

Polymorphisms in Ghrelin and Neuropeptide Y Genes Are Associated with Non-Hodgkin Lymphoma

Danica R. Skibola,¹ Martyn T. Smith,¹ Paige M. Bracci,² Alan E. Hubbard,¹ Luz Agana,¹ Shawn Chi,¹ and Elizabeth A. Holly²

¹School of Public Health, University of California, Berkeley, California and ²Department of Epidemiology and Biostatistics, University of California, San Francisco, California

Abstract

We previously reported a positive association among body mass index, single nucleotide polymorphisms (SNP) in the leptin and leptin receptor genes that are involved in body weight regulation, and non-Hodgkin lymphoma (NHL). Polymorphisms in the ghrelin (*GHRL*) and neuropeptide Y (*NPY*) genes were examined in the same population-based case-control study of NHL to further explore the role of genes involved in energy homeostasis and obesity in susceptibility to NHL. Ghrelin is an orexigenic hormone that induces NPY release and inhibits proinflammatory cytokines via its antagonistic relationship with leptin. NPY is a potent appetite stimulator controlled by ghrelin and leptin and also acts as a mediator of immune function. DNA from 458 cases and 812 controls was genotyped. Among genotyped *GHRL* SNPs, the variant allele for *GHRL* -4427G>A was inversely associated with all NHL [odds ratios (OR), 0.78; 95% confidence interval (95% CI), 0.59-1.0] and more

specifically with diffuse large cell lymphoma (DLCL; homozygous variant: OR, 0.31; 95% CI, 0.13-0.74). Another SNP, *GHRL* 5179A>G, decreased the risk of DLCL (homozygous variant: OR, 0.35; 95% CI, 0.10-1.2). *NPY* -485T>C, 1258G>A, and 5671C>T were in total linkage disequilibrium (D' = 0.99) and the homozygous variants were associated with an increased risk of NHL in *NPY* SNPs -485T>C (OR, 1.7; 95% CI, 1.1-2.5), 1258G>A (OR, 1.7; 95% CI, 1.1-2.5), and 5671C>T (OR, 1.9; 95% CI, 1.3-2.8). When stratified by subtype, the variant allele for *NPY* 1128T>C was positively associated with follicular lymphoma (OR, 2.3; 95% CI, 1.1-4.9) as were homozygous variants for *NPY* SNPs -485T>C (OR, 2.4; 95% CI, 1.3-4.4), 1258G>A (OR, 2.0; 95% CI, 1.1-3.5), and 5671C>T (OR, 1.8; 95% CI, 1.1-3.0). These results add further support for the hypothesis that SNPs in energy-regulating genes affect risk of NHL. (Cancer Epidemiol Biomarkers Prev 2005;14(5):1251-6)

Introduction

In developed Western countries, obesity has reached epidemic proportions due to the availability and overconsumption of high-fat, energy-dense foods combined with low physical activity. Obesity has been associated with an increased risk of a number of chronic diseases such as cardiovascular disease (1), type 2 diabetes (2), and some cancers (3), including non-Hodgkin lymphoma (NHL). However, the mechanism underlying this possible association with NHL remains unclear. A recent Canadian study that included >20,000 individuals with 19 different cancers, observed a 46% increased risk for NHL in people with a body mass index (BMI) exceeding 30 kg/m² (4). Conversely, a cohort study of 37,931 women in Iowa, found no evidence that BMI played a role in NHL overall or in diffuse or follicular lymphoma specifically (5, 6). We previously have shown a positive association between NHL and BMI and single nucleotide polymorphisms (SNP) in the leptin and leptin receptor genes that are involved in body weight regulation. We found that the obesity-related *LEP* 19A>G polymorphism and that interaction between the variants for *LEP* -2548G>A and *LEPR* 223Q>R, each were associated with an ~2-fold increased risk estimate for NHL (7). We hypothesized that the chronic states of inflammation and altered immune responses associated with obesity that influence B- and T-cell function may contribute to development of NHL.

We examined polymorphisms in the neuropeptide Y (*NPY*) and ghrelin (*GHRL*) genes to explore the association further between NHL and polymorphisms in genes related to energy homeostasis, obesity, and immune function in a population-based case-control study in the San Francisco Bay Area.

