Interleukin-6 and Interleukin-10 Gene Promoter Polymorphisms and Risk of Endemic Burkitt Lymphoma

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Abstract. Overexpression of interleukin-6 (IL-6) and IL-10 in endemic Burkitt lymphoma (eBL) may facilitate tumorigenesis by providing a permissive cytokine milieu. Promoter polymorphisms influence interindividual differences in cytokine production. We hypothesized that children genetically predisposed for elevated cytokine levels may be more susceptible to eBL. Using case-control samples from western Kenya consisting of 117 eBL cases and 88 ethnically matched healthy controls, we tested for the association between eBL risk and IL-10 (rs1800896, rs1800871, and rs1800872) and IL-6 (rs1800795) promoter single nucleotide polymorphisms (SNPs) as well as IL-10 promoter haplotypes. In addition, the association between these variants and Epstein Barr Virus (EBV) load was examined. Results showed that selected IL-10 and IL-6 promoter SNPs and IL-10 promoter haplotypes were not associated with risk eBL or EBV levels in EBV-seropositive children. Findings from this study reveal that common variants within the IL-10 and IL-6 promoters do not independently increase eBL risk in this vulnerable population.

INTRODUCTION

Burkitt lymphoma (BL) is a highly aggressive, germinal center-derived B-cell tumor characterized by translocation of the *c-myc* proto-oncogene on chromosome 8 to the immunoglobulin (Ig) heavy-chain locus on chromosome 14.^{1,2} There are three subtypes of BL: endemic BL (eBL), which is Epstein Barr Virus (EBV) -positive, is the most common pediatric cancer in equatorial Africa, where *Plasmodium falciparum* malaria is holoendemic with an annual incidence of 5–15 cases in 100,000 children^{3,4}; sporadic BL (sBL), which is an order of magnitude less frequent than eBL, is associated with EBV in only approximately 15% of cases and diagnosed in developed countries at any age⁵; and immune deficiency BL (idBL), which is also referred to as human immunodeficiency virus (HIV)-associated BL, occurs in adults who are immunocompromised as a result of HIV infection.⁶ BL has the highest proliferative index of any known human cancer; it progresses rapidly, with the tumors doubling in size every 24–26 hours.⁷ eBL is hypothesized to be of a multifactorial etiology, encompassing environmental factors, infectious factors (i.e., EBV and malaria infection), immune system disturbances, and potentially, host genetic predisposition.^{2,8,9} Although the precise pathogenic mechanisms for eBL have not been fully elucidated, genetic susceptibility has been found to play an important role in the development and progression of BL.^{10,11} Polymorphisms in cytokine genes can influence inflammation and immune response to infection and may be related to risk of eBL,^{12,13} but they have yet to be well-investigated within the context of epidemiologic studies.

The systematic overexpression of proinflammatory cytokines, such as interleukin-6 (IL-6), and a pleiotropic cytokine, such as IL-10, has been suggested to be responsible for the initiation and maintenance of B-cell malignancies.¹⁴ Both IL-6 and

IL-10 have been shown to be highly expressed in the BL tumor microenvironment, suggesting that they may be important signals in tumorigenesis.^{15,16}

IL-6 acts as a growth factor for EBV-infected B cells, resulting in increased Ig production and B-cell immortalization.¹⁶ It has also been shown to be highly expressed in EBV-immortalized B cells, thereby promoting tumor growth.¹⁷ Like IL-6, high levels of IL-10 in eBL tumor microenvironment also seem to be important in tumorigenesis and growth through multiple mechanisms.¹⁵ It has been shown to be an autocrine growth factor for Burkitt-derived cell lines,^{18,19} and it is frequently expressed in various B-cell malignancies.^{14,20} IL-10 has been well-characterized as a protumorigenic cytokine with immunosuppressive effects by way of inhibition of interferon- γ , proinflammatory T-helper-1 lymphocytes, and cytotoxic T cells,²¹ which are central mediators in control of EBV; hence, they potentially contribute to EBV and tumor escape from immune surveillance and enhance tumor growth.

The above information combined with the observation that interindividual variations in IL-6 and IL-10 production are genetically determined by single nucleotide polymorphisms (SNPs) within the gene promoter regions^{22,23} implicated these polymorphisms in eBL tumorigenesis and/or host response to EBV infection. The human IL-6 gene is located on the short arm of chromosome 7 (7p21). There are four common SNPs within the promoter region of *IL-6* at positions -597(G/A), -572(G/C), -373(A/G), and -174(G/C). The -174G/C polymorphism has been shown to influence IL-6 production *in vivo*, with decreased and increased expression associated with the G and C alleles, respectively.²⁴ The gene encoding IL-10 is located on the long arm of chromosome 1 (1q31–1q32). The promoter region contains three common SNPs at positions -1082A/G, -819C/T, and -592C/A. Only -1082 seems to affect cytokine levels, with the A and G alleles associated with lower and higher levels, respectively.²⁵ Specific haplotypes of these three SNPs are also associated with IL-10 production levels; for example, the IL-10 -1082G, -819C, -592C (GCC) haplotype of the promoter region is associated with high

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expression, whereas the ATA haplotype is associated with low IL-10 levels in children.²⁶

Given these associations, we hypothesized that polymorphisms in the IL-6 and IL-10 promoter, which influence their respective production, may predispose children to eBL as they cope with malaria and EBV coinfections. In this study, we focused on common promoter polymorphisms that influence expression levels of IL-10 (-1082A/G, -819C/T, and -592C/A) and IL-6 (-174G/C) to determine their association with susceptibility to eBL in children residing in a *P. falciparum* malaria-holoendemic region in western Kenya.

