



IL4-589C/T polymorphism and IgE levels in severe malaria[☆]

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Abstract

Previous studies identified an allelic variant of the *IL4* promoter region (*IL4*-589T) that appears to enhance the transcriptional activity of *IL4*, and is associated with increased IgE levels. Total serum IgE levels are elevated in malaria endemic regions, and higher in children with severe malaria. Here, we investigated the relationship of the *IL4*-589C/T polymorphism with severity of the disease in a case-control study of severe malaria in Burkina Faso, West Africa. No association between the *IL4*-589T and severe malaria was observed. No difference in *Plasmodium falciparum*-specific IgE was detected between severe and uncomplicated malaria patients. Among children with severe malaria, total IgE levels were significantly elevated in those carrying the *IL4*-589T allele ($P = 0.018$). In children with uncomplicated malaria, no significant difference was found. These results raise the possibility that there is a relationship between susceptibility to severe malaria, IgE production and genetic variation in the *IL4* region, which merits further investigation in other epidemiological settings.

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Interleukin-4, one of several cytokines involved in the modulation of the humoral immune response, is a key factor for the differentiation of precursors T helper cells into the Th2 subset that induces IgE production. Total serum IgE levels appear to be determined by a combination of genetic and environmental factors. Analysis of chromosome 5q31.1 markers and total serum IgE concentrations (Marsh et al., 1994) identified a peak of genetic linkage in the region of *IL4* and this could be relevant to malaria immunity in Burkina Faso, since in this population the 5q31–q33 region shows linkage to *Plasmodium falciparum* infection intensity (Rihet et al., 1998; Flori et al., 2003). *IL4* was shown to be involved in the response to *P. falciparum* malaria: in fact, a causal relationship was reported between the activation of *IL4* producing T cell subsets and the production of anti-Pf155/RESA-specific antibodies in malaria exposed individuals (Troye-Blomberg et al., 1990). Malaria parasitemia is associated with elevated serum levels of *IL4*, which are negatively correlated with parasite load, suggesting a role in parasite clearance (Mshana et al., 1991). These results are consistent with a broad scale survey indicating elevated levels of both total and *P. falciparum*-specific IgE are common in areas where malaria is endemic, with higher levels detected in severe malaria compared to uncomplicated malaria (Perlmann et al., 1994, 1997, 1999).

The *IL4* promoter region has a single nucleotide polymorphism (C/T) at 589 bp upstream of the transcriptional start site (Borish et al., 1994). Compared to *IL4*-589C allele, *IL4*-589T has been noted to have higher luciferase reporter activity, and a different pattern of protein binding on electrophoretic mobility shift assay (EMSA). This allele has been associated with elevated total serum IgE levels in asthma and atopy (Rosenwasser et al., 1995).

A recent study in Burkina Faso found that among the Fulani ethnic group, who have lower susceptibility to malaria than neighbouring ethnic groups (Modiano et al., 1995, 1996), *IL4*-589T is associated with elevated IgG levels against specific malaria antigens (Luoni et al., 2001). The above findings raise the question of whether the *IL4*-589 C/T polymorphism has any relationship to IgE levels and to malaria susceptibility, particularly in Burkina Faso where there is existing evidence for linkage of this gene region to the control of parasite density (Rihet et al., 1998;

Flori et al., 2003) and for association of this specific polymorphism with antibody production (Luoni et al., 2001). To address this question, we investigated this polymorphism with respect to the clinical presentation of the disease and to the parasite density, furthermore, we evaluated total and *P. falciparum*-specific IgE levels according to *IL4*-589T presence in a large case-control study of severe malaria in Burkina Faso. In order to minimize the influence of confounders such as genetic background and different levels of malaria transmission, only individuals belonging to the Mossi ethnic group and coming from the urban area of Ouagadougou were included in the present analysis. Inoculation rates values in the range of 1–10 bites per person per year are observed in the study area (Esposito et al., 1988) where the main malaria vectors are *Anopheles gambiae*, *A. arabiensis* and *A. funestus* (Petrarca et al., 1986). The protocol of the study was approved by the Centre National de Lutte contre le Paludisme of the Ministry of Health of Burkina Faso. Severe malaria cases were diagnosed by the presence of *P. falciparum* in the thick blood film associated with at least one of the following conditions: prostration (incapacity of the child to sit without help in the absence of coma), unrousable coma (score between 0 and 2 on the Glasgow modified coma scale), repeated generalized convulsions (more than two episodes in the preceding 24 h), severe anemia (hemoglobin < 5 g/dl), pulmonary edema/respiratory distress, spontaneous bleeding, and renal failure (plasma creatinine > 3 mg/dl). Children with other detectable infections or cause for the clinical presentation were not included in the study. Uncomplicated malaria cases were defined as a clinical illness characterized by an axillary temperature >37.5 °C associated with *P. falciparum* positive blood film. Patients were treated according to WHO guidelines with a complete regimen of drugs that were provided free of charge as a part of the study. After oral informed consent of the parents, a venous blood sample was drawn from children aged from 6 months to 15 years old for measurements of parasitemia, blood glucose level, plasma creatinine concentration, hemoglobin concentration, hematocrit, complete blood cell count and genetic analyses. Blood was collected onto EDTA, cell pellet and sera were separated and stored at –20 °C. Total IgE and *P. falciparum*-specific IgE were determined by ELISA as previously described

