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Polymorphic variability in the IL-1β promoter conditions susceptibility to severe malarial anemia and functional changes in IL-1β production

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Abstract

Interleukin (IL)-1β is a cytokine released as part of innate immune response to *Plasmodium falciparum*. Since the role of IL-1β polymorphic variability in conditioning the immunopathogenesis of severe malarial anemia (SMA) remains undefined, relationships between IL-1β promoter variants (-31C/T and -511A/G), SMA (Hb<6.0 g/dL), and circulating IL-1β levels were investigated in parasitemic children (n=566) from western Kenya. IL-1β promoter haplotype -31C/-511A (CA) was associated with increased risk of SMA (Hb<6.0 g/dL; OR; 1.98, 95% CI, 1.55-2.27; $P<0.05$) and reduced circulating IL-1β levels ($P<0.05$). The TA (-31T/-511A) haplotype was non-significantly associated with protection against SMA (OR; 0.52, 95% CI, 0.18-1.16; $P=0.11$) and elevated IL-1β production ($P<0.05$). Children with SMA had significantly lower IL-1β levels and non-significant elevations in both IL-1 receptor antagonist (Ra) and the IL-1Ra:IL-1β ratio compared to

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the non-SMA group. Results presented demonstrate that variation in IL-1β promoter conditions susceptibility to SMA and functional changes in circulating IL-1β levels.

Keywords

IL-1β; IL-1Ra; falciparum; high-density parasitemia; malarial anemia

INTRODUCTION

Malaria is one of the leading global causes of morbidity and mortality of infectious disease origin [1]. Approximately over 200 million clinical cases of malaria occur among populations residing in holoendemic malaria transmission regions, resulting in over one million deaths [2]. Severe malarial anemia (SMA) in infants and young children is the most common clinical manifestation of severe malaria in *Plasmodium falciparum* holoendemic transmission areas, such as western Kenya, and accounts for the greatest proportion of malaria-associated morbidity and mortality worldwide [3]. In western Kenya, cerebral malaria (CM), hyperparasitemia, hypoglycemia, and renal insufficiency occur rarely [4,5].

Although it is well-established that pediatric SMA results from both enhanced red blood cell (RBC) destruction and inefficient RBC production, the underlying molecular basis of the disease remains largely undefined. Dysregulation in pro-inflammatory cytokines such as interleukin (IL)-1 have been proposed to play an important role in immunopathogenesis of SMA [6-8]. The IL-1 gene family includes IL-1α, IL-1β, and IL-1 receptor antagonist (IL-1Ra) that form a cytokine gene cluster on human chromosome 2. IL-1 is a potent endogenous pyrogen that promotes an acute inflammatory response as part of the first line of defense against invading pathogens [9]. However, high levels of sustained IL-1β production in inflammatory diseases can induce hematological abnormalities such as anemia [10,11]. Although previous studies have reported elevated circulating IL-1β levels in individuals with CM and SMA [8, 12,13], additional studies found no significant changes in IL-1β in children with SMA [14]. In addition, since IL-1Ra is an important determinant of IL-1β availability, differential expression of IL-1Ra relative to IL-1β may impact on the immunopathogenesis of malaria [15]. Previous studies in Gambia [16] and Uganda [17] showed that increased concentrations of IL-1Ra were associated with enhanced malaria disease severity. Taken together, these investigations suggest that rapid induction of IL-1β may control invading pathogens, while overproduction of IL-1β may cause enhanced pathogenic effects by promoting anemia.

Since genetic variability in inflammatory genes influences susceptibility to polygenic infectious diseases such as malaria [18], an increased understanding of malaria pathogenesis can be achieved by identifying functional polymorphisms in those critical genes that mediate the development and clinical course of disease. Previous genetic studies have demonstrated significant associations between variability in IL-1β and infectious, neurological, and autoimmune diseases [19-22]. A study in The Gambia demonstrated significant associations between variation at IL-1β +4845G/T and IL-1β +3953C/T and susceptibility to clinical malaria [23]. Investigations in Thailand [24] and Ghana [25], examining a functional polymorphism at IL-1β -31C/T and variable number tandem repeat (VNTR) within the IL-1Ra, failed to demonstrate a significant relationship with severe malaria.

