

Association of AMP-Activated Protein Kinase with Risk and Progression of Non-Hodgkin Lymphoma

Aaron E. Hoffman¹, Kathryn Demanelis¹, Alan Fu², Tongzhang Zheng², and Yong Zhu²

Abstract

Background: Metabolic dysregulation has been identified as an "emerging hallmark" of cancer. The heterotrimeric AMP-activated protein kinase (AMPK) complex is a central regulator of the metabolic system and an important component of the mTOR pathway and the p53 axis, making it uniquely positioned to influence carcinogenesis through its canonical functions in the metabolic arena, as well as through more traditional mechanisms such as regulation of apoptosis and angiogenesis.

Methods: We conducted a population-based genetic association study to examine the impact of mutations in *AMPK* subunit genes on risk of non-Hodgkin lymphoma (NHL). We also analyzed public microarray data to determine the expression of AMPK in NHL cells and to assess the influence of AMPK expression on overall survival in patients with NHL.

Results: We identified an *AMPK* subunit haplotype, which was significantly associated with NHL [OR, 5.44, 95% confidence interval (CI), 2.15–13.75] in women with no family history of cancer. Haplotypes in two subunits, *PRKAA2* and *PRKAG3*, were nominally associated with the follicular and diffuse large B-cell lymphoma histologic subtypes, respectively, although these associations did not retain statistical significance after correction for multiple comparisons. Further, both of these subunits were differentially expressed ($P < 0.05$) in one or more lymphoma cell type, and higher expression of two versions of the *AMPK-β* subunit was significantly associated with increased 5-year survival among patients with NHL ($P = 0.001$ and $P = 0.021$).

Conclusion: These results provide evidence for *AMPK* involvement in the pathogenesis and progression of NHL.

Impact: These findings may lead to a novel area of research into NHL treatment and chemoprevention. *Cancer Epidemiol Biomarkers Prev*; 22(4); 736–44. ©2013 AACR.

Introduction

Metabolic pathways are integral for maintaining myriad cellular processes including energy allocation, cell growth, protein translation, and cell proliferation. As cell growth and protein translation are tightly regulated by intracellular metabolism, metabolic genes have been extensively researched as potential tumor suppressors and oncogenes. One such potential tumor suppressor is the master energy regulator AMP-activated protein kinase (AMPK). AMPK is a heterotrimeric protein that consists of an α -catalytic subunit and 2 regulatory subunits, β and γ . The α -subunit is encoded either by the

PRKAA1 or *PRKAA2* gene. The β - and γ -subunits are encoded by the *PRKAB1* or *PRKAB2* and the *PRKAG1*, *PRKAG2*, or *PRKAG3* genes, respectively (1). These subunits can assemble to form multiple isoforms of AMPK, and variation within these genes can affect the sensitivity and overall function of the AMPK complex (2).

AMPK regulates metabolic processes via its sensory capacity, detecting intracellular concentrations of ATP and AMP (3). When the cell is consuming energy at a faster rate than it is replacing energy, the concentration of AMP increases inside the cell, which prompts AMPK to allosterically change its conformation and enable its α -catalytic subunit to be phosphorylated by liver kinase B1 (LKB1; ref. 4). Once AMPK is activated via phosphorylation, it enhances catabolic pathways, such as glycolysis, and suppresses anabolic pathways such as lipid and glycogen synthesis and pathways that regulate cell growth, gene transcription, and protein translation (5). Dysregulation of AMPK can disrupt these downstream processes and the consequences of this cellular dysfunction could have relevance for tumorigenesis.

While there is limited evidence examining the biologic role of AMPK in NHLs, AMPK has been implicated in at least 2 important tumor-related pathways: the mTOR

Authors' Affiliations: ¹Department of Epidemiology, Tulane School of Public Health and Tropical Medicine and Tulane Cancer Center, New Orleans, Louisiana; and ²Department of Environmental Health Sciences, Yale University School of Public Health, New Haven, Connecticut

Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (<http://cebp.aacrjournals.org/>).

