IFN-λ4 Genetic Variants Influence Clinical Malaria Episodes in A Cohort of Kenyan Children

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Abstract

Background: Interferon (IFN)- λ4, a type III IFN, production is controlled by a dinucleotide frameshift variant (rs368234815-dG/TT) within the first exon of the *IFNL4* gene. Carriers of the *IFNL4*-dG allele but not the *IFNL4*-TT allele are able to produce the IFN-λ4 protein. Patients with hepatitis C virus that do not produce the IFN-λ4 protein have higher rates of viral clearance suggesting a potential inhibitory role of IFN-λ4 in liver-tropic infections.

Methods: In this study, we investigated whether children infected with *Plasmodium falciparum,* which has a well-characterized liver stage infection, would be more susceptible to clinical malaria relative to their *IFNL4*- rs368234815 allele. We analyzed a cohort of 122 children from a malaria holoendemic region of Kenya. Episodes of clinical malaria and upper respiratory tract infections (URTIs) were determined using information collected from birth to two years of age. The dinucleotide frameshift variant *IFNL4*-rs368234815-dG/TT was genotyped using a TaqMan assay.

Results: In this cohort, we found that 33% had the dG/dG genotype, 45 % had the dG/TT genotype, and 22% had TT/TT genotype. We evaluated the number and time to first episode of clinical malaria and URTIs with respect to the *IFNL4*-rs368234815 allele. We found that children that carried the *IFNL4*-rs368234815-dG allele had an increased number of clinical malaria episodes. In addition, there was a significant association between earlier age of first malaria infection with carriers of the *IFNL4*-dG allele (*p-value:* 0.021).

Conclusion: Our results suggest that the ability to produce IFN-λ4 negatively affects host immune protection against *P. falciparum* malaria in Kenyan children.

Classification: Immunology, Microbiology, Genetics

Background

Malaria remains a major global health burden. In 2018, there were 405,000 deaths due to malaria and more than 200 million cases worldwide, placing roughly half of the world's population at risk of infection [1]. The infection is caused by intracellular protozoan parasite of the genus *Plasmodium*, with the species *Plasmodium falciparum* contributing the greatest morbidity and mortality. Transmission occurs through the bite of an infected *Anopheles* mosquito. Sporozoites injected by the mosquito travel to and infect hepatocytes, where they develop to form thousands of merozoites that then infect erythrocytes, resulting in the clinical presentation of the disease.

Type III interferons (IFNs) are antiviral cytokines with a broad antiviral activity that induce hundreds of interferon-stimulated genes (ISGs) [2, 3]. They provide a localized immune response at epithelial surfaces and if this response is successful, type I and type II IFN responses are suppressed [2] There are four type III IFNs; IFN-λ1, IFN-λ2, IFN-λ3, and the most recently discovered IFN-λ4 [3, 4]. A dinucleotide frameshift variant (rs368234815-dG/TT) within the first exon of the *IFNL4* gene controls the production of IFN-λ4

protein. Roughly half of the world population are carriers of the *IFNL4*-dG allele and are able to produce the IFN-λ4 protein, whereas carriers *IFNL4*-TT allele cannot [4, 5]. The TT allele frequency is at its lowest in Africa (29%) and reaches near-fixation frequency in East Asia (94%) [6]. This is consistent with a population-specific natural selection, in which the TT allele appears beneficial outside of Africa [6]. The reason why the ancestral allele, *IFNL4*-dG, is still retained at high frequencies in the African populations remains unclear.

The effect of the *IFNL4*-rs368234815 frameshift variation has been studied in individuals infected with hepatitis C virus (HCV) [4]. Surprisingly, the *IFNL4*-dG allele (IFN-λ4 is produced) is associated with unfavorable clinical outcomes and the *IFNL4*-TT/TT genotype (IFN-λ4 null) with higher rates of viral clearance; the mechanism behind this paradoxical finding remains unknown [4, 7–9]. One possibility is that IFN-λ4 negatively regulates the Type I IFN responses critical for viral clearance[10]. More recently, it has also been reported that the *IFNL4*-dG allele is associated with reduced clearance of RNA viruses that cause respiratory infections [11]. These studies suggest that carriers of the *IFNL4*-dG allele have different disease risks compared to carriers of the *IFNL4*-TT/TT genotype. Because HCV is a hepatropic virus, we reasoned that carriers of the *IFNL4*-dG allele might also have differential risk of malaria infection where infection of hepatocytes occurs during the primary stage of infection. In this study, we investigated the association between IFNλ4 polymorphism and risk of clinical malaria and upper respiratory tract infections (URTIs) in a prospective birth cohort of Kenyan children.

