IFN-λ4 increases the risk of gastrointestinal infections and malaria in Malian children

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ABSTRACT

Genetic polymorphisms within the *IFNL3*/*IFNL4* genomic region encoding type III interferons have been strongly associated with impaired clearance of hepatitis C virus (HCV) infection. We hypothesized that this association might extend to the immune response to other pathogens as well. In a cohort of 914 Malian children enrolled at birth, we analyzed episodes of malaria, gastrointestinal and respiratory infections in relation to two genetic polymorphisms functionally affecting type III interferons - rs368234815 (IFN-λ4) and rs4803217 (IFN-λ3), using information for 30,626 clinic visits from birth through 1-5 years of follow-up. Compared to children with the rs368234815-TT/TT genotype (IFN-λ4-Null), each copy of the rs368234815-dG allele was associated with an earlier first episode of a gastrointestinal infection (p=0.004), malaria (p=0.044) or respiratory infection (p=0.045). The risk of ever experiencing an infection during the follow-up was also significantly increased with each copy of the rs368234815-dG allele – for gastrointestinal infections (OR=1.5, 95%CI (1.11-2.03), p=0.0079) and malaria (OR=1.32, 95%CI (1.04-1.68, p=0.022), but not respiratory infections (p=0.58). *IFNL4*-rs368234815 and *IFNL3*-rs4803217 were in moderate linkage disequilibrium in this population (r^2=0.78). All the associations for rs4803217 were weaker and lost significance after adjusting for rs368234815, implicating IFN-λ4 and not IFN-λ3 as the primary cause of these associations. Thus, our results suggest the role of IFN-λ4 in several infections in young children.

INTRODUCTION

The outcome of any infection is determined by a complex interplay between host and pathogen factors. One of the main host factors is the immune system, which can be modified by genetic polymorphisms in immune response genes. For example, variable ability to clear hepatitis C virus (HCV) infection spontaneously or after treatment has been significantly associated with polymorphisms in the genetic locus that encodes the family of type III interferons (IFNs) (1-5). Type III IFNs provide a localized immune response at the sites of pathogen entry, such as at the epithelial surfaces of the respiratory and gastrointestinal tracts, as well as in the liver (6, 7). If this first line of defense provided by type III IFNs is successful, it decreases the necessity of a much stronger and systemic immune response provided by type I IFNs (IFNα and IFNβ) and type II IFN (IFN□).

Humans have four type III IFNs, all encoded within the 70 kb region on chromosome 19. Three of these IFNs (IFN-λ1-3) can be inducibly generated in all the individuals, while IFN-λ4 is produced only in about 50% of the world population, including 90% of individuals of African ancestry, versus only 50% of Europeans and 10% of Asians (5, 8). The ability to produce IFN-λ4 is an ancestral trait, which appears to be under negative selection in humans (9). The reasons for this selection are unclear but could be related to poor clearance of some deadly infections in the past. Thus, historic infections might have shaped the genetic landscape of this locus. On the other hand, the variable production of IFN-λ4 might have influenced and still be influencing susceptibility to infections and their clearance.

The variable production of IFN-λ4 is controlled by a frameshift dinucleotide polymorphism, rs368234815-dG, within the first exon of the *IFNL4* gene. The dG allele creates the open reading frame for IFN-λ4 protein, whereas the TT allele results in the truncated, non-functional protein (IFN-λ4-Null) (5). Another polymorphism, rs12979860, also known as the *IL28B* (*IFNL3*) marker, is located within the first intron of *IFNL4* and often used as a proxy for the functional rs368234815 due to population-dependent moderate to high linkage disequilibrium between these variants. Although rs368234815-dG is generally considered the causal variant due to its direct functional effect on the production of IFN-λ4 protein (5), additional polymorphisms in the *IFNL3/IFNL4* region may also demonstrate significant associations because of considerable linkage disequilibrium. One of these linked variants is

a single nucleotide polymorphism (SNP) rs4803217, which is located within the 3'UTR of *IFNL3*; this variant was proposed to affect IFN-λ3 protein by modulating the stability of *IFNL3* mRNA (10).

The strong associations between the *IFNL3*/*IFNL4* genetic variants and HCV clearance have been well established and replicated by multiple studies. Additionally, a recent study reported an association between the *IFNL4* genetic polymorphisms (rs12979860 and rs368234815) and the clearance of respiratory RNA viruses in children from Rwanda (11). To further explore the hypothesis that type III IFNs might impact the immune response to diverse pathogens, we analyzed detailed health records of 914 Malian children and genotyped their DNA for the functional polymorphisms *IFNL4*-rs368234815 and *IFNL3*-rs4803217. These children had 30,626 scheduled and walk-in clinic visits from birth through 1-5 years of follow-up.

We observed that compared to children genetically unable to produce IFN-λ4 (carriers of the rs368234815-TT/TT genotype, IFN-λ4-Null), those with rs368234815-dG allele were significantly more likely to experience gastrointestinal and malarial infections, with the first episode of all infections tested occurring earlier in life. The associations for *IFNL3*-rs4803217 were weaker and not significant after adjustment for *IFNL4*-rs368234815. These results suggest the role of type III IFNs, and IFN-λ4, specifically, in genetic control of the immune response to diverse pathogens critically relevant for the health of infants and young children.

METHODS

The Mali longitudinal birth cohort

We used cord blood samples and data collected by the longitudinal birth cohort study conducted in Ouelessebougou, Mali, between 2010 and 2016. The goal of the cohort was to explore host and parasite factors that influence susceptibility to malaria infection and disease during pregnancy and early childhood. The study enrolled pregnant women aged 15-45 years presenting for antenatal consultations at the Ouelessebougou Community Health Center (CESCOM). The eligibility criteria included residency in the district of

Ouelessebougou for at least one year prior to enrollment and absence of a chronic, debilitating illness. Malaria history or diagnosis was not an exclusion criterion. Newborn children of the participating women were enrolled at delivery and were followed-up for 1-5 years. Prior to enrollment, written informed consent was obtained from the parents/guardians on behalf of their children after receiving written and oral study explanations from a study clinician in their native language. The protocol and study procedures were approved by the institutional review board of the National Institute of Allergy and Infectious Diseases at the US National Institutes of Health (ClinicalTrials.gov ID NCT01168271), and the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry at the University of Bamako, Mali.

The study site was located 80 km south of Bamako, an area of intense but highly seasonal malaria transmission. The follow-up protocol included visits to the clinic scheduled monthly during the high malaria transmission season (June-December) and every two months during the dry season (January-May), as well as *ad hoc* walk-in clinic visits for any acute symptoms. Clinical information was collected during all visits by project clinicians and recorded using standardized forms.

