Body Mass Index, Leptin and Leptin Receptor Polymorphisms, and Non-Hodgkin Lymphoma

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Abstract

In a population-based case-control study, obesity was associated with elevated odds ratios (ORs) for non-Hodgkin lymphoma (NHL), and the two major subtypes, diffuse large cell (DLCL) and follicular lymphoma (FL). Those who were obese (body mass index ≥ 30) were up to three times more likely to develop NHL or its major subtypes than persons with body mass index of 20 to <25. Obesity-related genetic factors including common polymorphisms in the leptin gene (*LEP* A19G and G-2548A) and its receptor (*LEPR* Q223R) were investigated in DNA available for 376 patients and 805 controls. Leptin is an adipocyte-derived hormone that regulates food intake and modulates immune and inflammatory responses through its receptor. Among those with the LEP 19G allele, an increased risk estimate was found for all NHL [OR = 1.6, confidence interval (CI) 1.1–2.3], DLCL (OR = 1.6, CI 0.86–3.0), and FL lymphoma (OR = 1.9, CI 0.98–3.6). Gene-gene interaction existed between the −G2548A and LEPR Q223R polymorphisms. Specifically, among those with LEPR 223RR, the risk estimate for NHL was increased in LEP −2548GA (OR = 1.7, CI 0.88–3.1) and LEP −2548AA (OR = 2.3,CI 1.1–4.6) relative to LEP −2548GG genotypes. These results suggest that genetic interactions between leptin and its receptor may promote immune dysfunction associated with obesity and NHL and that the emerging obesity epidemic is consistent with the increasing incidence of NHL in developed countries. (Cancer Epidemiol Biomarkers Prev 2004;13(5):779–86)

Introduction

Incidence rates of non-Hodgkin lymphoma (NHL) have increased dramatically over the past 40 years in the U.S. and other industrialized countries, making NHL one of the most commonly diagnosed cancers in the Western world. In the U.S., the major NHL histological subtypes are diffuse large cell lymphoma (DLCL) and follicular lymphoma (FL), accounting for approximately 40% and 20% of all B-cell lymphomas, respectively. Of these, the incidence of DLCL has increased the most, between 50% and 100% over the past few decades (1). Reasons for this increase are poorly understood and risk factors for the majority of NHL subtypes remain unidentified. Previous studies have reported an association between NHL and family history (2), immunosuppression related to rare inherited disorders and transplantation (3, 4), and infections related to HIV (5), human T-cell lymphotropic virus type-1 (HTLV-1) (6), EBV (7), and *Helicobacter pylori* infection (8). However, these risk factors account for only a small percentage of the total cases and cannot account for the magnitude of the increasing rates.

Obesity also has been increasing simultaneously in industrialized countries, mainly as a result of environmental and societal changes associated with low physical activity and the greater availability of high-fat, energy-dense foods. Obesity results in pathological states of inflammation and altered immune responses that can influence B- and T-cell function and it may therefore influence NHL risk (9). In the U.S., approximately 30–40% of adults (10) and 20–30% of children (11) fall into the WHO-defined obesity categories with a body mass index (BMI) ≥30. Similar increases in the prevalence of obesity have been observed in several European countries, the Eastern Mediterranean region, and urbanized Polynesian populations, while obesity remains fairly uncommon in Africa, Japan, and China (12).

Previous reports on the relationship between obesity and lymphoma have been inconsistent, with few studies having been performed in the case-control setting. An early cohort study reported an increased risk of NHL with higher ponderal index (13), but a hospital-based study in Italy found no association with BMI (14). Two large prospective studies that examined the role of obesity and NHL risk also have been inconsistent (15, 16). While no association between obesity and NHL risk was found in the Iowa Women's Health Study of

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37,392 women, a large Swedish hospital-based cohort of 28,129 obese patients found a 40% increased risk of lymphoma among women and no association in men when compared to cancer incidence in the general Swedish population. However, in the largest prospective study to date of more than 900,000 U.S. adults (404,576 men and 495,477 women), high BMI was associated with a significantly increased risk of death due to NHL (17). Thus, the relationship between obesity and NHL deserves further study, especially for the different subtypes.

