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# Homozygosity for the CD1E*02 allele is associated with a resistance to *Plasmodium falciparum* malaria infection in Gabonese school children

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ABSTRACT

**Objective:** To explore the possible association between polymorphisms in CD1 genes and both asymptomatic and mild *Plasmodium falciparum* infection.
**Methods:** Two clusters of 85 school children, from the village of Dienga (Gabon) were investigated. The first group was analysed for the prevalence and the multiplicity of asymptomatic *P. falciparum* infection, whereas the second group was screened for the frequency of malarial attacks.
**Results:** Our findings showed that homozygosity for the CD1E*02 allele was associated with a low frequency of malarial attacks. Furthermore, a strong association between CD1E*02 homozygotes and the resistance to multiple malarial attacks was identified. The CD1A*01 allele showed a weak association with a small number of malarial attacks.
**Conclusion:** Our results suggest a possible role of CD1E polymorphisms in malaria protection among school children and that CD1e molecules are involved in anti-malarial immunity.

## 1. Introduction

*Plasmodium falciparum* (*P. falciparum*) malaria remains one of the major causes of morbidity and mortality in tropical and sub-Saharan countries. Parasite-host genetic background has been shown to significantly impact the incidence and outcomes of malarial infection. Indeed, numerous markers of diverse influence have been implicated in the disease progression and development, implying that complete protection against malaria infection requires multifactorial immunity [1]. The disease caused by the invasion of erythrocytes by parasitic protozoa of the genus *Plasmodium* is characterized by clinical symptoms which arise through the release of parasite-derived toxins during blood-stage developmental cycle of the parasite, which are glycolipids, predominantly of the glycosylphosphatidylinositols (GPIs) class [2].

CD1 molecules present antigens, lipids and glycolipids (including GPIs) to a specific subset of T cells. CD1 proteins are encoded by five closely linked genes (CD1A to CD1E) [3]. Previously thought to be non-polymorphic, the CD1 loci has been showed to display some level of diversity, especially for CD1A and CD1E genes, with two and six alleles respectively raising the question on the potential implication of its polymorphism on CD1-restricted immune responses [4,5].

The role of CD1d-restricted NKT cells during both hepatocytic and erythrocytic cycles of malaria has been extensively studied in murine experimental models. Concerning long-lived malaria blood stages, CD1d-restricted NKT cells by their capacity to secrete large amounts of cytokine, have been showed to influence Th1/Th2 polarization, pathogenesis and fatality in murine model of the severe form of malaria [6,7].

The ability of CD1 molecules to bind and present GPIs antigens from *P. falciparum* parasites to T cells, in combination

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with the protective role CD1-restricted NKT cells mediated against malaria infection in mice models [7], led us to investigate whether polymorphisms in CD1A and CD1E genes are related to both asymptomatic and mild malaria.

## 2. Materials and methods

### 2.1. Patients' recruitment

To evaluate the influence of CD1A and CD1E gene polymorphisms on *P. falciparum* malaria, two groups of school children, 7–15 years old (age-group of children with similar immune status), from the village of Dienga were studied. This village is located in a mixed savannah/forest area (South-Eastern Gabon) where *P. falciparum* is endemic with the entomologic inoculation rate of one infective bite per person per day [8].

The first group, randomly recruited during a period of 4 months, corresponding to the main peak of malaria transmission occurring during the rainy season (February to May 1995), consisted of 85 children and was used to test the potential association of CD1 polymorphisms with asymptomatic *P. falciparum* infection (prevalence and multiplicity). The presence of parasites was determined by thick blood smear (parasite density). Secondly a nested-PCR determination using merozoite surface protein-2 (MSP-2) gene locus was tested in two cases: when thick blood film was negative, to confirm the absence of parasites or when parasite density was ≤800 parasites/μL, to establish the parasite infection profile by determining the mean number of parasite genotypes per infected sample (multiplicity). MSP-2 genotyping was done as previously described [9].

Uninfected children were defined as those that had no parasites in their blood (both thick blood film and nested-PCR are negative) during this period. Asymptomatic infection is defined by parasite detection (parasite density ≤800 parasites/μL) without malaria clinical symptoms.

In the second group, 85 school children also were clinically followed from February 1995 to March 1996, a period during which malarial attacks have been recorded when a febrile episode defined by an axillary temperature >37.5 °C, was associated with *P. falciparum* parasitemia >800 parasites/μL.

