Interleukin-17A Serum Level, IL-17A Genetic Polymorphisms and Severe Malaria Disease

Fatou THIAM *, Gora DIOP, Cedric COULONGES, Celine DERBOIS, Alassane THIAM, Abou Abdallah Malick Diouara, Mame New MBAYE, Mamadou DIOP, Cheikh Momar NGUER, Yakhya DIEYE, Babacar MBENGUE, Jean-François Zagury, Jean-François Deleuze, Alioune DIEYE

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Article

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Fatou Thiam [1,*], Gora Diop [2,3], Cedric Coulonges [4], Celine Derbois [5], Alassane Thiam [3], Abou Abdallah Malick Diouara [1], Mame Ndew Mbaye [1], Mamadou Diop [1], Cheikh Momar Nguer [1], Yakhya Dieye [1,6], Babacar Mbengue [7], Jean-Francois Zagury [4], Jean-Francois Deleuze [5] and Alioune Dieye [7]

[1] Groupe de Recherche Biotechnologies Appliquees & Bioprocedes environnementaux, Ecole Superieure Polytechnique, Universite Cheikh Anta Diop de Dakar, Corniche Ouest BP : 5085 Dakar-Fann, Senegal
[2] Unite postulante de Biologie Genetique, Genomique et Bio-informatique (G2B), Departement de Biologie animale, Faculte des Sciences et Techniques, Universite Cheikh Anta DIOP, Avenue Cheikh Anta DIOP, BP : 5005, Dakar, Senegal
[3] Pole d'Immunophysiopathologie & Maladies infectieuses (IMI), Institut Pasteur de Dakar, 36, avenue Pasteur, BP : 220, Dakar, Senegal
[4] Equipe GBA «Genomique, Bioinformatique & Applications», Conservatoire National des Arts et Metiers, 292, rue Saint Martin, 75141 Paris Cedex 03. Paris, France
[5] Centre National de Recherche en Génétique Humaine (CNRGH), Institut de Biologie François Jacob, 2 rue Gaston Crémieux, CP 5721, 91057, Evry Cedex, France
[6] Pôle de Microbiologie, Institut Pasteur de Dakar, 36 Avenue Pasteur, Dakar BP 220, Senegal
[7] Service d'Immunologie, Faculté de Médecine, de Pharmacie et d'Odontostomatologie, Université Cheikh Anta DIOP, Avenue Cheikh Anta DIOP, BP : 5005, Dakar, Senegal
* Correspondence: fatou54.thiam@ucad.edu.sn; Tel: +221786300621

Abstract: Malaria infection is a multifactorial disease partly modulated by host immuno-genetic factors. Recent evidence has demonstrated the importance of Interleukin-17 family proinflammatory cytokines and their genetic variants in host immunity. However, limited knowledge exists about their role in parasitic infections such as malaria. We aimed to investigate IL-17A serum levels in patients with severe and uncomplicated malaria, whether IL-17A gene polymorphisms are involved in severe malaria susceptibility and the polymorphism's influence on the IL-17A serum levels. 125 malaria patients and 48 free malaria controls were enrolled in this research. Malaria patients were classified into severe malaria (SM) and uncomplicated malaria (UM). IL-17A serum levels were measured with ELISA. PCR and DNA sequencing were used to assess host genetic polymorphisms in IL-17A. We performed a multivariate regression to estimate the impact of human IL-17A variants on IL-17A serum level and malaria outcome. Elevated serum IL-17A levels accompanied by increased parasitemia were found in SM patients compared to UM and controls ($P<0.0001$). Also, the IL-17A levels were lower in SM patients who were deceased than in those who survived. In addition, the minor allele frequencies (MAF) of two IL-17A polymorphisms (rs3819024 and rs3748067) were more prevalent in SM patients than UM patients indicating an essential role in SM. Interestingly, the heterozygous rs8193038 AG genotype was significantly associated with higher levels of IL-17A than the homozygous wild type (GG). According to our results, it can be concluded that IL-17A may play a role in protection against fatal malaria outcomes.

Keywords: IL17A; malaria; Plasmodium falciparum; serum level; polymorphism

1. Introduction

Malaria is an infectious disease, potentially fatal, caused by Plasmodium protozoan parasites and transmitted by Anopheles mosquitoes. According to the WHO, malaria is one of the leading

causes of death worldwide. Globally, there were an estimated 247 million malaria cases in 2021 in 84 malaria-endemic countries, increasing from 245 million in 2020, with most of this increase coming from countries in the African Region. Furthermore, in 2020, malaria deaths increased by 10% compared with 2019, and between 2019 and 2021, there were 63 000 deaths due to disruptions to essential malaria services during the COVID-19 pandemic (WHO). Thus, malaria remains a major public health problem, especially in Africa [1].