NPY acts on the central nervous system as a potent appetite stimulator controlled by the feedback action of both leptin from adipose tissue and ghrelin from the stomach (8). Recent studies show that these opposing hormonal signals regulate the secretion of *NPY* in the hypothalamus and that the *NPY* network is the primary pathway involved in appetite stimulation (9). Ghrelin stimulates appetite during fasting directly through increased gut motility and through the release of *NPY* (ref. 9; Fig. 1), leading to increased adiposity and energy intake (10, 11). Conversely, the anorexigenic properties of leptin, a hormone released after eating, results in inhibition of *NPY* secretion (ref. 8; Fig. 1). *NPY* also regulates immune function via its expression in primary and secondary lymphoid organs and expression of its receptors on macrophages, natural killer cells, peripheral blood mononuclear cells, and B and T lymphocytes (12). A polymorphism in the *NPY* gene (1128T>C: rs16139) that results in an amino acid change (Leu > Pro) at codon 7 is associated with elevated levels of *NPY*, exercise-induced growth hormone secretion (13), and high serum cholesterol and low-density lipoprotein cholesterol levels (14). We examined this SNP in the present study along with three other SNPs distributed across the *NPY* gene (Fig. 2). Additionally, we examined four SNPs across the *GHRL* gene (Fig. 2), including a nonsynonymous SNP (*GHRL* 408 C>A: rs696217) or (Leu72Met) that is associated with lower BMI, fat mass, and abdominal visceral fat (10) and higher levels of circulating lipoprotein A associated with the wild-type genotype in diabetics (15). Ghrelin is the endogenous ligand for the growth hormone secretagogue receptor and potentially

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Requests for reprints: Martyn T. Smith, Division of Environmental Health Sciences, School of Public Health, University of California, 140 Earl Warren Hall #7360, Berkeley, CA 94720-7360. Phone: 510-642-8770; Fax: 510-642-0427. E-mail: martyns@berkeley.edu

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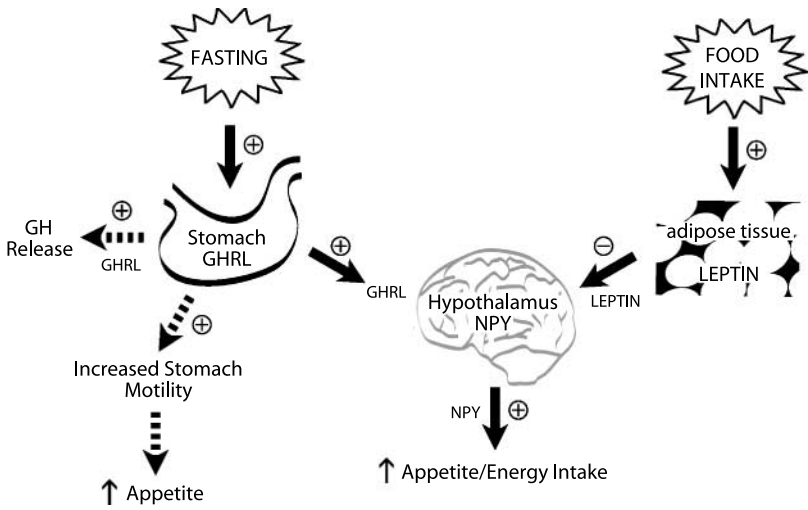


Figure 1. The effects of ghrelin and leptin on appetite and energy intake. Fasting causes the stomach to release GHRL, which stimulates hunger through increased stomach motility and up-regulates growth hormone (GH) release. GHRL also binds to its receptor in the hypothalamus increasing NPY release to further promote hunger. Conversely, food intake inhibits GHRL and NPY release but stimulates leptin production by adipose tissue thus decreasing appetite.

stimulates the release of growth hormone (ref. 10; Fig. 1). Ghrelin levels are inversely correlated with BMI and therefore are decreased in obese individuals and increased in individuals with anorexia nervosa (11). This down-regulation of ghrelin levels in human obesity may be the result of elevated leptin and insulin levels (16).

Materials and Methods

Study Population. Details of the study design and methods have been published (17-23) and will be presented briefly here. A population-based case-control study of NHL was conducted in the San Francisco Bay Area between 1988 and 1995. Cases were newly diagnosed NHL patients identified through the Northern California Cancer Center’s rapid case ascertainment, were residents of one of six Bay Area counties, and were between 21 and 74 years of age. Controls were identified by random digit dial (24, 25) enhanced by random sampling of Health Care Financing Administration (now the Center for Medicare and Medicaid Services) lists for participants ≥65 years of age and that include ~98% of U.S. residents ages ≥65 years. Controls were frequency matched to cases by 5-year age group, sex, and county. There were 1,593 eligible patients (72%) and 2,515 eligible controls (78%) who completed in-person interviews. Approximately 65% of patients who died before we could contact them were HIV-positive cases (23) and 12% (including eligibles and ineligibles) were HIV-negative cases. Therefore,

those who died before contact were unlikely to have biased the results presented here that focus on HIV-negative participants only.