Malaria infection was defined as positivity for *P. falciparum* malaria parasite on thick blood smear microscopy, with or without clinical symptoms. Clinical malaria was defined as fever >37.5^{0}C, presence of malaria-related symptoms and either a positive Rapid Diagnostic Test (RDT) for malaria antigens or visualized malaria parasites on thick blood smear microscopy. Severe malaria (SM) was defined as *P. falciparum* parasitemia and at least one of the following World Health Organization criteria for SM: 2 or more convulsions in the past 24 hours, prostration (inability to sit unaided or in younger infants inability to move/feed), hemoglobin < 5 g/dl, respiratory distress (hyperventilation with deep breathing, intercostal recessions and/or irregular breathing), or coma (Blantyre score < 3). The presence of SM symptoms but with negative tests for malaria antigens or parasites was attributed to other diseases. All other parasitemia episodes, including those that were asymptomatic, were classified as non-severe malaria (NSM). For ever/never analysis, the NSM group included children with malaria history who never had an SM episode, while the SM group included children with at least one SM episode, regardless of NSM episodes.

Gastrointestinal infections (GII) were defined by diarrhea; respiratory infections (RI) were defined by cough and chest-related signs and symptoms (sputum production, dyspnea, tachypnea, abnormal auscultatory findings). The infection etiology (bacterial, viral, parasitic, fungal) was not determined. All children received treatment as clinically indicated for their symptoms. Episodes of the same infection were considered independent if they occurred more than 28 days apart.

DNA extraction and genotyping

Genomic DNA was extracted from cord blood clots using the QIAsymphony automation system with protocol Blood_200_V7_DSP and the DSP DNA Mini Kit (Qiagen) by the Cancer Genome Research Laboratory of the DCEG/NCI. The DNA samples were tested by microsatellite fingerprinting (AmpFLSTR Indentifiler, ThermoFisher) to confirm gender concordance with study records and exclude any mixed up or contaminated samples.

DNA samples were genotyped for *IFNL4*-rs368234815 and *IFNL3*-rs4803217 by custom TaqMan genotyping assays as previously described (5, 12), (**Figure S1**). *HBB* polymorphisms rs334-T/A, *HBB*-Glu7Val (HbS, encoding hemoglobin variants A/A, A/S, and S/S) and rs33930165, Glu7Lys (HbC, encoding hemoglobin variants A/A, A/C, and C/C) were genotyped by Sanger sequencing as previously described (13), (**Figure S2**). Approximately 10% of samples were genotyped in technical duplicates, which were 100% concordant for all markers. The distributions of rs368234815 and rs4803217 were consistent with Hardy-Weinberg equilibrium (HWE). All genotyping and sequencing was done in the Laboratory of Translational Genomics (LTG, DCEG, NCI) blinded to demographic and clinical data. Of the 993 initial samples, 21 (2.1%) were excluded due to poor quantity/quality of the extracted DNA. Of the remaining 972 samples, 38 samples (3.9%) were excluded due to discordant *HBB* status between the study records (determined based on gel electrophoresis) and DNA testing, 18 samples (1.9%) were excluded due to sex discordance between study records and Identifiler profiles and 2 samples (0.2%) – due to failed rs368234815 genotyping. The final set included 914 children with genotype, demographic and clinical data for 30,626 clinic visits; 447 (48.9%) and 467 (51.1%) of children were females and males, respectively. The children were followed-up from birth for

an average of 147 weeks (range 52-264 weeks), with an average of 33.5 visits per child (range 5-81 visits).

Statistical analysis

Haplotypes and linkage disequilibrium metrics (D' and r2) between the markers were analyzed and plotted using Haploview 4.2. The associations for *IFNL4*-rs368234815 and *IFNL3*-rs4803217 were tested based on additive and genotypic genetic models, using the TT/TT genotype or TT allele (IFN-λ4-Null) for rs368234815 and G/G genotype or G allele for rs4803217 as reference groups.

The occurrence of infection episodes (never/ever) was analyzed for both polymorphisms separately and in the same model using binary logistic regression models and adjusting for the follow-up time (weeks). *HBB* status coded as 0, 1 and 2 for AA (wild-type) vs. sickle-cell trait (A/S or A/C) vs. sickle-cell disease (S/S, S/C or C/C) was used as a covariate for all malaria-related analyses; sex and birth month (as a proxy for seasonal effects) were tested but not significantly associated with any outcomes (data not shown). Logistic regression models were used to evaluate the associations of *IFNL4*-rs368234815 with infections of interest adjusting for the occurrence of other infections (ever/never) in order to examine whether these associations were specific to particular infection types.

Time to the first episode of each infection was analyzed with Cox proportional hazards regression models, with P-values calculated per rs368234815-dG and rs4803217-T alleles and presented with Kaplan-Meyer plots. The number of infection episodes during follow-up was analyzed using Poisson models with overdispersion (Quasi-likelihood), adjusting for the length of follow-up as an off-set in the model (offset = log(LastVisitAge)). All the analyses were done using R and SPSS v.25. The results were not adjusted for multiple testing.

RESULTS

Analysis of infection episodes in the Malian children

We analyzed information for 914 children based on health records for each of the 30,626 routine and walk-in clinic visits from birth through 1-5 years of follow-up. During follow-up, 76.1% of children had a malaria episode, including 11.1% children that had at least one SM episode (regardless of NSM episodes) and 65.0% of children that had only a NSM episode (and never an SM episode); 88.1% and 97.7% children experienced a GII and RI episode, respectively.

Episodes of any infection (malaria, GII or RI) were reported at 12.7% of the routine and 76.3% of the walk-in visits (**Table 1**). Thus, most infection episodes were acute and sufficiently severe to require an emergency visit to the clinic without waiting for the next scheduled regular check-up visit. The overall frequency of malaria infection at any visit was 10.6% (5.4% at routine and 19.9% at walk-in visits); GII was reported at 8.4% of all visits, (1.8% at routine and 20.2% at walk-in visits), and RI was reported at 21.0% of all visits (6.5% at routine and 47.4% at walk-in visits). No infection was reported at most visits (n=19,808, 64.7%), while one and two infection types were reported at 9,407 (30.7%) and 1,380 (4.5%) visits, respectively. Only 31 visits (0.1%) reported all three infection types at the same visit.

The most likely co-infection combination was malaria and RI, with 5.3% of routine and 8.2% of walk-in visits, and the least likely co-infection combination was malaria and GII, with 0.8% of routine and 1.6% of walk-in visits (**Table S1**). Logistic regression analysis showed that infections tended to be non-redundant, and any infection significantly decreased the risk of any other infection reported at the same visit (**Table S2**).