MATERIALS AND METHODS

Study site and participants. eBL is the most common pediatric cancer in sub-Saharan Africa. Its incidence closely parallels the level of malaria endemicity, which was originally described by Burkitt²⁷ and Burkitt and O'Connor.²⁸ All participants in this study were from Kisumu County in western Kenya, which is within equatorial Africa and burdened by holoendemic malaria transmission along with a high incidence of eBL.⁴

This study included 117 eBL patients between ages 5 and 13 years recruited from Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH; formerly named New Nyanza Provincial General Hospital) located in Kisumu City, a regional referral hospital for pediatric cancer cases in western Kenya, between December of 2007 and April of 2012. Histological, pathological, and cytological tests were done to confirm eBL diagnosis of all eBL patients recruited in this study. Histopathologic diagnosis of fine-needle aspirations stained with May-Grunewald Giemsa was confirmed by two independent pathologists, and only confirmed cases of eBL were used for this study. On admission of the patients, blood samples were drawn before chemotherapy induction, and the samples were then stored at -80°C while awaiting DNA extraction.

Eighty-eight malaria-exposed but healthy controls (ages 5–13 years) were recruited from Chulaimbo Sub-District Health Center in Kisumu County during the same time period as eBL sample collection. We used a rural health facility as a recruiting station to more appropriately enroll healthy children who reside in areas with malaria exposure and eBL incidence similar to cases.⁴ Controls were screened to ensure that they had never been diagnosed with any form of cancer. All the cases and controls were matched for sex, Luo ethnicity, and long-term residence within this malaria-holoendemic region. Although the cases and controls were not age matched, the median age range in both groups was within the age bracket of 5–9 years, an age with the peak eBL incidence.

Ethical approval. Ethical review and approval for this study was obtained from the Institutional Review Board at the University of Massachusetts Medical School and the Ethical Review Committee at the Kenya Medical Research Institute. Parents and legal guardians of the study participants provided written informed consent.

Genotyping. Genomic DNA was extracted from whole-blood samples using a Qiagen DNAeasy Kit (QIAGEN Sciences, Germantown, MD) in accordance with the manufacturer's protocol. The IL-10 -1082A/G (rs1800896; assay identification number C_1747360_10), -819C/T (rs1800871; assay identification number C_1747362_10), and -592C/A (rs1800872; assay identification number C_1747363_10)

promoter polymorphisms were genotyped using the high-throughput TaqMan 5' Allelic Discrimination Assay by Design method based on the manufacturer's instructions (Applied Biosystems, Foster City, CA). Genotyping of the SNP -174G/C (rs1800795) in the IL-6 gene was performed by the TaqMan Custom SNP assay using primer sequences 5'-CGACCTAA GCTGCACTTTTCC-3' (forward) and 5'-GGGCTGATTGG AACCTTATTAAGATTG-3' (reverse) and probe sequences CCTTTAGCATgGCAAGAC (for allele C) and CCTTTAG CATcGCAAGAC (for allele G).^{29,30} The fluorescence signals were detected on a StepOne Plus Real-Time PCR System (Applied Biosystems, Foster City, CA). All polymerase chain reaction (PCR) reactions were carried out with a final volume of 10 µL containing 3.5 µL molecular grade water, 0.5 µL 20× SNP assay mix, and 5 µL TaqMan Gene Expression Master Mix (Applied Biosystems, Foster City, CA) with 1.0 µL sample DNA. The alleles were then analyzed and determined using the graphical view from StepOne Software, version 2.1 (Applied Biosystems, Foster City, CA).

EBV viral load determination. DNA for cellular EBV load was extracted from (ethylenedinitrilo)tetracetic acid (EDTA) anticoagulated whole-blood cell pellets using QIAmp DNA Mini Kit (Qiagen Inc., Valencia, CA). Viral quantification was performed as previously described for EBV BALF5.³¹ The quantitative PCR (qPCR) cycle was 3 minutes at 95°C, 10 seconds at 95°C, and 30 seconds at 62.5°C repeated for 40 cycles and read by a Bio-Rad CFX96 Real-Time System C1000 Thermal Cycler Base (BioRad Laboratories, Hercules, CA), and it was performed for all the reactions. Standard curves were generated using EBV-positive Namalwa and EBV-negative BL41 cell lines (ATCC, Manassas, VA). Cell-associated viral loads were normalized based on EBV genome copies per microgram human β-actin DNA multiplexed in the qPCR assay as previously described.³¹

Statistical analysis. All statistical tests were carried out using SPSS Statistical Software Package, version 20.0 (IBM SPSS, Inc., Chicago, IL). χ^2 and Fisher's exact tests were used to evaluate case-control allele and genotype frequencies. The genotype proportions were tested for Hardy Weinberg equilibrium (HWE). The odds ratios (ORs) and their 95% confidence interval (95% CI) ranges were calculated using multivariate logistic regression analysis with adjustments for age and sex. The linkage disequilibrium of the three loci and haplotypes of IL-10 (-1082, -819, and -592) was conducted using the HPlus software, version 2.5. Kruskal-Wallis test was used to compare the median EBV loads and different genotypes within eBL patients. *P* values < 0.05 were considered statistically significant. Supplemental Table 1 contains the power calculations based on a sample size of 117 cases and 88 controls.

