Severity of malaria in relation to a complement receptor 1 polymorphism: a case–control study

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Background: Studies on the relationship between complement receptor 1 (CR1) polymorphisms in exon 29 encoding the Knops blood group antigens (Swain–Langley (*Sl*) and McCoy (*McC*)) and outcome of clinical malaria have produced inconsistent results.

Methods: Blood samples from Ghanaian children ($n=150$) aged 1–12 years with complicated and uncomplicated malaria were genotyped for the *Sl* and *McC* blood group alleles by polymerase chain reaction and restriction fragment length polymorphism. Effect of *Sl* and *McC* genotypes on the clinical outcome of malaria was evaluated using logistic regression.

Results: *McCa/b* genotype was significantly associated with more than two-fold increased susceptibility for severe malaria (OR=2.31; 95% CI: 1.03–5.20, $P=0.043$). However, *McCb/b* was associated with an 88% reduced risk of severe malaria (OR=0.12; 95% CI: 0.02–0.64, $P=0.013$). In contrast, there was no significant association between severe malaria and *Sl1/1*, *Sl1/2*, *Sl2/2* and *McCa/a* genotypes. There was a trend towards decreased susceptibility to both cerebral malaria (CM) (OR=0.13; 95% CI: 0.02–1.15, $P=0.07$) and severe malarial anaemia (SA) (OR=0.14; 95% CI: 0.02–1.19, $P=0.07$) for *McCb/b* genotype when compared with the *McCa/a* genotype. There were no significant associations between *Sl1/2* or *Sl2/2* genotype and CM or SA when compared with *Sl1/1* genotype.

Conclusions: *McCa/b* was associated with increased susceptibility to severe malaria and *McCb/b* associated with reduced risk of severe malaria. Further studies with large sample size in other malaria endemic regions in Africa are warranted to confirm these findings.

Keywords: Complement receptor 1, Swain–Langley, McCoy, Cerebral malaria, Severe malarial anaemia

Introduction

Malaria-related mortality is most common in children <5 years and pregnant women and is estimated to exceed 1 million deaths annually.¹ A majority of fatalities are a result of complications such as cerebral malaria (CM) and severe malarial anaemia (SA), as well as other debilitating effects caused by *Plasmodium falciparum*.^{2,3} Despite the overwhelming impact of the disease, the pathogenic basis of malaria, especially that caused by *P. falciparum*, is incompletely understood. The clinical outcome of malaria has been attributed to several factors such as host, parasite, geographic

and social characteristics.^{4,5} There have been indications from some studies regarding the role of complement receptor 1 (CR1, CD35) in the pathogenesis of malaria.^{6–9} However, the exact role played by CR1 is not fully understood.

Complement receptor 1 is a transmembrane protein found on some peripheral blood cells.¹⁰ Complement receptor 1 has decay accelerating factor (CD55) which serves as co-factor in the Factor I-mediated inactivation of C3b and C4b to C3bi and iC4i, respectively.¹¹ Complement receptor 1 on red blood cells (RBCs) also binds opsonised immune complexes and transports them to the spleen for removal. These functions collectively protect host tissues and organs from complement-mediated damage.⁸ Further, CR1 has been identified as a receptor for RBC invasion by *P. falciparum* and the receptor to

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which *P. falciparum* erythrocyte membrane protein 1 (*PfEMP1*) binds to form rosettes.^{12,13} Some studies have shown some correlation between *in vitro* rosette formation and severe malaria,^{14–16} and it has also been suggested that polymorphisms in *CRI* gene may be protective against severe malaria.¹⁷

The *CRI* gene exhibits a number of polymorphisms which includes the Knops blood group antigens. Polymorphism of the Knops blood group antigens includes the Swain–Langley (Sl: rs17047661), McCoy (McC: rs17047660) and their alleles.^{17,18} Red blood cells from individuals carrying the Sl (–1, 2) allele have been shown to form fewer rosettes *in vitro* which may affect the clinical outcome of malaria.^{17,18} Another allele, McC (a–b+) is dominant in ethnic groups of African descent and Africans living in malaria endemic regions.^{17,18}

Studies regarding the role of Sl and McC alleles in relation to the outcome of clinical malaria have been conflicting.^{8,9} For instance, while no association was observed between variants of the Sl or McC alleles and severe malaria, an association was observed between Sl2/2 and McCa/b genotypes and reduced susceptibility to CM when compared with Sl1/1 genotype.^{8,9} This study therefore examined further the relationship between polymorphisms of the Knops blood antigens and disease severity in Ghanaian paediatric malaria patients to establish a consistent body of evidence.

Materials and Methods

Patients and Sampling

Samples used in this study were selected from samples collected for a previous hospital-based case–control study.¹⁹ Participants were severe and uncomplicated malaria (UM) patients between the ages of 1 and 12 years who reported for medical care at the Korle-Bu Teaching Hospital, Ghana, between 2003 and 2005. The full details of the case–control study have been previously published.¹⁹ In brief, children with the following symptoms participated in the study: fever (>37.5°C), measured within 24 h of admission; asexual parasitaemia of >2500 parasites/μl; and at least one other sign of malaria, such as vomiting, diarrhoea, or malaise. In addition, patients had to be sickling negative and bacteraemia negative and have no other disease. Subjects were classified into CM, SA or UM as follows: CM was defined by an unrousable coma (score of <3 on the Blantyre coma scale) and no other neurological manifestation; SA was defined by a blood haemoglobin concentration of <5 g/dl, full consciousness and no other cause of anaemia; and UM was defined as described for SA, except that subjects had to have an Hb concentration of >8 g/dl and no other complication of malaria. The study received approval from the Institutional Review Board of the Noguchi Memorial

Institute for Medical Research and the Ethical and Protocol Review Committee of the University of Ghana Medical School.