Corresponding Author: Yong Zhu, Department of Environmental Health Sciences, Yale University School of Public Health, New Haven, CT 06520. Phone: 203-785-4844; Fax: 203-737-6023; E-mail: yong.zhu@yale.edu

doi: 10.1158/1055-9965.EPI-12-1014

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pathway and the p53 axis. The role of AMPK in the mTOR pathway begins when it is phosphorylated by LKB1. Once phosphorylated, AMPK then targets another molecule for phosphorylation: the TSC2 unit of the tuberous sclerosis complex (TSC1/TSC2; ref. 6). By phosphorylating TSC2, AMPK communicates the low energy status of the cell and thus prevents the activation of various cell growth pathways (7). Phosphorylated TSC2 inactivates the GTP-binding protein, Rheb, and prevents Rheb from promoting mTOR (8). mTOR is involved in protein translation, angiogenesis, and autophagy (9–11). As such, LKB1, AMPK, and TSC1/TSC2 serve as metabolic checkpoints to ensure that mTOR activity is permissible. Both LKB1 and TSC1/TSC2 are known tumor suppressors, and loss of function in these genes leads to increased tumor growth. Furthermore, germline mutations in *LKB1* predispose humans to Peutz–Jeghers syndrome, which increases the risk for lung, gastrointestinal, breast, and gynecological cancers (12). Mutations in *TSC1/TSC2* are associated with the inherited disorder tuberous sclerosis, which leads to widespread development of hamartous tumors (13). In addition, TSC1 is aberrantly expressed in breast tumors (14). Genetic alterations in the mTOR pathway are highly heterogeneous, but most are associated with disrupted cell growth (7). AMPK also independently interacts with p53, an important tumor suppressor, to halt aberrant cell-cycle progression (15). AMPK phosphorylates p53 and initiates AMPK-dependent cell-cycle arrest and allows the cell to survive in energy-depleted conditions. Persistent activation of AMPK can trigger p53-induced cellular senescence. As AMPK is a crucial component in both of these signaling pathways, its disruption could potentially lead to tumorigenesis.

In the current study, we use a population-based epidemiologic analysis to investigate the role of mutations in the *AMPK* subunits in lymphomagenesis. We also analyze public microarray expression data to determine the expression of *AMPK* in NHL cells and to assess the influence of *AMPK* expression on the overall survival of patients with NHL. Our data indicate an association between *AMPK* dysregulation and NHL, suggesting that *AMPK* may be an important contributor to lymphomagenesis.

Materials and Methods

Study population

The study population used in the genetic association component of the study has been described elsewhere (16). Briefly, all subjects were female residents of CT, and cases were incident and histologically confirmed NHL (ICD-o m-9590-9642, 9690-9701, 9740-9750) identified through Yale Cancer Center's rapid case ascertainment system from 1996–2000. Population-based controls were recruited through random digit dialing (for individuals younger than 65) or through Health Care Financing Administration files (for individuals 65 or older). Five-year age strata were constructed, and controls were frequency-matched to cases by adjusting the number of

controls selected from each stratum. Participation rates were 72% for cases, 69% for controls younger than 65, and 47% for controls older than 65.

Data collection

The study was approved by the Institutional Review Board at Yale University, the Connecticut Department of Public Health, and the National Cancer Institute. Participation was voluntary and informed consent was obtained for all study subjects. Interviews were conducted by trained study nurses and done either in the subject's home or at a convenient location. After the questionnaire was administered, subjects provided a 10-mL peripheral blood sample. gDNA was isolated from peripheral blood lymphocytes for each study subject.

Single-nucleotide polymorphism selection and genotyping

Single-nucleotide polymorphisms (SNPs) were identified by using the Tagger algorithm in the Haploview interface in HapMap's genome browser 22 (17). Note that although an updated version of the genome browser is currently available, release 22 was current at the time that the genotyping was conducted in 2008. A total of 19 SNPs were selected across the *AMPK* subunit genes. One SNP was identified in each of *PRKAA1* (rs11747210), *PRKAB1* (rs278145), *PRKAB2* (rs3766522), and *PRKAG1* (rs2293445). Two SNPs were identified in *PRKAG3* (rs6436094 and rs692243), and 3 SNPs were identified in *PRKAA2* (rs11206890, rs4912408, rs2796498). Finally, 10 SNPs were identified in *PRKAG2* (rs8961, rs5017429, rs2302532, rs17173197, rs2538039, rs2727572, rs2538042, rs6464156, rs2727565, rs4726070). Genotyping was conducted at Yale University's W.M. Keck Foundation Biotechnology Research Laboratory using the Sequenom MassARRAY multiplex genotyping platform. Duplicate samples from 100 study subjects and 40 replicate samples from each of 2 blood donors were interspersed throughout each batch for all genotyping assays to confirm accuracy. Concordance rates for the quality control samples were >95% for all assays. The average genotyping call rate was 95%, with the lowest call rate for rs11747210, 85.8%, and highest for rs2293445, 98.5%. Genotyping scores (including quality control data) were rechecked by different laboratory personnel to ensure the accuracy of each assay.