Materials And Methods

Study Design

We used samples and clinical data collected by the Chulaimbo Antenatal Postnatal (CHAP) study, a prospective cohort study conducted in Kisumu, Kenya between 2011 and 2015. Details of this cohort have been described elsewhere [12,13]. Briefly, the study enrolled pregnant women aged 18-45 years presenting for antenatal consultation at Chulaimbo County Hospital (CCH). The eligibility criteria included HIV negative, singleton pregnancy and residency within ten kilometers of the hospital. If the participating women gave birth at CCH, newborn children were enrolled at delivery and underwent a newborn exam that included laboratory testing, a physical exam and anthropometric measurements. Children were followed for up to two years. The protocol and study procedures were approved by the institutional review board of the SUNY Upstate Medical University (where the study was initiated), COMIRB at University of Colorado, and the Scientific and ethical review unit (SERU) at KEMRI.

A health questionnaire was completed each time the child came for a clinic visit; children then underwent a physical evaluation and any medical findings were included in these questionnaires. URTIs were diagnosed by physical exam by the study clinical officer. X-rays and other diagnostic tests such as respiratory antigen testing for respiratory syncytial virus, influenza, parainfluenza or adenovirus were not available. It is also relevant to mention that lower respiratory tract infections and pneumonia were

excluded. Clinical malaria episodes were diagnosed by clinical presentation and confirmed by blood smears to detect parasites in the blood.

Genotyping

Genomic DNA was extracted from blood using DNeasy Blood & Tissue Kit (Qiagen) and genotyped for *IFNL4*-rs368234815 by custom TaqMan genotyping assays, using Genotype Master Mix (Qiagen), on BioRad iQ5, with standard conditions as previously described [4] (Image S1). Testing was performed blinded to clinical phenotypes.

Glucose-6-Phosphate dehydrogenase deficiency (G6PDd) was characterized as previously published [14]. Briefly, two PCR products, 352bp and 295bp, within the *G6PD* gene were amplified by PCR using the following primers: A- Forward (5'-CAGCCACTTCTAACCACACACCT-3'), A- Reverse (5'-CCGAAGCTGGCCATGCTGGG-3'), A+ Forward (5'-CTGTCTGTGTGTCTGTCTGTCC-3') and A+ Reverse (5'-GGCCAGCCTGGCAGGCGGGAAGG-3'). The PCR amplicons were subsequently subjected to restriction enzyme digestion using NlaIII with resulting fragment sizes visualized by horizontal gel electrophoresis. For A- an uncut product was found from the normal locus, whereas two DNA fragments, 218- and 134-bp, were generated in the mutant locus. For A+ 2 DNA fragments, 243- and 52-bp, were found to be associated with the normal locus and 3 DNA fragments, 141-, 102-, 52-bp, were generated for the mutant locus.

Hb-A/S trait was characterized as previously published [15]. Briefly, a 772-bp PCR product within the human beta-globin gene was amplified from DNA extracted from whole blood using the following primers: HbB1 (5'-TCCTAAGCCAGTGCCAGAAG-3') and HbB2 (5'-GAATTCGTCTGTTTCCCATTCTAAAC-3'). The PCR amplicon was subsequently subjected to restriction enzyme digestion using Bsu361 with resulting fragment sizes visualized by horizontal gel electrophoresis. A 430-bp DNA fragment was found to be associated with the mutant locus, whereas 228- and 202-bp DNA fragments were generated from the normal locus.

Statistical Analysis

Using data from questionnaires collected on all clinic visits, the relationship between malaria episodes and upper respiratory tract infections (URTI) with respect to *IFNL4* alleles was evaluated. Only malaria episodes or URTIs that were reported on clinic visits forms were counted for. A Negative binomial regression model with an offset for the total number of sick and follow-up visits was used to evaluate the relationship between *IFNL4* alleles and the number of infections; malaria episodes and URTIs were modeled separately. Estimates from the negative binomial model were exponentiated and reported as rate ratios. To evaluate the time to first malaria infection, a Cox proportional hazards model was fit using the survival package (v 3.1-8) [16,17] and survival plots were created using the survminer package (v 0.4.6) [18] in R. The final Cox model included adjustments for *G6PDd* and sickle cell trait with right-censoring at the end of the two-year study; the proportional hazards assumption was checked and was not violated. Adjustments for gravidity and maternal malaria exposure did not improve model fit or

change conclusions about *IFNL4* associations, so they were not included in the final cox proportional hazards model. To evaluate time to first URTI, Kaplan Meier estimators were calculated, and a log rank test was used to test for differences between *IFNL4* alleles. All analyses were completed using R (version *3.6.0*) [19].