Biological Samples. Details of blood collection and specimen DNA isolation have been published (7) and will be presented briefly here. Blood specimens were obtained from 63% of eligible patients and 66% of controls for viral testing (individuals who had no chemotherapy within 3 months and no contraindications to venipuncture). However, because some specimen samples were depleted after testing that was part of the original laboratory analyses, we had fewer specimens with DNA available than originally were collected in the main study. The blood was processed using Ficoll-Paque separation and the lymphocytes were cryopreserved in liquid nitrogen. Coded specimens (458 cases, 812 controls), with all personal identifiers removed, were sent to the University of California at Berkeley laboratory for DNA isolation. 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. All protocols and procedures were approved by the University of California at San Francisco Committee on Human Research and by the University of California at Berkeley Committee for the Protection of Human Subjects. All participants provided written informed consent before interview and a separate consent before venipuncture.

Histopathology. NHL histologic 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 approximate the Revised European-American Classification of Lymphoid Neoplasms in these analyses, 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 follicular lymphoma subtype.

SNP Selection. SNPs were selected for the *GHRL* and *NPY* genes using the SNPper (<http://snpper.chip.org/>) and SNP500Cancer (<http://snp500cancer.nci.nih.gov/>) web sites and are described in Table 1. We attempted to cover the haplotype of each gene by choosing tag SNPs using Haploview (<http://www.broad.mit.edu/personal/jcbarret/haploview>) for haplotype analysis. All available Taqman assays designed by Applied Biosystems (Foster City, CA) were identified at <http://www.appliedbiosystems.com>. SNPs were chosen based

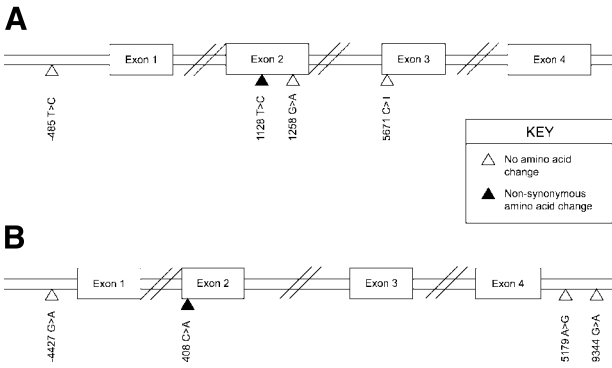


Figure 2. Genomic locations of *NPY* and *GHRL* polymorphisms analyzed in this study. **A.** *NPY*. **B.** *GHRL*.

Table 1. Primers and Taqman probes used for *GHRL* and *NPY* polymorphisms

SNP	RS no.	Probe/primer	5'-3' Sequence
<i>NPY</i> -485T>C	16147	F	CCACAAAGAGGATTCCAGGTGCTT
		R	GCAGCCCAGACGATTCTTGT
		A	VIC- ACAGGACTACCAACCCA
		G	6FAM-ACAGGACTACCAACCCA
<i>NPY</i> 1128T>C	16139	F	GCCTGCAGATGCTAGGTAACAA
		R	ACAGGGCGAGGGTCAGT
		A	VIC-ACAGCCCCAGTCGC
		G	6FAM-CAGCCCCGTCGC
<i>NPY</i> 1258G>A	11557492 aka 9785023	F	GCACCAGCGGAGGACAT
		R	CTGGTGATGAGGTTGATGTAGTGT
		G	VIC-CCAGATACTACTCGGCGCT
		A	6FAM-CCAGATACTACTCAGCGCT
<i>NPY</i> 5671C>T	5574	F	GCCTATTCCAAACTTGCTTTAAAAGACT
		R	CTCATCAAGAGGTCTGAAATCAGTGT
		G	VIC-TCTGGGCTGGATCG
		A	6FAM-CTCTGGGCTAGATCG
<i>GHRL</i> -4427G>A	1629816	F	GTGGGACGTGGCTTGGA
		R	ACACAACCCAACACAGGTCTG
		T	VIC-TATCTGGAAATTGAGATGAGAT
		C	6FAM-CTGGAAATTGAGACGAGAT
<i>GHRL</i> 408C>A	696217	F	ACAGAAGCATAAACTGCAGAGGTA
		R	GGAAGATGGAGGTCAAGCAGAAG
		C	VIC-CAGAGGATGAACTGGAAGT
		A	6FAM-CAGAGGATGAAATGGAAGT
<i>GHRL</i> 5179A>G	35684	F	GGGAAGACCAGTGTCTGTA
		R	AGCAGACACGAGCCTTGAC
		A	VIC-ATTAAGTTGTGGAACATAAGA
		G	6FAM-TTAAGTTGTGGAACGTAAGA
<i>GHRL</i> 9344G>A	2072578	F	GGGACTGCCGAGCATATCAA
		R	GATGGTTGTGCTGCAGITCAC
		G	VIC-TTGGTGACTATGAACACT
		A	6FAM-TTGGTGACTATAAACACT

