116/460	1.3 (1.0–1.8)	311/1,366	1.2 (0.98–1.4)
	TT	31/85	2.1 (1.3 –3.3)	72/242	1.6 (1.2–2.1)
	CT/TT	147/545	1.4 (1.1–1.9)	383/1,608	1.2 (1.0–1.4)
p trend			0.001		0.002
FL	CC	44/661	1.0	244/1,992	1.0
	CT	43/460	1.4 (0.91–2.2)	213/1,366	1.3 (1.1–1.6)
	TT	11/85	1.9 (0.96–3.9)	46/242	1.6 (1.1–2.2)
	CT/TT	54/545	1.5 (0.99–2.3)	259/1,608	1.3 (1.1–1.6)
p trend			0.04		0.001
CLL/SLL	CC	119/661	1.0	263/1,992	1.0
	CT	87/460	1.1 (0.79–1.5)	202/1,366	1.1 (0.92–1.4)
	TT	12/85	0.78 (0.41–1.5)	38/242	1.2 (0.81–1.7)
	CT/TT	99/545	1.0 (0.76–1.4)	240/1,608	1.1 (0.94–1.4)
p trend			0.84		0.19

Abbreviations: DLBCL: diffuse large B-cell lymphoma; FL: follicular lymphoma; CLL/SLL: chronic lymphocytic leukemia/small lymphocytic lymphoma.

[1] EpiLymph centers except Germany (Spain, France, Italy, Ireland and Czech Republic).

[2] Pooled data include also Germany center of EpiLymph and San Francisco Bay Area NHL1 and NHL2 study data.[5]

[3]Odds ratios and 95% confidence intervals are adjusted for age (continuous), sex and individual study center.