Results

Characteristics of study population

To evaluate whether genetic variants in IFN-λ4 play a role in *P. falciparum* and upper respiratory tract infection frequency, we analyzed clinical data collected from 122 children that were part of a previously described birth cohort based in Western Kenya where malaria transmission is holoendemic [12,13]. The demographics of this study population, including child gender, birth weight and median number of clinical visits that they had during the 2-year follow-up are described in Table 1. Genomic DNA was extracted from blood collected at 6 months of age and was genotyped for the *IFNL4*-rs368234815 polymorphism using a custom TaqMan genotyping assay (An additional figure shows the allelic discrimination plot generated from the genotyping assay [see Additional file 1]). In addition, we also genotyped them for glucose-6-phosphate dehydrogenase deficiency (G6PDd) and sickle cell trait (*HbB*-rs334-T/A); both of which are associated with resistance to blood stage malaria infection [15,20–23]. The observed *IFNL4*- rs368234815 dG allele (herein *IFNL4*- dG allele) frequency in our study population was 78% with a genotype frequency of 33%, 45% and 22% for dG/dG, dG/TT and TT/TT genotypes, respectively. This distribution was consistent with Hardy-Weinberg equilibrium (HWE *p-value:* 0.945), telling us that the genotype frequencies seen are a simple function of allele frequency and will remain constant from one generation to the next in the absence of evolutionary influence or other disturbing factors. In addition, 23 (19%) study participant had G6PD deficiency and 24 (20%) were found to be carriers of the sickle cell trait (Hb-A/S) (Table 2).

Carriage of the IFNL4-dG allele does not influence frequency of URTIs

To address whether there were effects on malaria or URTI, health records from a total of 1,520 clinic visits occurring between birth to two years of age were reviewed for the 122 children. We grouped children that were carriers of the *IFNL4*- dG allele (both dG/dG and dG/TT genotype) and compared them to those that did not have a dG allele (*IFNL4*-rs368234815 TT/TT genotype- herein *IFNL4*-TT/TT).

We used a negative binomial regression model to examine if there was an association between *IFNL4*-rs368234815 polymorphism and the number of URTIs during the first two years of life. We found that URTI's rate was 11.80% lower for those that had the *IFNL4*- TT/TT genotype relative to those that had an *IFNL4*-dG allele after adjusting for the number of visits (95% CI: -32.76%, 14.46%; *p-value:* 0.355) (Figure 1A). We also analyzed the association with time to first URTIs and found no significant difference between the *IFNL4*- dG allele and the *IFNL4*- TT/TT genotype with time to first URTIs (*p-value:* 0.512) (Figure 1B).

Carriage of the IFNL4-dG allele does influence age of first malaria infection

We also evaluated whether carriage of the *IFNL4*-dG allele was associated with frequency of malaria infections or time to first malaria infection in study participants (Figure 2), using the same regression framework. After offsetting for the number of visits, cases of malaria were found to be 38.75% lower for those with the *IFNL4*- TT/TT genotype relative to those that had a *IFNL4*-dG allele (95% CI: -66.70%, 10.14%; *p-value:* 0.111) (Figure 2A). Although the results are not significant, the trend suggests that those with the *IFNL4*- TT/TT genotype potentially have fewer cases of malaria. We next analyzed the association between *IFNL4*-rs368234815 polymorphism and time to first malaria episode. We found that children with an *IFNL4*-dG allele were more likely to have an earlier occurrence of the first malaria infection compared to children with the *IFNL4*- TT/TT genotype (*p-value:* 0.019) (Figure 2B). Because of the known association of G6PDd and sickle cell trait with protection from clinical malaria, we then performed a multivariable Cox proportional hazards model to control for the G6PDd and sickle cell trait. We found that the association remained statistically significant with children carrying the *IFNL4*-dG allele having a reduced hazard of earlier episodes of malaria compared to children with the *IFNL4*-TT/TT allele, (Hazards ratio: 0.39, 95% CI: 0.18, 0.87; *p-value*: 0.021(Figure 2C).

Discussion

Malaria remains a major burden worldwide but more so in the African population which has the highest malaria associated morbidity and mortality especially in children[1]. A number of studies have explored the driving force of malaria on human evolution with a specific focus on immune alleles[24]. In this study, we evaluated whether the ancestral *IFNL4*- dG allele was protective against *P. falciparum* malaria in an infant cohort in Kisumu, Kenya a malaria-endemic region with a high rate of transmission. We found that carriage of the *IFNL4*- dG allele (IFN-λ4 is produced), increased the number of clinical malaria episodes and was associated with an earlier time to first malaria infection during the first two years of life as compared to children with the *IFNL4*- TT/TT genotype. These suggests that the ability to produce IFN-λ4 negatively affects the ability to protect against *P. falciparum* in Kenyan children.

