

## ASSOCIATION OF Fc $\gamma$ RECEPTOR IIa (CD32) POLYMORPHISM WITH SEVERE MALARIA IN WEST AFRICA

GRAHAM S. COOKE,\* CHRISTOPHE AUCAN,\* ANDREW J. WALLEY, SHELLEY SEGAL, BRIAN M. GREENWOOD, DOMINIC P. KWIATKOWSKI, AND ADRIAN V. S. HILL

Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford, United Kingdom; Medical Research Council Laboratories, Banjul, The Gambia; London School of Hygiene and Tropical Medicine, London, United Kingdom

**Abstract.** Malaria continues to claim the lives of more children worldwide than any other infectious disease, and improved understanding of disease immunology is a priority for the development of new therapeutic and vaccination strategies. Fc $\gamma$ RIIa (CD32) contains a polymorphic variant (H/R131) that has been associated with variability in susceptibility to both bacterial diseases and *Plasmodium falciparum* parasitemia. We investigated the role of this polymorphism in West Africans with mild and severe malarial disease. The HH131 genotype was significantly associated with susceptibility to severe malaria ( $P = 0.03$ , odds ratio = 1.40, 95% confidence interval = 1.02–1.91). In contrast to studies of parasitemia, the presence of the R131 allele, rather than the RR131 genotype, appeared to be the important factor in protection from disease. This is the first evidence for an association between CD32 polymorphism and severe malaria and provides an example of balancing selective pressures from different infectious diseases operating at the same genetic locus.

### INTRODUCTION

Malaria continues to claim the lives of more children worldwide than any other infectious disease. A greater understanding of the mechanisms of malarial immunity would aid considerably the development of new therapeutic and vaccination strategies. The study of host genetics continues to provide important insights into such mechanisms of protection and improve our understanding of the pathogenesis of this complex disease.

The family of Fc receptors is believed to have an important role in immunity to many infections, including malaria. Fc $\gamma$ RIIa (CD32) is expressed on the surface of lymphocytes and monocytes/macrophages, and the molecule provides an important link between humoral and cellular immune systems. It is a low-affinity IgG receptor capable of binding immunoglobulin subtypes IgG<sub>1-4</sub> and it is able to bind C-reactive protein (CRP) with high affinity. However, both the recognition of IgG and CRP by CD32 are influenced by polymorphism within the gene.

A single nucleotide change within the CD32 molecule alters a histidine (H) to an arginine (R) residue at position 131 and changes its function *in vitro*. The presence of the H131 allele is essential for the efficient binding of IgG<sub>2</sub> subtypes.<sup>1</sup> In its absence, those with the RR131 genotype have preferential affinity for IgG<sub>3</sub> and IgG<sub>1</sub> subtypes. In addition to the differences in immunoglobulin affinity, the co-dominantly expressed H131 and R131 alleles differ in their ability to bind CRP. The R131 allele appears to be a high-affinity receptor for CRP, with the critical binding site lying near to that for IgG,<sup>2</sup> and the RR131 genotype has been shown to increase susceptibility to meningococcal and pneumococcal disease.<sup>3-5</sup>

Both CRP and immunoglobulin appear to play a role in malarial immunity. CRP has been shown to bind to *Plasmodium* species *in vitro* and might have a role in protection against the earliest stages of infection,<sup>6</sup> whereas immunoglobulin-transfer experiments have clearly demonstrated the importance of antibody-mediated immunity to malarial parasitemia.<sup>7</sup> Studies of protective antibody responses have shown that protection from disease correlates with antigen-specific

IgG<sub>1</sub> and, more particularly, IgG<sub>3</sub> immunoglobulin subtypes<sup>8</sup> that recognize specific malarial antigens including merozoite surface protein-1 (MSP-1) and MSP-2.<sup>9,10</sup> Furthermore, antigen-specific titers of IgG<sub>2</sub> specific for MSP-2 and glutamate-rich protein have been positively correlated with protection from malarial infection and disease.<sup>11,12</sup> However, there remains some uncertainty as to which of these IgG subclasses might be more important in malarial immunity, and the functional activity of these immunoglobulin subtypes is likely to depend, at least in part, on the CD32 genotype of the host.

A recent birth cohort study in western Kenya observed that the RR131 genotype offered protection against high levels of *P. falciparum* parasitemia in the first year of life.<sup>13</sup> In the only study of CD32 and malarial disease to date, a non-significant excess of HH131 homozygotes was seen in those with severe disease.<sup>14</sup> The objective of this study was to examine whether CD32 polymorphism is associated with susceptibility to either mild or severe *P. falciparum* disease in a large west African cohort.