Distribution of the HBB, IFNL4 and IFNL3 polymorphisms in the Malian children compared to the populations of the 1000 Genomes Project

The *HbB*-A/A genotype, representing wild-type hemoglobin, was present in 80.5% of children; sickle cell trait (SCT, HbS-A/S or HbC-A/C) – in 18.6%, and sickle cell disease – (SCD, S/S, S/C or C/C) - in 0.9% of the children. The *IFNL4*-rs368234815 alleles (dG-72.2% and TT- 28.8%) and *IFNL3*-rs4803217 alleles (G-66.7% and T-33.3%) were in moderate linkage disequilibrium (LD) in the total set of 914 Malian children (D'=0.98 and r^2=0.78), which was also reflected in the haplotype frequencies of these variants (**Figure S3**). The allele and genotype frequencies of *HBB, IFNL3* and *IFNL4* polymorphisms were

comparable to those in the populations of African ancestry in the 1000 Genomes Project, especially to the populations from West-Africa (**Table S3**).

Associations between the HBB, IFNL4 and IFNL3 polymorphisms and infection episodes in the Malian children

We analyzed the risk of reporting an infection episode at any clinic visit from birth through 1-5 years of follow-up (ever/never analysis). The higher SCT frequency in children with NSM (20.0%) and never malaria (21.1%) differed from the group of children with SM (4.9%) and was protective from SM (p=0.04, **Table 2**). Thus, *HBB* genotype status was included as a covariate in all the malaria-related analyses.

The rs368234815-dG allele was associated with an increased risk of malaria overall (with a per-allele OR=1.32, 95%CI (1.04-1.68), p=0.02), as well as with the risk of NSM (OR=1.30, 95%CI (1.02-1.65), p=0.035) and SM (OR=1.52, 95%CI (1.02-2.28), p=0.042). The risk of GII was significantly increased with each rs368234815-dG allele, OR=1.50, 95%CI (1.11-2.03), p=0.0079). However, the risk of RI was not associated with rs368234815, OR=1.20, 95%CI (0.63-2.29, p=0.58) (**Table 3**).

Gastrointestinal and respiratory symptoms might overlap with clinical manifestations of malaria. However, the associations of *IFNL4*-rs368234815 with these infections appeared independent as mutual conditioning on these infections did not significantly alter the results (**Table S4**).

We also analyzed the association between *IFNL4*-rs368234815 and time to the first episode of each infection, which represents the earliest occurrence of symptoms of sufficient severity to require a visit to the clinic. The most significant association was observed for the *IFNL4*-rs368234815 and the time to the first episode of GII (per allele HR=1.17, 95% CI (1.05-1.30, p=0.004) (**Figure 1**). The associations for malaria and RI were also significant but weaker (p=0.044 for malaria and p=0.045 for RI), but not significant for SM (**Figure 1**). The duration of follow-up did not differ significantly for children with different *IFNL4* genotypes (**Table S5**).

The number of episodes reported for each infection during follow-up was the lowest for children with the IFN-λ4-Null genotype, but this was significant only in the dominant

genetic model for GII (p=0.04, **Table S6**), suggesting that the increased susceptibility is related to infection overall and not its recurrence.

All the associations for *IFNL3*-rs4803217 were weaker than for rs368234815 (**Table S7**) and lost significance in the models that included both variants, while most of the associations for rs368234815 remained significant (**Table 4**).

DISCUSSION

In 2016, pneumonia, malaria and diarrhea were the three major infections that accounted for 36% of all deaths in African children under the age of 5 (14). Thus, it is critical to identify and understand the factors affecting susceptibility and the host response to these infections. We identified the genetic ability to produce IFN-λ4, a type III IFN, due to the presence of the *IFNL4*-rs368234815-dG allele as a factor significantly increasing the risk of gastrointestinal and malarial infections and earlier occurrence of these infections and RI in young Malian children.

Acute RI cause 1.9 million deaths annually in children under 5, with 70% of these deaths occurring in Africa and Southeast Asia (15). Several studies have explored the role of type III IFNs in the immune response to the respiratory syncytial virus (RSV) and influenza in the human and murine nasal epithelium. These studies suggested that type III IFNs represent the first line of defense from respiratory RNA viruses, and treatment with recombinant IFN-λs can protect from RI and stop their spread from upper to lower respiratory tract (16-19).

Recently, a genetic association was reported for *IFNL4*-rs12979860-T and rs368234815-dG alleles with impaired clearance of respiratory RNA viruses in nasal swabs of children from Rwanda (11). The observed effect was in the same direction as for clearance of HCV reported in many studies, suggesting that the negative impact of IFN-λ4 on clearance of RNA viruses is not limited by HCV but extends to respiratory RNA viruses. In our study, we observed an earlier occurrence of RI in children with rs368234815-dG allele, but the association with increased susceptibility to RI in these children did not reach statistical significance. This could be because respiratory symptoms recorded during clinic visits in our study were very common (were experienced by 97.7% of children during follow-up),

and occurred very early in life, with the median timing of the first RI episode being 16 weeks of age. Furthermore, RI episodes were defined very broadly, without stratifying by specific viral etiology, as was done in the study in Rwanda (11). In addition to bacterial and viral etiology, respiratory symptoms might also be caused by helminths (pinworms, roundworms, etc) transiting through the lungs during their life cycle. The more specific definition of RI etiology and surveillance of clearance is needed to clarify the association with genetic variants in the *IFNL3*/*IFNL4* region.

Episodes of acute gastroenteritis (diarrhea and vomiting) are commonly caused by infections with rotavirus (RoV) or norovirus (NoV). Rotavirus is considered the most important global cause of severe diarrhea in children under the age of 5, which, despite the global introduction of vaccinations a decade ago, still caused ~215,000 deaths in 2013 (https://www.who.int/immunization/monitoring_surveillance/burden/estimates/rotavirus/en/). Rotavirus was detected in rectal swabs of 36.9% of children under the age of 5 seeking clinical help for acute gastroenteritis in Rwanda (20).

