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IFNL4 Genotypes and Risk of Childhood Burkitt Lymphoma in East Africa

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Interferon lambda 4 (IFN-λ4) is a novel type-III interferon that can be expressed only by carriers of the genetic variant rs368234815-dG within the first exon of the *IFNL4* gene. Genetic inability to produce IFN-λ4 (in carriers of the rs368234815-TT/TT genotype) has been associated with improved clearance of hepatitis C virus (HCV) infection. The IFN-λ4-expressing rs368234815-dG allele (*IFNL4*-dG) is most common (up to 78%) in West sub-Saharan Africa (SSA), compared to 35% of Europeans and 5% of individuals from East Asia. The negative selection of *IFNL4*-dG outside Africa suggests that its retention in African populations could provide survival benefits, most likely in children. To explore this hypothesis, we conducted a comprehensive association analysis between *IFNL4* genotypes and the risk of childhood Burkitt lymphoma (BL), a lethal infection-associated cancer most common in SSA. We used genetic, epidemiologic, and clinical data for 4,038 children from the Epidemiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) and the Malawi Infections and Childhood Cancer case–control studies. Generalized linear mixed models fit with the logit link controlling for age, sex, country, *P. falciparum* infection status, population stratification, and relatedness found no significant association between BL risk and 3 coding genetic variants within *IFNL4* (rs368234815, rs117648444, and rs142981501) and their combinations. Because BL occurs in children 6–9 years of age who survived early childhood infections, our results suggest that additional studies should explore the associations of *IFNL4*-dG allele in younger children. This comprehensive study represents an important baseline in defining the health effects of IFN-λ4 in African populations.

Keywords: interferon Lambda 4, *IFNL4*, Burkitt Lymphoma, genetic susceptibility, infection

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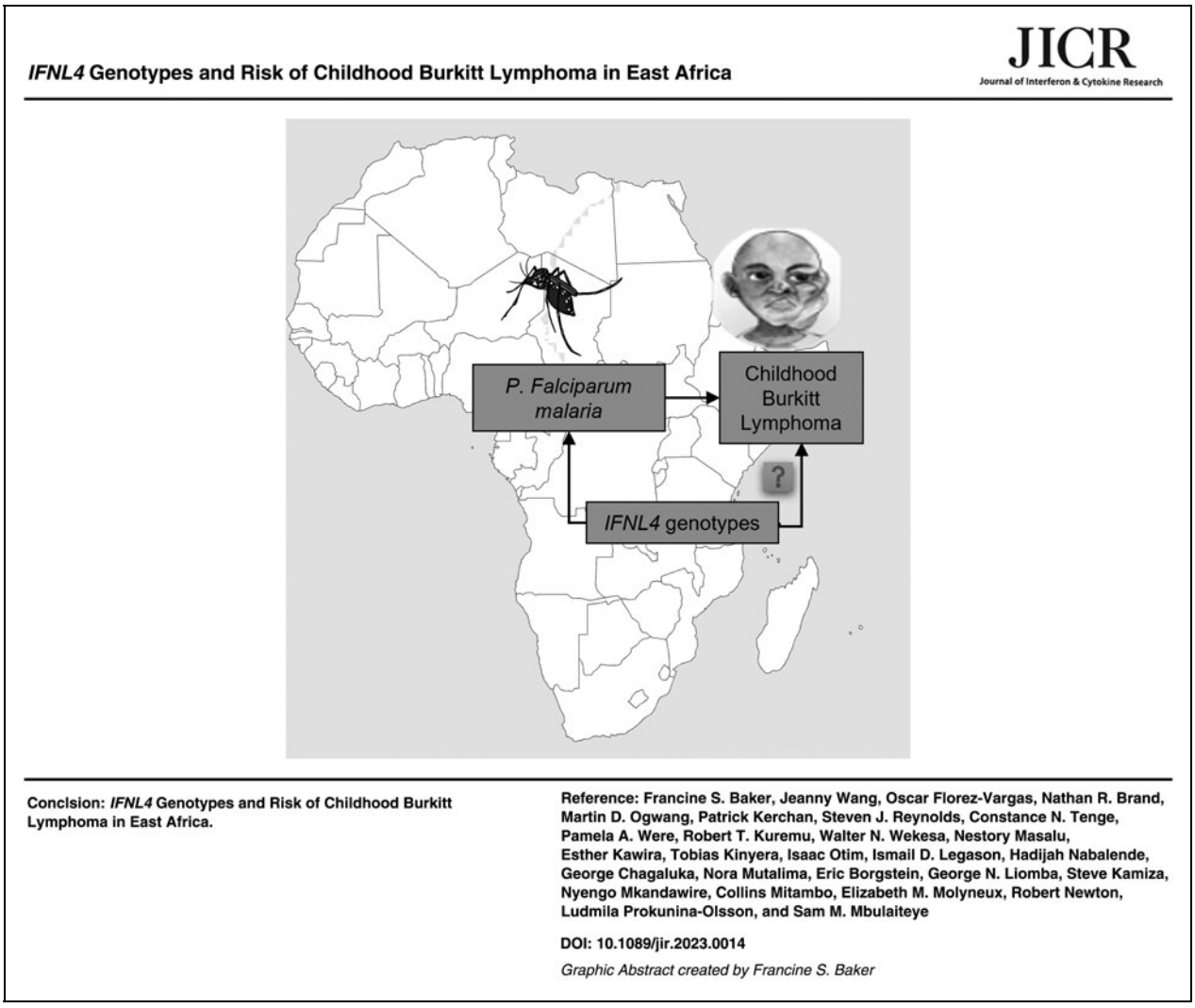