Obesity is a positive chronic imbalance between energy intake and expenditure mediated through the leptin-signaling pathway (18). Leptin is an adipose-derived cytokine present in the circulation in amounts proportional to fat mass that acts to reduce food intake and increase energy expenditure thereby regulating body weight homeostasis. The weight-regulating effects of leptin are mediated through the binding and activation of the long isoform of its receptor (LEPR-b) in the hypothalamus (19). Thus, the high circulating levels of leptin found in obese individuals suggest that obesity may be related to a leptin-resistant state. Genetic mutations in the leptin (*LEP*) gene of *ob/ob* mice and the leptin receptor (*LEPR*) gene in *db/db* mice produce a phenotype characterized by morbid obesity and immune dysfunction (20–22). In humans, several polymorphisms linked to an obese phenotype (23, 24) have been identified in the *LEP* and *LEPR* genes: an A to G nucleotide (nt) change at position 19 in the 5′-untranslated region (25), and a G to A substitution at nt −2548 upstream of the ATG start site (26), both in the *LEP* gene, and an A to G transition at nt 668 from the start codon that converts a glutamine to an arginine at codon 223 (Q223R) in the *LEPR* gene (27). This glutamine to arginine substitution occurs within the first of two putative leptin-binding regions and may be associated with impaired LEPR signaling capacity (19). Enhanced gene expression and increased circulating leptin levels have been reported in those with the *LEPR* 223RR and *LEP* −2548AA genotypes (19, 28), whereas the *LEP* 19GG variant has been reported to confer lower plasma leptin levels in morbidly obese women.

To examine the potential relationship between obesity and NHL, we studied the effects of increased BMI and NHL, DLCL, and FL in a large population-based case-control study conducted in the San Francisco Bay Area between 1988 and 1995 (29–34). In addition, to assess whether heritable traits associated with obesity affect odds ratio (OR) estimates for NHL, we studied polymorphisms in the *LEP* and *LEPR* genes in a subset of the cases and controls that had DNA available for analysis.

Materials and Methods

Study Population. In a study conducted in the San Francisco Bay Area between 1988 and 1995, NHL patients between 21 and 74 years old who were residents of one of the six Bay Area counties at the time of initial NHL diagnosis were identified by the Northern California Cancer Center's rapid case ascertainment. There were 1593 eligible patients (284 HIV-positive) who completed in-person interviews (72% response rate). Random-digit dial was used to identify control participants and was supplemented by random sampling of the Health Care Financing Administration files for participants aged ≥65 years. Controls were frequency-matched to patients by age within 5 years, sex, and county of residence. There were 2515 (78% response rate) eligible control participants (111 HIV-positive) who completed in-person interviews. Analyses on obesity-related genetic factors exclude all HIV-positive study participants. Additional details have been published in earlier manuscripts (29–34).

Biological Samples. The bloods originally were drawn for immunological studies and so patients who were receiving chemotherapy were excluded. Specifically, participants who had no history of chemotherapy within the past 3 months and met other requirements including no current use of blood thinners or having a portacath in place were eligible to participate in the laboratory portion of the study. Among eligible participants, blood specimens were obtained from 63% of patients and 66% of controls. Study protocols were approved by the UCSF Committee on Human Research and participants provided written informed consent before interview and collection of blood specimens. The blood was processed using Ficoll-Paque separation and the lymphocytes were cryopreserved in liquid nitrogen. Personal identifiers were removed from all samples sent by UCSF investigators to the lab in Berkeley for DNA isolation of 458 cases and 812 control participants. DNA was isolated using a modified QIAamp DNA Blood Maxi Kit protocol (QIAgen, Inc., Santa Clarita, CA), and was quantified using PicoGreen dsDNA Quantitation kits (Molecular Probes, Eugene, OR) according to the manufacturers' specifications.

Because some specimen samples were depleted after viral testing as part of the original laboratory analyses, we had fewer specimens with DNA available than originally were collected in the laboratory component of the main study. Therefore, to evaluate the generalizability of the remaining specimens, we compared demographic characteristic data of the laboratory participants with DNA to those without DNA. Results showed no differences by sex (χ^2 P = 0.58), race (χ^2 P = 0.37), education (χ^2 P = 0.97), or mean age (t test P = 0.84). Results were similar in further analyses stratified by case-control status. The similarity in these basic characteristics provides evidence that the subset for whom we have DNA is representative of the population who participated in the laboratory portion of the main study.

Histopathology. NHL histological subtype and grade were re-reviewed by an expert pathologist for 97% of all NHL study patients and classified using the Working Formulation (NHL Classification Project). To better reflect the Revised European American Lymphoma (REAL) Classification established in 2001 after the study recruitment was complete, Working Formulation diffuse large cell and immunoblastic lymphoma were combined for the DLCL subtype and Working Formulation follicular small, mixed and large cell lymphomas were combined for the FL subtype.