RESULTS

Clinical, demographic, and laboratory characteristics of the study participants. In total, 117 eBL patients (cases) and 88 controls were enrolled in this study. Table 1 shows their demographic characteristics. The cases and controls differed significantly with regards to EBV loads (*P* < 0.001). The proportions of males versus females were comparable between the cases and controls (*P* = 0.14). Median age differed between the groups, with the controls being, on average, 2 years older than eBL cases (*P* = 0.001).

TABLE 1

Clinical, demographic, and laboratory characteristics of the study participants

	eBL cases	Controls	P value
No. of participants (<i>N</i> = 205)	117	88	
Sex, <i>n</i> (%)			
Male	77 (65.8%)	49 (55.7%)	0.14*
Female	40 (34.2%)	39 (44.3%)	
Age, years	5	7	0.001†
EBV copies/ng DNA median rank	133.15	56.7	< 0.001†

*Statistical significance determined by the χ^2 analysis.†Statistical significance determined by the Mann-Whitney *U* test. Values were statistically significant at *P* < 0.05.

IL-10 polymorphisms (-1082A/G, -819C/T, and -592C/A) and eBL. Table 2 shows the distribution of IL-10 genotypes and alleles in cases and controls. No significant deviations of the genotype frequencies from the HWE were noted for the -1082A/G, -819C/T, and -592C/A SNPs in either the control group (*P* = 0.958, *P* = 0.311, and *P* = 0.311, respectively) or the eBL case group (*P* = 0.722, *P* = 0.663, and *P* = 0.663, respectively). To determine the role of the IL-10 promoter SNPs in conditioning susceptibility to eBL, multivariate logistic regression analyses were conducted while controlling for the confounding effects of age and sex. As shown in Table 2, there were no significant associations between any of the selected IL-10 promoter variants and eBL.

IL-6 polymorphism (-174G/C) and eBL. Table 3 shows the frequency of the IL-6 promoter polymorphism (-174G/C) in cases and controls. We observed no association between the IL-6 promoter SNP (*P* = 0.21) and eBL. All the controls carried the G/G genotype, whereas 96.6% of the cases carried the wild-type (GG) genotype. None of the cases or controls

TABLE 2

IL-10 (-1082A/G, -819C/T, and -592C/A) polymorphisms and risk of eBL

IL-10 genotype/allele	eBL cases <i>n</i> (%)	Controls <i>n</i> (%)	OR (95% CI)	P value
-1082A/G				0.90*
Genotype				
AA	53 (45.3)	39 (44.3)	1.00 (reference)	
AG	53 (45.3)	39 (44.3)	1.23 (0.54–2.78)	0.62†
GG	11 (9.4)	10 (11.4)	0.91 (0.24–3.46)	0.89†
Allele				
A	159 (67.9)	117 (66.5)		0.73*
G	75 (32.1)	59 (33.5)		
-819C/T				0.54*
Genotype				
CC	32 (27.4)	28 (31.8)	1.00 (reference)	
TC	61 (52.1)	39 (44.3)	1.68 (0.68–4.15)	0.26†
TT	24 (20.5)	21 (23.9)	1.15 (0.39–3.38)	0.80†
Allele				
C	125 (53.4)	95 (54.0)		0.92*
T	109 (46.6)	81 (46.0)		
-592C/A				0.55*
Genotype				
CC	32 (27.4)	28 (31.8)	1.00 (reference)	
AC	61 (52.1)	39 (44.3)	1.68 (0.68–4.15)	0.26†
AA	24 (20.5)	21 (23.9)	1.15 (0.39–3.38)	0.80†
Allele				
C	125 (53.4)	95 (54.0)		0.90*
A	109 (46.6)	85 (46.0)		

ORs and 95% CIs were determined using multivariate logistic regression controlling for age and sex. The reference groups in the multivariate logistic regression analysis were the homozygous wild-type genotypes and wild-type allele.

*Statistical significance determined by the χ^2 analysis.

†Statistical significance determined by the multivariate logistic regression analysis.

TABLE 3

IL-6 promoter polymorphism and eBL

IL-6 genotype/allele	eBL cases <i>n</i> (%)	Controls <i>n</i> (%)	OR (95% CI)	P value
-174G/C				0.14*
Genotype				
GG	113 (96.6)	88 (100.0)	1.00 (reference)	
GC	4 (3.4)	0 (0.0)	–	–
CC	0 (0.0)	0 (0.0)	–	–
Allele				0.13*
G	230 (98.3)	176 (100.0)	1.0 (reference)	
C	4 (1.7)	0 (0.0)	–	–

The reference groups in the multivariate logistic regression analysis were the homozygous wild-type genotypes and wild-type allele. ORs and 95% CIs were determined using multivariate logistic regression controlling for age and sex.

