Genetic variation in the immune system and malaria susceptibility in infants: a nested case–control study in Nanoro, Burkina Faso

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Abstract

Background: Genetic polymorphisms in the human immune system modulate susceptibility to malaria. However, there is a paucity of data on the contribution of immunogenetic variants to malaria susceptibility in infants, who present differential biological features related to the immaturity of their adaptive immune system, the protective effect of maternal antibodies and fetal haemoglobin. This study investigated the association between genetic variation in innate immune response genes and malaria susceptibility during the first year of life in 656 infants from a birth cohort survey performed in Nanoro, Burkina Faso.

Methods: Seventeen single nucleotide polymorphisms (SNPs) in 11 genes of the immune system previously associated with different malaria phenotypes were genotyped using TaqMan allelic hybridization assays in a Fluidigm platform. *Plasmodium falciparum* infection and clinical disease were documented by active and passive case detection. Case–control association analyses for both alleles and genotypes were carried out using univariate and multivariate logistic regression. For cytokines showing significant SNP associations in multivariate analyses, cord blood supernatant concentrations were measured by quantitative suspension array technology (Luminex).

Results: Genetic variants in IL-1β (rs1143634) and FcγRIIA/CD32 (rs1801274)—both in allelic, dominant and co-dominant models—were significantly associated with protection from both *P. falciparum* infection and clinical malaria. Furthermore, heterozygote individuals with rs1801274 SNP in FcγRIIA/CD32 showed higher IL-1RA levels compared to wild-type homozygotes (*P* = 0.024), a cytokine whose production is promoted by the binding of IgG immune complexes to Fcγ receptors on effector immune cells.

Conclusions: These findings indicate that genetic polymorphisms in genes driving innate immune responses are associated to malaria susceptibility during the first year of life, possibly by modulating production of inflammatory mediators.

Keywords: Malaria, *Plasmodium falciparum*, Immunogenetic variants, Cytokines, Innate immunity

Background

Malaria is a life-threatening infectious disease caused by *Plasmodium* protozoan parasites and transmitted by *Anopheles* mosquitoes. Despite global malaria control and elimination efforts, which reduced the number of malaria-related deaths by 50% since 2000, malaria
remains a major public health problem, particularly in pregnant women and children from sub-Saharan Africa [1, 2].

Individual risk for malaria infection and disease is complex and multifactorial, and is influenced/modulated by host genetic background [3, 4]. Quantitative genetics have estimated that human genetic factors could explain 25% of individual variation in susceptibility to clinical malaria in Africa [5]. An example are the numerous studies that have demonstrated a prominent role of red blood cell (RBC) polymorphisms, such as haemoglobin-inherited disorders (e.g. thalassaemia, sickle cell disease), erythrocyte membrane protein polymorphisms (e.g. ovalocytosis, spherocytosis, Duffy antigen) and erythrocyte enzymatic disorders (e.g. glucose-6-phosphate dehydrogenase) in malaria susceptibility [6, 7]. On the other hand, there is increasing evidence that identifies polymorphisms in genes related to the immune system as important determinants in susceptibility to malaria infection and disease. Immuno-genetic variants that have been associated with diverse degrees of malaria susceptibility include: (i) polymorphisms in the Human Leucocyte Antigen (HLA), which may affect recognition of parasite antigens [8–10]; (ii) polymorphisms in cytokine related genes, which may affect protein levels and downstream functions, such as production of C-reactive protein and immunoglobulin (Ig) isotype switching [11–16]; (iii) polymorphism in toll-like receptors (TLRs), which may impair the ability of individuals to respond adequately to TLR agonists [17–21]; and (iv) polymorphisms in IgG Fcy receptors, which may affect IgG immune complexes binding and the regulation of the IgG subclass production [22–26].

In Burkina Faso, genetic epidemiology has demonstrated that the wild-type R131 allele (rs1801274) of the FcyRIIA (CD32) and tumour necrosis factor (TNF)-238G allele (rs361525) were associated with protection from clinical malaria and high parasitaemia, respectively, in infants and children until 10 years of age [27–29]. In contrast, in a family based-study, TNF mutations rs3093664 and rs3093662 were associated with increased risk of parasitaemia and clinical malaria [27]. Overall, most of these studies have been conducted in children and adults, whereas the potential effect of immune genetic variants on infants, who are at great risk of malaria [30] and have particular immunologic characteristics (such as an immature adaptive immune system and the potential protective effect of maternal antibodies and fetal haemoglobin [31–35]), has not been investigated.

Previous studies by our group in Burkina Faso described that malaria infections and disease during the first year of life is high and has a marked age and seasonal-dependency [30], that individual heterogeneity in the risk of malaria in this age group is strongly influenced by in utero environment, with a profound effect of past placental malaria on fetal immune system [36]. The study hypothesis was that polymorphisms in genes driving Th1/Th2/innate immune response pathways may also affect the development of fetal innate immunity and thus, contribute to the heterogeneity in malaria susceptibility observed during the first year of life [30, 37, 38]. To address this question, 17 single nucleotide polymorphisms (SNPs) in 11 genes of the innate immune system previously associated with malaria-related phenotypes in African populations (including cytokines, TLRs, Fcγ receptors and nitrogen oxide synthase 2, NOS2) [4, 14, 39–42] were selected to investigated its association with malaria susceptibility in infants living in Nanoro (Burkina Faso), using a nested case–control study design.