Gene expression microarray data extraction

To assess the expression of each *AMPK* subunit gene in NHLs, we searched the GEO profile database in NCBI using the keywords "lymphoma" and the full name of each subunit. The search was then restricted to include only datasets that contained one or more subtype of human NHL and one or more subtypes of normal human lymphocytes and did not involve cells exposed to treatments or infectious agents. Common reasons for exclusion included no acceptable control (e.g., the study comparison involved analyses of treatment effects such as drug

response in tumor samples only, and contained no normal lymphocytes, which is uninformative for our purposes); unacceptable sample type (e.g., virus-infected cell lines rather than tumor samples); incorrect disease state (i.e., the outcome was something other than NHL); or incorrect organism (i.e., the cells were not from humans). Only one record fit these criteria (accession number GDS3516). Further information can be obtained from the original manuscript (18). Expression values for each gene were extracted for the following cell types: diffuse large B-cell lymphoma (DLBCL), follicular lymphoma (FL), memory B cells, naïve B cells, centrocytes, and centroblasts. Where multiple probes were included for a single gene, we used the probe which was labeled with the verified RefSeq identifier for the corresponding transcript. In one instance (PRKAA1), no probe was linked to a RefSeq number. In this case, we conducted a BLAST search comparing each of the 5 probes with the reference sequence, and the probe with the highest alignment score was used in the analysis.

Survival analysis

Using the OncoPrint database, we extracted expression data from datasets that included survival information and follow-up time. Initially, 9 studies reported data for our NHL subtypes of interest. Studies were then excluded if they did not include information about age, sex, chemotherapeutic regimen, and stage. In the 5 studies that remained (19–23), we selected the microarray reporter that was most commonly used across datasets. To reduce heterogeneity, which was considerable across array platforms, we further restricted the analysis to include only the studies (GSE10846 and GSE23501; refs. 22, 23) using a common array platform (AffymetrixU133 Plus 2.0). In both studies, all patients were treated with standard chemotherapy (CHOP), with all patients (GSE23501) and 56% of patients (GSE10846) also receiving rituximab (R-CHOP). Both studies obtained samples from patients with newly diagnosed DLBCLs before any treatment, and there was no difference between the studies in terms of follow-up time ($P = 0.2377$ for comparison of medians) and overall survival time ($P = 0.3501$).

Statistical analysis

All statistical analyses were conducted using SAS statistical software, version 9.2. All allelic distributions were tested using a goodness-of-fit χ^2 test for compliance with Hardy–Weinberg equilibrium (HWE). To determine the association between each SNP and NHL, an unconditional multivariate logistic model was generated and the adjusted ORs and 95% confidence intervals (CI) were reported. The model was adjusted for age, smoking status, alcohol consumption, education, and family history of any cancer. The most common allele was set as the referent, and adjusted ORs and 95% CIs were reported. For each gene harboring more than one tagging SNP (PRKAA2, PRKAG2, and PRKAG3), haplotypes were estimated using the PHASE program (24). To determine the association between each haplotype and NHLs, we conducted an

unconditional multivariate logistic regression analysis. Each haplotype with a frequency greater than 5% was analyzed individually, with rarer haplotypes grouped into an "other" category. To adjust for multiple comparisons within each SNP ($n = 57$) and haplotype ($n = 15$) analysis (including 2 additional stratified analyses involving subjects with and without a family history of NHL), we used a Benjamini–Hochberg false discovery rate (FDR) correction, as previously described (25). In the gene expression component of the study, median values were compared for each cell type, with P values obtained using the Wilcoxon rank-sum test. For the survival analysis, data were visualized using Kaplan–Meier curves, and equality of the survival functions in patients with high versus low expression (defined as having an expression value above or below the median expression for the full population, respectively, for each AMPK subunit) was tested using the log-rank test.

Results

Association between AMPK subunit variants and NHL risk

There were no significant differences in age, race, smoking status, and alcohol consumption between NHL cases and controls (Table 1), and none of the allelic distributions significantly deviated from the expected distribution under HWE among the controls (all adjusted $P > 0.05$). As expected, cases reported a significantly higher frequency of family history of NHLs and other cancers when compared with controls. In addition, significant effect modification by family history ($P < 0.01$) was noted for some SNPs, and thus stratum-specific data are presented for each association.