Norovirus is the second leading cause of acute gastroenteritis, accounting for up to 20% of all acute infections in all age groups worldwide (21). Although these infections are ubiquitous and spontaneously clear within days in healthy individuals, viral clearance may take longer in younger children, especially in those affected by malnutrition and other infections; extended severe dehydration due to diarrhea can be fatal, especially in infants. Human enteric viruses are difficult to study *in vitro*, but recent advances in human intestinal organoid models (22, 23) should facilitate these studies. In contrast to humans that can produce either 3 or 4 type III IFNs (IFN-λ1, 2, 3 in all and IFN-λ4 - in a subset of individuals), the mouse genome invariably encodes only IFN-λ2 and IFN-λ3, which are collectively referred to as IFN-λ. Although most conclusions on RoV and NoV infections have been drawn using murine viruses and based on mouse models that differ from human cells by the IFN-λ repertoire, these studies proposed an important role of type III IFNs in the control of GII. These conclusions were supported by the functional impact caused by the elimination of IFNLR1, which is a receptor expressed by epithelial cells and specific to the type III IFN family (24-29). However, the IFN-λ-related immune responses to enteric viruses appeared to be viral-strain and mouse-model specific (25), and could also be

affected by the intestinal microbiota (29, 30). Overall, clearance or persistence of enteric viruses likely results from the combination of factors contributed both by the host and the pathogen. Thus, although data to explore GII etiology was not available in our study, a large subset of these acute gastroenteritis episodes are likely to be caused by RoV and NoV - RNA viruses already linked with type III IFNs in animal models or *in vitro* studies. Our results present the first evidence on the impact of genetic variation affecting IFN-λ4, a type III IFN, on the occurrence of GII in African children. With the associations in the same direction as for HCV and respiratory viruses, GII can be added to the list of outcomes negatively affected by the ability to produce IFN-λ4. Clearance of several RNA viruses likely impaired by IFN-λ4 (HCV, respiratory viruses, RoV and NoV), suggests that clearance of other clinically relevant RNA viruses, such a coronavirus, might also be impaired in carriers of the *IFNL4*-rs368234815-dG allele.

Our results showing the association between type III IFNs and malaria infection are also novel. Although functions of type III IFNs are mostly recognized in relation to viral pathogens, the involvement of IFN-λs in bacterial and fungal infections have also been reported (31). Malaria is caused by plasmodium, an intracellular parasite, which initially invades hepatocytes and propagates in the liver where type III IFNs can be expressed after induction by different stimuli. Although the same clinical manifestations could be attributed to different infections, our mutual conditioning suggested that the association between rs368234815-dG allele and different infections is not redundant.

Although rs4803217 was previously suggested as a functional variant affecting the stability of *IFNL3* mRNA and possibly accounting for the association in the *IFNL3/IFNL4* region (10), this SNP appeared to be associated with all the outcomes in our study weaker than rs368234815, supporting *IFNL4*-rs368234815 as a primary associated and functional genetic variant in this region.

Our observations of earlier episodes of multiple infections in children with *IFNL4*-rs368234815-dG allele indicate weaker IFN-λ4-mediated protection from these infections. This protection might be unspecific and related to the generally weaker immune response that would manifest in more severe episodes requiring medical attention, but our study did not have information to examine the duration of each episode of infection.

The strength of our study is the longitudinal analysis of a cohort of children who were regularly examined from birth to up to 5 years of age, a large sample size with 30,626 clinic visits that captured most, if not all, episodes of common infections. Longitudinal studies of transient but recurrent infectious diseases with high spontaneous clearance are more informative than cross-sectional studies, which may miss a significant proportion of events not detectable at the time of the study. The limitations of our study include a broad definition of gastrointestinal and respiratory symptoms without specifying their infectious etiology. Due to the regular medical assistance provided to the children at the time of the routine and walk-in visits, the negative health impacts of these infections were likely to be diminished, which makes our findings conservative. Even though rs368234815-dG allele was associated with a higher risk of these infections in early childhood, our study did not explore the long-term health consequences of these associations. For example, we were not able to test whether earlier exposure to infections would affect immune fitness later in life and why the frequency of rs368234815-dG allele (the ability to produce IFN-λ4) remains very high in Africa. Our exploratory study tested associations between multiple infections and models, which might result in some of these results being false-positive due to multiple testing and unadjusted confounding factors. Further studies in comparable cohorts are warranted to confirm our findings and refine the etiology of these and other infections in different environmental and socio-economic settings. In conclusion, our study suggests that type III IFNs, and IFN-λ4, specifically, negatively affect the immune response to several infections in young children in Africa.

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Conflict of interest: L.P.-O. is a co-inventor on IFN-λ4-related patents issued to NCI/NIH, and receives royalties for monoclonal antibodies for IFN-λ4 detection.

TABLES

Table 1. Distribution of clinic visits for 914 children from the Mali birth cohort study

	Clinic visits, n			
	All visits#	Routine	Walk-in	Other
	30,626	18,654	10,884	1,088
Visits reporting any infection, n,	10,818	2,377	8,303	138
% of all visits	35.3	12.7	76.3	12.7
Visits with malaria, n,	3,260	1,015	2,167	78
% of malaria visits	100.0	31.1	66.5	2.4
% of all infections	10.6	5.4	19.9	7.2
Visits with GII, n	2,563	342	2204	17
% of GII visits	100.0	13.3	86.0	0.7
% of all infections	8.4	1.8	20.2	1.6
Visits with RI, n	6,437	1,221	5,161	55
% of RI visits	100.0	19.0	80.2	0.9
% of all infections	21.0	6.5	47.4	5.1

GII – gastrointestinal infections; RI – respiratory infections; Malaria – positivity by blood smear test with/without additional clinical symptoms, includes both severe (SM) and non-severe malaria (NSM).

Table 2. Associations between *HBB* genotypes and infections in 914 children from the Mali birth cohort study

Infections			*HBB* genotypes			Ever/never*	
	All children	Total n	A/A n , %	A/S or A/C n , %	S/S, S/C or C/C n , %	OR	P-val
	Total	914	736	170	8		
	% of total		80.5	18.6	0.88		
Malaria	Never	218	169	46	3	Ref	
	23.90		77.5	21.1	1.4		
	Any	696	567	124	5	0.83 (0.58-1.18)	0.30
	76.10		81.5	17.8	0.72		
	SM	102	94	5	3	**0.50** **(0.25-0.97)**	**0.04**
	11.20		92.2	4.90	2.9		
	NSM	594	473	119	2	0.89 (0.62-1.28)	0.52
	65.00		79.6	20.0	0.34		
GII	Never	109	90	18	1	Ref	
	11.90		82.6	16.5	0.92		
	Ever	805	646	152	7	1.21 (0.74-1.99)	0.45
	88.10		80.3	18.9	0.87		
RI	Never	21	16	4	1	Ref	
	2.3		76.2	19.1	4.76		
	Ever	893	720	166	7	0.70 (0.28-1.78)	0.46
	97.70		80.6	18.6	0.78		

*****Binary logistic regression analysis based on additive genetic models and adjusting for the duration of follow-up (weeks); in bold – significant associations; any malaria – positivity by blood smear test with/without additional clinical symptoms; SM – severe malaria; NSM – non-severe malaria.