Introduction

Type III interferons (IFNs) are antiviral cytokines that are critical to the innate immune response against different pathogens, including hepatitis C virus (HCV) (Hamming et al., 2013; Prokunina-Olsson et al., 2013), human immunodeficiency virus (HIV) (Freitas et al., 2020), cytomegalovirus (CMV) (Bibert et al., 2014; Wack et al., 2015), coronavirus (CoV) (Hamming et al., 2013), respiratory RNA viruses (Rugwizangoga et al., 2019), and infection with *Plasmodium (P.) falciparum*, a parasite that can cause clinical malaria in humans (Prokunina-Olsson et al., 2021; Samayoa-Reyes et al., 2021).

There are 4 type III IFNs: IFN-λ1 (IL-29), IFN-λ2 (IL28A), IFN-λ3 (IL-28B) (Kotenko et al., 2003; Sheppard et al., 2003), and IFN-λ4 (Prokunina-Olsson et al., 2013). Interferon-λ4 (IFN-λ4) can be produced only by carriers of the genetic variant rs368234815-dG (referred to as *IFNL4*-dG) within the first exon of the *IFNL4* gene, but not by individuals with the homozygous rs368234815-TT/TT genotype (Prokunina-Olsson et al., 2013). The IFN-λ4 activity might be further modified by the missense variants within the second exon of *IFNL4*: rs117648444-A/G (P70S) and rs142981501-C/G (R60P, African specific) (Prokunina-Olsson, 2019; Prokunina-Olsson et al., 2013; Terczyńska-Dyla et al., 2014). P70S attenuates the ability of IFN-λ4 to induce interferon-stimulated genes, whereas R60P might affect IFN-λ4 function through its glycosylation at an adjacent amino acid N61 (Bamford et al., 2018).

Similar to other interferons, *IFNL4* expression is induced in response to viral infection, but the rs368234815-dG/TT polymorphism within the first exon of *IFNL4* determines which version of the protein is produced from the induced transcripts—a functional IFN-λ4 protein (by the dG allele) or a truncated nonfunctional frame-shifted protein unrelated to IFN-λ4 (by the TT allele) (Prokunina-Olsson et al., 2013).

Thus, genotypes can be used to infer the possibility of producing IFN-λ4 by an individual. *IFNL4* variation emerged as a major genetic factor for HCV clearance in studies conducted in high-income countries (Prokunina-Olsson et al., 2013), with individuals carrying genotypes not supporting IFN-λ4 expression achieving better viral clearance. The frequency of the ancestral dG is the highest in Africa, reaching 78% in West Africa, while sharply lower in non-African populations (Prokunina-Olsson et al., 2013). This pattern was linked with negative selection for IFN-λ4 outside of Africa (Key et al., 2014), but the possible benefits of retaining IFN-λ4 in African populations remain undetermined.