*Statistical significance determined by the Fisher's exact test and the Freeman-Halton extension of the Fisher's exact test for a 2 × 3 table.

were homozygous for the mutant (C/C) allele. There was no significant deviation from the HWE detected for this SNP, although disequilibrium suggesting selection was observed for this polymorphism. The suggestive bias, where only cases had the IL-6 -174G allele, was not statistically significant within our study population.

IL-10 (-1082A/G, -819C/T, and -592C/A) haplotypes and eBL. Additional stratification of the children into haplotypic groups based on the three IL-10 promoter polymorphisms yielded three observed haplotypes (ATA, ACC, and GCC). As shown in Table 4, our analyses on the distribution of the three haplotypes revealed that there was no significant association between any of the three IL-10 haplotypes and susceptibility to eBL (-1082A/-819C/-592C [ACC]: OR = 0.841, 95% CI = 0.38–1.87, *P* = 0.67; ATA: OR = 1.489, 95% CI = 0.64–3.46, *P* = 0.36; GCC: OR = 1.160, 95% CI = 0.53–2.52, *P* = 0.71).

IL-6 (-174G/C) and IL-10 (-1082A/G, -819C/T, and -592C/A) polymorphisms and EBV load. To further interrogate the possible association of these genetic variations and eBL development, we next tested the association between the IL-6 and IL-10 promoter polymorphisms and EBV load separately within both the case and control groups. Across-genotype analysis using Kruskal-Wallis test was performed on the viral load. No significant difference was observed in the IL-6 and IL-10 promoter polymorphisms and EBV loads. Across-group analyses revealed that the EBV load did not vary across IL-10 -1082A/G (*P* = 0.98), -819C/T (*P* = 0.60), -592C/A (*P* = 0.60), and IL-6 -174G/C (*P* = 0.80) (Table 5). Collectively, these results suggest that IL-6 -174G/C and IL-10 (-1082A/G, -819C/T, and -592C/A) polymorphisms are not associated with EBV loads.

IL-10 haplotypes and EBV loads. To determine the association between IL-10 promoter haplotypes and EBV loads, a Mann-Whitney *U* test was performed between those with and without the IL-10 promoter haplotypes. These analyses revealed that there was no association between the three IL-10

TABLE 4

IL-10 (-1082/-819/-592) haplotypes and eBL

Haplotype (-1082/-819/-592)	OR	95% CI	P value
ACC	0.841	0.38–1.87	0.67
ATA	1.489	0.64–3.46	0.36
GCC	1.160	0.53–2.52	0.71

Children were grouped based on the presence or absence of eBL. ORs and 95% CIs were determined using multivariate logistic regression controlling for age and sex.^{3,47} The reference group in the logistic regression analysis is those individuals without the haplotypes.

TABLE 5

IL-6 (-174G/C) and IL-10 (-1082A/G, -819C/T, and -592C/A) genotypes and median EBV loads

SNPs	Median (interquartile range), EBV copies/ng DNA	P value
IL-10 -1082A/G		
AA	0.379 (18.8)	0.98*
AG	1.107 (9.66)	
GG	0.368 (9.24)	
IL-10 -819C/T		
CC	0.390 (16.41)	0.60*
TC	0.676 (14.67)	
TT	0.217 (12.15)	
IL-10 -592C/A		
CC	0.390 (16.41)	0.60*
AC	0.676 (14.67)	
AA	0.217 (12.15)	
IL-6 -174G/C		
GG	0.441 (12.6)	0.80*
CG	0.63	
CC	—	

EBV load data are represented as medians (interquartile ranges).

*Kruskal-Wallis test, with values being statistically significant at $P \leq 0.05$.

promoter haplotypes and the EBV load (ACC: $P = 0.83$; ATA: $P = 0.92$; GCC: $P = 0.85$) (Table 6).

DISCUSSION

Molecular mechanisms that give rise to eBL have yet to be completely determined. A distinct possibility exists for genetic variation that alters the balance between anti-inflammatory and proinflammatory cytokines to contribute to the immunologic dysregulation over EBV observed in eBL.^{32–34} IL-6 and IL-10 are two cytokines that have been implicated in the pathogenesis and progression of other lymphoproliferative disorders.^{14,35,36} We hypothesized that these cytokines could potentially provide a permissive milieu for eBL oncogenesis, and therefore, patients with eBL might be more likely to possess genotypes that predispose them to higher levels of inducible IL-6 and IL-10. We, therefore, examined SNPs with genetic variations that have been shown to alter their respective cytokine levels^{23,26} but found no association between the three IL-10 promoter SNPs (-1082A/G, -819C/T, and 592C/A) and the IL-6 promoter SNP (-174G/C) and the risk of eBL in children from western Kenya; also, they did not alter EBV loads.