Since previous genetic studies focused primarily on relationships between individual single nucleotide polymorphisms (SNPs) in the IL-1β gene and malaria disease severity, the current study investigated the role of IL-1β haplotypes in conditioning susceptibility to SMA [defined as hemoglobin (Hb) <6.0 g/dL, with any density parasitemia] [26]. However, children were also classified according to World Health Organization (WHO) definition of SMA [Hb <5.0

g/dL, with any density parasitemia] [27] to place the current findings into a broader geographic context. In addition, the relative levels of IL-1Ra to IL-1β were also investigated. The study was performed in a phenotypically well-characterized cohort (n=566) of children (aged <3 yrs) with falciparum malaria in a holoendemic *P. falciparum* transmission area of western Kenya. Results presented here demonstrate that polymorphic variability in the IL-1β promoter (-31C/T and -511A/G) is associated with increased susceptibility to SMA and functional changes in circulating IL-1β concentrations, and that elevated levels of IL-1Ra relative to IL-1β were associated with enhanced disease severity in children with malaria.

METHODS

Study participants

Children <3 years of age with acute malaria (n=566) were recruited at Siaya District Hospital, western Kenya, where *P. falciparum* transmission is holoendemic [26]. All study participants were from Luo ethnic group. Children with acute *P. falciparum* malaria (any density parasitemia) were categorized into two primary groups: SMA (Hb <6.0 g/dL) and non-SMA (Hb ≥6.0 g/dL). SMA in children from this geographic location is best defined as Hb <6.0 g/dL with any density parasitemia, and is based on >10,000 longitudinal Hb measurements in a matched reference population in western Kenya [26]. Additionally, parasitemic children were classified according to the WHO definition of SMA [27]. Children were excluded from the study if they had CM or non-*P. falciparum* malaria. In addition, since our previous studies [28] and those by others [29] demonstrate that HIV-1 status and bacterial co-infection are important determinants of malarial anemia severity, all children were tested for these co-infections (see procedures below). Pre- and post-test HIV counseling was provided for parents/guardians of all study participants and written informed consent in language of choice (i.e., English, Kiswahili, or Dholuo) obtained. The study was approved by the ethical and scientific review committees at Kenya Medical Research Institute and Institutional Review Boards in the USA.

Laboratory procedures

Venous blood (<3.0 mL) was obtained prior to administration of antimalarials and/or antipyretics. Asexual malaria parasites (trophozoites) were counted against 300 leukocytes in peripheral blood smears stained with 3% Giemsa. Parasite density was estimated as follows: parasite density/μL = white blood cell count/μL × trophozoites/300. Complete hematological parameters were determined with a Beckman Coulter® AcT diff2™ (Beckman-Coulter Corporation). HIV-1 status was determined according to our published methods [28]. Trimethoprimsulfamethoxazole was administered to all children that had serologically positive HIV-1 test results. At the time of sample collection, none of the HIV-1(+) study participants had been initiated on antiretroviral treatment. Bacteremia was determined using Wampole Isostat® Pediatric 1.5 system (Wampole Laboratories) and blood was processed according to manufacturer's instructions. API biochemical galleries (Biomerieux) and/or serology were used for identification of bacterial isolates.

IL-1β genotyping

DNA was extracted from blood spotted on FTA® Classic cards (Whatman® Inc.) using Gentra System (Gentra System, Inc.). IL-1β -31 genotyping was carried out as previously described [30]. The IL-1β -511A/G SNP was genotyped using a Taqman® 5' allelic discrimination Assay-By-Design™ method according to manufacturer's instructions (Assay ID: C_1839943_10; Applied Biosystems).

Circulating IL-1β and IL-1RA determination

Plasma samples obtained from venous blood were stored at -70°C until use. IL-1β and IL-1Ra concentrations were determined using Cytokine 25-plex Ab Bead Kit, Hu (BioSource™ International) according to manufacturer's instructions. Plates were read on a Luminex 100™ system (Luminex Corporation) and analyzed using Bio-Plex Manager™ Software (Bio-Rad Laboratories). The detection limit for IL-1β and IL-1Ra was 15 pg/mL and 30 pg/mL, respectively.

Statistical Analyses

Statistical analyses were performed using SPSS (Version 12.0). Chi-square (χ^2) and Mann-Whitney U tests were used to examine differences between proportions and for pairwise comparisons of medians, respectively. Since circulating IL-1β and IL-1Ra levels were non-normally distributed, levels were log-transformed prior to analyses. IL-1β haplotypes (-31C/T and -511A/G) were constructed using HPlus software (Version 2.5). Multivariate logistic regression, controlling for age, gender, HIV-1 status [including both HIV-1 exposure and definitively HIV-1(+) results], bacteremia, and prevalence of sickle-cell trait (HbAS), was used to examine associations of IL-1β genotypes and haplotypes with SMA. Statistical significance was defined as $P \leq 0.05$.