In view of the reports of associations between *IFNL4* genotypes and infections in African children—with respiratory RNA viruses (Rugwizangoga et al., 2019) and clinical malaria (Prokunina-Olsson et al., 2021; Samayoa-Reyes et al., 2021)—we wondered whether *IFNL4* genotypes might be associated with childhood Burkitt lymphoma (BL). BL is an aggressive B cell non-Hodgkin lymphoma, frequently presenting as jaw or abdominal tumors. BL incidence in children is high in countries where *P. falciparum* infection occurs year-round (Burkitt, 1969). BL risk is increased through dysregulation of the immune response to Epstein-Barr virus (EBV) infection (Burkitt, 1969; Epstein et al., 1964; Mbulaiteye and Devesa, 2022; Torgbor et al., 2014), a DNA gamma-2 herpesvirus that is ubiquitous and occurs predominantly in the settings of *P. falciparum* infection.

EBV and *P. falciparum* influence BL risk by promoting *IG*::*MYC* translocations (Alaggio et al., 2022; Mbulaiteye and Devesa, 2022), an early and possibly primary abnormality in BL (Basso and Dalla-Favera, 2015). BL occurs between 6 and 9 years of age in children with chronic infection exposure, who survived *P. falciparum* infection earlier in life. The association of the *IFNL4*-dG allele with increased risk of clinical malaria in children in Mali (Prokunina-Olsson et al., 2021) and Kenya (Samayoa-Reyes et al., 2021) prompted us to investigate the association between *IFNL4* genotypes with BL. Considering the strong associations reported with different viral pathogens (predominantly RNA viruses), we were also interested in exploring whether BL, a cancer associated with EBV (a DNA virus), might serve as a sentinel condition to detect other genetic associations for *IFNL4* in African populations.

We comprehensively investigated associations between *IFNL4* genotypes and the risk of childhood BL among 4,038 children (720 BL cases and 3,318 controls) from Uganda, Tanzania, Kenya, and Malawi in East Africa. We aimed to explore the patterns of *IFNL4* genotypes in a large pediatric population in Africa and investigate associations of these genotypes with BL risk in case–control studies.

Materials and Methods

Study population

The participants were from 2 previously described (Maziarz et al., 2018; Maziarz et al., 2017; Mutalima et al., 2008; Peprah et al., 2020, Peprah et al., 2019a, Peprah et al., 2019b; Simbiri et al., 2014) case–control studies—the Epidemiology of Burkitt Lymphoma in East African Children and Minors (EMBLEM) study (Simbiri et al., 2014) and the Malawi Infections and Childhood Cancer study (Mutalima et al., 2008). Briefly, The EMBLEM study enrolled population-based cases and controls younger than 16 years from Uganda, Kenya, and Tanzania (Peprah et al., 2020). The BL cases were enrolled at participating hospitals before the initiation of treatment. The controls were apparently healthy children enrolled from 295 villages randomly selected from the 6 enrollment regions, with frequency matching the historical case age (± 2 years) and sex distributions in each country (Peprah et al., 2020).

The Malawi study enrolled children with cancer from the Queen Elizabeth Hospital in Blantyre. Children with BL were categorized as cases, while children with solid cancers were enrolled as controls. Venous blood was collected in EDTA tubes and stored at −80°C until DNA extraction. *P. falciparum* infection status was considered positive based on results of thick blood film microscopy, antigen rapid diagnostic tests (RDT) in EMBLEM (Peprah et al., 2020), or polymerase chain reaction (PCR) for *P. falciparum*-specific sequences in Malawi study (Arisue et al., 2021), and negative otherwise.