Our results are consistent with several studies that have looked at the relationship between carriers of the *IFNL4*- dG allele and HCV infection and found that the ability to produce IFN-λ4 (e.g. in carriers of *IFNL4*- dG allele) has a negative effect on viral clearance either spontaneously or after treatment [4, 7–9]. Why expression of IFN-λ4 correlates with increased malaria disease risk in infancy remains unknown. This is counterintuitive to what we know of interferons' capacity to induce an antiviral state in infected and uninfected cells to block viral replication and spread of infection [2]. One possibility is that IFNs have a different effect on intracellular parasitic infection as compared to viral infections. Alternatively, recent studies have shown that IFN-λ4 acts faster than the other type III IFNs and that its extremely strong antiviral response induces negative regulators, like USP18, to prevent other IFN responses from being mounted [25]. Based on these it is likely that in *IFNL4*- dG allele carriers, IFN-λ4 is the first response mounted against *P. falciparum* in the liver but this response alone cannot clear infection and other IFN responses are inhibited. It was recently shown in a rodent malaria model using *Plasmodium berghei*, that

liver stage infection is sensed by the host and activates a type I IFN response that is able to control parasite load and mediate host resistance to reinfection [26]. This led us to hypothesize that in *IFNL4-* dG allele carriers (produce IFN-λ4) type I IFN response would be suppressed, via the negative regulators induced by IFN-λ4, and the host would not be able to control parasite load, leading to a higher number of malaria episodes.

Type III IFNs are known to be the first line of defense for respiratory RNA viruses [27]. For example, a recent study conducted with children from Rwanda showed a reduced clearance of RNA viruses that cause respiratory infections in children carrying the *IFNL4*-dG allele [11]. In this study, we found that carriers of the *IFNL4-* dG allele had more URTIs during the first two years of life, although this was not statistically significant. Our results are consistent with the study in Rwanda and suggests that the capacity to express IFN-λ4 may increase the risk of URTIs.

The reason why the ancestral, *IFNL4*-dG allele, is still conserved in the African population and has not changed to the derived human-specific allele, *IFNL4*-TT allele, remains unknown. Carriage of the *IFNL4-dG* allele is clearly not providing protection against HCV [4, 7–9], respiratory infections [11], gastrointestinal infections [28], human coronavirus, HCoV-229E or MERS-CoV [29], and now we have found similar results for *P. falciparum* malaria in children. Undoubtedly there are other selection pressures conserving this allele in this population.

Interestingly, in a study using the rodent *Plasmodium* species *P. yoelii* and infection of mice, it was reported that the absence of IFNλ signaling decreased parasite burden and increased early antibody titers [30]. This suggests that IFNλ receptor expression plays a role in suppressing the humoral immune response to blood-stage malaria and impedes acute parasite clearance during primary blood-stage malaria infection. This demonstrates that IFNλ signaling has a strong influence on acute response during blood-stage malaria infection [30]. This led us to ask if the detrimental role that expression of IFNλ4 (carriers of the *IFNL4*-dG allele) has during malaria infection is due to its effect on blood or liver stage of *P. falciparum* infection? We show that even after adjusting for G6PD deficiency (carriage of the A^- G6PD allele) or sickle cell trait (Hb-A/S allele), both associated with resistance to blood stage malaria infection, carriers of the *IFNL4* TT/TT genotype had a reduced risk for malaria infection. These results would argute that *IFNL4* action is more important for modulating the liver stage of malaria infection as compared to a role in blood stage infection.

A main strength of our study is that it was a prospective cohort study that followed children from birth to two years of age and this allowed us to measure malaria episodes over time. A limitation of this study was a small sample size, which in some cases (e.g. URTI's) hindered our ability to detect a significant difference between carriers of the different *IFNL4* alleles. In addition, the study was not designed to evaluate the cause of URTI's and therefore might have missed disease specific effects. There is also a likelihood that not all children came to the clinic when they were exhibiting symptoms potentially resulting in a failure to capture some episodes of infection. It is important to mention that since these children were part of a cohort study, they had access to regular medical care potentially reducing the

frequency of infections and the severity of malaria observed. One of the questions that we were not able to address in the 2-year study is if the ability to produce IFN-λ4, which correlates to earlier time to first infection and more clinical malaria episodes during infancy has any long-term detrimental effects on this pediatric study population.

Conclusion

In conclusion, this study suggests production IFN-λ4 (e.g. carriers of *IFNL4-* dG allele) negatively affects the ability to protect against *P. falciparum* malaria during infancy in children living in a malaria holoendemic region of East Africa. These results are consistent with a longitudinal study of children in Mali, West Africa [28]. The underlying mechanism as to why the ability to express a type III interferon is not protective against a parasitic infection remains an important question for future studies.

We demonstrated that production of IFN-λ4, a type III interferon (IFN), had a detrimental effect on carriers, increasing the frequency of infections and time to first malaria infection during infancy, presenting a risk for the host. This finding is paradoxical from the known role of interferons in inducing an antiviral state in cells suggesting a different mechanism for function of IFN-λ4 in parasitic infections. Production of IFN-λ4 suppresses the type I IFN response, via the induction of negative regulators. In rodent models, the type I IFN response can control Plasmodium parasite load; without type I IFN response due to production of IFN-λ4, the host would not be able to control parasite load potentially leading to a higher number of malaria episodes.

Declarations

Ethics approval and consent to participate

The protocol and study procedures were approved by the institutional review board of the SUNY Upstate Medical University (where the study was initiated), COMIRB at University of Colorado, and the Scientific and ethical review unit (SERU) at KEMRI.