The full genotyping results for individual SNPs are available as Supplementary Table S1. Multiple significant associations were noted, but only one remained significant after FDR correction. Individuals with no family history of cancer and heterozygous for rs2293445 in the PRKAG1 subunit had a significantly increased risk of NHL (OR, 2.80; 95% CI, 1.54–5.07; $P < 0.001$). Significant effect modification of this association was noted by family history ($P_{\text{interaction}} < 0.001$). In the haplotype analysis, the strongest association was noted in PRKAA2. A 3-SNP haplotype in this gene was associated with significantly increased risk of NHLs in the full population ($P < 0.001$; Table 2). As the effect differed significantly by family history of cancer ($P_{\text{interaction}} = 0.001$), the data are presented as stratified analyses. Among subjects who reported no history of cancer among first- or second-degree relatives, one PRKAA2 haplotype was associated with a 5-fold increase in NHL risk (OR, 5.44; 95% CI, 2.15–13.75; $P < 0.001$). An effect of similar magnitude was noted for this haplotype when restricting the cases to the follicular lymphoma subtype only (OR, 5.82; 95% CI, 1.41–23.96; $P = 0.015$), although this association was not significant after adjustment for multiple comparisons. Similarly, at the nominal α of 0.05, a haplotype in the PRKAG3 subunit was significantly associated with DLBCL (OR,

Table 1. Participant characteristics

Variable	Cases	Controls	<i>P</i> ^a
	(<i>n</i> = 456)	(<i>n</i> = 527)	
	<i>n</i> (%)	<i>n</i> (%)	
Mean age, y	61.88	62.34	0.607
Race			
Caucasian	439 (96.27)	496 (94.12)	
African-American	13 (2.85)	14 (2.66)	
Other	4 (0.88)	17 (3.22)	0.103
Smoking			
No	201 (44.08)	247 (46.87)	
Yes	255 (55.92)	280 (53.13)	0.381
Alcohol consumption			
No	165 (36.18)	168 (31.88)	
Yes	291 (63.82)	359 (68.12)	0.155
Family history			
None	96 (21.05)	130 (24.67)	
NHL	9 (1.97)	2 (0.38)	
Other cancer	351 (76.97)	395 (74.95)	0.029
Education			
1	13 (2.85)	11 (2.09)	
2	45 (9.87)	52 (9.87)	
3	127 (27.85)	131 (24.86)	
4	39 (8.55)	50 (9.49)	
5	115 (25.22)	106 (20.11)	
6	64 (14.04)	101 (19.17)	
7	51 (11.18)	71 (13.47)	
8	2 (0.44)	5 (0.95)	0.175
Case pathology			
All B cell	364 (79.82)		
DLBCL	135 (37.09)		
Follicular	105 (28.85)		
SLL/CLL	54 (14.84)		
Marginal zone	30 (8.24)		
Other	40 (10.99)		
All T cell	33 (7.24)		
Not otherwise specified	59 (12.94)		

^a*P* value for *t* test (age) or χ^2 test (all other variables).

0.28; 95% CI, 0.08–0.92; *P* = 0.035). No significant findings were identified among cases with marginal zone B-cell lymphoma, T-cell lymphoma, chronic lymphocytic leukemia, or among participants with a family history of cancer (data not shown).

To investigate the potential functional consequence of these variants, we analyzed each of the SNPs in the haplotype most strongly associated with NHL (composed of 3 SNPs: rs11206890, rs4912408, and rs2796498 in PRKAA2) using the HaploReg tool (26). We found that rs4912408 alters the motif for various forkhead box (FOX) transcription factor-binding sites. These proteins have been implicated in tumorigenesis (27), and high expression of FOXP1, a member of this family, is associated with

poor prognosis in patients with DLBCLs (28). As such, SNPs affecting FOX protein binding could potentially be a source of differential cancer susceptibility and/or an indicator of prognosis.

AMPK subunit gene expression in NHL

To further elucidate the potential molecular relevance of these associations, we extracted published microarray data from the GEO database (accession number: GDS3516). We obtained expression values for various normal cell types, including memory B cells, naïve B cells, centrocytes, and centroblasts; along with the NHL subtypes DLBCL and FL. Because the expression of memory B cells and naïve B cells was measured in the peripheral blood, we grouped these as "peripheral blood B cells." Centrocytes and centroblasts, potential histopathologic indicators of NHL, were grouped together as "follicle B cells." We compared the median expression between the NHL subtypes and peripheral blood B cells and follicle B cells using a Wilcoxon rank-sum test (Fig. 1). For PRKAA2, median gene expression in FL (median, 10.44; range, 8.67–11.33) was significantly higher than in follicle B cells (median, 8.75; range, 7.41–10.59; 19.3% increase, *P* < 0.05). For PRKAG3, the median expression in NHL overall (median, 13.89; range, 9.87–16.92) was significantly lower than in peripheral blood B cells (median, 15.05; range, 13.90–17.30) and follicle center B cells (median, 16.20; range, 11.28–17.75; 7.7% and 14.25% decrease, respectively, *P* < 0.05). Median PRKAG3 expression in the FL subtype (median, 12.58; range, 11.44–15.38) was also significantly lower than in follicle center B cells (22.4% decrease, *P* < 0.05), and the median expression in the DLBCL subtype (median, 13.83; range, 11.88–15.45) was significantly lower than both peripheral blood B cells and follicle center B cells (8.1% and 14.6% decrease, respectively, *P* < 0.05). No other AMPK subunits showed significant differential expression in lymphoma cells versus normal B cells.