on a minor allele frequency exceeding 5%, and preference was given to SNPs residing in the untranslated region or exonic coding regions. When no acceptable coding SNPs were found, intronic SNPs were used to ensure gene coverage.

Genotyping. We did genotyping using Assays-by-Design supplied by Applied Biosystems. Reactions were done with the following protocol on a 7700 Applied Biosystems Sequence Detection System: 95°C for 10 minutes, 40 cycles of 95°C for 15 seconds, and 60°C for 1 minute. Probes and primer sets used for analysis of polymorphisms in the *GHRL* and *NPY* genes are listed in Table 2. For quality control, we selected 5% of samples at random for repeat analysis using our standard Taqman genotyping protocol, which were in 100% concordance with the original calls. The assessment of genotypes included the same four independent control samples and one blank negative control analyzed on each 96-well plate. The call rate for all samples exceeded 99%.

Statistical Analyses. Results from analyses that compared demographic characteristics of study participants with DNA to those without DNA showed no difference by sex ($P = 0.58$), race ($P = 0.37$), education ($P = 0.97$), or mean age ($P = 0.84$). Overweight/obesity status (BMI >25) also was similar between those genotyped and not genotyped for the polymorphisms of interest ($P = 0.35$). Results also were similar in further analyses stratified by case-control status. Study design analyses were planned to address confounding and selection bias. Hardy-Weinberg equilibria for genotype frequencies among controls were determined using standard χ^2 statistics and for low-frequency alleles, an exact test using a Markov-chain method (Genepop v3.4). Odds ratios (OR) and 95% confidence intervals (95% CI) as estimates of the relative risk (hereafter referred to as risk) were computed from unconditional logistic regression. In genotype analyses, the wild-type category (chosen either as the most common homozygotic genotype or arbitrarily if the same) was the reference group

and models were adjusted for age and sex. BMI was categorized as <25 kg/m² (lean to reference range) versus ≥ 25 kg/m² (overweight to obese) or <30 kg/m² (nonobese) versus ≥ 30 kg/m² (obese) to evaluate gene-environment interactions. A likelihood ratio test (comparing nested models with and without the relevant interaction terms) was used to test for statistical evidence of gene-environment interactions between BMI and *NPY* or *GHRL* SNP genotypes. However, the power to detect interactions for all NHL and by NHL subtypes is low after adjusting for multiple testing. For each set of analyses (e.g., all tests of interaction between all pairs of SNPs, including *LEP* -2548G>A, *LEP* 19A>G, and *LEPR* Q223R from our previous analyses, ref. 7, and their association with NHL), the false discovery rate was estimated to adjust for multiple comparisons (26). The false discovery rate is the expected proportion of false positives among a set of tests where the hypotheses were rejected. Haplotype frequencies for case and control groups were estimated and the ORs for common haplotypes were computed with the reference group defined as the haplotype carrying the most frequent alleles at each loci. The probability of each haplotype, for each participant, was determined using the E-M algorithm as implemented in the *gap* package (*gc.em* function) available as an add-on in the statistical package, *R* (27). HIV-positive participants were excluded and all analyses were restricted to White non-Hispanic participants to avoid potential bias due to population stratification (28). Results from all statistical analyses were considered significant for two-sided $P_s \leq 0.05$ and were considered borderline associated for $0.05 < P \leq 0.10$.

Results

Demographic characteristics for all HIV-negative study participants have been published elsewhere (17). Among this

subset, 58% of cases and 68% of controls were men, and mean age was 57 years at diagnosis for patients and 52 years at interview for controls.

Effect of *NPY* and *GHRL* Genotypes on NHL Risk. Figure 2 displays the genomic locations of the individual polymorphisms genotyped in this study. Aside from *NPY* 1128T>C (borderline result from exact test $P = 0.05$), all control genotype distributions were in Hardy-Weinberg equilibrium.