BMI Calculation. Participants were asked to report their height, usual adult weight and their weight at age 25, excluding weight during pregnancy for women. BMI was computed using these self-reported values (weight

in kilograms divided by height in square meters), and WHO recommendations were used to categorize BMI scores as follows: lean (<20), normal range (20 to <25), class I overweight (25 to <30), class II and III obese (30+). There were 0.6% of control men and 2.6% of control women with BMI <18.5, considered underweight based on recent WHO BMI classifications, who were included in the lowest BMI group and may reflect the general tendency of participants to underreport their true weight. Because of the possibility of having included underweight participants and the small number of participants in this lowest BMI category, the normal range BMI 20 to <25 was selected as the referent group for analyses. However, based on results from analyses of BMI data included in the WHO Health Report 2003 that indicated 8–42% of cancers worldwide were attributable to BMI >21 (Fact Sheet—Obesity and Overweight http://www.who.int/hpr/gs.fs.obesity.shtml#::%20FACTS, WHO 2003), BMI <21 may be an important referent group for public health comparisons when sample size is sufficient. Detailed dietary, physical activity, and other data such as waist-to-hip ratio that could be used to supplement the BMI estimates of overweight and obesity were not collected in this study.

Genotyping. Genotyping was performed using Assays-by-Design supplied by Applied Biosystems (ABI) (Applied Biosystems, Foster City, CA). Reactions were performed with the following protocol on a 7700 ABI Sequence Detection System: 95°C for 10 min, then 40 cycles of 95°C for 15 s, and 60°C for 1 min. Probes and primer sets used for the *LEP* G-2548A, A19G and the *LEPR* Q223R polymorphisms are listed in Table 1.

Quality Control Procedures. The following quality assurance protocol was applied in the assessment of all genotyping data. Verification of TaqMan genotyping methodology was made by direct sequencing of not less than 10 different samples for each locus. To validate our TaqMan-based genotyping results, 5% of all genotypes were repeated using standard RFLP analysis. For added quality control, we selected 5% of samples at random for repeat analysis using our standard TaqMan genotyping protocol. The assessment of genotypes included four independent control samples analyzed on each 96-well plate.

Table 1. Primers and TaqMan$_2$ probes used for *LEP* G-2548A, *LEP* A19G, and *LEPR* Q223R polymorphisms

SNP	Probe/ primer	5′–3′ Sequence
LEP G-2548A	F	TCCCGTGAGAACTATTCTTCTTTTG
	R	CCTGCAACATCTCAGCACTTAGG
	G	VIC-AGGATCAGCGCAAC
	A	6FAM-ATCAGTGCAACCCT
LEP A19G	F	GGAGCCCCGTAGGAATCG
	R	CCAGCAGAGAAGGAGGAAGGA
	A	VIC-AACCGTTGGCGCTG
	G	6FAM-AACCGCTGGCGCT
LEPR Q223R	F	GTTTGAAAATCACATCTGGTGGAGTA
	R	CATATTTATGGGCTGAACTGACATTAG
	Q	VIC-AGGTGACTGGAAAAT
	R	6FAM-AGGTGACCGGAAAA

Statistical Analysis. Statistical analyses to detect associations between BMI and NHL were conducted using SAS statistical software (SAS v8, SAS Institute, Cary, NC). Unconditional logistic regression analyses adjusted for sex and age in 5-year groups were used to obtain ORs as estimates of the relative risk and associated 95% confidence intervals (CIs). Tests for trend of ordinal categorical variables were evaluated using Wald's χ^2 statistic from adjusted logistic models with $P \leq 0.05$ considered statistically significant. BMI was computed using usual adult weight and weight at age 25 for men and women.

Genetic analyses also were conducted using unconditional logistic regression to obtain ORs. Initially, we used univariate analyses to examine the possible association between each polymorphism and NHL. We also investigated possible interaction between genes by including multiplicative terms in a logistic regression model that contained dummy variables for two genes. We then treated the number of mutant variants as continuous variables. Haplotypes for the *LEP* G-2548A and A19G polymorphisms were investigated using the COCAPHASE program (35).