Median AMPK subunit gene expression and NHL survival

To determine whether AMPK expression influenced survival, we extracted published array data from the Oncomine database (29). We identified 2 studies that fit our inclusion criteria and reported AMPK expression. Both studies examined only the DLBCL subtype, used the same sample processing methods, and there were no differences in follow-up time (*P* = 0.2377 for comparison of medians) and overall survival time (*P* = 0.3501). As such, the studies were combined, resulting in a total of 436 subjects. We stratified the population into "high" or "low" expression of each AMPK subunit (i.e., above or below the median expression for the full population) and evaluated differences in survival using the log-rank test (Fig. 2). Higher expression of 2 subunits, PRKAB1 (*P* = 0.001) and PRKAB2 (*P* = 0.021), was associated with increased survival, and a similar trend was noted for PRKAA1, although this association did not reach statistical

Table 2. Haplotype analysis results in women with no family history of cancer

Gene	Freq (%)	Controls (n)	All		DLBCL		Follicular lymphoma	
			Cases (n)	OR (95% CI) ^a	Cases (n)	OR (95% CI)	Cases (n)	OR (95% CI) ^a
<i>PRKAA2</i>								
CGA	48.7	137	83	Reference	31	Reference	16	Reference
TAG	33.2	83	67	1.45 (0.93–2.25)	20	1.18 (0.61–2.28)	21	2.54 (1.21–5.32)
TGG	11.3	30	21	1.07 (0.56–2.03)	3	0.35 (0.10–1.26)	7	1.94 (0.69–5.44)
TGA	6.2	7	21	5.44 (2.15–13.75)	4	2.43 (0.54–11.03)	4	5.82 (1.41–23.96)
Other	0.7	3	0	n/a	0	n/a	0	n/a
Combined	51.3	123	109	1.51 (1.02–2.22)	27	0.94 (0.51–1.71)	32	2.44 (1.24–4.79)
<i>PRKAG2</i>								
CTTTCACAAG	18.8	50	35	Reference	9	Reference	10	Reference
TCTCTGAGCA	16.4	44	30	0.88 (0.46–1.72)	9	1.21 (0.41–3.58)	7	0.68 (0.23–2.04)
CTTCCACGCA	15.9	40	32	1.16 (0.60–2.24)	11	1.83 (0.64–5.20)	7	0.81 (0.27–2.44)
CTGTTGCACA	11.7	29	24	1.15 (0.56–2.35)	5	1.09 (0.31–3.78)	8	1.11 (0.37–3.36)
TTGCCGAACG	5.3	15	9	0.89 (0.34–2.35)	2	0.82 (0.15–4.47)	2	0.67 (0.12–3.58)
Other	31.9	82	62	0.99 (0.56–1.76)	22	1.82 (0.73–4.54)	14	0.71 (0.28–1.84)
Combined	81.2	210	157	1.02 (0.62–1.68)	49	1.50 (0.66–3.41)	38	0.78 (0.34–1.76)
<i>PRKAG3</i>								
AC	67.7	169	137	Reference	48	Reference	33	Reference
GG	17.9	48	33	0.89 (0.53–1.49)	6	0.47 (0.18–1.22)	12	1.33 (0.61–2.89)
GC	13.7	40	22	0.64 (0.34–1.20)	4	0.28 (0.08–0.92)	3	0.28 (0.07–1.13)
AG	0.7	3	0	n/a	0	n/a	0	n/a
Combined	32.3	91	55	0.75 (0.49–1.14)	10	0.36 (0.17–0.78)	15	0.82 (0.4–1.66)

NOTE: Significant associations at the nominal *P* value (0.05) are shown in bold. Associations that remain significant after correction for multiple comparisons are underlined.

^aAdjusted for age, smoking status, alcohol consumption, and education.

significance ($P = 0.075$). Interestingly, an opposite trend was observed for *PRKAG3*, with lower expression associated with a median survival time increase of 5.76 months ($P = 0.064$). This is particularly notable in light of the

previous results. In the genetic association analysis, *PRKAG3* was the only subunit to contain variants which were associated with protection, rather than increased risk, and in the expression analysis, *PRKAG3* was the only