RESULTS

Demographic, clinical, and laboratory characteristics of study participants

A total of 566 children (age, <3 yrs.) with *P. falciparum* parasitemia (any density) presenting at hospital with febrile illness were included in the study. Children were stratified into two clinical categories: non-SMA (Hb≥6 g/dL, n=349) and SMA (Hb<6 g/dL, n=217) [26]. Demographic, clinical, and laboratory characteristics of study participants are presented in Table 1. There were significant differences in age (mos.) and Hb concentrations (g/dL) between the groups ($P<0.001$ for both comparisons). Proportions of males vs. females, peripheral parasitemia (MPS/μL), high-density parasitemia (HDP, ≥10,000 parasites/μL) and geomean parasitemia (parasites/μL) were non-significantly different between the groups ($P=0.30$, $P=0.22$, $P=0.08$, and $P=0.78$, respectively). Overall, these results illustrate that SMA within this holoendemic region is largely unrelated to concomitant peripheral parasite density upon presentation at hospital.

Distribution of IL-1β genotypes

To investigate the role of variation in the IL-1β promoter in conditioning susceptibility to SMA, two IL-1β loci were examined: IL-1β -31C/T and -511A/G. The prevalence of IL-1β -31 genotypes in the population was 65.6% CC, 29.8% CT, and 4.6% TT with overall allele frequencies of C=0.81 and T=0.19. Genotypic distribution of IL-1β -31 in the non-SMA group was 64.5% CC, 30.8% CT, and 4.7% TT (Table 2) with allele frequencies of C=0.80 and T=0.20. The non-SMA group did not display departure from Hardy Weinberg Equilibrium (HWE; $\chi^2=0.616$, $P=0.50$). Genotypic distribution in SMA group was 67.5% CC, 28.2% CT, and 4.4% TT (Table 2) with allele frequencies of C=0.82 and T=0.18. There was no departure from HWE ($\chi^2=0.849$, $P=0.50$) in children with SMA.

Prevalence of IL-1β -511 genotypes was 37.4% AA, 48.3% AG, and 14.3% GG (Table 2) with overall allele frequencies of A=0.62 and G=0.38. Distribution of IL-1β -511 genotypes in non-SMA was 35.2% AA, 49.7% AG, and 15.2% GG (Table 2) with allele frequencies of A=0.60 and G=0.40, with no departure from HWE ($\chi^2=0.378$, $P=0.75$). In SMA group, the genotypic distribution was 41.8% AA, 45.6% AG, and 12.7% GG (Table 2) with allele frequencies of A=0.65 and G=0.35, with no departure from HWE ($\chi^2=0.0027$, $P=0.95$). Chi-squared analyses

revealed that distribution of IL-1β -31 and -511 genotypes did not differ significantly between non-SMA and SMA groups (P=0.77 and P=0.35, respectively, Table 2).

Role of individual IL-1β loci in SMA and HDP

Prior to investigating the role of IL-1β haplotypes in conditioning susceptibility to SMA and HDP, the relationship between variation at individual IL-1β loci and susceptibility to these two clinical presentations were examined by multivariate logistic regression analyses. Since our previous investigations, and those of others, demonstrated that age, gender, HIV-1 status, bacteremia, and sickle-cell trait influence susceptibility to SMA [28,31], the confounding effects of these variables were controlled for in the analyses. No significant relationships were obtained between variation at individual IL-1β loci and either susceptibility to SMA or HDP (Table 3).

IL-1β haplotypes distribution according to hematological characteristics

To further characterize the role of variability in IL-1β promoter in conditioning outcomes of severe malaria, haplotypic groups were constructed. These produced the following distribution: 87.2% CA (IL-1β -31C/-511A); 29.1% CG; 2.1% TA; and 30.9% TG. To investigate the potential role of haplotypic structures in conditioning hematological outcomes, median Hb levels and SMA (using both modified and WHO definitions) were compared between children with and without the haplotypic compositions. Results revealed that carriage of CA and CG haplotypes was associated with significantly lower Hb levels (Table 4). In addition, proportions of children with SMA using the modified definition [26] were 39.1% CA, 32.1% CG, 16.7% TA, and 37.1% TG, while proportions of children according to WHO definition of SMA [27] were 19.6% CA, 17.0% CG, 8.3% TA, and 20.0% TG (Table 4).