(Perlmann et al., 1994). Genomic DNA was extracted from leucocytes by a salt-chloroform method (Miller et al., 1988). PCR amplification of the *IL4* promoter region was performed with the following sense 5'-AAATAAAAATAAAAATGAGC-3' and antisense 5'-CTGGGGAAAGATAGAGTAACT-3' primers under the following conditions: 6 min at 96 °C, then 35 cycles of 94 °C for 30 s, 53 °C for 40 s, 72 °C for 40 s. The PCR fragment (of 512 bp) was digested with *BsmFI* and the restriction fragments were resolved on a 3% Metaphor agarose gel stained with ethidium bromide. Allelic distribution was tested according to a Hardy–Weinberg equilibrium by the χ^2 -test. *P*-values for the comparison of antibody levels means were obtained by univariate ANOVA using EpiInfo 6.0 (CDC, Atlanta, GA). The possible confounding effect of age on total IgE (high and low IgE titers) according to the *IL4*-589 genotype (CC homozygotes versus CT and TT) was evaluated by Mantel–Haenszel corrected χ^2 -test after stratification by age (age classes: 0–2; ≥ 2 –4; ≥ 4 –8; ≥ 8 –15 years old).

The study population comprised 300 cases of severe malaria and 280 controls with uncomplicated malaria. The two groups were well matched for age (5.2 ± 3.1 years versus 5.6 ± 3.5 years, $P = 0.2$). Cases and controls did not differ in the frequency of the *IL4*-589T allele (0.22 in both groups) or in the distribution of genotypes (Table 1). Furthermore, no difference as

regards to age was observed within each group among different genotype classes. The distribution of *IL4*-589 genotypes in the total study population was consistent with Hardy–Weinberg equilibrium.

Total IgE levels were marginally higher in cases compared to controls (2.32 log IU/ml, S.D. 0.70; versus 2.24 log IU/ml, S.D. 0.67, $P = 0.13$). Anti-*P. falciparum* IgE levels were similar in cases and controls (2.82 log IU/ml, S.D. 0.40; versus 2.80 log IU/ml, S.D. 0.42).

Our main interest was in the relationship between the *IL4*-589 genotype and total IgE levels. Analysis of variance showed a significant association within the cases ($P = 0.018$) but not in the controls (Table 1). Further analysis indicated that the main difference in total IgE levels lays between *IL4*-589CC and other genotypes ($P = 0.01$); the CT and TT genotypes were not significantly different. Within severe malaria cases total serum IgE differences according to the *IL4*-589 genotype (CC homozygotes versus CT and TT) were still statistically significant ($P = 0.03$) after adjustment for age.

A similar pattern was observed when we analysed the subset of severe malaria cases who were comatose (148 individuals). *IL4*-589CC homozygotes had a mean total IgE level of 2.21 log IU/ml (S.D. 0.69) compared to a 2.53 log IU/ml (S.D. 0.68) in the other genotypes ($P = 0.005$).

Table 1

Log means of total (IgE) and specific *Plasmodium falciparum* (IgEpf) serum IgE for each genotype at *IL4*-589C/T polymorphism of *IL4* promoter region in severe (cases) and uncomplicated (controls) malaria patients belonging to the Mossi ethnic group from the Central Hospital of Ouagadougou, Burkina Faso

	Cases (300)			Controls (280)		
	CC (181)	CT (105)	TT (14)	CC (176)	CT (87)	TT (17)
Mean age $\pm$ S.D.	4.98 $\pm$ 3.14	5.41 $\pm$ 3.01	5.50 $\pm$ 3.55	5.90 $\pm$ 3.47	5.19 $\pm$ 3.73	4.71 $\pm$ 2.75
IgE (μ g/ml) $\pm$ S.D.	2.24 $\pm$ 0.70	2.42 $\pm$ 0.70	2.70 $\pm$ 0.65	2.27 $\pm$ 0.68	2.17 $\pm$ 0.63	2.18 $\pm$ 0.77
Comparisons	P values					
CC vs. CT + TT	0.01			NS		
	Cases (286)			Controls (272)		
	CC (180)	CT (103)	TT (13)	CC (171)	CT (84)	TT (17)
Mean age $\pm$ S.D.	5.00 $\pm$ 3.14	5.47 $\pm$ 3.01	5.77 $\pm$ 3.54	5.94 $\pm$ 3.47	5.24 $\pm$ 3.73	4.71 $\pm$ 2.75
IgEpf (ng/ml) $\pm$ S.D.	2.80 $\pm$ 0.38	2.84 $\pm$ 0.35	2.94 $\pm$ 0.25	2.81 $\pm$ 0.39	2.77 $\pm$ 0.43	2.82 $\pm$ 0.69
Comparisons	P values					
CC vs. CT+TT	NS			NS		

Number of individuals tested are given in parentheses. S.D. = Standard Deviation.