### MATERIALS AND METHODS

**Population.** The subjects were recruited in The Gambia in western Africa and the study population has been described in detail elsewhere.<sup>15</sup> This study was reviewed and approved by the Gambian government/Medical Research Council joint ethical committee. All subjects or the parents or guardians of the children gave informed consent to participate in the study. Subjects were children ranging in age from birth to 10 years old (mean  $\pm$  SD age = 3.4  $\pm$  2.1 years) and the burden of severe malaria falls mainly on those less than five years of age (81.8% of the severe cases and 80.5% of the controls were less than five years old). Non-malarial controls were drawn from those with mild disease (generally attending an out-patient clinic for mild illness with a negative malaria blood film) and severe disease (generally in-patients with mainly infectious, non-malarial illnesses). Severe malaria was defined as the presence of one or more of the following: 1) cerebral malaria (Glasgow Coma Scale <3, or a prolonged seizure); 2) severe malarial anemia (hemoglobin level <5 g/dL); and 3) hypoglycemia (glucose level <2.2 mM). Mild malaria cases had parasitemias >2,500/ $\mu$ L. There was no significant difference in

\* These authors contributed equally to this work.

the representation of each ethnic group for mild malaria, severe malaria, or controls. Frequencies of ethnic groups were Fula (9.1% mild malaria, 12.6% severe malaria, and 11.9% controls), Jola (14.3%, 15.3%, and 13.8%), Mandinka (49.3%, 41.9%, and 44.8%), Serrahuli (5.2%, 5.3%, and 4.5%), Wolof (10.2%, 14.7%, and 13.7%) and Manjago (5.8%, 4.5%, and 5.9%).

**Genotyping.** The CD32 genotype was determined using a modified version of previously published methods.<sup>16</sup> The polymerase chain reaction conditions were as follows: one cycle at 96°C for five minutes, 35 cycles at 92°C for 40 seconds and 55°C for 30 seconds, and one cycle at 72°C for 10 minutes. Products were digested using *Bst* UI, which cuts once in the presence of the R131 allele and twice in the presence of the H131 allele. Fragments were resolved by electrophoresis on a 3% agarose gel.

**Statistical analysis.** Logistic regression analysis was performed using SPSS version 11 (SPSS, Inc, Chicago, IL) and included age, sex, geographic area of residence, and ethnicity as cofactors. Using the same software, we performed an overall comparison of genotype frequency using a 3 × 2 chi-square test and subsequent allele frequency using a 2 × 2 chi-square test.

## RESULTS

A total of 1,415 individuals were included in this study. Three hundred thirty-three (23.5%) were diagnosed with mild malaria, 524 (37.0%) with severe malaria, and 558 (39.4%) were non-malarial controls. Overall genotype frequencies did not vary significantly between different ethnic groups in the control population ( $P = 0.26$ ).

The HH131 genotype was at a higher frequency in those with severe malaria compared with controls (26.3% for severe malaria versus 21.7% for controls;  $P = 0.065$ ) (Table 1), and this result was significant in those less than five years of age (27.9% for severe malaria versus 21.9% for controls; odds ratio [OR] = 1.38, 95% confidence interval [CI] = 1.0–1.90,  $P = 0.04$ ). A similar trend was seen with each severe phenotype (24.2% for severe anemia and 25.6% for cerebral malaria versus 21.7% for controls;  $P = 0.68$  and  $P = 0.26$ , respectively). The HH131 genotype was more common in those with mild malaria than in controls, although this was not statistically significant (24% versus 21.7%, OR = 1.14, 95% CI = 0.82–1.60,  $P = 0.41$ ). A higher frequency of HR131 heterozygotes was observed in the control group (50.9%) than either the mild malaria (46.8%;  $P = 0.45$ ) or the severe malaria group (44.7%;  $P = 0.07$ ). No significant difference was seen in the frequency of RR131 genotype between those with severe malaria and the controls, (28.6% for severe malaria versus 28.0% for controls;  $P = 0.81$ ). Similar trends in data

were seen when analysis was restricted to infants less than one year of age and when the phenotypes of severe malarial anemia and cerebral malaria were considered separately.

Using a logistic regression model (taking into account ethnic group, geographic area, age, and sex), we found that the HH131 genotype was significantly associated with severe malarial disease (OR = 1.40 95% CI = 1.02–1.91,  $P = 0.034$ ) (Table 2). This result was more significant when those less than five years old were analyzed separately (OR = 1.50 95% CI = 1.06–2.12,  $P = 0.021$ ).

## DISCUSSION

These data demonstrate that the HH131 genotype is significantly associated with increased susceptibility to severe malarial disease in the West African population of The Gambia. A similar trend was seen towards susceptibility to mild malaria. The effect of this genotype was seen most clearly in those less than five years of age, the age group which carries the burden of severe disease in endemic areas, a finding consistent with previous evidence of age dependent effects.<sup>3</sup>

Given current understanding of the functional differences between H131 and R131 alleles, there are several potential explanations for the observed disease association. Individuals with the HH131 genotype have a low affinity for binding CRP,<sup>2</sup> and the susceptibility associated with this genotype is consistent with previous evidence for a protective role of CRP in malaria.<sup>6</sup> However, in a region of high malarial endemicity, CRP is likely to be less important than IgG in protection from malarial disease.