Role of IL-1β haplotypes in conditioning susceptibility to SMA

To further examine the relationship between IL-1β promoter haplotypic structures and severe malaria, multivariate logistic regression analyses were performed in a model controlling for appropriate confounders [28,31]. Analyses demonstrated that individuals with IL-1β CA haplotype were significantly more susceptible to SMA (Hb<6.0 g/dL) than individuals without the haplotype (OR; 1.98, 95% CI, 1.55-2.27; P<0.05, Table 5). Additional analyses based on the WHO definition of SMA [27] demonstrated an increased risk of SMA in children with CA haplotype (OR; 1.52, 95% CI, 1.36-2.66; P<0.05, Table 5). In contrast, carriage of TA haplotype was associated with non-significant protection against SMA using both modified [26] (OR; 0.52, 95% CI, 0.18-1.16; P=0.11, Table 5) and WHO [27] definitions of SMA (OR; 0.61, 95% CI, 0.18-1.31; P=0.20, Table 5). None of the other haplotypes showed a strong association with susceptibility to SMA (Table 5). In addition, no significant relationships were identified between any of the haplotypic structures and HDP (Table 5). To delineate the effects of heterozygosity between the two SNPs, the association between heterozygous individuals at both loci (-31CT and -511AG) and malaria disease severity were investigated. However, there were no significant associations between heterozygosity and malaria disease severity. Since the above haplotype reconstructions were based on a dominant model, effects of the recessive model (i.e. presence of combined recessive alleles) on malaria disease severity were also investigated. This model demonstrated that individuals with IL-1β CA/CA haplotype were significantly more susceptible to SMA (Hb<6.0 g/dL) than individuals without the CA/CA haplotype (OR; 1.53, 95% CI, 1.07-2.19; P<0.05), further supporting our finding that presence of CA haplotype increases susceptibility to SMA. In this model, there were no associations between CG/TG, CA/TA, CG/CG, and TG/TG haplotypes and either SMA or HDP.

Functional relationship between IL-1β haplotypes and circulating IL-1β levels

To determine if genotypes or haplotypes were associated with functional changes in IL-1β production, circulating concentrations of IL-1β were compared across genotypic and haplotypic groups. Results demonstrated no significant associations between variation at individual IL-1β loci and circulating IL-1β levels (data not presented). However, individuals with the CA haplotype had significantly lower circulating IL-1β levels [log median (interquartile range); 2.20 (1.95-2.16)] than those without the haplotype [2.35 (1.91-2.44), $P<0.05$, Figure 1]. In addition, individuals with the TA haplotype had significantly higher circulating IL-1β levels [log median (IQR); 2.67 (1.60-3.40)] relative to those without the haplotype [2.23 (1.97-2.17), $P<0.05$, Figure 1]. No significant differences in IL-1β production were observed for CG ($P=0.78$) and TG ($P=0.90$) haplotypes (data not presented). These results illustrate that CA and TA haplotypes are associated with significant differences in circulating IL-1β levels.

Circulating IL-1β and IL-1Ra levels in children with SMA and non-SMA

To determine if changes in IL-1β and IL-Ra levels were associated with SMA, circulating levels of these inflammatory mediators were compared between SMA and non-SMA groups. Results demonstrated that children with SMA had significantly lower IL-1β levels [log median (IQR); 2.27 (1.84-2.41)] than the non-SMA group [2.37 (1.93-2.45), $P<0.05$]. In contrast, circulating levels of IL-1Ra were non-significantly elevated in SMA [log median (IQR); 3.56 (3.31-3.64)] relative to the non-SMA group [3.46 (3.31-3.57), $P=0.63$]. Since IL-1Ra antagonizes IL-1β levels during active disease [15], the IL-1Ra:IL-1β ratio was also investigated. These analyses demonstrated that IL-1Ra:IL-1β ratio was non-significantly higher in children with SMA [log median (IQR); 1.49 (1.68-2.58)] compared to the non-SMA group [1.46 (1.67-2.33), $P=0.43$]. These results suggest that association between reduced circulating IL-1β levels and SMA may be due to elevated IL-1Ra production.