Data shows that individual risk for malaria infection and disease is multifactorial and modulated by host genetic factors [2]. However, the mechanisms underlying the differences in malaria susceptibility between individuals have yet to be fully understood. Quantitative genetics have estimated that human genetic factors could explain 25% of individual variation in susceptibility to clinical malaria in Africa [3]. Numerous studies have demonstrated a prominent role of red blood cell (RBC) Polymorphisms (SNP), such as haemoglobin-inherited disorders (thalassaemia, sickle cell disease), erythrocyte membrane protein polymorphisms (Duffy antigen) and erythrocyte enzymatic disorders (glucose-6-phosphate dehydrogenase (G6PD)) in malaria susceptibility [4–6]. On the other hand, increasing evidence identifies polymorphisms in genes related to the immune system as essential determinant in susceptibility to malaria infection and disease. Immuno-genetic variants associated with diverse degrees of malaria susceptibility include polymorphisms in cytokine-related genes, which may affect protein levels and downstream functions, such as the production of C-reactive protein and immunoglobulin (Ig) isotype switching [7–11].

The pathogenesis of malaria is complex and needs to be elucidated. During blood-stage infection, the host's immune system produces proinflammatory cytokines to eliminate the parasite, including IL-6, IFN-γ, and TNF, which are pivotal in controlling the parasite's growth and elimination. In many studies, the high levels of some pro-inflammatory cytokines have been protective in malaria [12,13]. Pro-inflammatory biomarkers were more elevated in cerebral malaria than in non-cerebral malaria patients [14]. Regulatory cytokines such as transforming growth factor-β (TGF-β) and IL-10 balance the pro-inflammatory and anti-inflammatory responses. However, in many cases, cytokines have a double role. On the one hand, they contribute to parasitic clearance; on the other, they are responsible for pathological changes encountered in malaria. Cytokine-modulating strategies may represent a promising modern approach to disease management [12].

Recently, the IL-17 cytokine has gained attention among malaria researchers because of its protective role in immunity against extracellular pathogens [15–17] and for the clearance of intracellular pathogens [18–20]. In addition to its essential role in protective immunity, IL-17 is critical in the pathogenesis of various autoimmune inflammatory diseases. IL-17 is a cytokine family that plays a vital role in innate and adaptive immune systems [21–24]. The IL-17 gene is located on chromosome 6p12, comprises three exons and two introns and is coded with six protein members (IL-17A-F). IL-17A is the most essential member of the IL-17 family. The IL-17 receptor family now comprises 5 members (IL-17RA, RB, RC, RD and RE) [25–27].

In mice, it has been demonstrated that elevated IL-17 levels and high IL-4, IL-12α and IFN-γ levels may be a marker of protection against Plasmodium berghei [28]. However, the role of IL-17 in human malarial infection outcomes is poorly described [28], even if increased IL-17 levels in vivax and falciparum malaria and disease severity have been reported [14,29]. Further studies are needed to evaluate the implication of IL-17 cytokines levels and polymorphisms in malaria protection and/or pathogenesis.

This work analyzed IL-17A levels and gene polymorphisms in a Senegalese cohort. IL-17A gene and its flanking regions were sequenced in samples from a cohort of individuals, including healthy controls (CTR), uncomplicated malaria (UM) and Severe Malaria (SM) subjects. The Genetic variations, including single nucleotide polymorphisms (SNPs), were analyzed among individuals concerning malaria disease status to detect their influence on the IL-17A serum levels and associations with malaria severity.

2. Materials and Methods

2.1. Study participants

Our cohort included black Senegalese-born individuals whose parents and grandparents were born in Senegal, a malaria-endemic country in the Sahelian zone of West Africa. Malaria patients were enrolled from participating hospitals and corresponded to subjects with Plasmodium-positive Quantitative Buffy Coat (QBC). This test is more sensitive than Giemsa-stained thick films [6,30]. The malaria patients were classified into two groups: Mild Malaria (MM) and Severe Malaria (SM), according to the criteria defined by Saissy. et al. 2003, and previously described [31]. To ensure homogeneity of data, inclusion criteria were: (1) Only black Senegalese individuals with P. falciparum infection confirmed in diagnosis; (2) persons born in Senegal; whose parents and grandparents were born in Senegal (3) individuals who have not travelled in the last three months of their hospital admission. Exclusion criteria were as follows: (1) other racial or ethnic group living in Senegal; (2) subjects with clinical signs of severity or any state that may interfere with the study, such as a recent pregnancy and childbirth; (3) previous use of antimalarial treatment or a possible stay out of town not older than three months;(4) people who travelled outside the areas few months before infection. The healthy control subjects corresponded to the exposed and uninfected subjects group in the same areas and belonging to Wolof ethnic group. A signed informed consent form was obtained from adult participants and parents or guardians of children involved in the study before blood sampling.