Methods
Study setting
The study was conducted in the rural health district of Nanoro (Burkina Faso), located in the central-west region at 85 km from the capital Ouagadougou. Nanoro health district (NHD) has approximately 166,683 inhabitants and comprises 21 peripheral health centres. Malaria transmission is seasonal and hyperendemic with peaks between July and December and overlapping with the rainy season (June to November). Malaria burden in infants is high and strongly dependent on age and season [30]. Between 2014 and 2016, the incidence of clinical malaria was estimated at 1.03 cases per child-year during the first year of life, whereas the age-specific prevalence of asymptomatic infections ranged from 17.7% at 3 months of age to 31.3% at 12 months [30].

Study visits and participants
A birth-cohort study (n = 734) was nested within the COSMIC clinical trial (NCT01941264) to investigate factors that modulate the risk of malaria during the first year of life [30]. COSMIC was a cluster-randomized controlled trial investigating the protective effect of adding community-scheduled screening and treatment of malaria during pregnancy (CSST) to the standard intermittent preventive treatment with sulfadoxine–pyrimethamine (CSST/IPTp-SP, intervention arm) compared to IPTp-SP alone (control arm) in Burkina Faso, Benin and The Gambia [43]. Infants from mothers participating into COSMIC trial in Burkina Faso were involved in the birth-cohort study. Infants were followed up until 12 months of age following procedures that have been described in detail elsewhere [30, 44]. In brief, malaria infections were detected actively and passively. Active case detection of asymptomatic infections consisted of 4 cross-sectional surveys conducted at 3, 6, 9, and 12 months of age. At
each survey, blood films and blood spots on filter papers (Whatman 3MM, GE Healthcare Life Sciences) were collected for examination by light microscopy (LM) and quantitative real-time polymerase chain reaction (qPCR), respectively. For passive clinical case detection, mothers were encouraged to bring their offspring to the health centre if they displayed any signs of illness. Infants presenting with fever (axillary temperature ≥37.5 °C) on examination or history of fever in the previous 24 h were screened for malaria infection using RDT (SD-Bioline Malaria Ag Pf, Standard Diagnostic, Korea) according to manufacturer’s instructions and, if positive, treated according to national guidelines. Malaria diagnosis was subsequently confirmed by LM on Giemsa stained blood films according to standard procedures [45] and qPCR (procedure see below). For the present study, available DNA samples from 694 unrelated singletons were used for SNPs typing.

Detection of *P. falciparum* infections by qPCR
Parasite and human genomic DNA were extracted from three punches (5 mm in diameter) of dried blood spots on filter papers with QIAamp 96 DNA blood kit (Qiagen, Germany) and eluted in 150µL of water, following the manufacturer’s recommendations. Five microliters of DNA were used as template for qPCR analysis targeting *P. falciparum* var genes acidic terminal sequence (varATS, ≈59 copies per genome) in StepOne Plus thermocycler (Applied Biosystems), as previously described [46]. The limit of detection of the varATS-qPCR was 0.1 parasite/µL as described elsewhere [47]. Samples with Ct value >39.7 were considered negative.

SNPs genotyping in fluidigm system
The SNPs selection for typing was performed based on existing published data. A SNP was included for analysis if: (i) had been previously associated with a malaria-related phenotype (malaria infection, clinical malaria, severe malaria, high parasites density), (ii) had an expected minor allele frequency (MAF) ≥5% in African populations, and (iii) had a functionally tested and/or validated TaqMan® SNPs genotyping assay. The final SNPs selected for analysis were: TLR1 (rs4833095), TLR4 (rs4986790), TLR9 (rs5743836, rs352139 and rs352140), interleukin (IL)-4 (rs2243250), IL-10 (rs1800890 and rs1800896), IL-17F (rs4715291), IL-1β (rs1143634), TNF (rs1800629 and rs3093664), interferon-γ receptor 1 (IFN1R1, rs10065633 and rs10213701), nitrogen oxide synthase 2 (NOS2, rs2297518) and FcγRIIA/CD32 (rs1801274). TaqMan® SNPs genotyping assays were obtained from Applied Biosystems, and genotyping assays performed in GT192.24 dynamic arrays using the BioMark® platform (Fluidigm) at the Department of Genomics of Common Disease, Imperial College (London, UK). All samples underwent 16 cycles of specific target amplification (STA) in a total reaction volume of 10µL. Eight control samples containing known combinations of mutations at selected loci were identified from the Gambian in Western Division (GWD) population based on data from 1000 Genomes Project (http://phase3browser.1000genomes.org/), and DNA obtained from Coriell Cell Repositories. Both GWD controls and duplicate samples were included in all dynamic arrays. PCR and image processing were carried out on the BioMark® HD80 system (version 4.1.2) to determine SNP genotypes from clusters in each assay. Plots generated by the Fluidigm analysis software were subsequently visually revised to check the cluster profile for each assay and how samples with different genotypes were separated. Allelic calls for all GWD controls were consistent across all runs, and all duplicate samples within each plate had identical allele calls. The genotyping success and the overall call rate was determined per SNP.