DISCUSSION

Cross-sectional results presented here in a phenotypically well-defined cohort of children (<3 years of age) demonstrate that presence of the IL-1β promoter haplotype -31C/-511A (CA) was associated with increased risk of developing SMA using both the modified (Hb <6.0 g/dL) [26] and WHO (Hb <5.0 g/dL) [27] definitions of disease. Consistent with these findings, individuals with the CA haplotype had significantly reduced Hb levels relative to individuals with other haplotypes. Children with the CA haplotype also had significantly reduced circulating levels of IL-1β. Additional results revealed that the -31T/-511A (TA) haplotype was associated with non-significant protection against SMA and significantly elevated IL-1β production. Taken together, these results suggest that protection against SMA may require an appropriate elevation in IL-1β production that is conditioned by genotypic combinations. Results presented here demonstrating significant associations between IL-1β promoter variants with SMA, but neither overall parasite burden nor HDP are expected since the current findings, as well as our previous studies in western Kenya, illustrate that concomitant parasite density and SMA are largely unrelated [5,28,32-35].

Infectious diseases such as malaria have exerted significant selective pressure on the human genome, particularly in host-immune response genes that mediate susceptibility and clinical outcomes of disease [18]. Allele frequencies of IL-1β -31 and -511 variants presented here are comparable to those observed in previous studies in African populations [23,25]. However, none of the previous studies examined frequencies of these variants in a population where SMA was the primary clinical manifestation. Genotypic distribution in the current cohort showed a non-significant departure from HWE for both IL1β -31 and -511 promoter variants, likely illustrating the importance of expanding current investigations to include a more inclusive

panel of immunoregulatory genes, since susceptibility to infectious diseases occurs through complex, multifactorial, and often contradictory selective forces [36].

Consistent with results presented here, previous cross-sectional studies in Thai adults [24], Gambian children (aged <5 years) [23], and Ghanaian children (aged 1-12 years) [25] showed that variation at IL-1β -31 was not associated with malaria disease outcomes. However, construction of IL-1β -31 and -511 haplotypes demonstrated the important role of these variants in conditioning susceptibility to SMA. Additional studies aimed at identifying the role of these haplotypic compositions in conditioning susceptibility to severe malaria should take into account the well-documented fact that clinical manifestations of disease differ according to the level of *P. falciparum* exposure. For example, in hyperendemic transmission areas, severe malaria is frequently characterized by an overlapping sequelae of CM, SMA, and hypoglycemia, whereas severe malaria in holoendemic transmission areas, such as western Kenya, is typically defined by SMA (with or without HDP) as a single disease entity [5,37, 38]. It will be important to expand the current studies to additional geographic locations to determine if the haplotypic structures examined here condition susceptibility to severe malaria in individuals with diverse clinical presentations of disease (e.g., CM, SMA, and hypoglycemia). Children in the present study were recruited during their first hospital contact for treatment of malaria, and, therefore, experienced few, if any, previous malaria episodes, making them largely immune-naïve. It remains to be determined if haplotypic structures identified here will condition susceptibility to severe malaria in older children and adults that may have acquired a greater degree of malarial immunity based on previous exposures.

Earlier investigations demonstrated that IL-1β -31T allele is associated with higher IL-1β expression and secretion [39] and a multi-fold induction of DNA binding in lipopolysaccharide-activated monocytes [40,41]. Elevated IL-1β production in the presence of the T allele is due to increased transcription factor binding at the TATA box sequence spanning position -31 within the promoter [42,43]. We did not find a significant association between variation at IL-1β -31 and circulating levels of IL-1β in children with malaria. However, haplotypic analyses revealed that individuals with the -31C/-511A (CA) haplotype produced significantly lower levels of IL-1β, while carriage of the TA haplotype was associated with significantly higher IL-1β production. Despite these significant relationships, it is important to note that *in vivo* IL-1β production is likely influenced by a number of polymorphic variants within the IL-1β gene, as well as additional genetic variation in other inflammatory mediators that regulate IL-1β gene expression, such as tumour necrosis factor (TNF)-a [8,44-47]. Additional studies are currently ongoing in our laboratory to identify genetic variation in an inclusive panel of innate immune response genes that can condition immune response to malaria.