Consent for Publication

Not Applicable

Availability of Data and Materials

Not Applicable

Competing interests

The authors declare that they have no competing interests

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Authors' contributions

GSR designed research, performed research, wrote the paper. CJ analyzed data, wrote the paper. SO designed research, performed research, wrote the paper. KRS analyzed data, wrote the paper. AO contributed analytic tools, wrote the paper. AED designed research, wrote the paper. LPO designed research, wrote the paper. RR designed the research, wrote the paper. All authors read and approved the final manuscript.

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References

1. World Health Organization. World malaria report. Geneva; 2019 Licence: CC BY-NC-SA .0 IGO.
2. Wack A, Terczyńska-Dyla E, Hartmann R. Guarding the frontiers: the biology of type III interferons. Nat Immunol. 2015 Aug;16(8):802–9.
3. Lazear HM, Nice TJ, Diamond MS. Interferon-λ: Immune Functions at Barrier Surfaces and Beyond. Immunity. 2015 Jul;43(1):15–28.
4. Prokunina-Olsson L, Muchmore B, Tang W, Pfeiffer RM, Park H, Dickensheets H, Hergott D, Porter-Gill P, Mumy A, Kohaar I, Chen S, Brand N, Tarway M, Liu L, Sheikh F, Astemborski J, Bonkovsky HL, Edlin BR, Howell CD, Morgan TR, Thomas DL, Rehermann B, Donnelly RP, O'Brien TR. A variant upstream of IFNL3 (IL28B) creating a new interferon gene IFNL4 is associated with impaired clearance of hepatitis C virus. Nat Genet. 2013 Feb;45(2):164–71.
5. Prokunina-Olsson L. Genetics of the Human Interferon Lambda Region. Journal of Interferon & Cytokine Research. 2019 Oct 1;39(10):599–608.
6. Key FM, Peter B, Dennis MY, Huerta-Sánchez E, Tang W, Prokunina-Olsson L, Nielsen R, Andrés AM. Selection on a Variant Associated with Improved Viral Clearance Drives Local, Adaptive Pseudogenization of Interferon Lambda 4 (IFNL4). Pritchard JK, editor. PLoS Genet. 2014 Oct 16;10(10):e1004681.
7. Meissner EG, Bon D, Prokunina-Olsson L, Tang W, Masur H, O'Brien TR, Herrmann E, Kottilil S, Osinusi A. IFNL4-ΔG Genotype Is Associated With Slower Viral Clearance in Hepatitis C, Genotype-1 Patients

Treated With Sofosbuvir and Ribavirin. The Journal of Infectious Diseases. 2014 Jun 1;209(11):1700–4.

8. Aka PV, Kuniholm MH, Pfeiffer RM, Wang AS, Tang W, Chen S, Astemborski J, Plankey M, Villacres MC, Peters MG, Desai S, Seaberg EC, Edlin BR, Strickler HD, Thomas DL, Prokunina-Olsson L, Sharp GB, O'Brien TR. Association of the IFNL4-ΔG Allele With Impaired Spontaneous Clearance of Hepatitis C Virus. The Journal of Infectious Diseases. 2014 Feb 1;209(3):350–4.
9. O'Brien TR, Pfeiffer RM, Paquin A, Lang Kuhs KA, Chen S, Bonkovsky HL, Edlin BR, Howell CD, Kirk GD, Kuniholm MH, Morgan TR, Strickler HD, Thomas DL, Prokunina-Olsson L. Comparison of functional variants in IFNL4 and IFNL3 for association with HCV clearance. Journal of Hepatology. 2015 Nov;63(5):1103–10.
10. Onabajo OO, Muchmore B, Prokunina-Olsson L. The IFN-λ4 Conundrum: When a Good Interferon Goes Bad. Journal of Interferon & Cytokine Research. 2019 Oct 1;39(10):636–41.
11. Rugwizangoga B, Andersson ME, Kabayiza J-C, Nilsson MS, Ármannsdóttir B, Aurelius J, Nilsson S, Hellstrand K, Lindh M, Martner A. IFNL4 Genotypes Predict Clearance of RNA Viruses in Rwandan Children With Upper Respiratory Tract Infections. Front Cell Infect Microbiol. 2019 Oct 4;9:340.
12. Ray JE, Dobbs KR, Ogolla SO, Daud II, Vulule J, Sumba PO, Rochford R, Dent AE. Reduced Transplacental Transfer of Antimalarial Antibodies in Kenyan HIV-Exposed Uninfected Infants. Open Forum Infectious Diseases. 2019 Jun 1;6(6):ofz237.
13. Daud II, Ogolla S, Amolo AS, Namuyenga E, Simbiri K, Bukusi EA, Ng'ang'a ZW, Ploutz-Snyder R, Sumba PO, Dent A, Rochford R. Plasmodium falciparum Infection is Associated with Epstein–Barr Virus Reactivation in Pregnant Women Living in Malaria Holoendemic Area of Western Kenya. Matern Child Health J. 2015 Mar;19(3):606–14.
14. Kotea R, Kaeda JS, Yan SLK, Sem Fa N, Beesoon S, Jankee S, Ramasawmy R, Vulliamy T, Bradnock RW, Bautista J, Luzzatto L, Krishnamoorthy R, Mason PJ. Three major G6PD-deficient polymorphic variants identified among the Mauritian population. Br J Haematol. 1999 Mar;104(4):849–54.
15. Mulama DH, Bailey JA, Foley J, Chelimo K, Ouma C, Jura WGZO, Otieno J, Vulule J, Moormann AM. 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: Sickle cell trait and EBV loads in endemic Burkitt lymphoma. Int J Cancer. 2014 Feb 1;134(3):645–53.
16. Therneau T. A Package for Survival Analysis in S. version 2.38, [Internet]. 2015. Available from: https://CRAN.R-project.org/package=survival
17. Therneau TM, Grambsch PM. Modeling Survival Data: Extending the Cox Model. Springer, New York.; 2000.
18. Kassambara A, Kosinski M, Biecek P. survminer: Drawing Survival Curves using "ggplot2". R package version 0.4.6. [Internet]. 2019. Available from: https://CRAN.R-project.org/package=survminer
19. Rich B. table1: Tables of Descriptive Statistics in HTML. R package version 1.1. [Internet]. 2018. Available from: https://CRAN.R-project.org/package=table1