Haemoglobin genotyping
Haemoglobin genotypes were determined by high resolution melting (HRM) analysis, adapting previously described methods [48]. Reactions were conducted in a LightCycler® 480 System (Roche) in the presence of LightCycler® 480 HRM- Dye (HRM Master 2X, Roche Diagnostics GmbH) in a reaction of 20µL. LightCycler® 480 Gene Scanning Software (version 1.5) was used to analyse the HRM curves data and determine haemoglobin genotypes. Controls with known haemoglobin genotypes (rs 334 AA [HbAA], rs334 AT [HbAS], rs334 TT [HbSS], rs33930165 GA [HbAC]) were added to every plate.

IL-1β and IL-1RA cytokine levels in cord blood
Levels of IL-1β and IL-1RA by cord blood cells unstimulated and stimulated with TLR7/8 agonist (a stimulant that demonstrated higher induction of cytokine production than TLR3 and TLR9 agonists in the same cohort) were determined as described previously [36]. Briefly, fresh whole cord blood samples diluted 1:1 with RPMI 1640 (1X, Gibco) were left unstimulated or stimulated with 10 µg/mL of imidazoquinoline (R848, TLR7/8 ligand; InvivoGen, San Diego, USA). Supernatant was collected following incubation for 24 h at 37 °C in 5% CO2, and then frozen at −80 °C. IL-1β and IL-1RA were quantified with the fluorescent bead-based multiplex immunoassay (Human Cytokine Magnetic 30-Plex Panel kits, Novex®, Life Technologies™, USA) and analysed on a Luminex® 100/200™ instrument using Xponent 3.1 software.
Definitions and genetic association analysis
The association between polymorphisms in immune genes and (a) malaria infection or (b) clinical malaria was investigated using case–control analysis. For malaria infection analysis, cases were infants who had at least one *P. falciparum* infection during the first year of life, regardless of symptoms; controls were infants who remained uninfected during the first 12 months of life. Uninfected infants who did not complete the 12-month follow-up were excluded from the analysis.

Clinical malaria cases were infants who experienced at least one clinical episode (presence of *P. falciparum* of any density by qPCR and an axillary temperature ≥ 37.5 °C or history of fever within the past 24 h) from birth to 12 months of age, whereas controls were who did not develop a clinical episode, irrespective of infection status.

Data was analysed using R statistical package version 3.2.3 [49]. Baseline characteristics of study participants was compared between case and control groups. Continuous data with normal distribution and categorical variables was analysed using Student *t*-test and Pearson's chi-square tests, respectively. Hardy–Weinberg Equilibrium (HWE) was examined using Pearson’s chi-square statistical test. SNPs were excluded from the analysis if they had more or equal 10% of genotype calls missing or a significant genotypic deviation from HWE (P < 0.001). Case–control association analyses for both SNPs alleles and genotypes were assessed using logistic regression in co-dominant, dominant, over-dominant and recessive models [50]. Maternal age, gravidity, infant birth season, sex, low birth weight (LBW), prenatal malaria exposure (defined as maternal peripheral and placental infections and considered as main risk factor regardless of the presence of symptoms; controls were infants who remained uninfected during the first 12 months of life. Uninfected infants who did not complete the 12-month follow-up were excluded from the analysis.

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Results
Characteristics of cases and controls groups
Of the 694 infants whose DNA samples were genotyped, 656 (94.5%) completed the 12 months follow-up and were included in the analysis. Overall, 80.3% (527/656) of infants experienced at least one *P. falciparum* infection during the first year of life, out of which 78% (411/527) developed at least one clinical episode. The baseline characteristics of the study participants (cases and controls) are presented in Table 1. There were no significant differences in maternal characteristics between cases and controls for both malaria infections and clinical episodes. The proportion of infants in different categories of prenatal malaria exposure was significantly different between cases and controls for both clinical cases and malaria infection (P = 0.021 and P < 0.001, respectively). In addition, the proportion of infants born during the malaria high-transmission season was significantly higher among clinical malaria controls than cases (P = 0.031). In contrast, the proportion of infants born with LBW was significantly higher among clinical malaria cases than controls (P = 0.015). There was an ethnic homogeneity among the study participants with most infants (90%) belonging to the Mossi ethnic group. The proportion of children whose mother received 3 or more SP doses during pregnancy was significantly higher among controls than cases for both malaria infection and clinical episode (P = 0.006 and P = 0.034, respectively). There were no significant differences in haemoglobin genotypes between cases and controls. All the participants were breast feed during the first year of life.

Association between immune genetic variants and malaria infection
Genotyping success per SNP was >94% for all SNPs selected with the exception of TNF rs3093662 SNP (59.7%), which had >10% missing data and was therefore removed from the analysis (Table 2). Call rates for all SNPs were >96% for most assays. TLR9 rs3521140 was removed as it deviated from HWE (P < 0.001). Among the 15 SNPs included in the final analysis, SNPs rs4833095 (TLR1), rs3093664 (TNF-α) and rs2297518 (NOS2A) showed a minor allele frequency below 10% (Table 2).