Although associations between IL-1β production and childhood malaria pathogenesis are largely unreported, a previous study demonstrated that circulating IL-1β levels were non-significantly different in Malian children with SMA vs. their age-matched controls without SMA [14]. Additional investigations in adult populations in low malaria endemic areas demonstrated significant associations between IL-1β production and development of CM [8, 13] and SMA [12]. Results presented here, however, definitively show that high IL-1β-producing TA haplotype is associated with elevated Hb concentrations and moderate protection against SMA, while low IL-1β producing CA individuals have an increased risk of developing SMA. Although the mechanism(s) by which enhanced IL-1β production may protect against SMA remain to be determined, previous *in vitro* findings showed that IL-1 promotes enhanced production of hematopoietic factors including, granulocyte-macrophage colony-stimulating factor (GM-CSF) [48], G-CSF, IL-6, and expression of their respective receptors on bone marrow cells [49,50]. We, therefore, hypothesize that the TA haplotype conditions increased

production of IL-1β levels during acute malaria that, in turn, protects against SMA by inducing a bone marrow milieu that fosters enhanced erythropoiesis.

Since IL-1Ra is a potent anti-inflammatory mediator that regulates activities of IL-1β [15], additional experiments investigated IL-1Ra levels in children with and without SMA. Consistent with previous studies in The Gambia [16] and Uganda [17] showing that children with CM had elevated IL-1Ra levels relative to other parasitemic groups, results presented here demonstrate that children with SMA also have increased circulating concentrations of IL-1Ra compared to the non-SMA group. In addition, the IL-1Ra:IL-β ratio was higher in the SMA versus the non-SMA group, further emphasizing that elevated production of IL-1Ra may be an important factor in malaria disease severity. Contrary to findings presented here, previous studies in Thailand [24] and Ghana [25] found no functional associations between IL-1β and IL-1Ra polymorphisms and malaria disease severity. Although presently unclear, differences between populations may be because children in western Kenya develop SMA as the primary manifestation of severe malaria, whereas the severe disease presentation in West Africa was primarily CM. This rationale, however, fails to explain similarities in our study population with SMA and those in The Gambia [16] and Uganda [17] with CM. As such, the most plausible explanation for differences in the study populations may be due to genetic variation in IL-1β and IL-1Ra that differentially affects IL-1β and IL-1Ra production in diverse populations. Additional investigations are currently ongoing in our laboratory to determine the molecular mechanisms by which IL-1β and IL-1Ra condition anemia outcomes in children with falciparum malaria.

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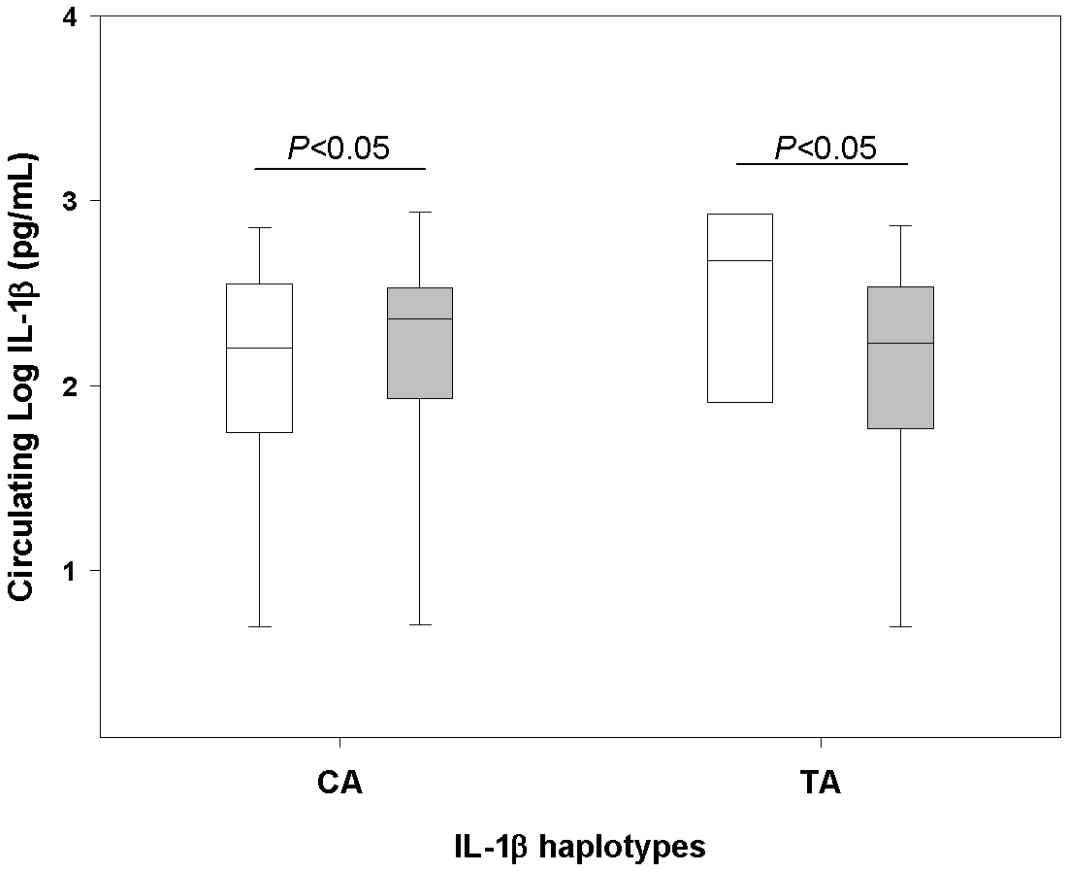