Table 3. Associations between *IFNL4*-rs368234815 and infections in 914 children from the Mali birth cohort study

Infections			rs368234815 genotypes			rs368234815 alleles		Ever/never*	
	Total % of total	Total n, %	TT/TT n , %	TT/dG n , %	dG/dG n , %	TT n, %	dG n, %	OR	P-val
		914	77	373	464	527	1301		
			8.4	40.8	50.8	28.8	71.2		
Malaria#	Never	218	24	99	95	147	289	Ref	
	23.9		11.0	45.4	43.6	33.7	66.3		
	Any	696	53	274	369	380	1012	**1.32 (1.04-1.68)**	**0.022**
	76.1		7.6	39.4	53.0	27.3	72.7		
	SM	102	4	44	54	52	152	**1.53 (1.01-2.30)**	**0.044**
	11.2		3.9	43.1	52.9	25.5	74.5		
	NSM	594	49	230	315	328	860	**1.30 (1.02-1.65)**	**0.035**
	65.0		8.3	38.7	53.0	27.6	72.4		
GII	Never	109	16	49	44	81	137	Ref	
	11.9		14.7	45.0	40.4	37.2	62.8		
	Ever	805	61	324	420	446	1164	**1.50 (1.11-2.03)**	**0.0079**
	88.1		7.6	40.3	52.2	27.7	72.3		
RI	Never	21	4	7	10	15	27	Ref	
	2.3		19.1	33.3	47.6	35.7	64.3		
	Ever	893	73	366	454	512	1274	1.20 (0.63-2.29)	0.58
	97.7		8.2	41.0	50.8	28.7	71.3		

*****Binary logistic regression analysis based on additive genetic models and adjusting for the duration of follow-up (weeks); in bold – significant associations; #results for malaria are adjusted for *HBB* status; GII – gastrointestinal infections; RI – respiratory infections; any malaria – positivity by blood smear test with/without additional clinical symptoms; SM – severe malaria; NSM – non-severe malaria.

Table 4. Associations between *IFNL4*-rs368234815 and *IFNL3*-rs4803217 with infections in 914 children from the Mali birth cohort study

Infections		*IFNL4*-rs368234815		*IFNL3*-rs4803217		Models with both variants$			
						rs368234815		rs4803217	
		OR, 95%CI	P-val	OR, 95%CI	P-val	OR, 95%CI	P-val	OR, 95%CI	P-val
Malaria #	Never	Ref		Ref		Ref		Ref	
	Any	**1.32 (1.04-1.68)**	**0.022**	1.20 (0.95-1.52)	0.122	**1.79 (1.0-3.21)**	**0.049**	0.72 (0.41-1.27)	0.26
	SM	**1.53 (1.01-2.30)**	**0.044**	**1.56 (1.04-2.33)**	**0.032**	1.07 (0.39-2.96)	0.9	1.47 (0.54-3.99)	0.45
	NSM	**1.3 (1.02-1.65)**	**0.035**	1.16 (0.92-1.47)	0.22	**1.92 (1.07-3.47)**	**0.03**	0.66 (0.37-1.16)	0.15
GI I	Never	Ref							
	Ever	**1.5 (1.11-2.03)**	**0.0079**	**1.34 (0.99-1.80)**	**0.055**	**2.24 (1.01-4.98)**	**0.048**	0.65 (0.30-1.43)	0.29
RI	Never	Ref							
	Ever	1.2 (0.63-2.29)	0.58	1.24 (0.66-2.33)	0.51	0.94 (0.23-3.90)	0.93	1.31 (0.32-5.31)	0.71

*****Binary logistic regression analysis based on additive genetic models and adjusting for the duration of follow-up (weeks); in bold – significant associations; #results for malaria are adjusted for *HBB* status, $ - results for models including both variants; any malaria – positivity by blood smear test with/without additional clinical symptoms; SM – severe malaria; NSM – non-severe malaria.

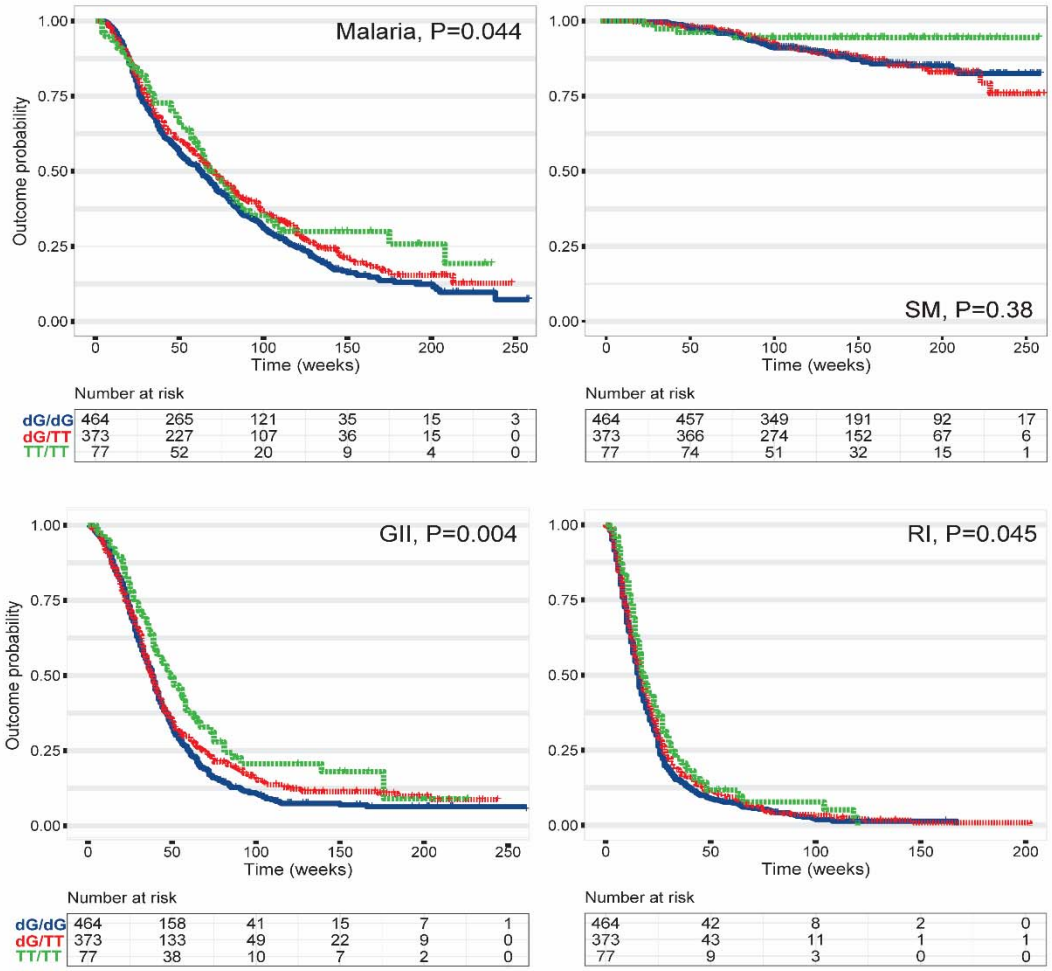

Figure 1. Time to the first episode of infections in relation to *IFNL3*- rs368234815 polymorphism in 914 children from the Mali birth cohort study. Malaria – positivity by blood smear test with/without additional clinical symptoms, includes both severe (SM) and non-severe malaria; GII – gastrointestinal infections; RI – respiratory infections. The plots are for Kaplan-Meyer analysis, P-values are for Cox proportional hazards regression models with per-allele linear trends, for malaria – adjusting for *HBB* status.