The *NPY* -485T>C, 1258G>A, and 5671C>T SNPs were found to be in total linkage disequilibrium ($D' = 0.99$), and there also was evidence of linkage disequilibrium between these SNPs and the low frequency nonsynonymous *NPY* -1128T>C (Leu > Pro) polymorphism ($P < 0.01$). In univariate analyses, homozygous variant genotypes for *NPY* -485T>C, 1258G>A, and 5671C>T were overrepresented in the NHL cases resulting in increased risk for NHL in *NPY* -485T>C (OR, 1.7; 95% CI, 1.1-2.5), 1258G>A (OR, 1.7; 95% CI, 1.1-2.5), and 5671C>T (OR, 1.9; 95% CI, 1.3-2.8; Table 2). In analyses stratified by NHL subtype, results were even stronger for follicular lymphoma in homozygous variants for *NPY* -485T>C (OR, 2.4; 95% CI, 1.3-4.4), *NPY* 1258G>A (OR, 2.2; 95% CI, 1.1-4.1), and *NPY* 5671C>T (OR, 2.4; 95% CI, 1.3-4.3). The variant *NPY* -1128C allele also was associated with an increased risk for follicular lymphoma (OR, 2.3; 95% CI, 1.1-4.9;

Table 2). No associations between *NPY* SNPs and DLCL were observed in these analyses (Table 2).

Strong linkage disequilibrium was found between the nonsynonymous *GHRL* 408C>A SNP and both the -4427A>G ($D' = 0.84$) and 5179A>G SNPs ($D' = 1.0$). The *GHRL* -4427GG genotype was associated with a lower risk of all NHL, but confidence limits overlapped unity (OR, 0.77; 95% CI, 0.51-1.2). However, in analyses stratified by NHL subtype, the homozygous variant *GHRL* -4427AA genotype was associated with a reduced risk for DLCL (OR, 0.31; 95% CI, 0.13-0.74; Table 2), with heterozygotes having an intermediary reduced level of risk (OR, 0.71). Few participants were homozygous variant for *GHRL* 5179A>G, but a borderline reduced risk for DLCL was associated with this genotype. No associations between *GHRL* SNPs and follicular lymphoma were observed in these analyses.

We found no interaction between BMI and variant allele frequencies. We did find significant two-way SNP-SNP interactions between *NPY* 1128T>C and *GHRL* 9344G>A and between *GHRL* 5179G>A and *LEP* -2548G>A; however, none of these results were significant after adjusting for multiple comparisons using the false discovery rate (the false discovery rate in no set of tests of interaction was <0.45) indicating relatively weak evidence of statistical interaction in this data set.

Table 2. ORs and 95% CIs for all NHL, follicular lymphoma, and DLCL associated with SNPs in *NPY* and *GHRL* genes: White, non-Hispanic, HIV-negative women and men