A total of 48 CTR, 54 UM and 71 SM subjects were included in the study. The study's objectives have been explained clearly using the local dialect before including patients in hospital centers. The protocol has been reviewed according to the rules issued by the National Committee for Ethics for Health Research (CNERS) of Senegal and according to the procedures established by the Cheikh Anta Diop University of Dakar (UCAD) for the ethical approval of any research involving human participants. Written informed consent was obtained from adult participants and parents or legal representatives of children. In addition, based on the information provided, UCAD's Committee on Research and Ethics (CER) considers that the research proposed respects the appropriate ethical standard and, as a result, approves its execution under "Protocole 0344/2018/CER-UCAD". Furthermore, all patients enrolled in the cohort/or legal representative gave signed and informal written consent to provide a blood sample for further studies.

2.2. Serum collection and IL-17A quantification

Blood samples were collected from malaria patients and controls and drawn into EDTA vacutainer tubes. Samples were centrifuged, plasma aliquoted, and stored at −20°C until testing. Serum levels of IL-17A were quantified by enzyme-linked immunosorbent assays (ELISA) using the pre-designed kit (Bioassays Technology, China) as per the manufacturer. The sensitivity of the assay protocol was 2.38 pg/mL, and the coefficient of variation (CV) for intra-assays and inter-assays was <8% and <10%, respectively.

2.3. Genotyping

DNA was extracted from the peripheral blood of each subject by using standard Qiagen Kits according to the manufacturer's recommendations. The concentration and quality of the extracted DNA were measured by using a NanoDrop spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The IL-17A polymorphisms were genotyped using the polymerase chain reaction method. The Oligonucleotide primers used to amplify promoters and exon regions are listed in Table 1. The PCR reactions were performed using a Gotaq®Green Master Mix (Promega, Germany) in a total volume of 25 µl containing 25 ng of genomic DNA (5 ng/µl) and 2.5 µL of each primer (10 µM). The PCR conditions were initial denaturation at 95 °C for 5 min, 35 cycles at 95 °C for 30 s, 60 °C for 30 s, and 72 °C for 5 min, with a final extension at 72 °C for 10 min. The amplicons were purified using BioGel P100 gels (Bio-Rad). Sequencing reactions (2 µL of PCR product) were performed using the

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dye terminator v3.1 method in an ABI PRISMs 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequencing conditions were: 96 °C for 5 min, 25 cycles of 96 °C for 10 s, 60 °C for 4 min and 15 °C forever, and PCR products were purified with Sephadex G50 superfine columns (GE Healthcare). Alignment of acquired sequences and SNP discovery were performed using NC_000014.9 as a reference. Analysis was performed with Genalys version 2.0b software [32].

Table 1. List of Primers used to amplify the exons of IL17A by polymerase chain reaction (PCR).

PRIMERS	Sequence (5′ > 3′)	Primer Orientation	Product Lenght (bp)
IL17APromPF	CCAAGTTGCTTGGTAGCATG	Sense	557
IL17APromPR	CAGTGGGTTCAGGGGTGACA	Antisense	
IL17AEx1PF	TAGCAGCTCTGCTCAGCTTC	Sense	563
IL17AEx1PR	CTTCTTGTGTGGTTTAGCCC	Antisense	
IL17AEx3_1PF	TTGGTCTTCTTCTGTCTGTC	Sense	291
IL17AEx3_1PR	AGTCAAACCTTCCTTCTTGG	Antisense	
IL17AEx3_3PF	ATAATGGCCCTGAGGAATGG	Sense	554
IL17AEx3_3PR	ACCCCTGGATTTGGAATAGG	Antisense	

PF, Primer Forward; PR, Primer Reverse; bp, base pair.

2.4. Statistical analysis

GraphPad Prism v9.5.1 was employed for all statistical analyses. Serum levels of IL-17A in malaria groups and controls were compared by one-way variance analysis (ANOVA). First, allelic frequencies and Hardy-Weinberg equilibrium were calculated, as described [33]. Then, as reported previously, the differences in allelic frequencies between the three groups (SM, UM, CTR) were determined using the logistic regression analysis method [34,35]. Next, associations between SNPs and IL-17A serum levels and malaria outcomes were performed using the Mann–Whitney test, and then associations with P values < 0.05 were considered statistically significant.