A first analysis investigated the association between immune genetic variants (allelic and genotypic) and *P. falciparum* infections as detected by qPCR (Table 3).

Both univariate and multivariate allele-based analysis showed that infants carrying the mutant allele A in IL-1β (rs1143634, adjusted odds ratio (AOR) = 0.52, 95%CI 0.34–0.80, *P* = 0.003) and the mutant allele A in FCyRIIA/CD32 (rs1801274, AOR = 0.51, 95%CI 0.37–0.70, *P* < 0.001, respectively) were more likely to be protected against malaria infection than infants with the wild type G allele. The genotypic analysis (univariate and multivariate) confirmed that polymorphisms in IL-1β (dominant model: AG/GA vs GG, AOR = 0.54, 95%CI 0.33–0.88, *P* = 0.014) and iFCyRIIA/CD32 (dominant model: GA/AA vs GG, AOR = 0.44, 95%CI 0.28–0.70, *P* < 0.001) were significantly associated with protection from malaria infection. Furthermore, protection was enhanced among homozygotes individuals in
Natama et al. Malar J (2021) 20:94

Table 1 Characteristics of study participants

| Variables | Malaria infection | Clinical malaria |
|-----------|-------------------|------------------|
|           | Cases (527)       | Controls (129)   | P-value | Cases (N = 411) | Controls (N = 245) | P-value |
| Maternal characteristics |                   |                  |         |               |                   |         |
| Age (years, mean ± SD)     | 26.5 ± 6.3        | 26.2 ± 6.2       | 0.641   | 26.4 ± 6.2    | 26.4 ± 6.3        | 0.988   |
| Gravidity (no. (%))        | 0.176             | 0.250            |         | 0.520         |                     |         |
| Primigravida                | 88 (16.7)         | 25 (19.4)        | 0.355   | 212 (51.6)    | 120 (49.0)         | 0.520   |
| Secundigravida              | 92 (17.5)         | 14 (10.8)        | 0.006   | 223 (54.3)    | 112 (45.7)         | 0.034   |
| Multigravida                | 347 (65.8)        | 90 (69.8)        | 0.152   | 326 (79.3)    | 195 (79.6)         | 0.934   |
| MIP preventive strategy    |                   |                  |         |               |                   |         |
| Standard IPTp-SP           | 262 (49.7)        | 70 (54.3)        | 0.021   | 242 (58.9)    | 165 (67.4)         | 0.031   |
| CSST/IPTp-SP               | 265 (50.3)        | 59 (45.7)        | 0.443   | 413 (78.4)    | 108 (83.7)         | 0.410   |
| SP doses uptake            |                   |                  |         |               |                   |         |
| <3 doses                   | 283 (53.7)        | 52 (40.3)        | 0.006   | 223 (54.3)    | 112 (45.7)         | 0.034   |
| ≥3 doses                   | 244 (46.3)        | 77 (59.7)        | 0.014   | 188 (45.7)    | 133 (54.3)         | 0.934   |
| ITN usagea                 | 413 (78.4)        | 108 (83.7)       | 0.152   | 326 (79.3)    | 195 (79.6)         | 0.934   |
| Infant’s characteristics   |                   |                  |         |               |                   |         |
| Birth season (no. in malaria high-transmission season (%)) | 323 (61.3) | 84 (65.1) | 0.422 | 242 (58.9) | 165 (67.4) | 0.031 |
| Gender (no. females (%))   | 272 (51.6)        | 64 (49.6)        | 0.684   | 201 (48.9)    | 135 (55.1)         | 0.125   |
| LBW (< 2500 g) (no. (%))   | 45 (8.5)          | 6 (4.6)          | 0.140   | 40 (9.7)      | 11 (4.5)           | 0.015   |
| PME (no. (%))              | < 0.001           |                  |         | 0.021         |                   |         |
| Active PM                  | 113 (23.7)        | 16 (13.8)        |         | 87 (23.1)     | 42 (19.4)          |         |
| Past PM                    | 223 (46.8)        | 43 (37.0)        |         | 177 (47.1)    | 89 (41.0)          |         |
| MPI                        | 53 (11.1)         | 19 (16.4)        |         | 47 (12.5)     | 25 (11.5)          |         |
| Non-exposed                | 88 (18.4)         | 38 (32.8)        |         | 65 (17.3)     | 61 (28.1)          |         |
| Ethnicity (no. (%))        | 0.443             |                  |         | 0.410         |                   |         |
| Mossi                      | 471 (89.4)        | 122 (94.6)       |         | 366 (89.1)    | 227 (92.7)         |         |
| Gourounsi                  | 52 (9.9)          | 6 (4.6)          |         | 42 (10.2)     | 16 (6.5)           |         |
| Fulani                     | 3 (0.6)           | 1 (0.8)          |         | 2 (0.5)       | 2 (0.8)            |         |
| Somo                       | 1 (0.2)           | 0 (0.0)          |         | 1 (0.2)       | 0 (0.0)            |         |
| Haemoglobinb               |                   |                  |         | 0.428         |                   |         |
| AA                         | 293 (56.8)        | 75 (58.6)        | 0.960   | 235 (58.3)    | 133 (55.2)         | 0.428   |
| AC                         | 137 (26.5)        | 34 (26.6)        |         | 100 (24.8)    | 71 (29.5)          |         |
| AS                         | 67 (13.0)         | 16 (12.5)        |         | 53 (13.2)     | 30 (12.4)          |         |
| SC                         | 12 (2.3)          | 2 (1.5)          |         | 8 (2.0)       | 6 (2.5)            |         |
| SS                         | 7 (1.4)           | 1 (0.8)          |         | 7 (1.7)       | 1 (0.4)            |         |