20. Guindo A, Fairhurst RM, Doumbo OK, Wellems TE, Diallo DA. X-Linked G6PD Deficiency Protects Hemizygous Males but Not Heterozygous Females against Severe Malaria. Krishna S, editor. PLoS Med. 2007 Mar 13;4(3):e66.
21. Aidoo M, Terlouw DJ, Kolczak MS, McElroy PD, ter Kuile FO, Kariuki S, Nahlen BL, Lal AA, Udhayakumar V. Protective effects of the sickle cell gene against malaria morbidity and mortality. The Lancet. 2002 Apr;359(9314):1311–2.
22. Williams TN. Human red blood cell polymorphisms and malaria. Current Opinion in Microbiology. 2006 Aug;9(4):388–94.
23. Gilles H, Fletcher K, Hendrickse R. Glucose-6-phosphate-dehydrogenase deficiency, sickling, and malaria in African children in South Western Nigeria. Lancet. 1967;(1):138–40.
24. Stevenson MM, Riley EM. Innate immunity to malaria. Nat Rev Immunol. 2004 Mar;4(3):169–80.
25. Obajemu AA, Rao N, Dilley KA, Vargas JM, Sheikh F, Donnelly RP, Shabman RS, Meissner EG, Prokunina-Olsson L, Onabajo OO. IFN-λ4 Attenuates Antiviral Responses by Enhancing Negative Regulation of IFN Signaling. JI. 2017 Dec 1;199(11):3808–20.
26. Liehl P, Zuzarte-Luís V, Chan J, Zillinger T, Baptista F, Carapau D, Konert M, Hanson KK, Carret C, Lassnig C, Müller M, Kalinke U, Saeed M, Chora AF, Golenbock DT, Strobl B, Prudêncio M, Coelho LP, Kappe SH, Superti-Furga G, Pichlmair A, Vigário AM, Rice CM, Fitzgerald KA, Barchet W, Mota MM. Host-cell sensors for Plasmodium activate innate immunity against liver-stage infection. Nat Med. 2014 Jan;20(1):47–53.
27. Mordstein M, Neugebauer E, Ditt V, Jessen B, Rieger T, Falcone V, Sorgeloos F, Ehl S, Mayer D, Kochs G, Schwemmle M, Günther S, Drosten C, Michiels T, Staeheli P. Lambda Interferon Renders Epithelial Cells of the Respiratory and Gastrointestinal Tracts Resistant to Viral Infections. JVI. 2010 Jun 1;84(11):5670–7.
28. Prokunina-Olsson L, Morrison RL, Obajemu A, Mahamar A, Kim S, Attaher O, Florez-Vargas O, Sidibe Y, Onabajo OO, Hutchinson AA, Manning M, Kwan J, Brand N, Dicko A, Fried M, Albert PS, Mbulaiteye SM, Duffy PE. IFN-λ4 increases the risk of gastrointestinal infections and malaria in Malian children [Internet]. Genetics; 2020 Feb [cited 2020 Mar 26]. Available from: http://biorxiv.org/lookup/doi/10.1101/2020.02.24.962688
29. Hamming OJ, Terczyńska-Dyla E, Vieyres G, Dijkman R, Jørgensen SE, Akhtar H, Siupka P, Pietschmann T, Thiel V, Hartmann R. Interferon lambda 4 signals via the IFNλ receptor to regulate antiviral activity against HCV and coronaviruses: Recombinant IFNλ4 is antiviral. The EMBO Journal. 2013 Nov 27;32(23):3055–65.
30. Hahn WO, Pepper M, Liles WC. B cell intrinsic expression of IFNλ receptor suppresses the acute humoral immune response to experimental blood-stage malaria. Virulence. 2020 May 14;21505594.2020.1768329.