SNP*	Genotype	All NHL, cases (n = 308)			Follicular lymphoma, cases (n = 112)		DLCL, cases (n = 98)	
		Controls (n = 684) n (%)	n (%)	OR (95% CI)†	n (%)	OR (95% CI)†	n (%)	OR (95% CI)†
NPY -485T>C	TT	170 (25)	56 (18)	1.0	16 (14)	1.0	20 (21)	1.0
	TC	340 (50)	157 (51)	1.5 (1.0-2.1)	57 (51)	1.8 (1.0-3.3)	48 (49)	1.2 (0.71-2.2)
	CC	174 (25)	93 (30)	1.7 (1.1-2.5)	38 (34)	2.4 (1.3-4.4)	29 (30)	1.4 (0.77-2.6)
	TC/CC	514 (75)	250 (82)	1.5 (1.1-2.2)	95 (86)	2.0 (1.2-3.6)	77 (79)	1.3 (0.77-2.2)
	$P_{\text{trend}}^{\ddagger}$			0.01		0.008		0.26
NPY 1128T>C	TT	653 (96)	286 (93)	1.0	102 (91)	1.0	92 (96)	1.0
	TC	28 (4)	20 (7)	1.7 (0.92-3.0)	10 (9)	2.4 (1.1-5.2)	4 (4)	1.1 (0.36-3.1)
	CC	2 (0.3)	0 (0)	—	0 (0)	—	0 (0)	—
	TC/CC	30 (4)	20 (7)	1.6 (0.88-2.9)	10 (9)	2.3 (1.1-4.9)	4 (4)	1.0 (0.35-3.0)
	$P_{\text{trend}}^{\ddagger}$			0.01		0.02		0.31
NPY 1258G>A	GG	168 (25)	54 (18)	1.0	16 (14)	1.0	20 (21)	1.0
	GA	344 (50)	161 (53)	1.5 (1.1-2.2)	61 (54)	1.9 (1.1-3.5)	49 (51)	1.2 (0.71-2.2)
	AA	172 (25)	91 (30)	1.7 (1.1-2.5)	35 (31)	2.2 (1.1-4.1)	28 (29)	1.4 (0.74-2.6)
	GA/AA	516 (75)	252 (82)	1.6 (1.1-2.2)	96 (86)	2.0 (1.1-3.5)	77 (79)	1.3 (0.76-2.2)
	$P_{\text{trend}}^{\ddagger}$			0.01		0.004		0.22
NPY 5671C>T	CC	202 (30)	66 (22)	1.0	21 (19)	1.0	24 (24)	1.0
	CT	340 (50)	157 (51)	1.4 (1.0-2.0)	56 (50)	1.6 (0.94-2.7)	50 (51)	1.2 (0.74-2.1)
	TT	140 (20)	84 (27)	1.9 (1.3-2.8)	34 (31)	2.4 (1.3-4.3)	24 (24)	1.5 (0.80-2.7)
	CT/TT	480 (70)	241 (78)	1.6 (1.1-2.2)	90 (81)	1.8 (1.1-3.0)	74 (76)	1.3 (0.80-2.1)
	$P_{\text{trend}}^{\ddagger}$			0.001		0.004		0.22
GHRL -4427G>A	GG	254 (37)	134 (44)	1.0	44 (39)	1.0	48 (50)	1.0
	GA	321 (47)	130 (42)	0.79 (0.58-1.1)	47 (42)	0.88 (0.56-1.4)	42 (44)	0.71 (0.45-1.1)
	AA	105 (15)	42 (14)	0.77 (0.51-1.2)	21 (19)	1.2 (0.68-2.1)	6 (6)	0.31 (0.13-0.74)
	GA/AA	426 (63)	172 (56)	0.78 (0.59-1.0)	68 (61)	0.96 (0.63-1.4)	48 (50)	0.61 (0.40-0.94)
	$P_{\text{trend}}^{\ddagger}$			0.12		0.72		0.005
GHRL 408C>A	CC	574 (84)	257 (84)	1.0	92 (82)	1.0	85 (88)	1.0
	CA	104 (15)	48 (16)	1.0 (0.69-1.5)	20 (18)	1.2 (0.71-2.1)	12 (12)	0.77 (0.41-1.6)
	AA	6 (1)	0 (0)	—	0 (0)	—	0 (0)	—
	CA/AA	110 (16)	48 (16)	0.96 (0.66-1.4)	20 (18)	1.1 (0.67-1.9)	12 (12)	0.73 (0.38-1.4)
	$P_{\text{trend}}^{\ddagger}$			0.24		0.72		0.09
GHRL 5179A>G	AA	333 (49)	164 (53)	1.0	56 (50)	1.0	55 (56)	1.0
	AG	295 (43)	121 (39)	0.83 (0.62-1.1)	42 (38)	0.86 (0.56-1.3)	40 (41)	0.84 (0.54-1.3)
	GG	53 (8)	22 (7)	0.84 (0.49-1.4)	13 (12)	1.4 (0.72-2.8)	3 (3)	0.35 (0.10-1.2)
	AG/GG	348 (51)	143 (47)	0.83 (0.63-1.1)	55 (50)	0.95 (0.63-1.4)	43 (44)	0.76 (0.50-1.2)
	$P_{\text{trend}}^{\ddagger}$			0.24		0.72		0.09
GHRL 9344G>A	GG	558 (82)	252 (82)	1.0	85 (76)	1.0	85 (87)	1.0
	GA	120 (17)	55 (18)	1.0 (0.72-1.5)	26 (23)	1.4 (0.89-2.4)	13 (13)	0.72 (0.39-1.3)
	AA	6 (1)	1 (0.3)	0.31 (0.04-2.7)	1 (1)	0.91 (0.10-7.8)	0 (0)	—
	GA/AA	126 (18)	56 (18)	0.98 (0.70-1.40)	27 (24)	1.4 (0.88-2.3)	13 (13)	0.68 (0.37-1.3)
	$P_{\text{trend}}^{\ddagger}$			0.79		0.20		0.20

*Row 1, homozygous wild type; row 2, heterozygous; row 3, variant; row 2/row 3, heterozygous + variant.