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SUPPLEMENTARY MATERIALS

Table S1. Analysis of clinic visits reporting co-infections in 914 children from the Mali birth cohort study

Visit types	**Visits, N**	**Malaria & GII**	**Malaria & RI**	**GII & RI**
Routine visits with at least 1 infection	2377	19 (0.8%)	127 (5.3%)	58 (2.4%)
Walk-in visits with at least 1 infection	8303	136 (1.6%)	677 (8.2%)	444 (5.3%)

Table S2. Probability of co-infections reported at clinic visits, Log Odds in 914 children from the Mali birth cohort study

Routine visits with at least one infection

	Second infection		
First infection	Malaria	GII	RI
Malaria		-2.79	-3.35
GII	-2.79		-1.88
RI	-3.35	-1.88	

Walk-in visits with at least one infection

	Second infection		
First infection	Malaria	GII	RI
Malaria		-2.03	-1.79
GII	-2.03		-2.60
RI	-1.79	-2.6	

All logistic regression tests reported $P < 2e\text{-}16$ for Log Odds

Table S3. Distribution of *IFNL4*-rs368234815, *IFNL3*-rs4803217 and *HBB* polymorphisms in populations of African ancestry from the 1000 Genomes Project and 914 children from the Mali birth cohort study

Gene Variant	Alleles Genotypes	ACB N= 96	ASW N= 61	LWK N= 99	ESN N= 99	GWD N= 113	MSL N= 85	YRI N= 108	Mali N= 914
IFNL4 **rs11322783** corresponds to rs368234815 in 1000 Genomes	TT	0.26	0.30	0.44	0.28	0.22	0.29	0.27	0.29
	dG	0.75	0.70	0.56	0.72	0.78	0.71	0.73	0.71
	TT/TT	0.08	0.10	0.14	0.09	0.05	0.07	0.09	0.08
	TT/dG	0.34	0.41	0.61	0.38	0.34	0.44	0.35	0.41
	dG/dG	0.57	0.49	0.25	0.53	0.61	0.49	0.56	0.51
IFNL3 **rs4803217**	G	0.29	0.34	0.47	0.28	0.30	0.38	0.33	0.33
	T	0.71	0.66	0.53	0.72	0.70	0.62	0.67	0.67
	G/G	0.10	0.15	0.18	0.07	0.09	0.15	0.12	0.11
	T/G	0.52	0.48	0.24	0.52	0.50	0.39	0.45	0.45
	T/T	0.38	0.37	0.58	0.41	0.41	0.46	0.43	0.44
LD **between rs11322783 and rs4803217**	D'	0.97	1.0	0.85	0.92	1.0	1.0	1.0	0.98
	r^2	0.78	0.86	0.66	0.83	0.67	0.65	0.73	0.78
HBB **rs334**	T	0.95	0.98	0.90	0.88	0.89	0.88	0.86	0.95
	A	0.05	0.02	0.10	0.12	0.12	0.12	0.14	0.05
	T/T	0.91	0.97	0.80	0.76	0.77	0.75	0.72	0.91
	A/T	0.09	0.03	0.20	0.24	0.23	0.25	0.28	0.09
	A/A	0	0	0	0	0	0	0	0.0011
HBB **rs33930165**	G	0.96	0.98	1.0	1.0	1.0	0.99	0.97	0.95
	A	0.04	0.02	0	0	0.004	0.006	0.03	0.05
	G/G	0.93	0.97	1.0	1.0	0.99	0.99	0.94	0.90
	G/A	0.07	0.03	0	0	0.009	0.01	0.06	0.10
	A/A	0	0	0	0	0	0	0	0.0011

HBB status: WT – wild-type; SCT – sickle cell trait; SCD – sickle cell disease

1000 Genomes populations of African ancestry (AFR) include Yoruba in Ibadan Nigeria (YRI), Luhya in Webuye, Kenya (LWK), Mandinka in The Gambia (MAG), 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/. LD between markers was analyzed with LDLink (https://ldlink.nci.nih.gov/?tab=home).

Table S4. Time (weeks) from birth to the end of follow-up in relation to *IFNL4*-rs368234815 in 914 children from the Mali birth cohort study

All children, weeks of follow-up Median (95% CI)	*IFNL4*-rs368234815 genotypes, weeks of follow-up, Median (95% CI)			P-value for non-parametric one-way ANOVA, Kruskal-Wallis test
	dG/dG	dG/TT	TT/TT	
140.0 (143.5-150.5)	142.5 (144.0-154.0)	140 (140.4-151.2)	143 (127.8-154.3)	0.537
Min-max time range, weeks 52-264	52-262	52-264	53-261	

Table S5. Numbers of infection episodes per year in relation to *IFNL4*-rs368234815 genotypes during 1-5 years of follow-up in 914 children from the Mali birth cohort study

Infection	Max episode counts, N	Geometric mean*, N				Additive model#, Per-dG allele		Dominant model# dG/dG and dG/TT vs. TT/TT	
		All genotypes	dG/dG	dG/TT	TT/TT	Relative rate	P-value	Relative rate	P-value
Malaria	22	2.82	2.84	2.86	2.45	1.09	0.10	1.19	0.18
SM	3	1.18	1.23	1.13	1.19	1.24	0.20	2.16	0.13
NSM	22	2.75	2.76	2.79	2.44	1.08	0.13	1.17	0.24
GII	14	2.49	2.51	2.54	2.20	1.04	0.29	1.21	**0.04**
RI	25	5.33	5.43	5.30	4.80	1.00	0.84	1.04	0.49

Malaria – includes both severe (SM) and non-severe malaria (NSM); GII – gastrointestinal infections; RI – respiratory infections. For malaria episodes – adjusted for *HBB* status. Geometric means were calculated excluding children who never experienced an infection episode (zero episodes). *Geometric mean is computed by exponentiating the average log-transformed number of episodes. Relative rate represents an increase in the number of episodes per year compared to the reference category, according to additive or dominant genetic models.