3. Results

3.1. Characteristics of malaria patients and healthy controls

A total of 125 malaria patients and 48 controls were included in this retrospective study. The clinical characteristics of the malaria groups and the control group are summarized in Table 2. A significant difference was observed in parameters while comparing malaria cases and controls, such as age, hemoglobin level, blood cell parameters and leukocyte cells. However, there were no statistically significant differences in monocytes, Basophils.

Table 2. Characteristics of malaria patients and healthy controls.

Characteristics	CTR (n= 48)	UM (n= 54)	SM (n= 71)	P value
Age, (M ± SD, year)	30,54 (16,14)	13,72 (17,56)	20,76 (20,17)	P<0,0001
Gender, (%)				
Male	21 (43.75)	19 (35.20)	37 (52.11)	
Female	27 (56.25)	31 (57.40)	31 (43.67)	
Hb (M ± SD, g/dl)	12,95 (1,930)	12,49 (2,855)	8,631 (3,150)	P<0,0001
Hematocrit (M ± SD, %)	38,76 (5,762)	37,65 (8,382)	25,22 (9,631)	P<0,0001
MCV (M ± SD, fL)	83,25 (5,687)	88,60 (12,90)	79,14 (13,25)	P=0,0009
RBCs (M ± SD, $\times 10^6/\mu L$)	4,699 (0,6322)	4,145 (0,7966)	3,149 (1,162)	P<0,0001
MCHC (M ± SD, pg/cell)	33,60 (0,6265)	33,74 (3,159)	33,33 (1,536)	P=0,6685

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Leucocyte (M ± SD, $^{x}10^{3}/\mu L$)	5,933 (2,405)	8,217 (4,351)	12,40 (6,952)	P<0,0001
Neutrophil (M ± SD, %)	43,71 (10,64)	59,95 (15,36)	54,05 (25,60)	P=0,0068
Lymphocyte (M ± SD, %)	43,21 (10,89)	29,27 (13,13)	22,86 (17,03)	P<0,0001
Monocyte (M ± SD, %)	7,970 (2,887)	7,312 (5,597)	6,662 (4,691)	P=0,5191
Eosinophil (M ± SD, %)	4,210 (3,829)	3,200 (2,815)	0,4608 (0,4506)	P<0,0001
Basophil (M ± SD, %)	0,8925 (0,5581)	0,7179 (0,5755)	0,6858 (0,7290)	P=0,3369

M ± SD, mean ± standard deviation; CTR, Control; UM, Uncomplicated Malaria; SM, Severe Malaria; Hb, Hemoglobin; MCV, Mean Corpuscular Volume; RBCs, Red Blood Cells; MCHC, Mean Corpuscular Hemoglobin Concentration.

3.2. Severe malaria patients displayed higher serum IL-17A compared to uncomplicated malaria and controls

ELISA quantified the level of IL-17A. As shown in Figure 1, the mean level of IL-17A was significantly higher in the SM group (mean±SE: 37.74±140 pg/mL) compared to that of the UM and control groups (mean±SD:1.69±2.4 pg/ml and 0.67±1.07 pg/mL, respectively; P<0.001) (Figure 1a). In the SM group, we observed a lower level of IL-17A in patients who were deceased compared to those who survived (mean±SD: 25.92±46.48 and 40.78±150.67 pg/mL, respectively). Still, the difference was not significant (P=0.56); the lack of significance may be partly due to the small sample size (Figure 1b). The parasitemia at the time of diagnosis in SM patients was higher than that of the UM patients (mean±SD: 9039.54±17891 and 3084±14092, respectively; P=0.0022). In addition, there was a decrease in parasitemia in the deceased compared to the survivors, but the difference was not significant (mean±SD: 9915.54±19185 and 6496±7161, respectively; P=0.8) (Figure 1c,d).