TABLE 6

IL-10 (-1082/-819/-592) haplotypes and EBV load (EBV copies per nanogram DNA)

Haplotype (-1082/-819/-592)	EBV load (EBV copies/ng DNA) median (IQR)	P value
ACC		
1	0.474 (23.9)	0.83*
0	0.423 (9.82)	
ATA		
1	0.4871 (12.34)	0.92*
0	0.39 (16.41)	
GCC		
1	0.496 (9.66)	0.85*
0	0.379 (18.83)	

EBV viral loads are medians (interquartile range [IQR]), and the comparisons between carriers and non-carriers of the IL-10 promoter haplotypes were computed using the Mann-Whitney U test (1 = carrier of the haplotype; 0 = non-carrier).

*Statistical significance was determined by the Mann-Whitney U test.

IL-10 may have opposing effects on eBL risk. On one hand, IL-10 may inhibit the activation of T cells, macrophages, and monocytes and is thought to facilitate the development of cancers by supporting tumor escape from immune surveillance.³⁷ On the other hand, its antiangiogenic effects may prevent or reduce tumor growth.³⁷ A number of case-control studies have found associations between IL-10 promoter polymorphisms and the risk of cancers, including B-cell lymphoma.^{12,38,39} Other investigations have failed to implicate IL-10 promoter polymorphisms in susceptibility. Thus, the observations are conflicting.¹² At a biological level, it seems plausible that variability of IL-10 expression levels could contribute to lymphoma genesis, but we did not identify any significant evidence to link selected IL-10 promoter SNPs (-1082A/G, -819C/T, and -592C/A) and eBL risk.

Our findings contradict a recent case-control study conducted in Brazil that reported a positive association between the IL-10 promoter polymorphisms (-1082, -819, and -592) and BL risk.³⁹ However, in the Brazilian study,³⁹ only 54.1% of the BL patients were EBV-positive (defined as intermediate BL), whereas in our study, all cases were EBV-positive.³¹ In addition, they had a smaller sample size, and their controls were from different genetic backgrounds, which could have contributed to the discrepancies between the two studies. Furthermore, the previous study did not select age-matched controls, and instead, they included adults with a median age of 36 years old.

In addition to EBV, holoendemic *P. falciparum* malaria exposure is considered a risk factor for eBL.^{4,27} It is postulated that malaria interferes with EBV-specific immunosurveillance⁴⁰; however, it is unknown whether the severity or duration of malaria infections contributes to eBL pathogenesis. Past studies have shown that common IL-10 promoter variants (-1082A/G, -819T/C, and -592A/C) enhance IL-10 production and are associated with protection against severe malaria anemia.²⁶ Based on this observation, we hypothesized that, if severe malaria contributed to eBL, then children diagnosed with this cancer would be less likely to have protective polymorphisms against severe malaria. The lack of association with IL-10 promoter variants observed in our study is consistent with a lack of protection against eBL associated with the sickle cell trait, another host mutation associated with protection against severe malaria.³¹ These studies do not rule out host genetic variants influencing immune regulation as part of the etiologic pathway to eBL but suggest that future case-control studies use an unbiased approach to uncover novel host genetic factors associated with eBL.

As an EBV immortalized B-cell growth factor,⁴¹ high expression of IL-6 could facilitate eBL tumor progression. Its role in lymphoblastoid cells (to increase tumor burden by facilitating escape from tumor immune surveillance⁴²) may also be one of its effects in eBL development. Thus, it would be expected that an increased frequency of children bearing IL-6 high-expression genotypes would be among the eBL cases. However, a striking bias favoring the low-expressing -174G allele over the high-expressing -174C allele was seen in both groups, which suggests that there is a likely selective advantage for the G allele within this population. Because of the limited sample size available for this study, we had minimal power to detect a relative risk of 1.5 for the -174C allele at 0.7% frequency. However, given the low allele frequency in our study population, we can conclude

that this SNP is not associated with a very strong effect for eBL risk.

Previous studies have also reported that elevated IL-6 and IL-10 levels facilitate survival of EBV-infected B cells through interference with the innate immune defense mechanism against EBV infection.^{43,44} In addition, EBV encodes a functional homologue of IL-10 (BCRF1 protein), which is thought to modulate the immune system to allow persistent viral infection.^{45,46} We, therefore, speculated that children genetically predisposed to produce high levels of these two cytokines would be unable to clear the virus more effectively. However, this study did not observe any association between common IL-6 and IL-10 polymorphisms (and haplotypes for IL-10) and EBV load.