Ethics approvals

Ethical approval for the EMBLEM study was granted by the Uganda Virus Research Institute Research and Ethics Committee, Uganda National Council for Science and Technology (H816), Tanzania National Institute for Medical Research (NIMR/HQ/R.8c/Vol. IX/1023), Moi University/Moi Teaching and Referral Hospital Institutional Research and Ethics Committee (000536), and National Cancer Institute (NCI) Institutional Review Board (10-C-N133). The Infections and Childhood Cancer study received approval from the Malawian College of Medicine Research and Ethics Committee (P.03/04/277R) and the Office of Human Subjects Research at the National Institutes of Health (Exemption #: 4742). Written informed consent was obtained from guardians of all the children and from children 7 years of age or older.

DNA extraction and genotyping

DNA extraction was performed using the Qiagen QIAsymphony automated instrument at the NCI Cancer Genomics Research (CGR) Laboratory (Legason et al., 2017). *IFNL4* variants rs368234815, rs117648444, and rs142981501 were genotyped using custom TaqMan genotyping assays at the Laboratory of Translational Genomics, as previously described (Prokunina-Olsson et al., 2013), using QuantStudio 7 Flex Real-Time PCR System, in 384-well plates, with 5 ng of DNA per 5-μL reactions. Genotyped duplicates showed 100% concordance. The distribution of all 3 genotyped markers was consistent with Hardy-Weinberg equilibrium (HWE, $P < 0.05$) both in cases and controls.

Statistical analysis

Generalized linear mixed models (GLMM) were fit using the generalized linear mixed model association test (GMMAT) R package version 1.3.2 with the logit link to assess the associations of genotypes with BL as odds ratios (OR) and 95% confidence intervals (CI), controlling for age, sex, country, *P. falciparum* infection status (positive/negative), the first 3 population-specific principal components (PCs) as fixed effects, and the genetic relationship matrix (GRM) as a random variable. PCs and GRM were generated in our previous studies (Chen et al., 2016; Hong et al., 2022) to control for potential population stratification and increased relatedness among the participants. *IFNL4* genotypes were coded as 0, 1, and 2 for TT/TT, TT/dG, and dG/dG, respectively.

As rs117648444 and rs142981501 are missense variants that reside on mutually exclusive haplotypes with the dG allele (IFN-λ4 is produced) (Prokunina-Olsson, 2019), we used the genotype combinations of these alleles together with rs368234815 to infer IFN-λ4-P70S and IFN-λ4-R60P groups (Table 3). As in previous analyses (Gadalla et al.,

2020; Hamming et al., 2013), the P70S group, defined based on the combination of rs368234815 and rs117648444, comprised 4 categories (IFN-λ4-null, weak IFN-λ4-70S, moderate IFN-λ4-70S/70P, and strong IFN-λ4-70P). IFN-λ4-R60P was not previously studied, but based on the combination of rs368234815 and rs142981501, we defined categories as IFN-λ4-Null, IFN-λ4-60R (one copy), IFN-λ4-60R (2 copies), and IFN-λ4-60P/60R or 60P/60P (this group included one or 2 copies of the uncommon African-specific 60P allele).

Two-sided Wald-test $P < 0.05$ was considered statistically significant. To assess the validity of our *IFNL4* association analyses, we used a positive control, carriage of the sickle cell trait (SCT), which is associated with protection against severe malaria and BL (Legason et al., 2017). The study was designed to detect a minimum OR = 1.2 with 87% power, assuming alpha = 0.05, an additive genetic model, and a minor allele frequency of 40% for an effect allele. Thus, our sample size (720 cases and 3,318 controls) is well powered to detect with confidence any effect with OR >1.2, but might miss weaker effects.

The estimated age of the *IFNL4* genetic variants was looked up in the Human Genome Dating database https://human.genome.dating/ (Albers and McVean, 2020).