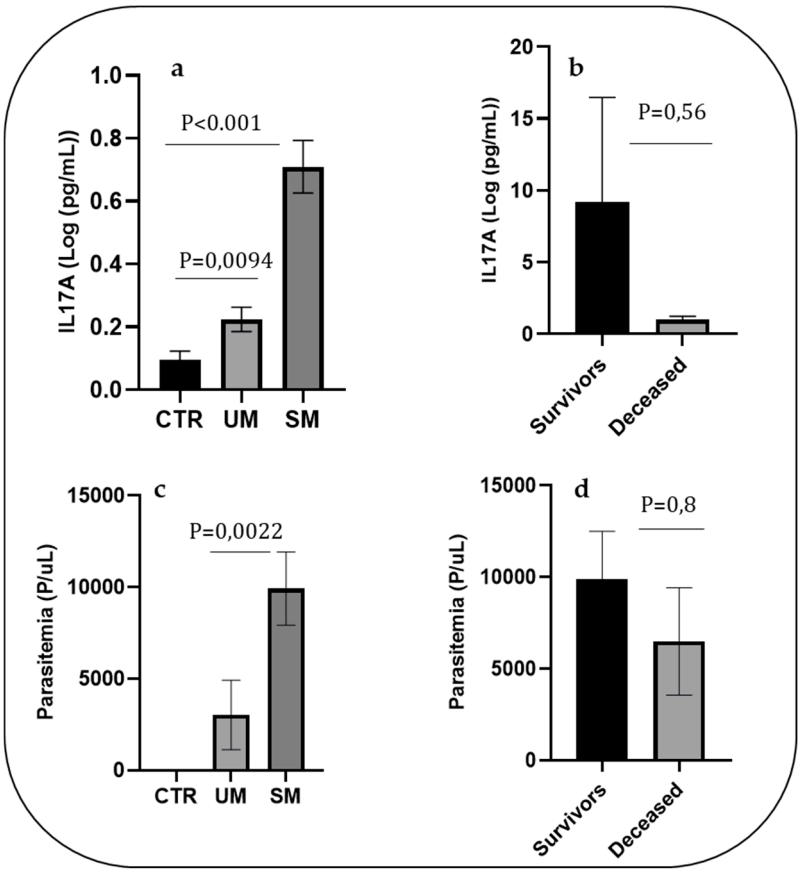

Figure 1. Parasite density and IL17A levels in different categories of enrolled subjects.(a, b) Serum level IL-17 between SM, UM and Control group, survivors, and deceased SM patients (c, d).

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Parasitemia between SM, UM and Control group, survivors, and deceased SM patients. Data representing mean pg/ml±SE for IL17A and mean P/uL for parasitemia were compared with one-way ANOVA. P<0.05 was considered statistically significant.

3.3. Distribution of IL-17A polymorphisms and association with the risk of severe malaria outcome

We analyzed genetic variations on the IL-17A gene in the promoter region and all the coding regions by sequencing and identified eight SNPs, including: 4 SNPs located in the 5′UTR: IL-17A +521A/C (rs9791323), IL-17A +606A/G (rs3819024), IL-17A +849G/A (rs2275913), IL-17A +973G/A (rs8193037); two SNP located within inton: IL-17A +1090G/A (rs3819025), IL-17A +1198A/G (rs8193038); one located within exon 3: IL-17A +3840G/A (rs17880588) and one located in the 3′UTR: IL-17A +5151C/T (rs3748067) (Figure 2).