†Adjusted for age and sex.

‡ P_{trend} for wt/wt, wt/var, var/var; based on χ^2 test for the β estimate from multivariable logistic models with genotype as an ordinal variable.

Haplotype Analyses. Common haplotypes (>5%) frequency was estimated for *GHRL* and *NPY* using tagSNP implementation of the EM algorithm (Table 3). Five common haplotypes were predicted for *GHRL*. Haplotypes containing variant alleles for any of the *GHRL* SNPs were associated with reduced risk for NHL and DLCL, although all estimates were imprecise. However, using the model where HapA was compared with all other haplotypes, HapA was associated with NHL (OR, 1.8; 95% CI, 1.2-2.8). For *NPY*, due to the low frequency of the *NPY* 1128T>C SNP and the tight linkage disequilibrium across the gene, the haplotype analysis did not reveal any further relevant information beyond that found in the univariate analysis.

Discussion

Previously, we reported a positive association between BMI and NHL and identified SNPs in the leptin and leptin receptor genes, associated with an obese phenotype, as risk factors for NHL (7). We hypothesized that leptin promotes lymphomagenesis through direct mitogenic and antiapoptotic effects in B-cell populations mediated through the leptin receptor (7). In the present study, we report additional evidence of a link between NHL and polymorphisms in genes involved in energy homeostasis and regulation that may lead to immune dysfunction. Specifically, we found that the linked *NPY* variant alleles at the four genotyped SNPs yielded a >2-fold risk for NHL, particularly for follicular lymphoma. We also found that variant alleles in the linked *GHRL* SNPs -4427A>G and 5179A>G were associated with reduced risk for NHL, especially for DLCL, where an ~65% decreased risk was observed for homozygous variant genotypes. These results may suggest alternative mechanisms in the etiology of follicular lymphoma and DLCL related to the actions of *NPY* and *GHRL* in immunoregulation. However, the associations in *NPY* and *GHRL* by NHL subtype should be interpreted with caution given the wide confidence intervals and number of participants in some genotype categories when stratified by disease entity. These results warrant exploration in larger data sets and in multiple studies.

Whereas there has been major interest in the actions of *NPY* as a neurotransmitter, there is increasing evidence of the role of *NPY* in immune modulation. In immune cells such as B cells, monocytes, and macrophages, *NPY* levels are up-regulated upon cell activation (29). In macrophages, *NPY* increases adhesion, chemotaxis, phagocytosis, and superoxide anion production. However, *NPY* also suppresses innate immunity by inhibiting natural killer cell activity (30). Studies show *NPY*-induced reduction in natural killer activity in tumor cells of young animals (31), and that elevated *NPY* levels associated with stress and depression lead to reduced natural killer cytotoxicity (32). Furthermore, *NPY* stimulates lymphocyte proliferation and the release of interleukin 4,

interleukin 6, and tumor necrosis factor- α cytokines (12), whereas it inhibits IFN- γ production (33). Previous studies have shown that the nonsynonymous *NPY* Leu⁷Pro polymorphism (1128T>C) is associated with increased *NPY* secretion, elevated cholesterol levels, enhanced angiogenesis, and lymphocyte proliferation (14). Thus, in the present study, the associations found in the four *NPY* SNPs may be driven by the functional SNP 1128T>C, given that the four SNPs are in close linkage disequilibrium. Whether elevated cholesterol is a risk factor for NHL remains to be explored further, but this would be consistent with our previous results (21) and those of others (34) that show an inverse relationship between cholesterol-lowering drugs and NHL.

Because *NPY* suppresses natural killer cell activity, the increased *NPY* secretion associated with the 1128T>C variant could hinder normal actions of the innate immune system, which is the first line of defense against viral and bacterial infections. *NPY* also stimulates estradiol release by human granulosa cells in a dose-dependent fashion (35). Previous studies have shown that estradiol promotes B-cell proliferation and T-cell suppression and inhibits apoptosis (36). These actions could enhance the growth of transformed B-cells that may favor lymphomagenesis.