PME prenatal malaria exposure, PM Placental malaria, MPI maternal peripheral infection during pregnancy, IPTp-SP Intermittent preventive treatment during pregnancy with sulfadoxine–pyrimethamine, CSST/IPTp-SP community-based-scheduled screening and treatment of malaria in addition to the standard IPTp-SP

a ITN usage the last night before delivery

b Haemoglobin genotyping was successful for 644 (98.2%) with a total of 516 infants who experienced P. falciparum infection while 403 developed a clinical case

co-dominant models when comparing the genotypes carrying the mutant allele versus the wild-type genotype for both IL-1β (AA vs GG, AOR = 0.16, 95%CI 0.04–0.74, P = 0.019 and AG vs GG, AOR = 0.57, 95%CI 0.35–0.93, P = 0.026) and FcyRIIA/CD32 (AA vs GG, AOR = 0.28, 95%CI 0.14–0.55, P < 0.001 and GA vs GG, AOR = 0.50, 95%CI 0.31–0.81, P = 0.004) SNPs (Additional file 1: Table S1). On the contrary, the presence of IL-10 rs1800896 tended to increase the risk of malaria infection in over-dominant model (CT vs TT/CC, AOR = 1.60, 95%CI 0.99–2.61, P = 0.057).

Association between immune genetic variants and clinical malaria

The allelic analysis showed that carriage of the mutant allele A in IL-1β rs1143634 SNP (AOR: 0.66, 95%CI 0.46–0.97; P = 0.032) and mutated allele A in FcyRIIA/CD32 rs1801274 SNP (AOR: 0.68; 95%CI 0.52–0.89;
## Table 2  Call rate, HWE and minor allele frequencies in selected SNPs in immune genes

| Gene | SNPs   | Genotyping success N (%) | Overall call rate<sup>a</sup> | Maj/MinA | MAF     | HWE (P value) | Malaria infection | Clinical malaria |
|------|--------|--------------------------|-------------------------------|----------|---------|--------------|-------------------|------------------|
|      |        |                          |                               |          |         |              |                   |                  |
|      |        |                          |                               |          |         |              | MAF Cases         | MAF Controls     |
|      |        |                          |                               |          |         |              | MAF Cases         | MAF Controls     |
|      |        |                          |                               |          |         |              |                   |                  |
| TLR1 | rs4833095 | 645 (98.3)             | 98.8                          | C/T      | 9.5     | 0.600        | 9.1               | 11.1             |
| TLR4 | rs4986790 | 654 (99.7)             | 98.4                          | A/G      | 9.0     | 0.999        | 9.2               | 8.1              |
| TLR9 | rs5743836 | 653 (99.5)             | 98.9                          | A/G      | 42.3    | 0.600        | 42.4              | 42.2             |
| TLR9 | rs352139  | 654 (99.7)             | 99.3                          | C/T      | 43.0    | 0.018        | 43.6              | 40.6             |
| TLR9 | rs352140  | 645 (98.3)             | 98.9                          | C/T      | 24.2    | <0.001       | 24.5              | 23.1             |
| IL-4 | rs2242350 | 655 (99.8)             | 96.4                          | T/C      | 22.1    | 0.600        | 21.8              | 23.3             |
| IL-10| rs1800896 | 619 (94.4)             | 98.6                          | T/C      | 31.1    | 0.024        | 31.4              | 29.9             |
| IL-10| rs1800890 | 650 (99.1)             | 97.8                          | A/T      | 20.1    | 0.114        | 20.3              | 19.7             |
| IL-17F| rs4715291 | 655 (99.8)             | 99.0                          | C/T      | 15.6    | 0.045        | 16.2              | 13.6             |
| IL-1B| rs1143634 | 654 (99.7)             | 99.0                          | G/A      | 11.7    | 0.000        | 10.5              | 16.7             |
| TNF  | rs1800629 | 655 (99.8)             | 98.8                          | G/A      | 14.5    | 0.061        | 14.1              | 16.0             |
| TNF  | rs3093664 | 656 (100)              | 98.9                          | A/G      | 5.6     | 0.000        | 5.5               | 6.2              |
| TNF  | rs3093662 | 392 (59.7)             | 97.4                          | A/G      | 5.7     | –            | –                 | –                |
| IFNRI| rs10213701| 655 (99.8)             | 98.6                          | T/A      | 36.4    | 0.063        | 36.5              | 36.0             |
| IFNRI| rs10066533| 656 (100)              | 99.0                          | T/C      | 50.8    | 0.061        | 51.3              | 48.4             |
| NOS2A| rs2297518 | 656 (100)              | 99.4                          | G/A      | 8.2     | 0.180        | 8.5               | 6.6              |
| FcγRIIA/CD32 | rs1801274 | 648 (98.8)         | 98.9                          | G/A      | 32.6    | 0.961        | 30.4              | 41.4             |