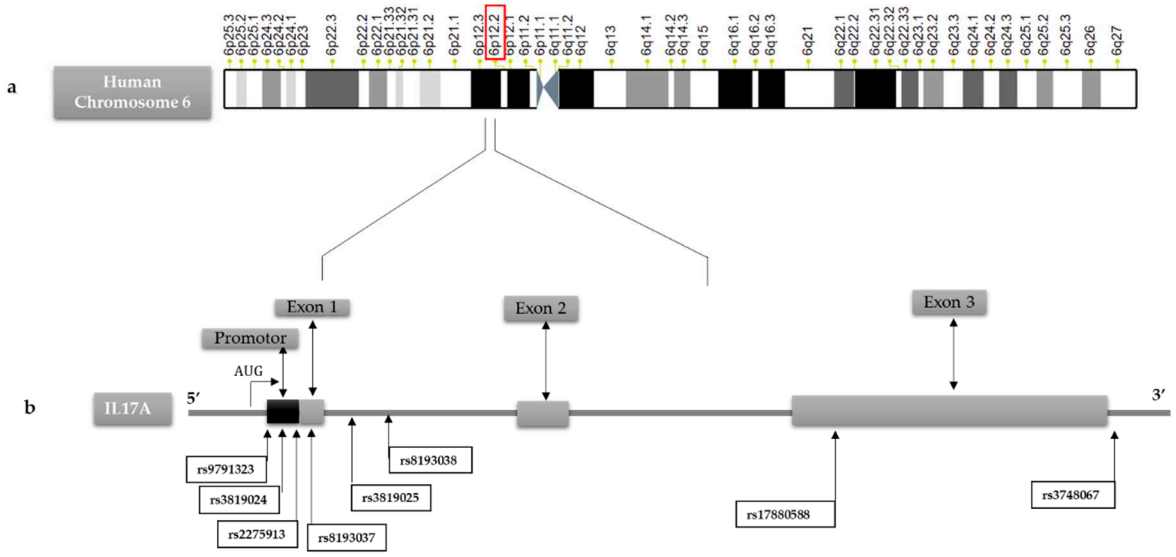

Figure 2. Mapping of Interleukin -17A (IL-17A) gene and the polymorphisms genotyped. Schema of the gene encoding Interleukin 17 A (IL17A) (a) The human IL17A gene (located at 6p12-2) contains 3 exons that span 4 kb. Exons are described by number. AUG: the transcription start site. Black arrows mark eight specific polymorphisms identified in the IL-17A gene.

The Minor Allele frequencies (MAF) of the eight SNPs loci of the IL-17A gene were identified using the Hardy–Weinberg equilibrium test (Table 3). Among them, 6 SNP (rs9791323, rs3819024, rs2275913, rs3819025, rs2233860 and rs3748067) were detected with high frequencies (with MAF > 3%), unlike 2 other SNPs (rs8193037 and rs8193038) with MAF < 3% were observed (Table 3). First, comparisons were performed among the three groups to test whether polymorphisms were associated with malaria severity. Then, statistical IL-17A polymorphisms analysis were performed using logistic regression tests with an adjustment for potential confounders such as Hb polymorphisms. The SNPS rs3819024 and rs3748067 yielded a significant association with Severe Malaria. For SM vs UM, the p-value =0.007 (OR 2.61, 95% CI 0.35– 0.91), and p value= 0.04 (OR 0.32, 95% CI 1-2.1), respectively.

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Table 3. Frequencies and single nucleotide polymorphism (SNP) of *IL17-A* gene and association analysis with susceptibility to severe malaria (SM).

SNP	Gene location	NCBI dbSNP number	MAF				HWE, exact P-value (OR)		
			CTR	UM	SM	Global	SM vs UM	UM vs CTR	SM vs CTR
+521 (A>C)	5 Prime UTR	rs9791323	0.033	0.113	0.075	0.075	0.37 (0.64)	**0.05** 3.78	0.2477 (2.43)
+606 (A>G)	5 Prime UTR	**rs3819024**	0.239	0.113	0.25	0.203	**0.007** (2.61)	0.02 0;40 ()	0.876 (1.06)
+849 (G>A)	5 Prime UTR	**rs2275913**	0.053	0.028	0.079	0.055	0.15 (2.96)	0.48 (0.52)	0.59 (1.53)
+973 (G>A)	5 Prime UTR	rs8193037	0.021	0.009	0.024	0.018	0.63 (2.56)	0.60 (0.44)	1 1.1
+1090 (G>A)	intron_variant	**rs3819025**	0.085	0.104	0.095	0.096	0.83 0.91 ()	0.81 1.2	1 1.1
+1198 (A>G)	intron_variant	rs8193038	0.021	0.009	0.024	0.018	0.63 2.56 ()	0.60 0.44	1 1.1
+3840 (G>A)	coding_sequence	rs17880588	0.053	0.034	0.043	0.043	1 1.27 ()	0.67 0.63	1 0.8
+5151 (C>T)	3 Prime UTR Variant	**rs3748067**	0.125	0.055	0.155	0.112	**0.04** 0.32 ()	**0.10** 2.43	0.54 0.78

The p values for statistical tests were performed using the linear regression model analysis for each polymorphisms. Association analysis IL-17A was performed separately, and corrections to each other were applied to reflect the real effect of each. Analysis has been carried out by comparing SM vs UM, UM vs CTR, and SM vs CTR. Borderline ($0.05 \leq p \leq 0.1$) and significant ($0- \leq p \leq 0.05$) p values are in bold. The OR (odds ratio) and CI (Confidence intervals) were shown when p values were significant. SM, Severe Malaria; UM, Uncomplicated Malaria; CTR, Control group; MAF, Minor Allele Frequency; HWE, Hardy–Weinberg.

3.4. Association of SNP IL-17A+1198 A/G (rs8193038)) and serum IL-17A concentration

The relationship between the IL-17A polymorphisms and serum IL-17A concentration was analyzed regardless of the differences in the study groups. As shown in Figure 3, the heterozygous rs8193038 AG genotype is significantly associated with higher levels of IL-17A amongst the whole study groups compared to the homozygous rs8193038 AA genotype (OR = 4.9, 95% CI = (2.01- 8.13), P<0.001).