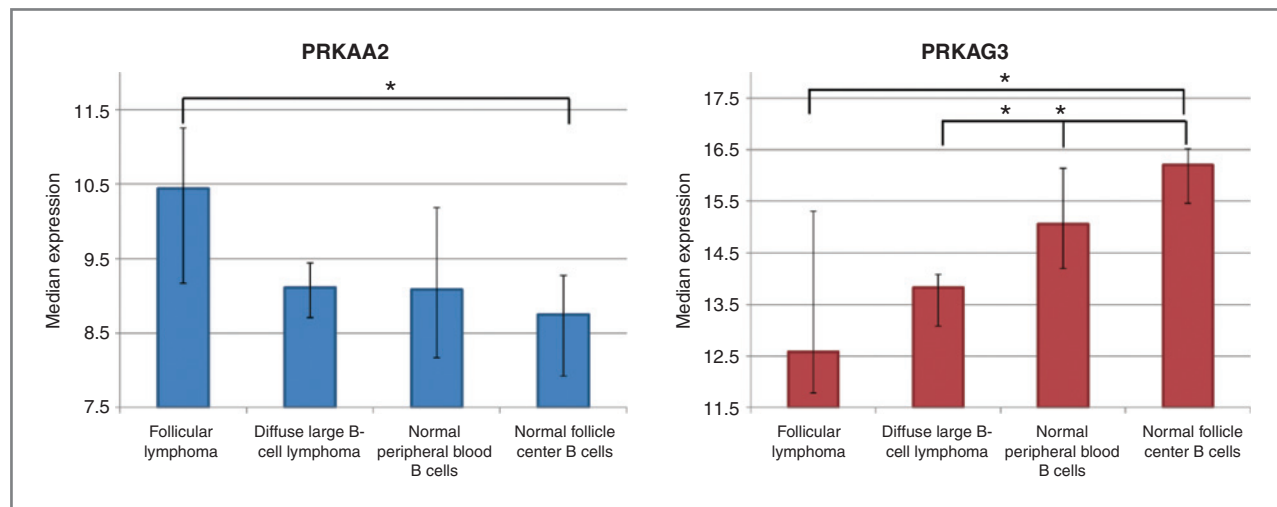


Figure 1. Expression analysis of *PRKAA2* and *PRKAG3* in lymphoma subtypes and normal B cells. *, significant differences in expression ($P < 0.05$). Error bars represent the interquartile range. No other AMPK subunits showed significant differential expression in lymphoma cells versus normal B cells.