Results

Obesity and Risk Estimates for Lymphoma. Results from adjusted logistic regression analyses that included BMI as a continuous variable, in nearly all instances, showed a significant positive trend with increasing BMI based on self-reported usual adult weight and weight at age 25 (Table 2). In general, compared with those of a BMI 20 to <25, those who were obese (≥30) were up to three times more likely to have been diagnosed with NHL or with one of the two major subtypes, DLCL and FL. In general, ORs consistently were decreased for those with BMI <20 compared with those with BMI 20 to <25. No associations were found between height and risk estimates for NHL subtypes among women and men separately or combined (data not shown).

Polymorphisms in the *LEP* and *LEPR* Genes and Risk Estimates for Lymphoma. The genotype distribution and allele frequencies for the *LEP* A19G, *LEP* G-2548A, and *LEPR* Q223R polymorphisms in all NHL, and by the DLCL and FL subtypes are presented in Table 3. Analyses showed that there was no strong statistical evidence of a lack of Hardy-Weinberg equilibrium (HWE) for *LEPR* Q223R ($P = 0.12$) and *LEP* G-2548A ($P = 0.16$), but HWE was rejected for *LEP* A19G ($P = 0.01$), due mainly to an excess of AA homozygotes (119 observed, 102 expected).

In univariate regression analyses, we found increased risk estimates for NHL among those with the *LEP* 19G allele (AG or GG *versus* AA) (OR = 1.6, CI 1.1–2.3, Table 3). Stratifying by histological subtype, we found increased risk estimates for DLCL (OR = 1.6, CI 0.86–3.0) and FL (OR = 1.9, CI 0.98–3.6) in those with the *LEP* 19G allele. No significant associations were observed between risk for all NHL or by NHL subtype with either the *LEP* G-2548A or *LEPR* Q223R polymorphisms.

Using the COCAPHASE program, there was evidence of strong linkage disequilibrium, D, between LEP

Table 2. BMI and risk of NHL for all NHL and by REAL subtypes,* adjusted ORs,† and 95% CIs for HIV-negative women and men

BMI	Cases (n = 725)		Controls (n = 1566)		Men and women combined	Men	Women
	n	%	n	%	OR (95% CI)	OR (95% CI)	OR (95% CI)
Usual adult weight							
All NHL							
WHO categories							
<20	78	6	161	7	0.91 (0.68–1.2)	0.60 (0.31–1.2)	0.92 (0.66–1.3)
20 to <25	655	50	1335	56	1.0‡	1.0‡	1.0‡
25 to <30	450	35	759	32	1.2 (1.1–1.5)	1.3 (1.0–1.5)	1.2 (0.90–1.5)
≥30	118	9	145	6	1.7 (1.3–2.2)	1.7 (1.2–2.4)	1.5 (0.97–2.3)
P for trend					<0.0001§	<0.0001§	0.01§
Follicular							
WHO categories							
<20	25	7	161	7	0.99 (0.63–1.6)	0.22 (0.03–1.6)	1.1 (0.69–1.9)
20 to <25	178	51	1335	56	1.0‡	1.0‡	1.0‡
25 to <30	117	33	759	32	1.3 (0.97–1.6)	1.2 (0.88–1.7)	1.2 (0.80–1.8)
≥30	31	9	145	6	1.6 (1.1–2.5)	1.6 (0.91–2.8)	1.6 (0.85–3.0)
P for trend					0.001§	0.006§	0.07§
Diffuse large cell							
WHO categories							
<20	26	5	161	7	0.76 (0.49–1.2)	0.76 (0.30–2.0)	0.70 (0.42–1.2)
20 to <25	251	49	1335	56	1.0‡	1.0‡	1.0‡
25 to <30	185	36	759	32	1.4 (1.1–1.7)	1.4 (1.1–1.9)	1.3 (0.91–1.8)
≥30	48	9	145	6	1.8 (1.3–2.6)	2.3 (1.5–3.6)	1.1 (0.60–2.1)
P for trend					<0.0001§	<0.0001§	0.03§
Weight at age 25							
All NHL							
WHO categories							
<20	220	17	489	20	0.72 (0.60–0.87)	0.82 (0.60–1.1)	0.66 (0.52–0.84)
20 to <25	800	62	1493	62	1.0‡	1.0‡	1.0‡
25 to <30	236	18	364	15	1.4 (1.1–1.7)	1.4 (1.1–1.7)	1.2 (0.80–1.9)
≥30	41	3	54	2	1.5 (1.0–2.4)	1.4 (0.81–2.3)	2.0 (0.95–6.8)
P for trend					<0.0001§	0.001§	0.0002§
Follicular							
WHO categories							
<20	56	16	489	20	0.60 (0.44–0.83)	0.54 (0.29–1.0)	0.66 (0.45–0.96)
20 to <25	223	64	1493	62	1.0‡	1.0‡	1.0‡
25 to <30	61	17	364	15	1.3 (0.98–1.8)	1.2 (0.87–1.8)	1.4 (0.74–2.6)
≥30	11	3	54	2	1.5 (0.75–2.9)	0.64 (0.19–2.1)	3.3 (1.3–8.3)
P for trend					0.002§	0.07§	0.02§
Diffuse large cell							
WHO categories							
<20	84	17	489	20	0.70 (0.54–0.92)	0.97 (0.63–1.5)	0.58 (0.41–0.81)
20 to <25	309	61	1493	62	1.0‡	1.0‡	1.0‡
25 to <30	95	19	364	15	1.4 (1.1–1.9)	1.6 (1.2–2.1)	1.1 (0.61–2.0)
≥30	18	4	54	2	1.8 (1.1–3.2)	2.4 (1.3–4.4)	0.86 (0.24–3.1)
P for trend					<0.0001§	0.003§	0.003§