<sup>a</sup> Determined by the Fluidigm SNP genotyping analysis
were associated with protection from clinical malaria. The same associations were observed in the genotype-based analysis showing that individuals with AA genotype in IL-1β rs1143634 SNP (recessive model: AA vs AG/GG, AOR = 0.14, 95%CI 0.03–0.78, \( P = 0.024 \)) and those with GA/AA genotypes in FcγRIIA/CD32 rs1801274 SNP (dominant model: GA/AA vs GG, AOR = 0.58, 95%CI 0.41–0.84, \( P = 0.003 \)) were more likely to be protected against clinical malaria (Table 4).

In the co-dominant model, the risk of clinical malaria was only reduced among homozygote AA carriers of IL-1β rs1143634 SNP (AA vs GG, AOR = 0.14, 95%CI 0.03–0.75, \( P = 0.022 \)), while both AA mutant homozygote and GA heterozygote individuals for FcγRIIA/CD32 rs1801274 SNP were significantly protected (AA vs GG, AOR = 0.48, 95%CI 0.26–0.87, \( P = 0.017 \) and GA vs GG, AOR = 0.61, 95%CI 0.42–0.89, \( P = 0.010 \)) (Additional file 2: Table S2). No significant association was found between the selected SNPs with increased risk of clinical malaria during the first year of life. However, a marginal increase risk was observed among infants carrying the TA genotype for rs1800890 SNP in IL-10 locus.
(over-dominant model: TA vs TT/AA, AOR = 1.48, 95%CI 0.99–2.19, P = 0.052) (Table 4).

**Association between genetic polymorphisms and cytokine levels in cord blood**

Next it was assessed whether polymorphisms in IL-1β (rs1143634) were associated with immune effector phenotypes. Therefore, IL-1β levels were quantified in a sub-group of infants) for whom un-stimulated and TLR7/8-stimulated cord blood samples were available (N = 313). Figure 1 shows that IL-1β levels did not differ across rs1143634 genotypic groups for both un-stimulated and TLR-7/8-stimulated samples (P = 0.682 and P = 0.707, respectively).

The association between FcγRIIA/CD32 rs1801274 SNP and IL-1RA levels was also investigated, since binding of FcγRIIA/CD32 to immune complexes induces IL-1RA production [52, 53]). In this case, significant differences in IL-1RA levels across FcγRIIA/CD32

| Gene  | SNPs   | Alleles/genotypes | Model               | Univariate analysis | Multivariate analysisa |
|-------|--------|-------------------|---------------------|---------------------|------------------------|
|       |        |                   |                     | OR (95%CI)          | P                      | OR (95%CI)          | P                      |
| Allele-based analysis |        |                   |                     |                     |                        |                        |                        |
| TLR1  | rs4833095 | T vs C          | –                   | 0.90 (0.62–1.33)    | 0.608                  | 0.96 (0.63–1.46)    | 0.844                 |
| TLR4  | rs4986790 | G vs A          | –                   | 1.04 (0.70–1.54)    | 0.838                  | 1.19 (0.76–1.87)    | 0.442                 |
| TLR9  | rs5743836 | G vs A          | –                   | 0.89 (0.71–1.12)    | 0.324                  | 0.82 (0.64–1.06)    | 0.137                 |
| TLR9  | rs352139  | T vs C          | –                   | 1.13 (0.90–1.42)    | 0.296                  | 1.09 (0.84–1.40)    | 0.524                 |
| IL-4  | rs2243250 | C vs T          | –                   | 1.04 (0.79–1.36)    | 0.772                  | 0.97 (0.72–1.31)    | 0.857                 |
| IL-10 | rs1800896 | C vs T          | –                   | 1.05 (0.82–1.35)    | 0.676                  | 1.00 (0.76–1.33)    | 0.982                 |
| IL-10 | rs1800890 | T vs A          | –                   | 1.17 (0.88–1.56)    | 0.268                  | 1.14 (0.83–1.56)    | 0.423                 |
| IL-17F| rs4715291 | T vs C          | –                   | 1.12 (0.82–1.53)    | 0.462                  | 0.98 (0.70–0.97)    | 0.918                 |
| IL-1β | rs1143634 | A vs G          | –                   | 0.68 (0.48–0.95)    | 0.025                  | 0.66 (0.46–0.97)    | 0.032                 |
| TNF-α | rs1800629 | A vs G          | –                   | 0.97 (0.70–1.33)    | 0.843                  | 1.08 (0.76–1.53)    | 0.682                 |
| TNF-α | rs3093664 | G vs A          | –                   | 0.82 (0.51–1.32)    | 0.406                  | 0.73 (0.43–1.25)    | 0.259                 |
| IFNRI | rs10213701| A vs T          | –                   | 1.11 (0.88–1.41)    | 0.361                  | 1.15 (0.89–1.50)    | 0.283                 |
| IFNRI | rs10065633| C vs T          | –                   | 1.02 (0.82–0.82)    | 0.843                  | 1.06 (0.82–1.36)    | 0.644                 |
| NOS2A | rs2297518 | A vs G          | –                   | 1.09 (0.72–1.65)    | 0.682                  | 1.32 (0.83–2.10)    | 0.239                 |
| FcγRIIA/CD32 | rs1801274 | A vs G          | –                   | 0.71 (0.56–0.90)    | 0.005                  | 0.68 (0.52–0.89)    | 0.005                 |