Results

Study population characteristics

Table 1 shows the characteristics of 720 BL cases and 3,318 controls. There were 454 (63.1%) males among cases compared with 1,720 (52.1%) among controls. Most of the children were between 3 and 11 years of age, 9.7% were younger than 2 years, and 16.1% were older than 12 years. Positivity to *P. falciparum* was detected in 256 (35.6%) BL cases compared with 1,636 (49.3%) controls. The sickle cell

Table 1. Characteristics of the Study Population

Study characteristics	*Cases* N = *720 (%)*	*Controls* N = *3,318 (%)*	*Total* N = *4,038 (%)*
Sex			
Male	454 (63.1)	1,730 (52.1)	2,184 (54.1)
Female	266 (36.9)	1,588 (47.9)	1,854 (45.9)
Age, years			
0–2	64 (8.9)	326 (9.8)	390 (9.7)
3–5	196 (27.2)	812 (24.5)	1,008 (25.0)
6–8	213 (29.6)	952 (28.7)	1,165 (28.9)
9–11	152 (21.1)	674 (20.3)	826 (20.5)
12–15	95 (13.2)	554 (16.7)	649 (16.1)
Country			
Uganda	182 (25.3)	1,524 (45.9)	1,706 (42.2)
Tanzania	96 (13.3)	745 (22.5)	841 (20.8)
Kenya	227 (31.5)	858 (25.9)	1,085 (26.9)
Malawi	215 (29.9)	191 (5.8)	406 (10.1)
P. falciparum infection			
Negative	449 (62.4)	1,658 (50.0)	2,107 (52.2)
Positive	256 (35.6)	1,636 (49.3)	1,892 (46.9)
Unknown	15 (2.1)	24 (0.7)	39 (1.0)
HBB-rs334			
A/A (wild type)	644 (89.4)	2,728 (82.2)	3,372 (83.5)
A/T (sickle cell trait)	75 (10.4)	569 (17.1)	644 (15.9)
T/T (sickle cell disease)	1 (0.1)	21 (0.6)	22 (0.5)
IFNL4 variants			
rs368234815			
TT/TT (IFN-λ4-Null)	122 (16.9)	519 (15.6)	641 (15.9)
TT/dG (IFN-λ4, 1 copy)	343 (47.6)	1,550 (46.7)	1,893 (46.9)
dG/dG (IFN-λ4, 2 copies)	255 (35.4)	1,249 (37.6)	1,504 (37.2)
TT	587 (41.0)	2,588 (39.0)	3,175 (39.0)
dG	853 (59.0)	4,048 (61.0)	4,901 (61.0)
rs117648444			
G/G	635 (88.2)	2,916 (87.9)	3,551 (87.9)
A/G	85 (11.8)	391 (11.8)	476 (11.8)
A/A	0 (0.0)	11 (0.3)	11 (0.3)
G	1,355 (94.0)	6,223 (94.0)	7,578 (94.0)
A	85 (6.0)	413 (6.0)	498 (6.0)
rs142981501			
C/C	649 (90.1)	2,985 (90.0)	3,634 (90.0)
C/G	71 (9.9)	322 (9.7)	393 (9.7)
G/G	0 (0.0)	11 (0.3)	11 (0.3)
C	1,369 (95.0)	6,229 (95.0)	7,661 (95.0)
G	71 (5.0)	344 (5.0)	415 (5.0)

allele *HBB*-rs334-T was present in 77 (5.0%) cases compared with 611 (9.0%) controls. The distribution of *IFNL4* genotypes in our study population was comparable to that reported in Kenya's Luhya (LWK) population in the 1000 Genomes Project (56%, Table 2). Notably, the *IFNL4*-dG allele frequency was much lower (58%–64%) in all East-African countries in our study compared to 71%–78% in West-African countries in the 1000 Genomes project (Table 2).

IFNL4 genetic variants and BL risk

Results of the association for *IFNL4* genotypes with BL risk are shown in Table 3. Compared with those carrying *IFNL4*-TT/TT genotype (IFN-λ4-Null group), the ORs for BL among those with TT/dG and dG/dG genotypes were 0.91 (0.70–1.18) and 0.86 (0.66–1.13), respectively. Compared to the same IFN-λ4-Null reference group, the ORs for BL risk were 0.80 (0.51–1.26) for weak (IFN-λ4-70S), 0.90 (0.70–1.17) for moderate (IFN-λ4-70S/70P), and 0.88 (0.67–1.17) for strong IFN-λ4-70P protein (Table 3). Similarly, compared to the IFN-λ4-Null reference group, the ORs were 0.89 (0.68–1.15) for one copy of IFN-λ4-60R, 0.88 (0.67–1.16) for 2 copies of IFN-λ4-60R, and 0.94 (0.65–1.36) for the uncommon group with one or 2 copies of IFN-λ4-60P (Table 3).