*To better reflect the REAL Classification, Working Formulation diffuse large cell and immunoblastic lymphoma were combined for the DLCL subtype and Working Formulation follicular small, mixed, and large cell lymphomas were combined for the FL subtype.
†Adjusted for age in analyses stratified by sex, and for age and sex in analyses of women and men combined.
‡Referent group is BMI 20 to <25.
§P for trend obtained from analysis of BMI as a continuous variable in a model adjusted for age and sex (sex included only for men and women combined).

G-2548A and A19G polymorphisms (D' = 0.99, where $D' = D/D_{max}$). Estimated haplotype frequencies were −2548G/19A, 35.4%; −2548G/19G, 20.3%; −2548A/19G, 44.0%, and −2548A/19A, 0.2%. However, no evidence of associations between haplotypes and NHL was found.

In multivariable analyses, we observed statistical interaction between the *LEP* G-2548A and *LEPR* Q223R polymorphisms (interaction P = 0.02). When we estimated ORs for one gene within strata of the other, we found that among *LEPR* 223RR variants, risk estimates for NHL were increased in those with *LEP* −2548GA (OR = 1.7, CI 0.88–3.1) and those with *LEP* −2548AA (OR = 2.3, CI 1.1–4.6) relative to the *LEP* −2548GG genotype. There were no significant associations of the *LEP* −2548 and NHL within other strata of LEPR 223 (estimates not shown). There was insufficient power to explore interactions by NHL subtype.

Obesity and *LEP/LEPR* Variants. To determine the effect of these polymorphisms on the relationship between obesity and NHL, we compared those with a BMI ≥30 (obese) to those with a BMI <25 (lean to normal) and with a BMI 25–30 (overweight) stratified by the wild-type and variant genotypes for *LEP* A19G, *LEP* G-2548A, and *LEPR* Q223R (Table 4). No obvious

Table 3. *LEP* A19G, G-2548A, and *LEPR* Q223R genotype frequencies, OR,* and 95% CIs in NHL cases and controls using *LEP* 19AA, *LEP* −2548GG, and *LEPR* 223QQ as the reference group

	Control	All NHL cases (%)		DLCL (%)		FL (%)		Other (%)	
Genotype data for LEP A19G									
AA	119 (15)	36	(10)	11	(9)	11	(9)	14	(12)
AG	335 (42)	169	(46)	57	(46)	67	(52)	45	(37)
GG	351 (44)	168	(45)	55	(45)	52	(40)	61	(50)
SNA	0	2		1		1		0	
Total	805	376		124		131		121	
OR (95% CI)	AG/GG *versus* AA	1.6	(1.1–2.3)	1.6	(0.86–3.0)	1.9	(0.98–3.6)	1.3	(0.73–2.4)
Genotype data for LEP G-2548A									
GG	259 (32)	118	(31)	38	(31)	44	(34)	36	(30)
GA	376 (47)	167	(44)	55	(44)	60	(46)	52	(43)
AA	167 (21)	91	(24)	31	(25)	27	(21)	33	(27)
SNA	3	0		0		0		0	
Total	805	376		124		131		121	
OR (95% CI)	GA/AA *versus* GG	1.1	(0.81–1.4)	1.1	(0.73–1.7)	0.95	(0.64–1.4)	1.1	(0.75–1.7)
Genotype data for LEPR Q223R									
QQ	226 (28)	115	(31)	38	(31)	33	(25)	44	(37)
QR	379 (47)	173	(46)	56	(45)	65	(47)	52	(43)
RR	198 (25)	87	(23)	30	(24)	33	(25)	24	(20)
SNA	2	1		0		0		1	
Total	805	376		124		131		121	
OR (95% CI)	QR/RR *versus* QQ	0.89	(0.68–1.2)	0.91	(0.60–1.4)	1.2	(0.77–1.8)	0.68	(0.46–1.0)