### 2.2. CD1 genotyping

CD1A and CD1E polymorphism was investigated in each child in both groups as previously described [4,5]. Analysis of PCR fragments by *HphI* (codon 13) and *HaeIII* (codon 51) restriction enzymes showed the two different CD1A alleles, CD1A*01 (Cd 13 ATC and Cd 51 TGG) and CD1A*02 (Cd 13 ACC and Cd 51 TGC). For the genotyping of the six CD1E alleles, restriction fragment length polymorphism (RFLP) with one of the primers having an introduced mismatch to create a Rsa I restriction site was conducted as previously reported [5].

### 2.3. Statistical analysis

The statistical analysis was done using *Chi*-square and Mann–Whitney tests.

### 2.4. Ethical considerations

Informed consent was obtained from the parents or guardians of children before sampling. This study was approved by the institutional ethical committee of the Centre International de Recherches Medicales (Franceville, Gabon).

## 3. Results

### 3.1. CD1 genotypes

Polymorphisms of CD1 genes may affect susceptibility to infection with *P. falciparum*, thus we examined CD1A and CD1E genotypes by RFLP. CD1E genotypes obtained have permitted to indicate individuals homozygous for the CD1E*02 allele (E*02/E*02) and those heterozygous for the CD1E*02 allele (E*01/E*02, E*02/E*05 and E*02/E*06). CD1 allele frequencies in the two groups were 5.3% for CD1E*01; 87.6% for CD1E*02; 6.5% for CD1E*05; 0.6% for CD1E*06; 8.8% for CD1A*01 and 91.2% for CD1A*02.

### 3.2. CD1 genotypes and asymptomatic P. falciparum infection

In the first group, the statistical analysis failed to reveal any association between the CD1A and CD1E polymorphisms and the prevalence of asymptomatic infection, although the number of patients with asymptomatic infection is higher in individuals homozygous for the CD1E*02 allele than in those non-homozygous (53.8% *vs.* 40.0% respectively). Moreover, in this group, analysis of the multiplicity of asymptomatic infections related to the different CD1 genotypes did not showed significant difference between different CD1A and CD1E genotypes, even if a greatest number of multiple infections in individuals homozygous for the CD1E*02 allele compared to the heterozygous one was noted (2.2 *vs.* 1.6 respectively) ($P$ = 0.4 by Mann–Whitney test), suggesting that patients homozygous for this allele may have more immune protection against *Plasmodium*. These results are summarized in Table 1.

**Table 1**
Distribution of CD1 genotypes related to prevalence and multiplicity of *P. falciparum* infections ($n$ = 85).

| *P. falciparum* infections | CD1*E02 | | CD1*A02 | |
|---|---|---|---|---|
| | CD1*E02 homozygote | CD1*E02 heterozygote | CD1*A02 homozygote | CD1*A02 heterozygote |
| Uninfected | 17 (26.2%) | 9 (45.0%) | 22 (31.4%) | 4 (26.7%) |
| Asymptomatic | 35 (53.8%) | 8 (40.0%) | 34 (48.6%) | 9 (60.0%) |
| Symptomatic | 13 (20.0%) | 3 (15.0%) | 14 (20.0%) | 2 (13.3%) |
| Multiplicity (asymptomatic) | 2.2 | 1.6 | 2.01 | 2.00 |
| *P* value (*Chi*-square test) | 0.3 | | 0.9 | |
| *P* value (Mann–Whitney test) | 0.4 | | >0.9 | |

**Table 2**
Distribution of CD1 genotypes related to malarial attacks of *P. falciparum* during 14 months ($n$ = 85).

| *P. falciparum* malarial attacks | CD1*E02 | | CD1*A02 | |
|---|---|---|---|---|
| | CD1*E02 homozygote | CD1*E02 heterozygote | CD1*A02 homozygote | CD1*A02 heterozygote |
| Without malarial attack | 34 (52.3%) | 4 (20%) | 31 (44.3%) | 7 (46.7%) |
| With 1 malarial attack | 20 (30.8%) | 5 (25%) | 18 (25.7%) | 7 (46.7%) |
| With 2 malarial attacks or more | 11 (16.9%) | 11 (55%) | 21 (30.0%) | 1 (6.6%) |
| Mean number of malarial attacks | 0.77 | 1.85 | 2.0 | 1.1 |
| $P$ value (*Chi*-square test) | <0.005 | | 0.15 | |
| $P$ value (Mann–Whitney test) | <0.05 | | 0.05 | |

### *3.3. CD1 genotypes and mild form of P. falciparum infection*

As shown in Table 2, the most striking finding concerns the group followed long-term, in which CD1E*02 homozygosity is associated with a low frequency of malarial attacks (47.7% *vs.* 80.0%; $P = 0.015$, *Chi*-square test). In addition, the distribution of CD1 genotypes in children with 0.1 and more than 1 malarial attacks, shows strong association between the CD1E*02 allele at homozygous state and resistance to multiple malarial attacks ($P < 0.005$, *Chi*-square test). A low mean number of malarial attacks was associated with the CD1E*02 allele at homozygous state (0.77 *vs.* 1.85; $P < 0.05$; Mann–Whitney test).