In line with the results of our previous studies (Hong et al., 2022; Legason et al., 2017), BL risk was inversely associated with the carriage of the SCT (OR = 0.67, 95% CI: 0.51–0.88, $P = 3.83E\text{-}03$). Including the sickle cell status in statistical models did not change associations for *IFNL4* variants (data not shown). Similarly, the inclusion of *IFNL4* variants did not affect the association between BL risk and the SCT (OR = 0.67, 95% CI: 0.54–0.87, $P = 3.07E\text{-}3$).

In this study, *P. falciparum* infection status was ascertained once, only at enrollment. Cross-sectional infection in older children (median age >7 years in our study) is a poor predictor of the burden of multiple prior infections. Thus, we did not test for the association between *P. falciparum* infection and *IFNL4* variants as was done in previous studies of children 0–5 years of age (Prokunina-Olsson et al., 2021; Samayoa-Reyes et al., 2021).

Discussion

In this study, we report the results from a large association study between *IFNL4* genetic variants and BL risk conducted in 4,038 children (720 with BL and 3,318 controls) from 4 countries in East Africa. Although we found no significant association between *IFNL4* variants and BL risk in East African children, the findings represent important baseline research to define the potential health effects of IFN-λ4 in African populations. The study was sufficiently large and incorporated careful adjustment for population substructure, genetic relatedness, and *P. falciparum* infection. The results indicate that strong effects, such as association between BL and carriage of the SCT allele (Legason et al., 2017), were not missed by our study, but weaker effects might still be undetectable.

Interest in understanding the health effects of IFN-λ4 is stimulated by findings in evolutionary studies reporting that the *IFNL4*-dG allele, which defines the production of IFN-λ4, is retained at a high frequency in populations in Africa, suggesting that it might confer benefit in those settings (Prokunina-Olsson et al., 2013). In contrast, the *IFNL4*-TT allele, which abrogates IFN-λ4 production, has been rapidly selected in populations that migrated out of Africa, suggesting that environmental factors necessitating *IFNL4*-dG allele retention are enriched in Africa (Key et al., 2014).

We note that other explanations, such as migration, admixture, and genetic drift, might have also influenced the distribution of *IFNL4* alleles in human populations. Currently, known associations with the *IFNL4*-dG allele, such as the reduced clearance of HCV (Aka et al., 2014; O'Brien et al., 2014; Obajemu et al., 2017; Prokunina-Olsson et al., 2013), increased risk of virus-related upper respiratory tract infections among children in Rwanda (Rugwizangoga et al., 2019), sexually transmitted infections in African American men with prostate cancer (Jenkins et al., 2022; Minas et al., 2018; Rugwizangoga et al., 2019), and the absence of association with HIV-related opportunistic infections and cancers (Fang et al., 2022), do not explain why this allele is retained at a high frequency in African populations.

Thus, given that BL is a highly lethal condition that is more than 20-fold common in Africa compared to Asia and Europe (López et al., 2022) and is also associated with *P. falciparum* infection (WHO, 2021), we were interested in ascertaining whether *IFNL4* may contribute to the geographical patterns of BL. However, the results of our association analysis do not support the presence of a strong association between *IFNL4* variants and BL risk.

Because BL predominantly occurs at 6–9 years of age (Redmond et al., 2020), yet most malaria mortality occurs below 5 years, the *IFNL4*-dG allele might provide survival benefits at an earlier age, not covered by our study. For example, while *P. falciparum* is a strong environmental risk factor for BL, our study includes cases and controls that were well matched on age and, therefore, likely similar regarding factors that influence survival against early-life malaria-related deaths (WHO, 2021). Thus, future studies on the health effects of *IFNL4*-dG should focus on younger children where differences in genotypes, including for *IFNL4* variants, may influence survival. Although the *IFNL4*-dG is overall most common in individuals of African ancestry compared to all other ancestries, within Africa, its frequency is the highest in West Africa (71–78%).