The strengths of our study included an ethnicity- and malaria endemicity-matched case-control design, data on potential effect modifiers, and also, a large number of eBL cases. Although age differed between the groups, with the controls being slightly older than the cases, both groups were within the age range of children at risk of developing eBL (i.e., 5–9 years of age).³² There were several limitations to this study. Because of this study being conducted in a limited-resource setting, diagnosis was not confirmed by immunohistochemistry; therefore, it is possible but highly improbable that BL patients may have been misclassified. This study was limited to testing the association between common genotypic and haplotypic polymorphisms previously identified within the IL-10 and IL-6 promoters^{22,23}; therefore, we were unable to determine if novel polymorphisms were present in our study population. It is also possible that children with very aggressive eBL did not reach the hospital and therefore, were not available for study. Because of the relatively low incidence of pediatric cancer, cases were hospital-based, whereas to match controls by age, ethnicity, and malaria exposure, we selected controls from the community. Sibling comparison studies might diminish confounding, with the caveat that immunity to malaria changes with age and cumulative exposure. We were unable to test the effect of these variants on the circulating levels of IL-10 and IL-6 because of the retrospective nature of this study. *In vitro* studies are underway to determine whether IL-6 and IL-10 production *in vivo* differs between cases and controls to further elucidate the role of these cytokines in eBL pathogenesis. In addition, future unbiased genome-wide studies are being pursued to identify candidate genetic variation, which may uncover novel mechanisms specific to eBL.

CONCLUSION

Our study explored the frequency of three previously identified SNPs in the IL-10 gene and a single SNP in the IL-6 gene promoter in an eBL case-control study of children from western Kenya. We found no allelic or haplotypic associations of IL-6 and IL-10 promoters and risk to eBL. Furthermore, there was no association between these IL-10 and IL-6 promoter SNPs and EBV loads. This study suggests that the IL-10 and IL-6 promoter polymorphisms investigated in this study are not relevant for eBL risk. However, more in-depth human genome studies could reveal immune gene polymorphisms important for eBL pathogenesis in children coinfecting with *P. falciparum* and EBV infection.

Received October 24, 2013. Accepted for publication June 30, 2014.

Published online July 28, 2014.

Note: Supplemental table appears at www.ajtmh.org.

Acknowledgments: The authors thank the parents and guardians for enrolling their children in this study. We are also indebted to the medical staff at Jaramogi Oginga Odinga Teaching and Referral Hospital and the field teams in Nyanza for assisting in both recruitment and blood sample collection. This work is published with the permission of the Director of the Kenya Medical Research Institute.

Financial support: This work was supported by National Institutes of Health Grants 1KL2RR031981 (to J.A.B.), R01AI099473 (to J.A.B.), and R01CA134051 (to A.M.M.) and the Thrasher Research Fund (to A.M.M.).

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REFERENCES

1. Bhatia K, Huppi K, Spangler G, Siwarski D, Iyer R, Magrath I, 1993. Point mutations in the c-Myc transactivation domain are common in Burkitt's lymphoma and mouse plasmacytomas. *Nat Genet* 5: 56–61.
2. Brady G, MacArthur GJ, Farrell PJ, 2007. Epstein-Barr virus and Burkitt lymphoma. *J Clin Pathol* 60: 1397–1402.
3. Bishop PC, Rao VK, Wilson WH, 2000. Burkitt's lymphoma: molecular pathogenesis and treatment. *Cancer Invest* 18: 574–583.
4. Rainey JJ, Omenah D, Sumba PO, Moormann AM, Rochford R, Wilson ML, 2007. Spatial clustering of endemic Burkitt's lymphoma in high-risk regions of Kenya. *Int J Cancer* 120: 121–127.
5. Bornkamm GW, 2009. Epstein-Barr virus and the pathogenesis of Burkitt's lymphoma: more questions than answers. *Int J Cancer* 124: 1745–1755.
6. Orem J, Mbide EK, Lambert B, de Sanjose S, Weiderpass E, 2007. Burkitt's lymphoma in Africa, a review of the epidemiology and etiology. *Afr Health Sci* 7: 166–175.
7. Iversen OH, Iversen U, Ziegler JL, Bluming AZ, 1974. Cell kinetics in Burkitt lymphoma. *Eur J Cancer* 10: 155–163.
8. Chène A, Donati D, Guerreiro-Cacais AO, Levitsky V, Chen Q, Falk KI, Orem J, Kironde F, Wahlgren M, Bejarano MT, 2007. A molecular link between malaria and Epstein-Barr virus reactivation. *PLoS Pathog* 3: e80.
9. Epstein MA, 1984. Burkitt's lymphoma: clues to the role of malaria. *Nature* 312: 398.
10. Richter J, Schlesner M, Hoffmann S, Kreuz M, Leich E, Burkhardt B, Rosolowski M, Ammerpohl O, Wagener R, Bernhart S, Lenze D, Szczepanowski M, Paulsen M, Lipinski S, Russell R, Adam-Klages S, Apic G, Claviez A, Hasenclever D, Hovestadt V, Hornig N, Korb J, Kube D, Langenberger D, Lawerenz C, Lisfeld J, Meyer K, Picelli S, Pischmarov J, Radlwimmer B, Rausch T, Rohde M, Schilhabel M, Scholtysik R, Spang R, Trautmann H, Zenz T, Borkhardt A, Drexler H, Möller P, MacLeod R, Pott C, Schreiber S, Trümper L, Loeffler M, Stadler P, Lichter P, Eils R, Küppers R, Hummel M, Klapper W, Rosenstiel P, Rosenwald A, Brors B, Siebert R; MML-Seq Project ICGC, 2012. Recurrent mutation of the ID3 gene in Burkitt lymphoma identified by integrated genome, exome and transcriptome sequencing. *Nat Genet* 44: 1316–1320.