In the presence of the HH131 genotype, IgG<sub>1</sub>, IgG<sub>2</sub>, and IgG<sub>3</sub> immunoglobulin subtypes are able to activate the immune system using the FcγRIIa molecule, and the presence of the H131 allele is essential for effective IgG<sub>2</sub>-mediated cellular activation through this mechanism. There is accumulating evidence that some IgG<sub>2</sub> antigen-specific responses correlate with disease protection, but one plausible interpretation of these data is that the clinical importance of IgG<sub>2</sub> subtypes is less than that of IgG<sub>1</sub> and IgG<sub>3</sub>. This is consistent with early immuno-epidemiologic studies, which first found that disease protection correlated most clearly with IgG<sub>1</sub> and IgG<sub>3</sub> antigen-specific immunoglobulins.<sup>8</sup> An alternative and more speculative explanation for the findings could be that in the presence of the HH131 genotype, a greater quantitative activation of the innate immune system can be achieved by a broader repertoire of antibodies, increasing the risk of immunopathology and disease.

Shi and others, in their study of infant parasitemia in East Africa, observed that the RR131 genotype appeared to protect from high levels of parasitemia.<sup>13</sup> We observed no evidence for a protective effect of the RR131 genotype against

TABLE 1  
CD32 genotype distribution according to malarial phenotype\*

Genotype	Severe malaria	Cerebral malaria	Severe malarial anemia	Mild malaria	Controls
RR131	150 (28.6%)	94 (29.4%)	39 (29.5%)	97 (29.1%)	156 (28.0%)
HR131	236 (45.0%)	144 (45.0%)	61 (46.2%)	156 (46.8%)	281 (50.4%)
HH131	138 (26.3%)	82 (25.6%)	32 (24.2%)	80 (24.0%)	121 (21.7%)
Total	524	320	132	333	558

\* Cerebral malaria and severe malarial anemia are subgroups of severe malaria.

TABLE 2

Logistic regression analysis of CD32 genotypes on the risk of severe malaria\*

Genotype	Odds ratio (95% CI)	P
HR131	1.00	
RR131	1.16 (0.84–1.68)	0.34
HH131	1.40 (1.02–1.91)	0.03

\* CI = confidence interval.

mild malaria or malarial disease in this West African population. Rather, the data here suggest that it is the presence of the R131 allele that is protective against severe malaria. This discrepancy might well be due to differences in study design, particularly the different phenotype studied, and the contrasting sample sizes. In this study, children less than one year of age showed a similar trend in genotype distribution to the overall data set, suggesting that the age of study participants is unlikely to be an explanation for the differing results. An alternative possibility, in the absence of a protective effect from the RR131 genotype, is that heterozygosity at this locus might be protective against severe malaria. However, although there is a consistent trend within the data sets, heterozygotes were not significantly more common among the controls.

If the HH131 genotype alone was a major susceptibility factor for severe malaria, it might be expected that the H131 allele would be at lower frequency than the 47% observed in this population. However, the H131 allele might be playing an important protective role in protection from other diseases, particularly those caused by capsulated bacteria. Bredius and others found that the RR131 genotype was more frequent among survivors of severe meningococcal disease than in controls,<sup>17</sup> suggesting it might be a risk factor for severe disease. A similar association was identified with severe meningococcal disease<sup>3</sup> (where the effect was only clearly seen in those more than five years of age) and bacteremic pneumococcal pneumonia.<sup>5</sup> IgG<sub>2</sub>, which requires the presence of the H131 allele for efficient activity, is known to be important for protection from infection with capsulated bacteria including *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*,<sup>18</sup> and a reduction in this opsonic capacity is the most likely mechanism for genotypic effects observed. These bacterial diseases cause significant mortality in West Africa,<sup>19,20</sup> and the data here suggest that these potentially deleterious effects attributable to the R131 allele are, in this population at least, partly balanced by a protective effect against severe malaria.

The frequencies of the H131/R131 alleles differ significantly between populations and the selective pressures responsible have been the subject of some debate. In Caucasians in the United Kingdom, each allele is present at similar frequency (47% for R131 versus 53% for H131, Segal S, unpublished data), figures typical of European populations<sup>21</sup> and similar to those described here. In the Asian populations of Japan and China, the R131 allele is comparatively rare (22–28%) and invasive bacterial infections are relatively uncommon in populations of the Far East, an observation that some have linked to the low frequency of the RR131 genotype.<sup>21</sup> In regions where malaria is not endemic, it is possible that the lack of this selective pressure has allowed the frequency of the R131 allele to decrease relative to that of H131.

In summary, the association of HH131 genotype with severe malaria offers insight into immune mechanisms that might be important in protecting from severe malaria and provides evidence of balancing forces of natural selection, from different infectious diseases, operating at a single genetic locus.

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Authors' addresses: Graham S. Cooke, Christophe Aucan, Andrew J. Walley, Shelley Segal, Dominic Kwiatkowski, and Adrian V. S. Hill, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom. Brian M. Greenwood, Medical Research Council Laboratories, Banjul, The Gambia and London School of Hygiene and Tropical Medicine, London WC1E 7HT, United Kingdom.

Reprint requests: Graham S. Cooke, Wellcome Trust Centre for Human Genetics, University of Oxford, Oxford OX3 7BN, United Kingdom, E-mail: grahamc@well.ox.ac.uk.

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