The lower frequency of *IFNL4*-dG in our study in East Africa (58–64%), and similar to that of the Luhya (LWK) population in the 1000 Genomes Project (56%), might be reflective of variable selection forces on this allele, perhaps due to local infectious environments. Notably, the derived allele rs368234815-TT (represented by rs74597329-T in the 1000 Genomes Project) that eliminates IFN-λ4 emerged ~1,349,333 years (~54 K generations) ago. The other variants also potentially attenuating IFN-λ4 activity emerged on the rs368234815-dG haplotype later—the derived allele rs117648444-A emerged 259,798 years (~10.4 K generations) ago and the derived rs142981501-G allele only 142,820 years (~5.7 K generations) ago. The age of these alleles might also reflect their current geographical distribution pattern.

The strengths of our study include large sample size, high-quality genomic data, control for population substructure, genetic relatedness, and important confounders. In addition, this is the first genetic analysis of an *IFNL4* missense variant (rs142981501, R60P), which is present at up to

Table 2. Distribution of IFNL4 Variants rs368234815, rs117648444, and rs142981501 in Populations of African Ancestry from the 1000 Genomes Project and 4,038 Children from the EMBLEM and Childhood Infections and Cancer Case–Control Study in Malawi

		ACB	*ASW*	*LWK*	*ESN*	*GWD*	*MSL*	*YRI*	*EMBLEM*			*Malawi*
		Caribbean	*USA*	*Kenya*	*Nigeria*	*The Gambia*	*Sierra Leone*	*Nigeria*	*Uganda*	*Tanzania*	*Kenya*	*Malawi*
Gene-variant	*Alleles or genotypes*	N = *96*	N = *61*	N = *99*	N = *99*	N = *113*	N = *85*	N = *108*	N = *1466*	N = *838*	N = *1071*	N = *351*
IFNL4-	TT	0.26	0.3	**0.44**	0.28	0.22	0.29	0.27	0.39	0.41	**0.42**	0.37
rs368234815	dG	0.75	0.7	**0.56**	0.72	0.78	0.71	0.73	0.62	0.60	**0.58**	0.64
(represented by rs74597329	TT/TT	0.08	0.1	**0.14**	0.09	0.05	0.07	0.09	0.16	0.15	**0.19**	0.12
in 1000 Genomes Project)	TT/dG	0.34	0.41	**0.61**	0.38	0.34	0.44	0.35	0.45	0.51	**0.45**	0.49
	dG/dG	0.57	0.49	**0.25**	0.53	0.61	0.49	0.56	0.39	0.34	**0.35**	0.39
IFNL4-	A	0.09	0.06	**0.07**	0.05	0.11	0.04	0.10	0.05	0.06	**0.07**	0.08
P70S	G	0.91	0.94	**0.93**	0.95	0.89	0.96	0.90	0.95	0.94	**0.93**	0.92
rs117648444	A/A	0.01	0	**0.01**	0.03	0.01	0	0	0.001	0.005	**0.004**	0.003
	A/G	0.16	0.12	**0.11**	0.03	0.20	0.08	0.19	0.10	0.11	**0.14**	0.16
	G/G	0.83	0.88	**0.88**	0.94	0.79	0.92	0.81	0.9	0.89	**0.86**	0.84
IFNL4-	C	0.97	0.93	**0.95**	0.97	0.94	0.94	0.97	0.95	0.94	**0.95**	0.95
R60P	G	0.03	0.07	**0.05**	0.03	0.06	0.06	0.03	0.05	0.06	**0.05**	0.05
rs142981501	C/C	0.95	0.87	**0.90**	0.94	0.88	0.89	0.94	0.90	0.88	**0.91**	0.90
	C/G	0.05	0.13	**0.10**	0.06	0.12	0.09	0.06	0.10	0.11	**0.09**	0.1
	G/G	0	0	**0**	0	0	0.01	0	0.004	0.002	**0.002**	0

Data for the 1000 Genomes Project populations of African ancestry (AFR) include Yoruba in Ibadan Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Mandinka in The Gambia (GWD), Mende in Sierra Leone (MSL), Esan in Nigeria (ESN), Americans of African Ancestry in South-West USA (ASW), and African Caribbean in Barbados (ACB), based on information from https://www.internationalgenome.org/1000-genomes-browsers/. EMBLEM and Malawi datasets include data for all 4,038 children (cases and controls). In bold, data from Kenya as the 1000 Genomes population most similar to our study populations from East Africa.