Tables

Due to technical limitations, table 1,2 is only available as a download in the Supplemental Files section.

Figures

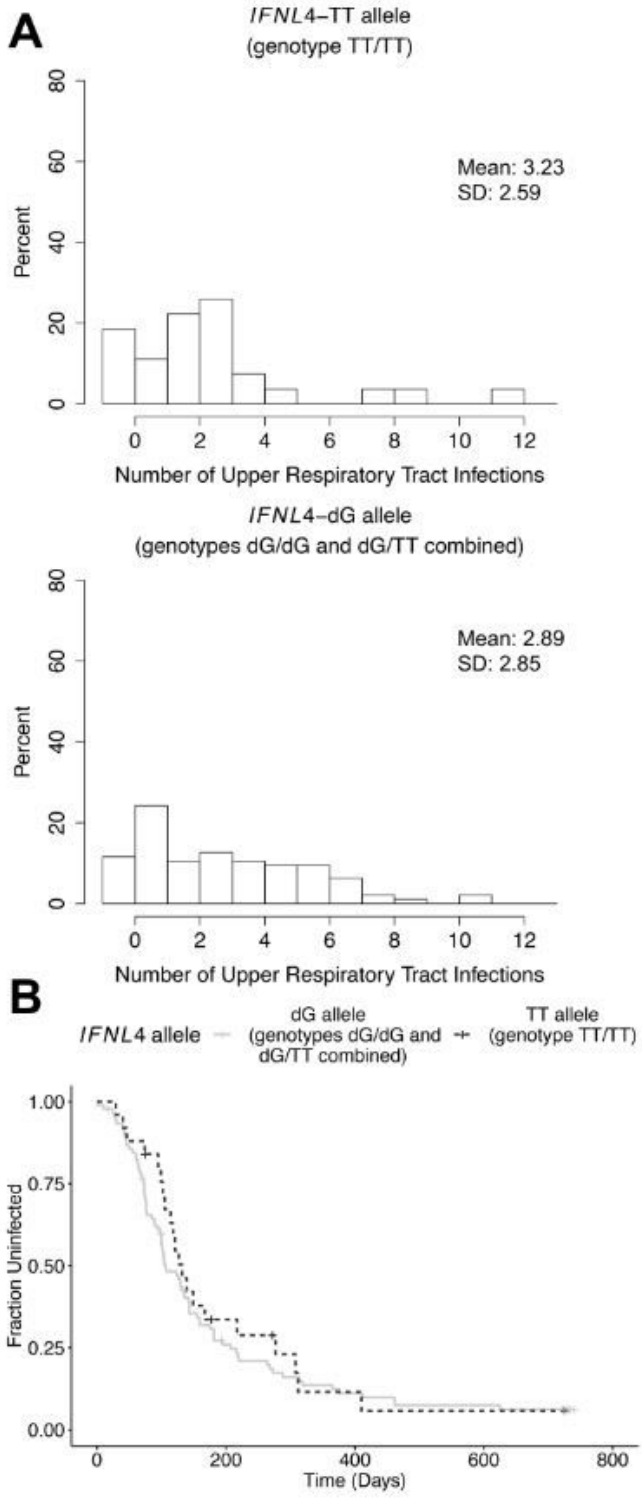

Figure 1

Incidence of URTIs and time to first infection in children from the CHAP prospective cohort study - The study subjects were grouped based on presence of a IFNL4-dG allele, dG/dG and dG/TT genotypes (n=95) and compared with children that did not carry a dG allele, TT/TT genotype (n=27). (A) Histograms

showing the distribution of URTIs in the study population during the first 2 years of life presented based on IFNL4-rs368234815 polymorphism. The mean number of URTIs and standard deviation (SD) is included. We show that the mean number of URTI's decreased for children with the TT/TT genotype relative to those that a dG allele. (B) Kaplan-Meier survival curve showing time to first URTIs based on IFNL4-rs368234815 polymorphism. No significant difference between IFNL4-rs368234815 genotype was found (p-value:0.512).