Anti-*P. falciparum*-specific IgE showed no statistically significant difference between the genotypes (Table 1).

The analysis of the relationship between *IL4*-589 polymorphism and parasite density in line with the suggested role of *IL4* in parasite clearance (Mshana et al., 1991) did not show any significant reduction in those individuals with *IL4*-589T allele both homozygotes and heterozygotes neither in severe nor in uncomplicated malaria cases.

This study did not detect any association between the *IL4*-589 polymorphism and severe malaria. However, among children with severe malaria, we found that *IL4*-589T allele was associated with significantly elevated levels of total serum IgE. This finding was still observed after adjustment on age ruling out the possible involvement of marginal differences in age among the different genotype classes. We found no significant association between this polymorphism and IgE levels among the group of children with uncomplicated malaria. The absence of a similar pattern between *IL4*-589 polymorphism and IgE levels in uncomplicated malaria cases is intriguing since we observed considerably high levels of IgE in both categories differently from previous studies reporting significantly higher levels of IgE in severe malaria when compared with uncomplicated malaria cases (Perlmann et al., 1994, 1997, 1999). A possible explanation for the discrepancy observed in this study is that our sample of uncomplicated malaria cases coming from Ouagadougou University Hospital, the last level in the therapeutic itinerary of the patient, might represent a biased group of higher severity within uncomplicated malaria patients. These results raise the question of whether the immunological changes associated with severe malaria cases might amplify the effect of the *IL4*-589C/T polymorphism on IgE production, or alternatively that there is some other genetic determinant which both predisposes to severe malaria and acts to modify *IL4* function. Another layer of complexity is represented by the variation in gene expression levels which might be different between cases and controls, thus, ultimately affecting antibodies levels. It is worth noting that *P. falciparum*-specific IgE represent a very small fraction of total IgE accounting for less than 1% of total IgE (Perlmann et al., 1994). Therefore, the effect of the *IL4*-589C/T polymorphism on this portion of the

total IgE might be relatively small and difficult to measure in our sample. Higher levels of both total and specific *P. falciparum* IgE have been detected in cerebral malaria compared to uncomplicated malaria (Perlmann et al., 1997, 1999). In cerebral malaria a local overproduction of TNF is supposed to take place in the small brain vessels, possibly due to monocytes reaction with deposited immune complexes containing malaria antigens and *P. falciparum*-specific IgE (Maeno et al., 2000). In fact, there is evidence that activated monocytes of patients with higher IgE levels produce higher levels of TNF (Perlmann et al., 1997). It is possible that in the severe patients an unbalance of the immune system towards Th2 response may amplify the effect of the *IL4* promoter polymorphism. Furthermore, severe malaria is the outcome of a complex cascade of cytokines production and cellular activation determining a wide spectrum of clinical symptoms; therefore, it is expected that other key genes may be involved whereby epistatic interactions may play a role.

Another aspect which requires careful assessment in severe malaria case-control studies is the parasite virulence heterogeneity (Gupta et al., 1994): although much effort has been employed so far in excluding host factors which might act as confounders, the phenotypic heterogeneity of the parasite as an important determinant of severe malaria has not yet been addressed (Nacher et al., 2001). The report of wide variation in TNF-inducing activity among wild parasite isolates (Allan et al., 1995) led to the hypothesis that cerebral malaria might be associated with strains of *P. falciparum* that induce an abnormally high TNF response. The different ability of pathogenic cytokines induction by different parasite strains would introduce a bias in our settings if it happens to be unevenly distributed in cases and controls. Effective markers for parasite virulence need to be developed in order to avoid this possible confounder.

In conclusion, this study highlights some of the pitfalls of association studies which require not only a careful assessment of the genetic background and the epidemiological context but also the analysis of other immuno-pathogenetic factors which may have an impact on the outcome of the specific SNP evaluated. It is possible that *IL4*-589T is in linkage disequilibrium (LD) with a functional SNP in another part of *IL4* or in neighbouring genes such as *IL13*

which also affects B cell activity and IgE production. Thus, we are currently working to obtain a first generation LD map of the 5q31–q33 region in West African populations to explore this problem in greater detail.

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