11. Love C, Sun Z, Jima D, Li G, Zhang J, Miles R, Richards K, Dunphy C, Choi W, Srivastava G, Lugar P, Rizzieri D, Lagoo A, Bernal-Mizrachi L, Mann K, Flowers C, Naresh K, Evens A, Chadburn A, Gordon L, Czader M, Gill J, Hsi E, Greenough A, Moffitt A, McKinney M, Banerjee A, Grubor V, Levy S, Dunson D, Dave S, 2012. The genetic landscape of mutations in Burkitt lymphoma. *Nat Genet* 44: 1321–1325.
12. Howell WM, Rose-Zerilli MJ, 2007. Cytokine gene polymorphisms, cancer susceptibility, and prognosis. *J Nutr* 137: 194S–199S.
13. Lech-Maranda E, Baseggio L, Bienvenu J, Charlot C, Berger F, Rigal D, Warzocha K, Coiffier B, Salles G, 2004. Interleukin-10 gene promoter polymorphisms influence the clinical outcome of diffuse large B-cell lymphoma. *Blood* 103: 3529–3534.
14. Cooper M, Caligiuri M, 2003. *Cytokines and Cancer*. The Cytokine Handbook (Fourth Edition). Elsevier Ltd., Academic Press, 1213–1232. ISBN: 978-0-12-689663-3.
15. Ogden CA, Pound JD, Batth BK, Owens S, Johannessen I, Wood K, Gregory CD, 2005. Enhanced apoptotic cell clearance capacity and B cell survival factor production by IL-10-activated macrophages: implications for Burkitt's lymphoma. *J Immunol* 174: 3015–3023.
16. Yokoi T, Miyawaki T, Yachie A, Kato K, Kasahara Y, Taniguchi N, 1990. Epstein-Barr virus-immortalized B cells produce IL-6 as an autocrine growth factor. *Immunology* 70: 100–105.
17. Cordano P, Lake A, Shield L, Taylor GM, Alexander FE, Taylor PRA, White J, Jarrett RF, 2005. Effect of IL-6 promoter polymorphism on incidence and outcome in Hodgkin's lymphoma. *Br J Haematol* 128: 493–495.
18. Benjamin D, Knobloch TJ, Dayton MA, 1992. Human B-cell interleukin-10: B-cell lines derived from patients with acquired immunodeficiency syndrome and Burkitt's lymphoma constitutively secrete large quantities of interleukin-10. *Blood* 80: 1289–1298.
19. Stewart JP, Behm FG, Arrand JR, Rooney CM, 1994. Differential expression of viral and human interleukin-10 (IL-10) by primary B cell tumors and B cell lines. *Virology* 200: 724–732.
20. Cunningham LM, Chapman C, Dunstan R, Bell MC, Joske DJL, 2003. Polymorphisms in the interleukin 10 gene promoter are associated with susceptibility to aggressive non-Hodgkin's lymphoma. *Leuk Lymphoma* 44: 251–255.
21. Liu J, Song B, Bai X, Liu W, Li Z, Wang J, Zheng Y, Wang Z, 2010. Association of genetic polymorphisms in the interleukin-10 promoter with risk of prostate cancer in Chinese. *BMC Cancer* 10: 456.
22. Ferrari SL, Ahn-Luong L, Garnerio P, Humphries SE, Greenspan SL, 2003. Two promoter polymorphisms regulating interleukin-6 gene expression are associated with circulating levels of C-reactive protein and markers of bone resorption in postmenopausal women. *J Clin Endocrinol Metab* 88: 255–259.
23. Wang J, Ding Q, Shi Y, Cao Q, Qin C, Zhu J, Chen J, Yin C, 2011. The interleukin-10-1082 promoter polymorphism and cancer risk: a meta-analysis. *Mutagenesis* 27: 305–312.
24. Gourley IS, Kurtis JD, Kamoun M, Amon JJ, Duffy PE, 2002. Profound bias in interferon- γ and interleukin-6 allele frequencies in western Kenya, where severe malarial anemia is common in children. *J Infect Dis* 186: 1007–1012.
25. Baran W, Szepietowski JC, Mazur G, Baran E, 2008. IL-6 and IL-10 promoter gene polymorphisms in psoriasis vulgaris. *Acta Derm Venereol* 88: 113–116.
26. Ouma C, Davenport GC, Were T, Otieno MF, Hittner JB, Vulule JM, Martinson J, Ong'echa JM, Ferrell RE, Perkins DJ, 2008. Haplotypes of IL-10 promoter variants are associated with susceptibility to severe malarial anemia and functional changes in IL-10 production. *Hum Genet* 124: 515–524.
27. Burkitt D, 1962. A lymphoma syndrome in African children. *Ann R Coll Surg Engl* 30: 211–219.
28. Burkitt D, O'Connor G, 1961. Malignant lymphoma in African children. I. A clinical syndrome. *Cancer* 14: 258–269.