*OR estimated using multiple logistic regression analyses adjusted for continuous BMI.

increase in the effect of BMI was observed at different levels for both LEP G-2548A and LEPR Q223R. While there is an increase in the risk estimate for the LEP 19AG/GG genotype (OR = 1.7, CI 1.1–2.7), the test for interaction yielded a P value of 0.47, suggesting that the association with BMI did not differ significantly by levels of LEP A19G and that these genetic polymorphisms did not affect the obesity and NHL association in this population. However, when obesity was included as a linear term in the regression model, the observed statistical interaction between LEP and LEPR genes remained unchanged, suggesting that factors other than obesity also are involved.

Table 4. ORs and 95% CIs for the association between all NHL and BMI* ≥30 stratified by wild type and variants for *LEP* A19G, *LEP* G−2548A, and *LEPR* Q223R

SNP genotype	NHL cases/ controls	OR (95% CI)
LEP 19AG/GG		
BMI < 25 (lean to normal)	189/403	1.0
BMI 25–30 (overweight)	110/228	1.0 (0.77–1.4)
BMI ≥ 30 (obese)	39/49	1.7 (1.1–2.7)
LEP 19AA (wild type)		
BMI < 25 (lean to normal)	25/72	1.0
BMI 25–30 (overweight)	8/35	0.66 (0.27–1.6)
BMI ≥ 30 (obese)	3/10	0.86 (0.24–3.2)
LEP −2548GA/AA		
BMI < 25 (lean to normal)	147/325	1.0
BMI 25–30 (overweight)	83/178	1.0 (0.74–1.4)
BMI ≥ 30 (obese)	28/30	1.6 (0.97–2.7)
LEP −2548GG (wild type)		
BMI < 25 (lean to normal)	67/154	1.0
BMI 25–30 (overweight)	36/84	1.0 (0.61–1.6)
BMI ≥ 30 (obese)	15/21	1.6 (0.81–3.3)
LEPR 223QR/RR		
BMI < 25 (lean to normal)	148/351	1.0
BMI 25–30 (overweight)	83/185	1.1 (0.78–1.5)
BMI ≥ 30 (obese)	29/40	1.7 (1.03–2.9)
LEPR 223QQ (wild type)		
BMI < 25 (lean to normal)	65/129	1.0
BMI 25–30 (overweight)	36/77	0.93 (0.56–1.5)
BMI ≥ 30 (obese)	14/19	1.5 (0.70–3.1)

*BMI in regression model calculated using usual adult weight.

Discussion

Results from our epidemiological and genetic analyses suggest that there is an association between obesity and NHL, and that leptin may be a mediator in the pathogenesis of this disease. In the present study, we found that obese individuals were more likely to have been diagnosed with NHL and its two major subtypes, DLCL and FL, than those with a BMI 20–<25. Furthermore, we observed that genetic polymorphisms in the *LEP* and *LEPR* genes that are associated with an obese phenotype were associated with increased risk estimates for NHL. Specifically, we found an association with NHL in those possessing the *LEP* 19G allele with an approximate 2-fold increased risk estimate for DLCL and FL. In examining joint effects between the *LEP* and *LEPR* polymorphisms, we also found a 2-fold elevated OR for NHL among *LEPR* 223RR/*LEP* −2548AA variants. Studying interactions by NHL subtypes may provide further insight into disease mechanisms; however, small sample sizes within NHL subtypes precluded us from further exploring this interaction. Although these results need to be reproduced in other data sets, the strengths of our study include its size, it is population-based, has a broad range of BMI levels, and it incorporates a genetic component

of obesity to assess the association with NHL. Because obesity and lymphoma are becoming increasingly more prevalent throughout the world, the observed associations may have important public health implications.

Study limitations include loss to follow up of some patients. However, most of the nonparticipating patients in the main study were HIV-positive and thus do not impact these analyses that include only HIV-negative participants. Patients >55 years old with high-grade NHL, although few in number, also were less likely to have been interviewed (36). Immunoblastic lymphomas, which compromise a small percentage of all lymphomas, were the most likely to have been underrepresented and these were combined for all analyses to create the REAL large-cell category. Despite the loss of these cases, the proportion of high-grade Working Formulation cases by subtype in our population reflects that published for other developed western populations (37).