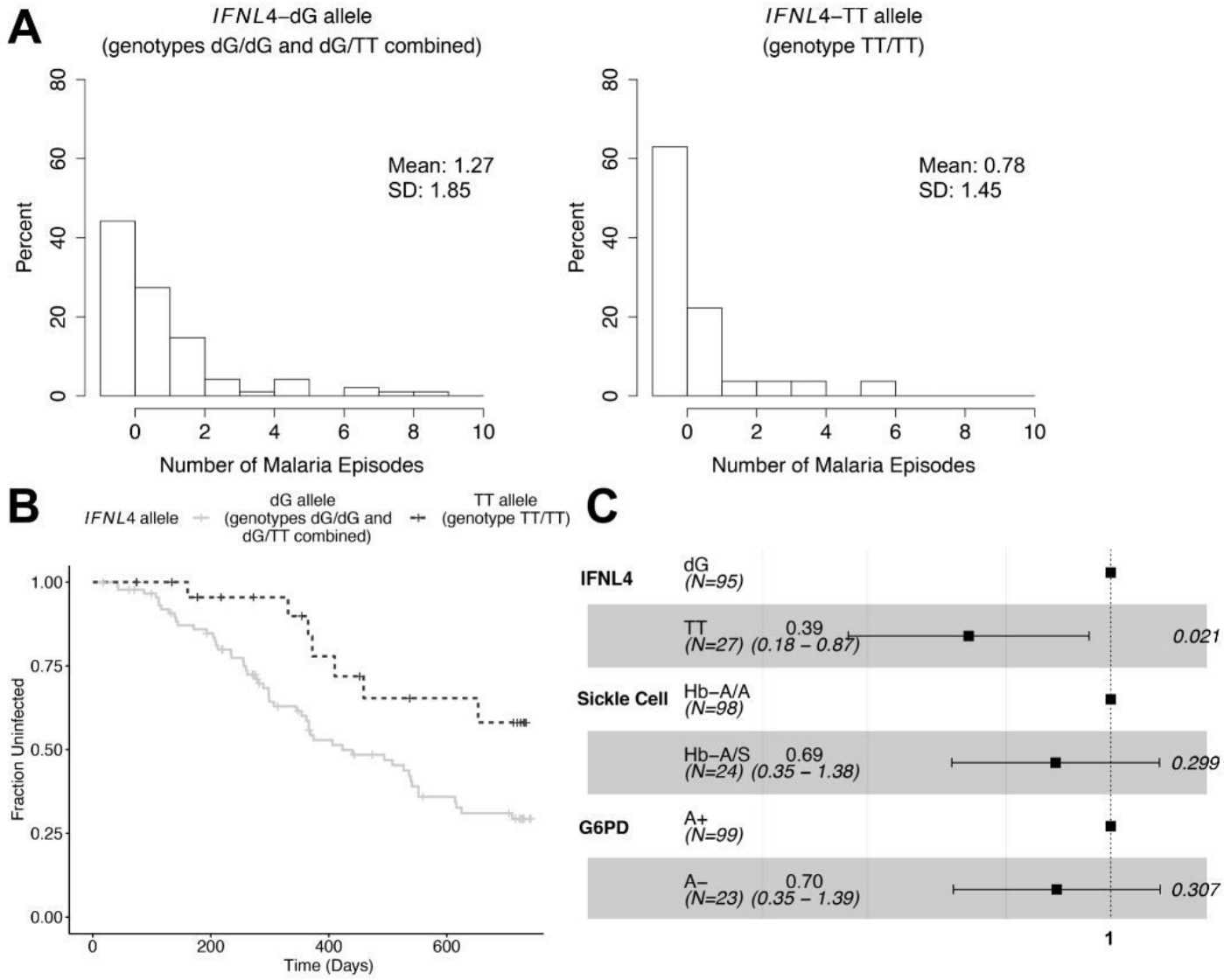

Figure 2

Frequency and time to first Malaria episodes in relation to IFNL4-rs368234815 polymorphism - The 122 study subjects were grouped based on presence of a IFNL4-dG allele, dG/dG and dG/TT genotypes (n=95) and compared with children that did not carry a dG allele, TT/TT genotype (n=27). (A) Histograms showing the distribution of malaria episodes in the study population during the first 2 years of life presented based on IFNL4-rs368234815 polymorphism. The mean and standard deviation (SD) of malaria episodes is included. We show that children that are carriers of the IFNL4-dG allele have a higher number of cases than those with the IFNL4-TT/TT genotype. (B) Kaplan-Meier survival curve showing

time to first malaria episode based on IFNL4-rs368234815 polymorphism. Earlier timing of the first malaria infection was associated with IFNL4-dG allele (p-value: 0.019) as compared to children with the IFNL4-TT/TT genotype. (C) Forest plot showing the results of a Cox proportional hazard model that takes into consideration the effect of two genetic traits that are known to be associated with resistance to blood stage malaria infection, G6PDd and sickle cell, on time to first episode. Adjustments for gravidity and maternal malaria exposure did not improve model fit, so they were not included for Cox proportional hazards model. Hazard ratios are reported along with confidence intervals and p-values for IFNL4 (dG allele, dG/dG and dG/TT genotypes and TT allele, TT/TT genotype), G6PD alleles and sickle cell genotypes. A significant hazard ratio (p-value: 0.021) is observed for the IFNL4 genotype, indicating reduced malaria risk for those with the IFNL4-TT/TT genotype.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table1.JPG
- Table2.JPG
- Additionalfile1SamayoaReyes.docx