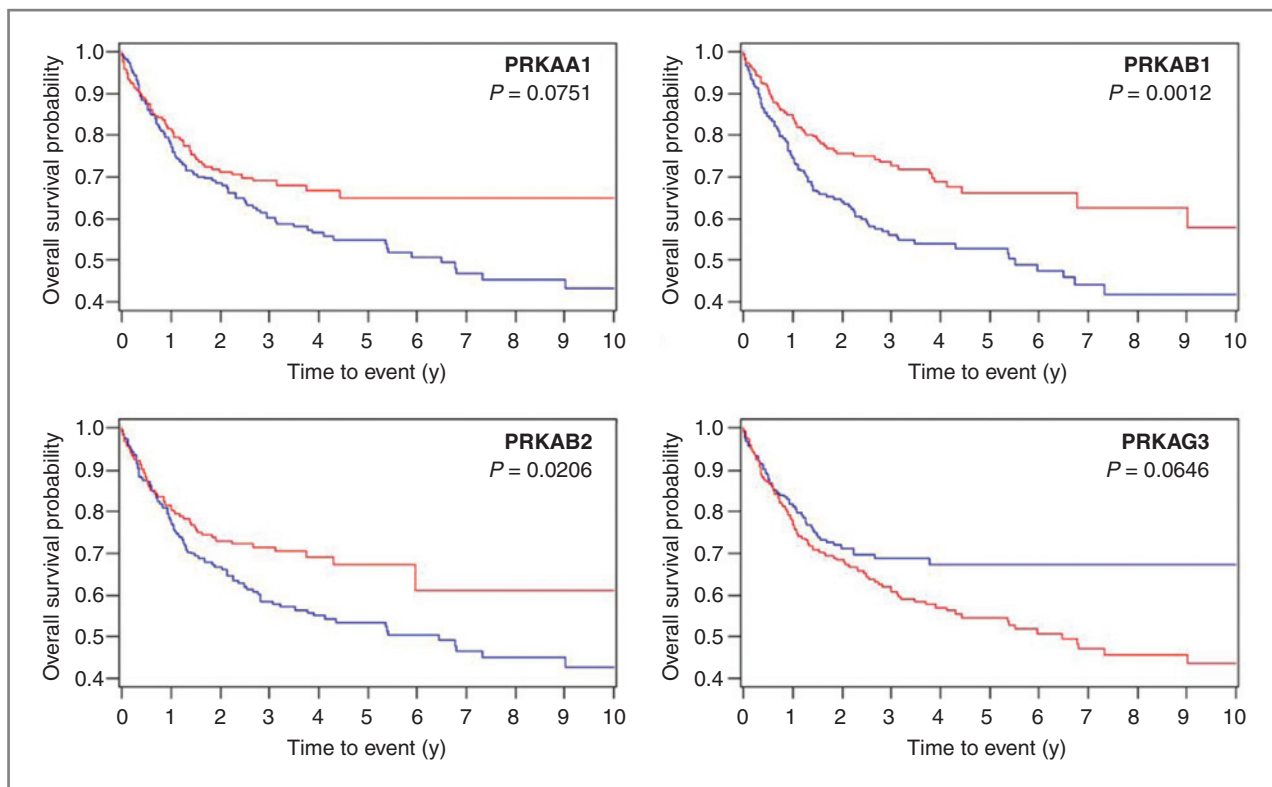


Figure 2. Ten-year overall survival probability by median expression of AMPK subunit genes in patients with DLBCLs. Red lines are for individuals with high expression (i.e., above the median), and blue lines are for individuals with low expression.

subunit with lower expression in FL and DLBCL cells relative to normal B cells.

Discussion

Although previous studies have pointed to the potential for AMPK involvement in lymphomagenesis, particularly through its involvement in the mTOR pathway, few studies have examined the association directly. Here, we investigated the relationship between NHL and AMPK and found strong associations with a variant in *PRKAG1* and haplotypes in *PRKAA2* and *PRKAG3*. Many genetic association studies have explored the role of AMPK in other cancers and diseases, suggesting that variation in AMPK may affect metabolic and cell growth pathways, influencing individual susceptibility to cancer and other endocrine disorders. Numerous SNPs in *PRKAG2* and *PRKAA1* were associated with colorectal cancer risk (30), and in a more recent study, rs11584787 in the 3'-untranslated region (UTR) of *PRKAB2* was associated with an increase in breast cancer risk, although this association did not meet the author's criteria for study-wide significance (31). Type II diabetes incidence was also associated with variation in *PRKAG2* (32). Finally, carrying one or both minor alleles of rs692243 in *PRKAG3* was nominally associated with increased risk of polycystic ovary syndrome, but this association did not stay significant after correction for multiple comparisons (33). To the best of

our knowledge, this is the first association study exploring variations in AMPK and NHL risk.

Our analysis suggests an interesting correlation between the expression data extracted from GEO and the associations identified in our haplotype analysis. We found a protective association between a relatively common (13.7%) haplotype in *PRKAG3* and DLBCL and also noted that median expression of this subunit was significantly lower in DLBCL cells than in peripheral blood B cells and follicle center B cells. Conversely, we found 2 variant haplotypes in *PRKAA2*, which were associated with increased risk of follicular lymphoma, and the median gene expression of this subunit was significantly higher in follicular lymphoma cells relative to normal follicle center B cells. While we have no information on the functional consequences of these haplotypes, the nature of these associations may be of interest for future research.

In FL cells in culture, mTOR has been shown to be active, and treatment with rapamycin, an inhibitor of mTOR, leads to significantly reduced clone formation (34). Rapamycin also causes mantle cell lymphoma (MCL) cells to enter cell-cycle arrest and enhances their sensitivity to other chemotherapeutic agents (35–37). In addition, chronic lymphocytic leukemia/small lymphocytic lymphoma (CLL/SLL) cells entered cell-cycle arrest after being treated with rapamycin, and various cell-cycle