Figure 1. Functional association between IL-1β haplotypes and circulating IL-1β levels

Circulating IL-1β levels for children (aged <3 years) with malaria were measured using the Human Cytokine Twenty-Five-Plex Antibody Bead Kit. Data are presented as box-plots where the box represents the interquartile range, the line through the box is the median, and whiskers show the 10th and 90th percentiles for haplotypic combinations of the IL-1β -31 and -511 variants. Open boxes represent individuals with the respective haplotype, while shaded boxes represent individuals without the haplotype. Statistical significance set at P<0.05 was determined by Mann Whitney U tests.

Table 1

Demographic, clinical, and laboratory characteristics of the study participants.

Characteristic	Non-SMA	SMA	*P*
No. of participants	349	217	
Gender (n, %)			
Male	186 (62.8)	110 (37.2)	0.30[a]
Female	163 (60.4)	107 (39.6)	
Age (mos.)	11.0 (9.75)	8.0 (8.00)	**<0.001**[b]
Hemoglobin (g/dL)	8.1 (2.80)	4.9 (1.20)	**<0.001**[b]
Parasite density (MPS/μL)	20, 345 (43,641)	17, 168 (41,638)	0.22[b]
Geomean parasitemia (/μL)	14, 614	11, 744	0.78[c]
HDP (≥10,000 parasites/μL), n (%)	229 (65.6)	129 (59.4)	0.08[a]

Data are the median (interquartile range; IQR) unless otherwise noted. Parasitemic children (n=566) were categorized according to a modified definition of SMA based on age- and geographically-matched Hb concentrations (i.e., Hb<6.0 g/dL, with any density parasitemia) [26] and Non-SMA (Hb≥6.0 g/dL, with any density parasitemia).

[a] Statistical significance determined by the Chi-square analysis.

[b] Statistical significance determined by Mann-Whitney U test.

[c] Statistical significance determined by student t-test.

Table 2

Distribution of IL-1β genotypes in the clinical categories.

	Genotype	Non-SMA	SMA	Total	*P*
IL-1β -31 C→T	CC, n (%)	218 (64.5)	139 (67.5)	357 (65.6)	
	CT, n (%)	104 (30.8)	58 (28.2)	162 (29.8)	0.77[a]
	TT, n (%)	16 (4.7)	9 (4.4)	25 (4.6)	
IL-1β -511 A→G	AA, n (%)	109 (35.2)	66 (41.8)	175 (37.4)	
	AG, n (%)	154 (49.7)	72 (45.6)	226 (48.3)	0.35[a]
	GG, n (%)	47 (15.2)	20 (12.7)	67 (14.3)	

Data are presented as numbers (proportions). Parasitemic children were categorized based on the presence of SMA (i.e., Hb<6.0 g/dL with any density parasitemia) [26] or Non-SMA (Hb≥6.0 g/dL with any density parasitemia).

[a]Statistical significance determined by the Chi-square analysis.

Table 3

Relationship between individual IL-1β loci, SMA and HDP.