29. Cozen W, Gill PS, Ingles SA, Masood R, Martínez-Maza O, Cockburn MG, Gauderman WJ, Pike MC, Bernstein L, Nathwani BN, Salam MT, Danley KL, Wang W, Gage J, Gundell-Miller S, Mack TM, 2004. IL-6 levels and genotype are associated with risk of young adult Hodgkin lymphoma. *Blood* 103: 3216–3221.
30. López RVM, Zago MA, Eluf-Neto J, Curado MP, Daudt AW, da Silva-Junior WA, Zanette DL, Levi JE, de Carvalho MB, Kowalski LP, Abrahão M, de Góis-Filho JF, Boffetta P, Wünsch-Filho V, 2011. Education, tobacco smoking, alcohol consumption, and IL-2 and IL-6 gene polymorphisms in the survival of head and neck cancer. *Braz J Med Biol Res* 44: 1006–1012.
31. Mulama DH, Bailey JA, Foley J, Chelimo K, Ouma C, Jura WGZO, Otieno J, Vulule J, Moormann AM, 2014. Sickle cell trait is not associated with endemic Burkitt lymphoma: an ethnicity and malaria endemicity-matched case-control study suggests factors controlling EBV may serve as a predictive biomarker for this pediatric cancer. *Int J Cancer* 134: 645–653.
32. Moormann AM, Chelimo K, Sumba OP, Lutzke ML, Ploutz-Snyder R, Newton D, Kazura J, Rochford R, 2005. Exposure to holoendemic malaria results in elevated Epstein-Barr virus loads in children. *J Infect Dis* 191: 1233–1238.
33. Moormann AM, Chelimo K, Sumba PO, Tisch DJ, Rochford RA, Kazura JW, 2007. Exposure to holoendemic malaria results in suppression of Epstein-Barr virus-specific T cell immunosurveillance in Kenyan children. *J Infect Dis* 195: 799–808.
34. Moormann AM, Lozada JS, 2009. Burkitt lymphoma in Uganda: 50 years of ongoing discovery. *Pediatr Blood Cancer* 52: 433–434.
35. Caligiuri MA, Lotze MT; SpringerLink, 2007. *Cytokines in the Genesis and Treatment of Cancer*. Totowa, NJ: Humana Press.
36. Voorzanger N, Touitou R, Garcia E, Delecluse HJ, Rousset F, Joab I, Favrot MC, Blay JY, 1996. Interleukin (IL)-10 and IL-6 are produced *in vivo* by non-Hodgkin's lymphoma cells and act as cooperative growth factors. *Cancer Res* 56: 5499–5505.
37. Mocellin S, Marincola FM, Young HA, 2005. Interleukin-10 and the immune response against cancer: a counterpoint. *J Leukoc Biol* 78: 1043–1051.
38. da Silva GN, Bacchi MM, Rainho Cu A, de Oliveira DE, 2007. Epstein-Barr virus infection and single nucleotide polymorphisms in the promoter region of interleukin 10 gene in patients with Hodgkin lymphoma. *Arch Pathol Lab Med* 131: 1691–1696.
39. Minnicelli C, Barros MHM, Klumb CE, Romano SO, Zalberg IR, Hassan R, 2012. Relationship of Epstein-Barr virus and interleukin 10 promoter polymorphisms with the risk and clinical outcome of childhood Burkitt lymphoma. *PLoS ONE* 7: e46005.
40. Whittle HC, Brown J, Marsh K, Greenwood BM, Seidlin P, Tighe H, Wedderburn L, 1984. T-cell control of Epstein-Barr virus-infected B cells is lost during *P. falciparum* malaria. *Nature* 312: 449–450.
41. Tosato G, Tanner J, Jones KD, Revel M, Pike SE, 1990. Identification of interleukin-6 as an autocrine growth factor for Epstein-Barr virus-immortalized B cells. *J Virol* 64: 3033–3041.
42. Tanner J, Tosato G, 1991. Impairment of natural killer functions by interleukin 6 increases lymphoblastoid cell tumorigenicity in athymic mice. *J Clin Invest* 88: 239–247.
43. Biron CA, Brossay L, 2001. NK cells and NKT cells in innate defense against viral infections. *Curr Opin Immunol* 13: 458–464.
44. Helminen M, Lahdenpohja N, Hurme M, 1999. Polymorphism of the interleukin-10 gene is associated with susceptibility to Epstein-Barr virus infection. *J Infect Dis* 180: 496–499.
45. Liu Y, de Waal Malefyt R, Briere F, Parham C, Bridon JM, Banchereau J, Moore KW, Xu J, 1997. The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10 receptor. *J Immunol* 158: 604–613.
46. Zeidler R, Eissner G, Meissner P, Uebel S, Tampé R, Lazis S, Hammerschmidt W, 1997. Downregulation of TAP1 in B lymphocytes by cellular and Epstein-Barr virus-encoded interleukin-10. *Blood* 90: 2390–2397.
47. Ogwang MD, Bhatia K, Biggar RJ, Mbulaiteye SM, 2008. Incidence and geographic distribution of endemic Burkitt lymphoma in northern Uganda revisited. *Int J Cancer* 123: 2658–2663.