In the main study, if interviewed cases were more prone to overweight and obesity than those not interviewed, the reported estimates of the association between BMI and NHL may be artificially inflated. In addition, differential recall of weight with cases reporting greater weights than controls is possible but evidence shows that weight tends to be underestimated rather than overestimated especially by those who are overweight (38). If NHL patients underreported their weight relative to controls, our risk estimates would be biased toward the null. Estimates also would be biased toward the null if self-reported weight measurements used in this study were subject to non-differential misclassification. Limitations that pertain to the laboratory study also may have affected the observed results. Similar proportions of case and control participants who were eligible for the laboratory portion of the study provided blood specimens, whereas, although this number was small, a greater proportion of patients than controls were ineligible for venipuncture because they had received chemotherapy within the previous 3 months. If those who had recent chemotherapy were more ill than those patients who did not report recent chemotherapy, our results may be generalizable to a 'healthier' NHL population. However, if obese patients with NHL were more likely to have been excluded from these analyses

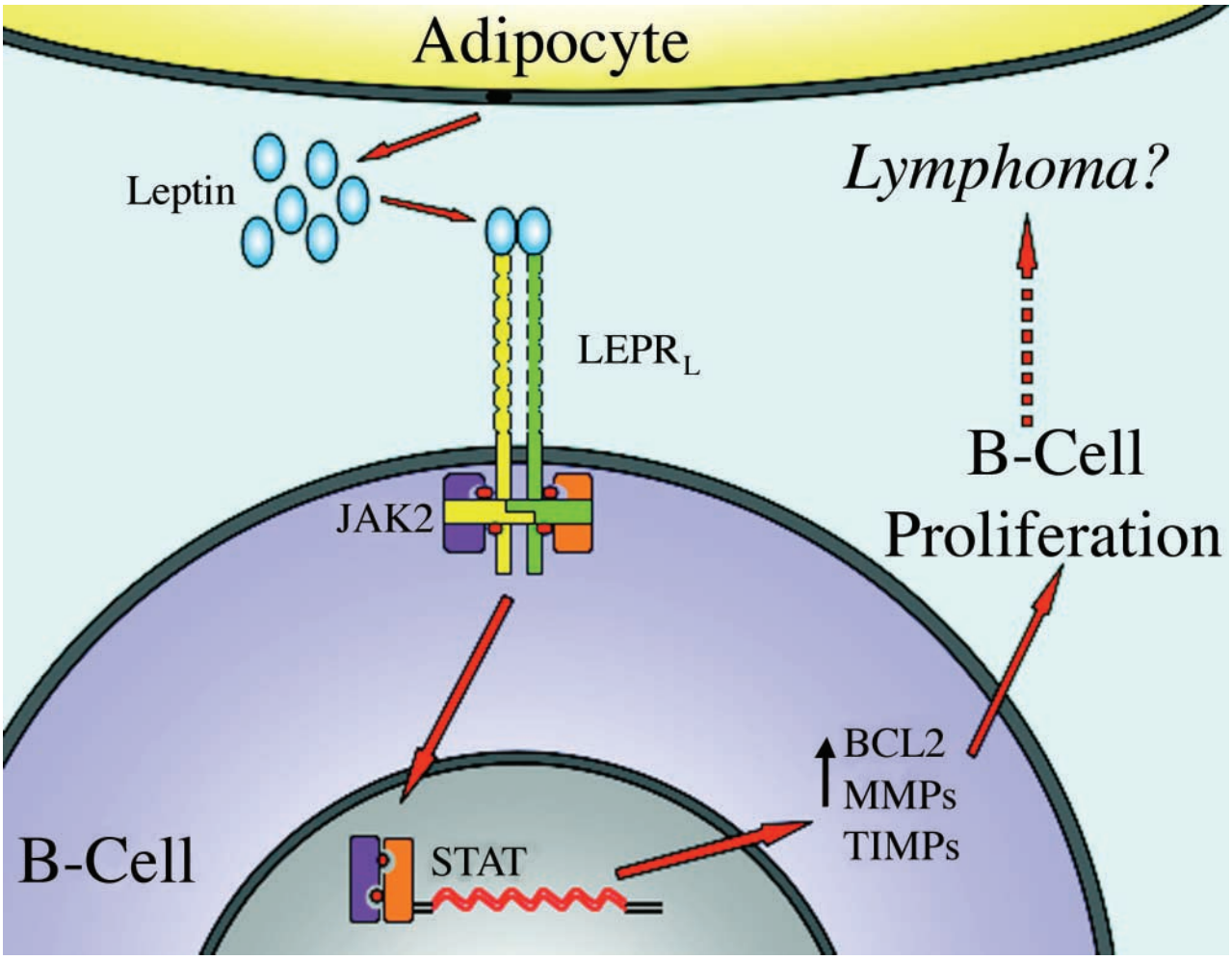

Figure 1. B-cell proliferation and enhanced survival through leptin activation of the JAK/STAT pathway. A tetrameric complex composed of two long isoforms of the leptin receptor ($LEPR_L$) and two leptin molecules binds JAK2 that phosphorylates the cytoplasmic tails of the LEPRs resulting in dimerization of STATs. STATs then migrate into the nucleus and activate gene transcription of MMPs, TIMPs, and BCL-2, resulting in B-cell proliferation and enhanced cell survival.

because they had shorter survival or were more likely to have been placed on chemotherapy earlier than nonobese patients, our ORs would be biased toward the null.

Our results suggest that the mechanisms underlying the relationship between obesity and NHL may involve leptin and its receptor in the regulation of immune function. Present in the circulation in amounts proportional to fat mass, the weight-regulating effects of leptin are mediated through LEPR. LEPR in turn activates the Janus kinase (JAK)/signal transducer and activator of transcription (STAT) system to alter expression of hypothalamic neuropeptides (19). However, LEPR also is expressed in lymphocytes (39) and CD 34+ progenitor cells, in the bone marrow and lymph nodes (40), mediating leptin-dependent cell proliferation. Thus, leptin may promote lymphomagenesis via direct mitogenic and anti-apoptotic effects on B-cell populations through LEPR-mediated activation of JAK/STAT pathways (Fig. 1) that result in increased expression of *BCL-2*, matrix metalloproteinases (MMPs), and tissue inhibitors of metalloproteinases (TIMPs) (41, 42). BCL-2 is an anti apoptotic protein and provides a distinct survival signal to the cell. Clinical studies have reported that most FL and approximately 20–30% of DLCL have a t(14;18) gene translocation resulting in disregulation and overexpression of the *BCL-2* gene. MMPs and TIMPs have been implicated in the B-cell lymphomas, Hodgkin disease, and Burkitt lymphoma, and have been associated with aggressive disease, neoplastic growth, and angiogenesis (43). Consequently, elevated leptin levels may provide another means to promote proliferation and enhanced B-cell survival in those who are obese.

Leptin's role in the balance of T-cell-derived cytokines in favor of a T-helper cell type 1 (TH1) response by enhancing the synthesis of interleukin (IL)-2, IFN-γ, and inhibiting IL-4 production suggests that leptin may bias T-cell responses toward a pro-inflammatory phenotype (26). Chronic inflammatory conditions such as pyelonephritis, inflammatory bowel disease, and chronic bacterial diseases including *H. pylori* infection have been associated with an increased risk of lymphoma (44, 45). Given that NHL has been associated with altered immune function, a heightened TH1 response may play an important role in lymphocyte- and macrophage-mediated inflammation. Moreover, pro-inflammatory cytokines can up-regulate the production of adhesion molecules such as vascular cell adhesion molecule-1 (VCAM-1) and intercellular adhesion molecule (ICAM)-1 that are vital to innate immune function in the binding and movement of lymphocytes and mononuclear cells during inflammation or other immunological stimuli (46) resulting in a persistent antigenic response that can promote NHL risk.

These data suggest that genetic interactions between leptin and its receptor may promote immune dysfunction associated with the pathogenesis of lymphoma. While no functional studies have been performed to determine the phenotypic effects of this interaction, it may be that those who are homozygous variant for both polymorphisms exhibit higher leptin levels than those who are homozygous variant for only one. Functional studies are needed to determine whether a multiplicative effect on leptin gene expression exists.

In conclusion, our results are consistent with an association between NHL and environmental and genetic factors related to energy homeostasis and altered immune function. Our data suggest that the emerging obesity epidemic may contribute to the increasing incidence of NHL. Further studies of the association between obesity, diet, and lymphoma are warranted to confirm our results and to clarify the biological mechanism that underlies the observed associations.

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