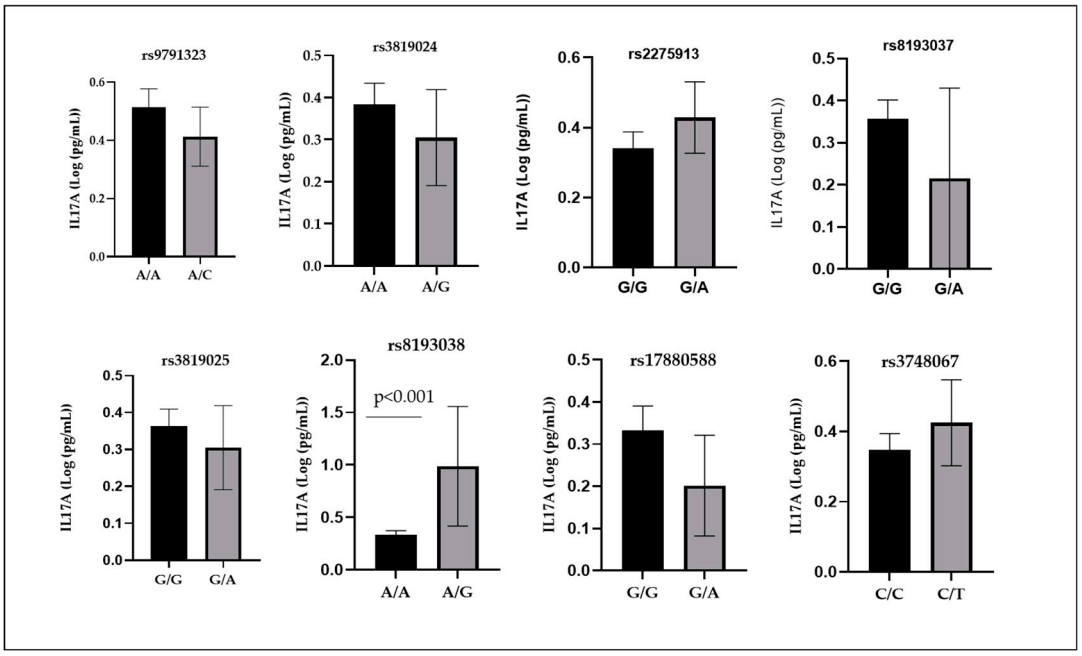

Figure 3. Concentration of IL-17A according to its genotypes.

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4. Discussion

The analysis of the genetic effects of inflammatory response gene variants is a key step in malaria research to motivate experimental investigations of the underlying pathogenesis mechanisms. This knowledge will be critical to identify rational adjuvant therapies to prevent fatality or undesired malaria complications and subsequent long-term sequels that represent a high burden in endemic regions [36]. Human populations display differences in susceptibility to infectious diseases such as malaria, and the basis for this differential susceptibility is due, at least in part, to genetic factors [3,37,38]. It is understood that cytokine gene polymorphisms could affect the serum levels of cytokines by influencing transcriptional regulation. The role of single-nucleotide polymorphisms (SNPs) in some infectious diseases and immunological disorders has been previously reported [39–41], and some exact genetic polymorphisms have been identified about malaria [42–44]. Significant associations between cytokine polymorphisms and diseases support that cytokine gene polymorphisms have an unquestionable role in the orchestration of the immune response, leading to the different functional scenarios, which in turn influence the outcome of disease establishment and evolution [36,45,46].

Thus, the present study explored IL-17A cytokine levels and polymorphisms in the Senegalese cohort and their association with malaria outcomes. In recent years, evaluating SNPs has been considered a common approach for testing human genetic variation [47]. The IL-17 cytokine family is a relatively new family linked to adaptive and innate immune systems. IL-17A are members of the IL-17 cytokine family, essential for the pathogenic activity of IL-17 cells and the production of various proinflammatory mediators in the body [18,48].