Ghrelin is a peptide hormone that is recognized as an important regulator of growth hormone release and energy homeostasis. As the only known circulating orexigen, ghrelin exerts antagonistic effects on the leptin-induced decrease in food intake through activation of the *NPY* pathway. Interestingly, as modulators in immune function, a mutually antagonistic relationship exists between ghrelin and leptin. Ghrelin inhibits the proliferation of inflammatory cytokines such as interleukin 6, interleukin 1 β , and tumor necrosis factor- α , whereas leptin promotes proinflammatory cytokine release (37). In addition, ghrelin levels are decreased in obese individuals, whereas leptin resistance results in elevated leptin levels found among obese individuals. Hypothetically, low levels of ghrelin and high levels of leptin present in obese individuals may induce a chronic proinflammatory state, potentially increasing NHL risk. Previous studies have shown that the homozygous variant of *GHRL* 408C>A (Leu72Met) is associated with lower BMI, fat mass, and abdominal visceral fat (10), but we did not detect a similar association between *GHRL* SNPs and BMI in the present study. The small number of carriers of the variant 408C>A allele in our study population may have limited our ability to detect an association if one exists.

Strengths of this study included the population-based design that used cancer registry data to identify incident NHL patients shortly after diagnosis and centralized expert review of diagnostic pathology materials for 97% of cases. These data were supplemented by receipt of the Surveillance, Epidemiology, End Results abstracts to identify additional cases that may have been missed by rapid case ascertainment. Controls from the same population base as the cases were

Table 3. Associations between *GHRL* haplotypes and risk of NHL

GHRL	Haplotype	Controls (<i>n</i> = 684)		All NHL (<i>n</i> = 308)		DLCL (<i>n</i> = 98)		Follicular lymphoma (<i>n</i> = 112)	
		Haplotype frequencies	Haplotype frequencies	OR (95% CI)	Haplotype frequencies	OR (95% CI)	Haplotype frequencies	OR (95% CI)	
A	0-0-0-0	0.38	0.43	1.00 (reference)	0.53	1.00 (reference)	0.36	1.00 (reference)	
B	1-0-0-0	0.22	0.20	0.81 (0.55-1.19)	0.18	0.56 (0.31-1.04)	0.21	0.99 (0.55-1.68)	
C	0-0-1-0	0.22	0.20	0.81 (0.56-1.16)	0.17	0.61 (0.31-1.07)	0.23	1.08 (0.62-1.78)	
D	1-0-1-0	0.07	0.07	0.78 (0.42-1.42)	0.03	0.34 (0.09-1.13)	0.07	1.05 (0.38-2.36)	
E	1-1-0-1	0.07	0.06	0.77 (0.39-1.39)	0.04	0.34 (0.09-1.15)	0.08	1.09 (0.41-2.39)	
Other	Pooled (low frequency)	0.039	0.043	0.92 (0.40-1.87)	0.042	0.26 (0.00-1.55)	0.051	1.23 (0.34-3.34)	

identified through random digit dial supplemented by random sampling of Healthcare Financing Administration lists that include ~98% of U.S. residents ≥ 65 years. No proxy interviews were conducted. Bias associated with exclusion of potential cases due to death is a concern in studies of cancer. However, ~65% of patients who died before we could contact them were HIV-positive cases (23) and therefore are unlikely to have biased these results that focused on HIV-negative participants only. There is a potential for bias associated with misclassification of BMI given that self-reported usual adult weight and height used to compute BMI are subject to misclassification. The National Health and Nutrition Examination Survey data showed that the prevalence of overweight U.S. adults is likely to be underestimated because both men and women tend to overestimate their height whereas men overestimate and women underestimate their weight, especially among those who are overweight or obese and among those >60 years old resulting in underestimates of overweight (38). If this misclassification was nondifferential then the estimates for BMI may be biased toward the null. Analyses were restricted to white non-Hispanic participants to control for the potential effects of population stratification (28). Due to few participants with homozygous variant genotypes, estimates associated with this genotype are unstable and may be due to chance, especially for histologic subtypes. In addition, there is some uncertainty in haplotypes from association studies because they must be inferred from unphased genotype data. However, based on a recent comparison of standard haplotype analysis techniques that showed that the E-M algorithm method used in our analyses performs well for uncomplicated haplotypes and haplotype risk estimates of ≤ 2 (39), the potential bias for these haplotype estimates is likely to be small. Despite concerns regarding small sample size in some analyses and multiple testing issues, results tended to be consistent and warrant replication and confirmation in larger studies.

In conclusion, this study provides further evidence of a relationship between NHL and genes associated with energy regulation and homeostasis and suggests that elevated NPY levels may promote immune dysfunction that could enhance the growth of transformed B-cells that may progress to lymphoma. Larger studies are needed to replicate these findings, to further explore potential interactions among NPY, GHRL, and LEP polymorphisms, and to clarify the interplay between these genes and environmental factors. Animal studies and functional studies of specific SNPs also are needed to identify the mechanistic pathways to explain these observations.

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