Table 3. Odds Ratios and 95% Confidence Intervals for the Associations Between IFNL4 Variants and Burkitt Lymphoma Risk

IFNL4 Genotypes rs368234815		*Frequency (n, %)*			
		Cases	*Controls*	*OR (95% CI)*[a]	P-*value*[a]
IFN-λ4-Null	TT/TT	122 (16.9)	519 (15.6)	ref	
IFN-λ4	TT/dG	343 (47.6)	1,550 (46.7)	0.91 (0.70–1.18)	0.48
IFN-λ4	dG/dG	255 (35.4)	1,249 (37.6)	0.86 (0.66–1.13)	0.28
P70S Group rs368234815 and rs117648444					
IFN-λ4-Null	TT/TT + G/G	122 (16.9)	519 (15.6)	ref	
Weak IFN-λ4-70S	TT/dG + A/G dG/dG + A/A	42 (5.8)	177 (5.3)	0.80 (0.51–1.26)	0.34
Moderate IFN-λ4-70S/70P	TT/dG + G/G dG/dG + A/G	344 (57.8)	1609 (48.5)	0.90 (0.70–1.17)	0.44
Strong IFN-λ4-70P	dG/dG + G/G	212 (29.4)	1013 (30.5)	0.88 (0.67–1.17)	0.39
R60P Group rs368234815 and rs142981501					
IFN-λ4-Null	TT/TT + C/C	122 (16.9)	519 (15.6)	ref	
IFN-λ4-60R, one copy	TT/dG + C/C	309 (42.9)	1434 (43.4)	0.89 (0.68–1.15)	0.36
IFN-λ4-60R, 2 copies	dG/dG + C/C	218 (30.3)	1032 (31.1)	0.88 (0.67–1.16)	0.37
IFN-λ4-60R/60P or 60P/60P	TT/dG + C/G dG/dG + C/G dG/dG + G/G	71 (9.9)	333 (10.0)	0.94 (0.65–1.36)	0.74

[a]Adjusted for age, sex, country, malaria, PCs, and relatedness.

7% allele frequency only in individuals of African ancestry, although we still had limited power to detect significant associations. The thorough adjustment of local geographical factors between BL cases and the controls further strengthened our study. This analysis helped to exclude *IFNL4* genetic variants as strong co-factors for BL; however, it did not address associations between *IFNL4* and other severe illnesses that occur in the region, especially at a younger age.

We note that the lack of direct measures of immune response to *P. falciparum* or EBV limits inferences that can be made about associations with immune responses to these infections. While our results are from a large sample, they are limited to individuals from East Africa and may not be generalizable to other areas not covered by the study. Nonetheless, our results represent an important baseline on the distributions of *IFNL4* genetic variants in a large set of apparently healthy children and BL cases in 4 East African countries. Our study increases the amount of data from SSA, which should be leveraged when designing future studies. While our results indicate that *IFNL4* markers are not associated with BL risk in children from East Africa, they are important for continuing to define the spectrum of conditions for which IFN-λ4 might be biologically relevant.

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Authors' Contribution

L.P.O. and S.M.M. conceived and designed the study, supervised its implementation, and finalized the article. F.S.B. and N.R.B. performed TaqMan genotyping; F.S.B. analyzed the data and wrote the first draft. O.F.V. and J.W. performed statistical and genetic analyses and contributed to the writing. M.D.O., C.N.T., P.A.W., T.K., I.D.L., C.G., E.B., G.N.L., S.K., N.M., C.M., R.T.K., H.N., W.N.W., E.M.M., N.M., E.K., S.J.R., R.N., and I.O. performed and supervised fieldwork. All authors read and approved the final article.

Author Disclosure Statement

No competing financial interests exist.

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