Genotype	SMA (Hb<6.0 g/dL)			SMA (Hb<5.0 g/dL)			HDP (≥ 10,000 MPS/μL)		
	OR	**95%CI**	***P***	**OR**	**95%CI**	***P***	**OR**	**95%CI**	***P***
IL-1β -31 C→T									
CC	1.00			1.00			1.00		
CT	0.85	0.60 - 1.19	0.33	0.93	0.62 - 1.41	0.74	0.83	0.57 - 1.21	0.34
TT	0.63	0.30 - 1.32	0.22	0.61	0.24 - 1.57	0.30	1.39	0.59 - 3.28	0.45
IL-1β -511 A→G									
AA	1.00			1.00			1.00		
AG	1.29	0.76 - 2.20	0.34	1.38	0.70 - 2.71	0.35	1.01	0.57 - 1.77	0.98
GG	1.46	0.85 - 2.54	0.17	1.43	0.71 - 2.86	0.31	0.94	0.52 - 1.68	0.82

Parasitemic children (n=566) were categorized according to a modified definition of SMA based on age- and geographically-matched Hb concentrations (i.e., Hb<6.0 g/dL, with any density parasitemia) [26] and the WHO definition of SMA (i.e., Hb<5.0 g/dL, with any density parasitemia) [27]. In addition, the children were further categorized into those with high-density parasitemia (HDP; ≥ 10,000 MPS/μL). Odds Ratios (OR) and 95% confidence interval (CI) were determined using logistic regression controlling for age, gender, HIV-1 status, bacteremia and sickle-cell trait (HbAS).

Table 4

Hematological characteristics of the study participants stratified according to IL-1β promoter haplotypes.

Haplotype		Hb (g/dL)	SMA (<6.0 g/dL), n (%)	SMA (<5.0 g/dL), n (%)
CA	**1**	6.50 (3.20)[a]	193/494 (39.1)	97/494 (19.6)
	0	7.00 (3.25)	24/72 (33.3)	12/72 (16.7)
CG	**1**	6.40 (3.10)[a]	53/165 (32.1)	28/165 (17.0)
	0	7.05 (3.20)	164/401 (40.9)	81/401 (20.2)
TA	**1**	6.95 (2.40)	2/12 (16.7)	1/12 (8.3)
	0	6.60 (3.25)	215/554 (38.8)	108/554 (19.5)
TG	**1**	6.60 (3.50)	65/175 (37.1)	35/175 (20.0)
	0	6.60 (3.10)	152/391 (38.9)	74/391 (18.9)

Data for hemoglobin (Hb, g/dL) are median (IQR) unless otherwise stated. Parasitemic children (n=566) were categorized according to a modified definition of SMA based on age- and geographically-matched Hb concentrations (i.e., Hb<6.0 g/dL, with any density parasitemia) [26] and the WHO definition of SMA (i.e., Hb<5.0 g/dL, with any density parasitemia) [27] and further stratified based on their IL-1β promoter haplotypes (-31/-511).

[a]Statistically significant at $P<0.05$ (Mann-Whitney U test) for those with versus those without the haplotype. 1=Presence of haplotype, 0=Absence of haplotype.

Table 5

Relationship between IL-1β haplotypes, SMA and HDP.

Genotype	SMA (Hb<6.0 g/dL)			SMA (Hb<5.0 g/dL)			HDP (≥10,000 MPS/μL)		
	OR	**95%CI**	***P***	**OR**	**95%CI**	***P***	**OR**	**95%CI**	***P***
Haplotype (n=566)									
CA (n=494)	1.98	1.55 - 2.27	**<0.05**	1.52	1.36 - 2.66	**<0.05**	0.85	0.51 - 1.41	0.53
CG (n=165)	0.87	0.63 - 1.22	0.42	1.06	0.71 - 1.59	0.76	1.38	0.94 - 2.02	0.09
TA (n=12)	0.52	0.18 - 1.16	0.11	0.61	0.18 - 1.31	0.20	0.79	0.24 - 2.54	0.68
TG (n=175)	0.84	0.61 - 1.17	0.30	0.89	0.60 - 1.33	0.56	0.89	0.62 - 1.28	0.53

Parasitemic children (n=566) were categorized according to a modified definition of SMA based on age- and geographically-matched Hb concentrations (i.e., Hb<6.0 g/dL with any density parasitemia) [26]; and the WHO definition of SMA (i.e., Hb<5.0 g/dL with any density parasitemia) [27]. In addition, the children were further categorized into those with high-density parasitemia (HDP; ≥ 10,000 MPS/μL). Odds Ratios (OR) and 95% confidence interval (CI) were determined using logistic regression controlling for age, gender, HIV-1 status, bacteremia and sickle-cell trait (HbAS). In order to determine the impact of each haplotype on disease phenotypes, individuals without the haplotype were used as the reference group in the multivariate analyses.