genes were also downregulated (38). More recently, AICAR, a chemotherapeutic agent and molecular target of AMPK, was shown to induce apoptosis in CLLs (39). After being treated with an mTOR inhibitor, DLBCL cells also entered cell-cycle arrest, cyclins D3 and A were downregulated, and the cytotoxicity of rituximab was enhanced (40). In a recent phase II trial, relapsed aggressive DLBCLs, MCLs, and FL responded to everolimus, another mTOR inhibitor, showing the clinical relevance of the mTOR pathway in lymphoma (41). This suggests a potential mechanism through which AMPK could influence lymphomagenesis, as previous reports have shown that AMPK is an important component of the mTOR pathway and the p53 axis (15). Importantly, a recent comprehensive analysis of copy number alterations (CNA) in DLBCLs showed that CNA-associated p53 deficiency was common in DLBCLs and that patients with the disrupted CNA signature had significantly poorer prognosis relative to patients without the CNA-disrupted pattern (42). In addition, the tumor suppressor activity of the LKB1 kinase may be mediated, at least in part, through its activation of AMPKs (43). For example, LKB1 has been shown to influence B-cell differentiation in response to DNA double-strand breaks by inactivating CRTC2 through AMPK family members (44). These associations, in addition to the role of AMPK as a master metabolic regulator, may make it an attractive target for new cancer therapies. At least 2 existing small molecules are known to influence AMPK directly, including metformin, an anti-diabetic drug that upregulates AMPK and increases glucose intake into cells (45), and AICAR, a selective AMPK activator (46). In p53-deficient tumor cells, metformin has been shown to be an effective proapoptotic agent (47), and a recent systematic review determined that metformin significantly reduced cancer risk by 31% in patients with type II diabetes (48). However, metformin did not significantly improve DLBCL survival in individuals undergoing chemoimmunotherapy (49). A phase I clinical trial (NCT00659568) examined the effectiveness of metformin in combination with temsirolimus, an mTOR inhibitor, against lymphoma and other solid tumors and found that among 11 patients with advanced tumors, 1 patient experienced a partial response and 5 had stable disease (50). In B-cell CLLs, AICAR was shown to induce apoptosis in all cell samples tested (51). Later, these same authors showed that AICAR also induced apoptosis in splenic marginal zone and MCL (52). Many tumor cells undergo metabolic reprogramming, described as the Warburg effect (53), and AMPK has been shown to be activated in some proliferating cells rather than inhibited (54, 55). As such, additional research on the role of AMPK in NHLs is necessary to determine whether AMPK activators may be potentially useful chemotherapeutic agents for immune-related tumors.

Although the findings from our genetic association analysis suggest a link between AMPK dysfunction and lymphomagenesis, some important limitations of our study should be noted. One such limitation is the im-

putation of haplotypes using PHASE, which relies on haplotype predictions based on the distribution of the known homozygotic phases, the frequency of the genotype at a particular locus, and the linkage disequilibrium between the loci. As such, heterozygotes and individuals with missing allele information for a particular locus could be misclassified. However, the haplotype model that is generated is an efficient and relatively accurate way to represent genetic variation in a given region, and PHASE is a widely accepted method for inferring haplotypes. We also imposed a strict FDR correction, which reduces the potential for false-positive findings, but increases the likelihood that true biologic associations for some SNPs could be mislabeled as having no association with disease. In addition, our SNP selection was conducted using an older release of HapMap, and thus the selected SNPs are not representative of all currently known variants within each gene. Furthermore, no direct functional data were available for these SNPs, as RNA was not collected in this study, and these patients were not followed up to determine survival characteristics. As such, further study is necessary to evaluate the impact of each variant on AMPK function. Finally, our study population was comprised exclusively of female participants and we do not have access to a replication sample. As such, it remains possible that the biologic consequences of the mutations and their role in tumorigenesis may differ by sex, and it is therefore not possible to generalize these findings to males. Indeed, recent trials have shown that women with B-cell lymphomas may have differential metabolic responses (56) and have significantly better progression-free survival (57) after treatment with immunochemotherapy. Future studies will be necessary to replicate these findings and determine whether they extend to males as well as females.

In summary, the findings from our population-based genetic association analysis suggest that variants in AMPK may influence lymphoma risk in women with no family history of cancer. In addition, our expression analysis showed differential expression of some AMPK subunits in lymphoma tissue relative to normal immune cells, and survival analyses revealed that AMPK expression influences DLBCL survival. As several lines of evidence have suggested metabolic dysregulation as an "emerging hallmark of cancer" (58), future studies may focus on understanding the role of AMPK in regulating the cellular transformation from the normal metabolic phenotype to the tumor-associated program of aerobic glycolysis and determining the ways in which AMPK may be targeted in new chemotherapeutic strategies. Although AMPK has been extensively studied in liver and muscle cells, relatively little is known about the role of AMPK in other tissues, nor is it clear whether reduced function in any one subunit would be more deleterious relative to other subunits, or whether any subunit has the potential to provide full or partial functional rescue for loss-of-function in another subunit. Advances in these areas could provide novel avenues for research into tumor biology and chemoprevention.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: A.E. Hoffman, T. Zheng, Y. Zhu
Development of methodology: A.E. Hoffman, T. Zheng
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): A.E. Hoffman, T. Zheng
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): A.E. Hoffman, K. Demanelis, A. Fu, T. Zheng
Writing, review, and/or revision of the manuscript: A.E. Hoffman, K. Demanelis, T. Zheng, Y. Zhu
Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): A.E. Hoffman
Study supervision: T. Zheng, Y. Zhu

Acknowledgments

The authors thank Irina Tikhonova at Yale University's W.M. Keck Foundation Biotechnology Research Laboratory for conducting the Sequenom genotyping analysis.

Grant Support

This work was supported by the NIH (grants CA122676 and CA154653). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received August 30, 2012; revised December 7, 2012; accepted January 22, 2013; published OnlineFirst February 8, 2013.

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