We have determined the serum IL-17A levels and genotyped IL-17A variants in Senegalese severe and uncomplicated malaria patients and controls. We observed elevated IL-17A levels in SM patients compared to the UM and healthy cases. The high parasitemia in the SM group accompanied the IL-17A increase. This indicates that IL-17A has an essential regulatory role in malaria infection, controlling the intensity of the immune response, as described in the experimental model and human malaria and several other infectious diseases [14,28,49,50]. IL-17 production is associated with a very high occurrence of chronic inflammation and immunopathological conditions [48]. Recent data suggest that IL-17 contributes to host protection against diverse infectious organisms during sepsis while inducing hyperinflammation with detrimental outcomes for the host under certain conditions [51]. Earlier investigations in the experimental model have deciphered the essential role of IL-17. In P. vivax infection, authors suggest that increasing serum IL-17 levels in malaria patients could be considered a host adaptation mechanism to control changes in blood viscosity, and IL-17 could thus be used as an immunomodulatory agent [52]. IL-17 appears to act on erythrocytes by remodeling their cell membrane; it is well-known that erythrocytes in malaria are very sensitive to osmotic shock [52].

We found an elevated level of IL-17A in severe malaria patients whom survivors compare to those who were deceased. Our results seem to confirm the results of Helegbe et al., which showed elevated IL-17 levels together with high IL-4, IL-12α, and IFN-γ levels may be a marker of protection, and the mechanism may be controlled by host factors [28]. Thus, pro-inflammatory IL-17A cytokine seems to have been protective against fatal malaria. Furthermore, the data agree with the observations of Oyegue-Liabagui et al. [29], who noted a correlation between Th17 cell count and overall survival in patients with malaria in children.

Immuno-genetic variants are associated with diverse degrees of malaria susceptibility, including cytokine gene polymorphisms that modify their expression and their circulating protein levels to reflect inflammatory or anti-inflammatory responses [53–55]. Polymorphisms in the IL-17A cytokine can impact the activity and expression of inflammatory mediators, which can affect interleukin-17 activity [56,57]. IL-17A polymorphisms have been linked to several malignancies, including gastric and breast cancer [58,59]. But little is known about the association between IL-17 gene variation and malaria.

One previous study has reported that IL-17F (rs6913472 and rs4715291) and IL-17RA (rs12159217 and rs41396547) polymorphisms independently modulate susceptibility to Cerebral Malaria and

provide evidence that IL-17F protects against CM [8]. In this study, we performed a genetic analysis of the variations of the IL-17A gene. For the first time, we identified two variants, rs3819024 and rs3748067, that are significantly associated with SM risk in the Senegalese population. For SM vs UM, the p-value =0.007 (OR 2.61, 95% CI 0.35– 0.91) and p value= 0.04 (OR 0.32, 95% CI 1-2.1), respectively. We also found that the heterozygous rs8193038 AG genotype is significantly associated with higher levels of IL-17A amongst the whole study groups compared to the homozygous rs8193038 AA genotype (OR = 4.9, 95% CI = (2.01- 8.13), P<0.001). These data suggest that the IL-17A gene rs8193038 polymorphism significantly affects IL-17A gene expression. Genetic variants controlling inflammatory responsiveness are proposed determinants of malaria clinical outcomes dependent on a history of exposure to infection [2,36].

A limitation of our study could be the need for a statistical correlation between IL-17A level, genotypes and malaria outcomes (SM and UM); this is due to a few numbers of patients. Therefore, further recruitment is needed to increase SM and UM patients.

5. Conclusions

The current report revealed an essential role of IL-17A in the pathogenesis of SM in Senegalese patients. Furthermore, heterozygous mutant and minor alleles of IL- rs3819024 and rs3748067 polymorphisms predisposed subjects for the development of SM. Interestingly, the current report further validated the functional relevance of IL-17A (rs8193038) variants and demonstrated the association of mutants with elevated IL-17A levels. However, further studies, including more significant sample-sized in the different populations, are required to validate the observations of the present study. In addition, further investigation on the role of IL-17 and its interplay with other immune factors needs to be conducted in clinical settings.

Author Contributions: **FT** conceived the study and the methodology and drafted the manuscript. **GD** conceived the study and the methodology. **JFZ** and **CC** conducted the data analysis, revised the manuscript and approved the final version. **CD** conducted the methodology, performed Sanger Sequencing and molecular biology experiments and approved the final version. **BM** conducted the malaria cohort recruitment, revised the manuscript from the hospital center, and approved the final version. **AAMD** contributed to the correction and revision of the manuscript and approved the final version. **AT** contributed to the correction and revision of the manuscript and approved the final version. **MDM** contributed to the correction and revision of the manuscript and approved the final version. **MD** contributed to the correction and revision of the manuscript and approved the final version. **CMN** contributed to the correction and revision of the manuscript and approved the final version. **YD** contributed to the correction, revising the manuscript and approving the final version. **JFD** & **AD** coordinated this study, revised the manuscript, and approved the final version. All authors read and approved the final manuscript.

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