Table S6. Associations between *IFNL4*-rs368234815 and mutually adjusted risk of infections in 914 children from the Mali birth cohort study

Infections	Total % of total	Ever/never*		Ever/never, adjusting for episodes of other infections					
		OR (95%CI)	P-val	OR (95%CI)	P-val	OR (95%CI)		OR (95%CI)	P-val
Malaria#	Never 23.9	Ref		Ref		Ref		Ref	
	Any 76.1	**1.32 (1.04-1.68)**	**0.022**	**1.33 1.05-1.70** Adjusted by GII	**0.019**	**1.32 1.04-1.70** Adjusted by RI	**0.022**	**1.33 1.05-1.70** Adjusted by GII and RI	**0.019**
GII	Never 11.9	Ref		Ref		Ref		Ref	
	Ever 88.1	**1.5 (1.11-2.03)**	**0.0079**	**1.52 1.12-2.05** Adjusted by Malaria	**0.0069**	**1.49 1.1-2.01** Adjusted by RI	**0.01**	**1.50 1.11-2.03** Adjusted by Malaria and RI	**0.0091**
RI	Never 2.3	Ref		Ref		Ref		Ref	
	Ever 97.7	**1.2 0.63-2.29**	0.58	1.2 0.63-2.29 Adjusted by Malaria	0.58	1.12 0.58-2.13 Adjusted by GII	0.73	1.12 0.59-2.14 Adjusted by Malaria and GII	0.73

Table S7. Associations between *IFNL4*-rs4803217 and infections in 914 children from the Mali birth cohort study

Infections			rs4803217 genotypes			rs4803217 alleles		Ever/never*	
	Total % of total	Total n, %	G/G n , %	T/G n , %	T/T n , %	G n, %	T n, %	OR	P-val
		914	101 11.5	407 44.5	406 44.4	609 33.3	1219 66.7		
Malaria#	Never 23.9	218	29 13.3	102 46.8	87 39.9	160 36.7	276 63.3	Ref	
	Any 76.1	696	72 10.3	305 43.8	319 45.8	449 32.3	943 67.7	1.20 (0.95-1.52)	0.122
	SM 11.2	102	4 3.9	48 47.1	50 49.0	56 27.5	148 72.5	**1.56** **(1.04-2.33)**	**0.032**
	NSM 65.0	594	68 11.4	257 43.3	269 45.3	393 33.1	795 66.9	1.16 (0.92-1.47)	0.22
GII	Never 11.9	109	16 14.7	54 49.5	39 35.8	86 39.4	132 60.6	Ref	
	Ever 88.1	805	85 10.6	353 43.9	367 45.6	523 32.5	1087 67.5	1.34 (0.99-1.80)	0.055
RI	Never 2.3	21	4 19.0	9 42.9	8 38.1	17 40.5	25 59.5	Ref	
	Ever 97.7	893	97 10.9	398 44.6	398 44.6	592 33.1	1194 66.9	1.24 (0.66-2.33)	0.51

SUPPLEMENTARY FIGURES

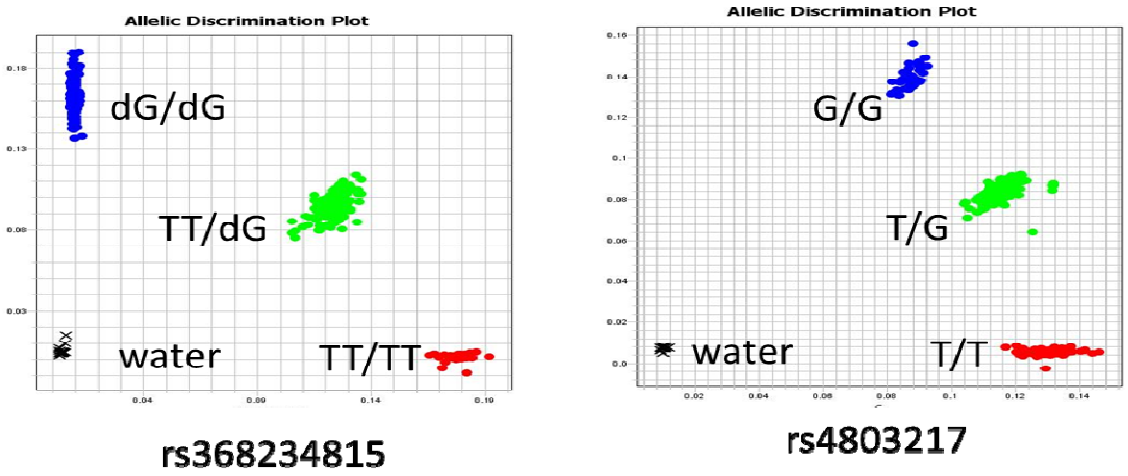


Figure S1. Representative allelic discrimination plots for genotyping of *IFNL4*-rs368234815 and *IFNL3*-rs4803217 polymorphisms in the Mali birth cohort study samples by custom TaqMan genotyping assays.

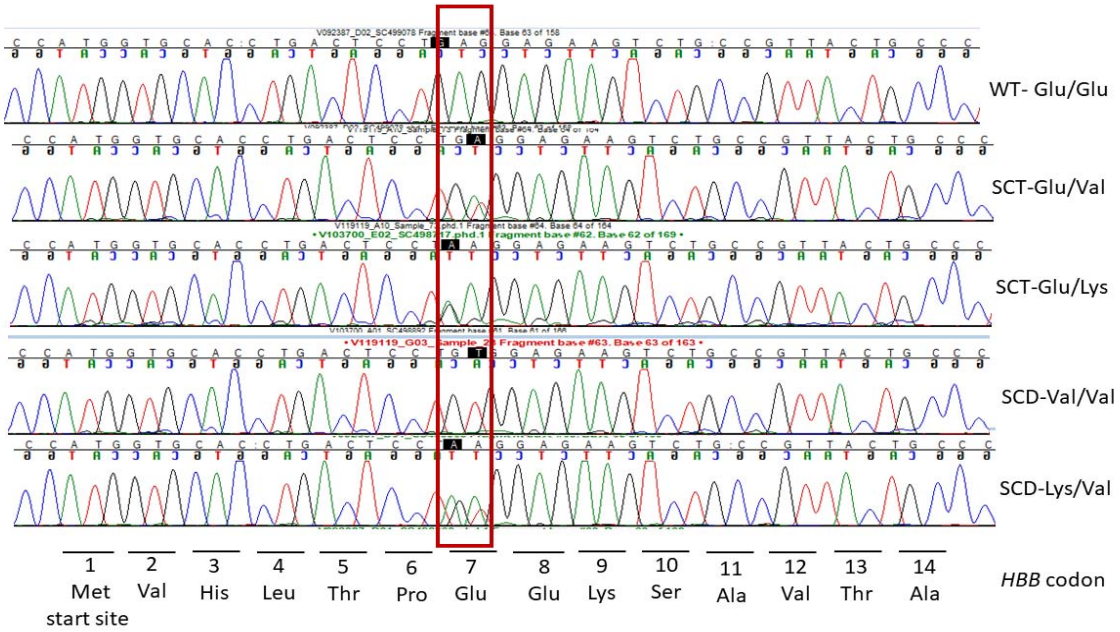
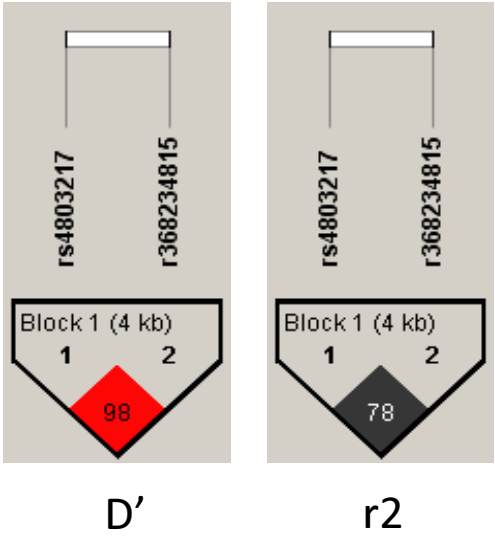