Significant P values < 0.05 are indicated in italics

a Adjusted by mother’s age, gravidity, birth season, baby’s gender, low birth weight, Prenatal malaria exposure, ethnicity, Haemoglobin variant

b Genotype-based analysis for dominant, over-dominant and recessive models. Models that showed lowest P values (P) are shown

**Table 4 SNPs included in the analysis and association with clinical malaria**
rs1801274 genotypes upon TLR7/8 stimulation of cord blood samples were observed ($P = 0.016$, Fig. 2b). Individuals with GA genotype had higher levels of IL-1RA compared with that of infants carrying the wild-type GG genotype ($P = 0.024$), while no significant difference was observed for individuals carrying the AA genotype ($P = 0.487$). Differences of IL-1RA levels between genotypes in un-stimulated cord blood samples was not significant ($P = 0.619$, Fig. 2a).

**Discussion**

This study investigated associations between genetic variants in immune genes and malaria susceptibility in infants in Burkina Faso. The study focused on cytokine, TLR, FcγRIIA/CD32 and NOS2A genes given their key role in innate immune responses against pathogens in early infancy. SNPs in FcγRIIA/CD32 (rs1801274) and in IL-1β (rs1143634) were significantly associated with protection from both malaria infection and clinical disease during the first year of life and FcγRIIA/CD32 SNP rs1801274 genotypes were associated with IL-1RA levels, a cytokine involved in inflammatory responses to malaria infection.

A number of studies have explored the association between FcyRIIA/CD32 rs1801274 SNP and malaria susceptibility resulting in contradictory findings [28, 29, 40, 54–59]. However, only a limited number included infants and the majority of those focused on severe malaria (e.g. severe malarial anaemia and high-density parasitaemia) [25, 60]. The strongest evidence of FcyRIIA/CD32 rs1801274 (A allele; variant H) protection against blood-stage malaria infections comes from a meta-analysis study on 6928 subjects from Africa and Asia [40]. Results in this study show a similar protective effect of the A allele of the FcyRIIA/CD32 rs1801274 SNP against both malaria infection and disease, with associations confirmed in both co-dominant (AA vs GG and GA vs GG) and dominant (GA/AA vs GG) models after adjusting by potential confounding factors.

Fcγ receptors (FcγRI, FcγRII, FcγRIII) bind to the Fc domain of IgG on immune cells and thereby mediate the initiation of a variety of immunological responses.
including antigen presentation, phagocytosis, cytotoxicity, release of inflammatory mediators and the modulation of immune responses [61, 62]. Therefore, FcγRs are an important link between cellular and humoral immunity in host defence against malaria infection [62]. FcyRIIA, which is expressed on the surface of all types of cells of the immune system, is a low affinity receptor for monomeric IgG, but binds IgG immune complexes efficiently [63]. FcyRIIA/CD32 rs1801274 has been found to alter the gene function in vitro, by extending the preferential affinity of the wild-type G allele for IgG1 and IgG3 towards affinity for IgG2 [57, 64]. Therefore, it’s reasonable to believe that the effec-tive binding to IgG2 while retaining its affinity to IgG1 and IgG3 increase the antibody mediated protective effect against malaria. It has been also suggested that differential binding between immune complexes and Fcy receptors due to polymorphisms in FcyRIIA induce changes in the ability of immune cells to respond to P. falciparum infection through production of inflammatory mediators [58, 62, 64]. Here, the effect of rs1801274 SNP in FcyRIIA/CD32 gene on unstimulated and TLR-stimulated IL-1RA production in cord blood samples was tested, as it has been previously demonstrated that FcyRIIA/CD32 plays a key role in IL-1RA production through binding of immune complexes to monocyte, macrophage and neutrophil lineage cells [52, 53]. Several studies have demonstrated the clinical relevance of IL-1RA in immune responses to malaria [65–68], which acts modulating disease severity by competing with the pro-inflammatory cytokines IL-1α and IL-1β for binding sites on the IL-1 type 1 receptor and inhibits IL-1 signalling [69]. While there were no significant differences in the spontaneous production of IL-1RA by cord blood cells across FcyRIIA/CD32 rs1801274 genotypes, upon TLR7/8 stimulation, individuals with GA genotype had significantly higher levels of IL-1RA compared with those with the wild type GG genotype. Remarkably, in infants from this cohort TLR7/8-induced IL-1RA production in cord blood was an independent predictor of malaria protection during the first year of life [36]. Therefore, study findings support the hypothesis that variability in susceptibility to malaria across FcyRIIA/CD32 rs1801274 genotypes is mediated, at least partially, by changes in immune complex-mediated cytokine production. In addition to the protective effect of IgG1 and IgG3, FcgR2A mutant carriers are likely to be protected due to the high affinity of the A allele product to IgG2 possibly by improving the antibody mediated protective effect. Given that this protective effect could be effective through the mediation of a variety of immunological responses including inflammatory mediators, level of IL1-RA could represent one of the changes in innate immune response to malaria infection between the mutant A and the wild-type G allele carriers. The fact that a significant difference was not observed for homozygous allele could be due to limited number of individuals carrying the AA genotype in the subset of data analysed.