In this group, the distribution of CD1A alleles related to mild malaria reveals no association between these alleles and the prevalence of malarial attacks. However, the weak association of the low mean number of malarial attacks with the CD1-A*01/A*02 genotype (2.0 *vs.* 1.1; $P = 0.05$; Mann–Whitney test) is likely related with a strong linkage disequilibrium between the CD1A*01 and CD1E*02 alleles [data not shown].

## 4. Discussion

In this present investigation, we sought to investigate the role of CD1 polymorphisms in the resistance of *P. falciparum* in an area at high risk of malaria infection. The functional importance of CD1 molecules in the host immune system is an area of extensive investigation [10].

The nucleotide sequence alignment of the CD1E variants revealed that the difference between the CD1E*01 and CD1E*02 alleles is due to the substitution of glutamine by arginine at position 79 (Q79R) in the heavy chain α1-domain of the molecule. Interestingly, a study has showed that Arg79 is a crucial amino acid of the mouse CD1d molecule for glycolipid ligand presentation to NKT cells, involved both in antigen binding and in TCR recognition [11]. Accounting also for its importance, the Arg79 is shared by both CD1b and CD1c molecules, which are known to be involved in CD1 restricted immune response against various pathogens [12]. Hence it seems reasonable to conceive that the amino acid difference observed between CD1E*01 and CD1E*02 is tightly related with resistance to malaria either by the selection of an efficient parasitic epitope or by a better T-cell interaction or by both by modulating antigen loading by CD1e molecules.

Another potential implication of CD1 molecules in anti-malarial immunity is that CD1d-restricted NKT cells have been found to recognise GPIs from plasmodial origin and to provide help for antibody formation against GPI-anchored proteins [2]. Naik *et al.* have demonstrated that individuals residing in malaria-endemic areas develop an age-dependant *P. falciparum*-specific anti-GPI antibody response correlated with protection against malaria-related febrile illness [13]. They showed also that the lipid moiety of GPIs is the antigenic structure against which specific anti-GPI antibodies are directed, opening hence the question about the potential involvement of CD1 molecules in this anti-malarial humoral response.

We found that homozygosity for CD1E*02 is associated with resistance to malarial attacks. Malarial attacks are defined by fever and fever is the result of cytokine release and symptoms. Association with CD1E genotypes present in the mild form of malaria, and missing in asymptomatic infection, has supported the hypothesis that CD1e molecules play a role in the cytokine-dependent immune responses. Cytokine responses by Th1/Th2 polarization are predominant in immunity against both *Plasmodium* liver and erythrocyte stages.

Concerning the clinically silent and short-lived cycle of *Plasmodium* (liver cycle), NKT cells increase in the liver after a primary infection and CD1d-restricted NKT cells, which secrete IFN-γ, were critical in reducing liver-stage burden of a secondary infection [6], hypothesizing the low number of malarial attacks.

Sequestration (adherence of infected erythrocytes to the vascular endothelium) is known to be influenced by cytokines. Through their capacity to secrete large amounts of regulatory cytokines, CD1d-restricted NKT cells have a protective role against *Plasmodium berghei*-mediated cerebral malaria in mice [7]. Following these findings, we anticipated that the CD1e molecules might influence cytokine rates by altering the lipid antigen repertoire.

In a recent study, Li *et al.* have demonstrated that humanized mice can possess human NKT cells that are functionally able to respond to human CD1d-binding and NKT-cell stimulatory glycolipids [14]. This anti-malarial immunity is effective by augmenting malaria-specific human CD8+ T cell response [14].

This idea of collaboration between CD1e and other CD1 molecules is the main suggestion of a study on the dynamics of the cellular distribution of CD1e molecules [15]. In fact, the absence of a cell-surface distribution of CD1e molecules at any stage of dendritic cell maturation argues against the capacity of these molecules to capture antigenic ligands in the secretory pathway, and for a biological function only in endocytic compartments [15].

In conclusion, this study suggests a possible role of CD1E polymorphisms in malaria protection among school children in Gabon. Further studies are necessary in large-scale cohort to confirm our findings.

## Conflict of interest statement

The authors do not have a commercial or any other association that might pose a conflict of interest.

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