Figure S2. Representative Sanger sequencing chromatograms for genotyping of the *HBB* polymorphisms rs33930165-A/G (Glu7Lys) and rs334-A/T (Glu7Val) in the Mali birth cohort study samples, as has been previously described (13).



Haplotypes of *IFNL4*-rs368234815 and *IFNL3*-rs4803217 in 914 children from the Mali birth cohort study				
Haplotype	*IFNL4* frame-shift rs368234815	*IFNL3* 3'UTR rs4803217	Frequency, %	IFN-λ4 protein
1	dG	T	66.4	produced
2	TT	G	28.5	not produced
3	dG	G	4.8	produced

Figure S3. HaploView linkage disequilibrium (LD) plots and haplotypes of the *IFNL4*-rs368234815 and *IFNL3*-rs4803217 polymorphisms in 914 children from the Mali birth cohort study. The plots show LD metrics - D'=0.98; r^2=0.78.

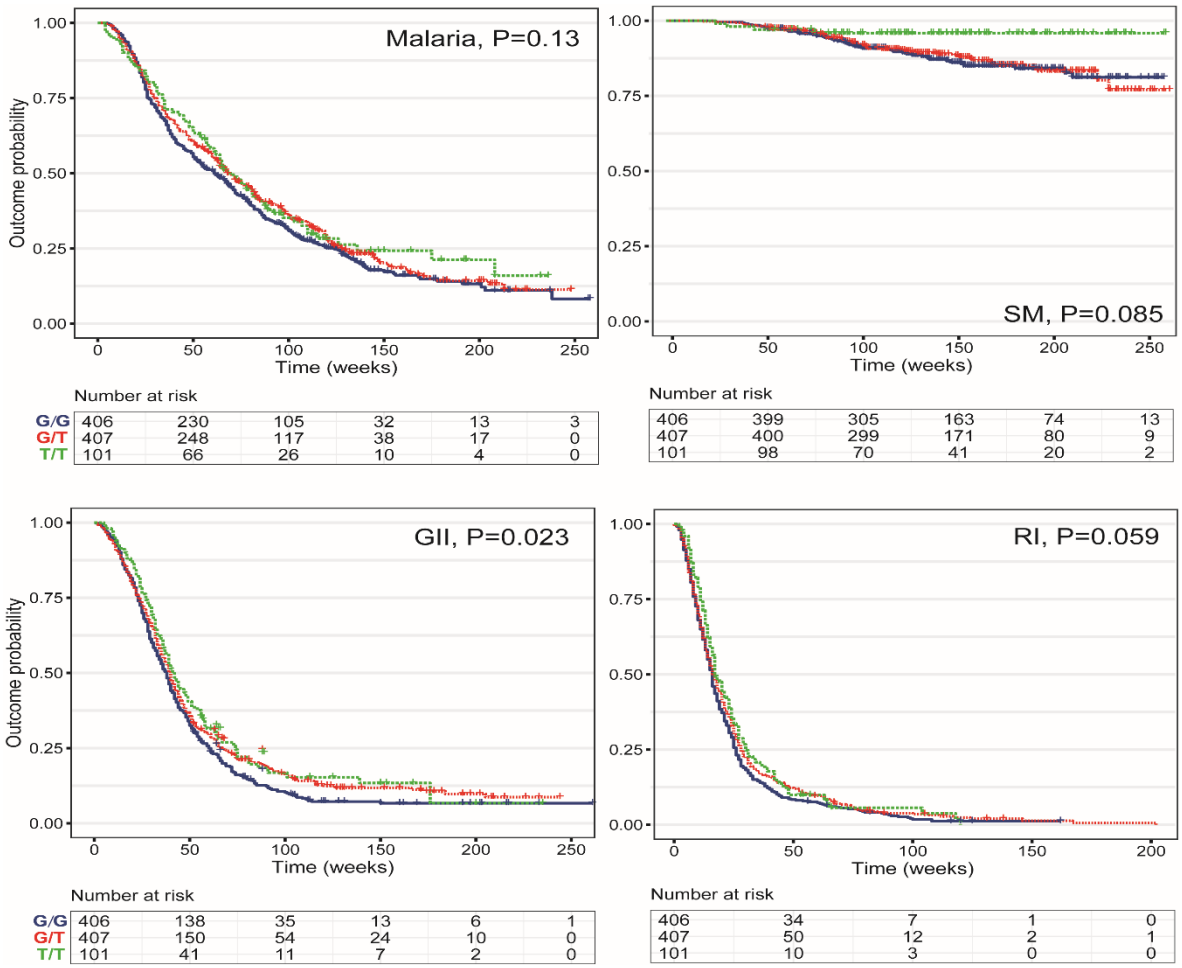


Figure S4. Time to the first episode of infections in relation to *IFNL3*-rs4803217 polymorphism in 914 children from the Mali birth cohort study. Malaria – positivity by blood smear test with/without additional clinical symptoms, includes both severe (SM) and non-severe malaria; GII – gastrointestinal infections; RI – respiratory infections. The plots are for Kaplan-Meyer analysis, P-values are for Cox proportional hazards regression models with per-allele linear trends, for malaria – adjusting for *HBB* status.