The second genetic variant in immune genes associated with malaria protection in this study was the IL-1β rs1143634 SNP. The carriage of IL-1β rs1143634 mutated allele A (both in homozygosity and heterozygosity) was associated with a decreased risk of malaria infection (84% and 43% reduction, respectively). In addition, AA homozygote carriers (but not AG) had a significantly reduced risk of clinical malaria, suggesting an enhanced protective effect of AA homozygosity. This is the first study to report a protective effect of IL-1β rs1143634 SNP against malaria infection and uncomplicated disease in infants. Previous studies assessing associations of IL-1β rs1143634 and malaria susceptibility reported heterogeneous findings in children including an association with higher peripheral parasitaemia in Ghanaian children (n=461; aged 1–12 years) [70], an association with severe malaria in Gambian children (n=1420; aged<5 years) [71], or no significant association with malaria susceptibility in Cameroonian children (n=1862; aged 1–14 years) [72]. These contrasting results may be attributed to differences in age groups, ethnicity and/or malaria phenotype evaluated.

IL-1β is a pro-inflammatory cytokine that is implicated in the first line of defence against pathogens, including the hepatic and erythrocytic stages of malaria parasites [73–75]. However, high levels of sustained IL-1β production may induce pathogenic effects that promote disease manifestation and severity [76–78]. Previous studies have already demonstrated a correlation between IL-1β rs1143634 SNP and increased in vitro production of IL-1β [79, 80]. To investigate whether the observed protective effect of the rs1143634 SNP in this study was related to functional changes in IL-1β production, IL-1β levels in cord blood were compared across genotypic groups but no significant differences were observed neither in un-stimulated or TLR7/8-stimulated samples.

Genetic variations occurring in genes encoding inflammatory cytokines can have a direct effect on the innate immune responses to malaria infection and disease manifestation. In this regard, the protective effect of the IL-1β rs1143634 could be due to the modulation of IL-1β level towards a protective effect instead to levels that could be harmful.

However, these findings should be interpreted with caution due to very low number of individuals carrying the AA genotype among the subset of infants that could
be analysed (n = 4) and, therefore, potential functional changes in IL-1β production by IL-1β rs1143634 SNP cannot be ruled out. Likewise, it is worth noting that IL-1β production is likely to be influenced by other SNPs in IL-1β gene, as well as polymorphisms in inflammatory mediators such as TNF [81–84] that were not assessed in this study. Correlation between SNP of interest and cytokine levels in different genotypes should be further explored in different malaria phenotypes (severe, uncomplicated, infection) in the field to elucidate these contrasting results.

Other genes coding for major TLRs (i.e. TLR1, 4 and 9) and cytokines (i.e. IL-4, IL-10, IL-17F and TNF) did not show significant association with malaria infection and clinical episodes, although rs1800890 SNPs in IL-10 gene showed a trend towards increased susceptibility to malaria infection or clinical episode in multivariate analysis (P = 0.057 and P = 0.052, respectively). IL-10 is a key immuno-regulator of immunity to infections [85] and has been associated with various malaria-related phenotypes, such as uncomplicated malaria, severe malaria, severe malaria anaemia and high parasite density [86–91]. In addition, a number of studies have reported associations between IL-10 SNPs and susceptibility to malaria with either protective effects in some SNPs and increased susceptibility in others [72, 92–95]. Therefore, this tendency of an increased risk of malaria among carriers of rs1800890 SNP in IL-10 gene, could results from changes that do not allow adequate modulation of inflammatory responses since IL-10 is an anti-inflammatory cytokine.

Conclusion
This study showed that the mutated A allele of IL-1β rs1143634 and mutated A allele of FcγRIIA/CD32 rs1801274 are associated with protection against both malaria infections and uncomplicated disease during the first year of life. These findings suggest that genetic polymorphisms in genes driving innate immune responses could condition malaria susceptibility during the first year of life, possibly by modulating production of inflammatory mediators. Future investigations on the epistatic effects of immune genes polymorphisms described here and functional studies may contribute to further understand how host genetic variation influences malaria susceptibility in infancy.

Supplementary Information
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