A total of 150 blood samples collected into EDTA tubes were processed and buffy coats separated for analysing white blood cells (WBCs). After separation, samples were frozen at –80°C until analysis.

Parasitological and Haematological Measurements

The haematological parameters, including Hb levels, total WBC counts, total RBC counts and mean corpuscular volume, were measured with an automated haematological analyser (Sysmex KX-21, Sysmex Co., Kobe, Hyogo, Japan). Thick and thin blood films were stained with Giemsa and examined for malaria parasites, which were counted against 200 WBCs. Parasite densities were obtained by multiplying the total WBC counts by the number of parasites/200 WBCs. Sickling test was done using the sodium metabisulphite test, and sickling positive patients were excluded from the study.

Polymerase Chain Reaction

Genomic DNA was extracted from EDTA-preserved buffy coat samples using QIAamp DNA Mini kit (Qiagen Co., Valencia, CA, USA) according to the manufacturer's protocol. After extraction, genomic DNA was stored at –20°C for later analysis.

The polymerase chain reaction–restriction fragment length polymorphism (PCR–RFLP) method was used for the detection of both single nucleotide polymorphisms (SNPs) at the Sl and McC loci as previously described.²⁰ The forward primer 24KnNde (No. 3558001): 5'-ACC AGT GCC ACA CTG GAC CAG ATG GAG AAC AGC TGT TTG AGC AT-3' and the reverse primer 25Rb: 5'-GGA GGA GTG TGG CAG CTT G-3' (Eurogentec SA, Seraing, Belgium) were used to amplify a 305 bp fragment of CR1 exon 29. The amplification reactions were carried out on genomic DNA in a 50-μl final volume containing 10 μl of 5x Green GoTaq Reaction Buffer/MgCl₂ (Promega Co., USA) and 0.5 μl of 10 mM each dNTP (Invitrogen, Carlsbad, CA, USA), 1 μl 10 μM each primer and 0.25 μl of 5 U GoTaq DNA Polymerase (Promega Co., Madison, WI, USA). The cycling parameters for the reaction were as follows: denaturation of the DNA extracts at 95°C for 9 minutes, followed by 1 minute of denaturation at 94°C, annealing at 58°C for 1 minute, extension at 72°C for 1 minute for 44 cycles and a final extension at 72°C for 10 minutes concluded the reaction.

For RFLP analysis, 10 μl of each PCR product was digested with the restriction enzyme *MfeI* for the detection of Sl1/Sl2 and *BsmI* for McC^a/McC^b, which are SNPs at positions R1601G and K1590E in exon 29 of *CRI*, respectively. The final reaction

volume of 20 µl contained 10 µl of amplified product, 2 µl of NE buffer 4, 0.5 µl of *MfeI* or *BsmI* (20U) (New England Biolabs Inc., USA) and 7.5 µl of double distilled water and digested at 37°C (*MfeI*) or 65°C (*BsmI*) for 2 hours.

Statistical Analysis

The data was entered in STATA (Version 12, Stata Corporation, College Station, Texas, USA). Descriptive statistics were calculated for baseline characteristics—age, haemoglobin concentration and parasite density. Frequencies of SI and McC alleles among malaria phenotypes were compared with χ^2 tests. A *P*-value of ≤ 0.05 was considered statistically significant. Odds ratio with corresponding 95% confidence intervals and *P*-values were tested for the effects of genotypes in the case-control study using unconditional logistic regression analysis at each locus (SI and McC). Hochberg correction was used to correct for multiple comparisons.²¹

Results

Characteristics of study participants

A total of 150 children between the ages of 1 and 12 years comprising 100 severe malaria cases and 50 UM cases were used for this study (Table 1). Among those with severe malaria, there were 50 who were classified as CM (mean age=4.41 years) and 50 who were classified as SA (mean age=3.23 years). Fifty UM patients (mean age=6.03 years) were included as controls.

Distribution of genotype and allelic frequencies in malaria phenotypes

Severe malaria subjects and uncomplicated controls were genotyped for the SI (R1601G) and McC (K1590E) polymorphisms in the *CR1* gene. Distributions of genotypic and allelic frequencies of both polymorphisms are summarised in Table 2. Alleles at both the SI and McC loci were in Hardy-Weinberg equilibrium (SI χ^2 (2df)=2.23, *P*-value=0.328; McC χ^2 (2df)=0.00, *P*-value=1.00).

Association between SI, McC and severe malaria

Comparison of SI allele frequency between severe malaria cases (0.78) and UM control (0.77) showed no

statistical significance (*P*=0.92). McC allele frequency between severe malaria cases (0.25) and UM controls (0.29) was not significantly different (*P*=0.40).

Table 3 presents the relationship between SI, McC and clinical outcome of malaria among participants with cerebral and SA as cases (100 cases) and UM as control (50 controls) group. From the logistic regression analysis, McCa/b was significantly associated with increased susceptibility to severe malaria. This was still significant after adjusting for age and sex. McCb/b was, however, associated with an 88% reduced risk of severe malaria, which was robust to adjustment for age and sex. In contrast, there was no significant association between severe malaria and SI1/1, SI1/2, SI2/2 and McCa/a genotypes.

Association between SI and McC genotypes (R1601G and K1590E) with SA and CM

The authors examined whether the SI2 or McCb alleles confer resistance or susceptibility to severe malaria when compared with the SI1 and McCa alleles (Table 4). There was a trend towards decreased susceptibility to both CM and SA for McCb/b genotype when compared with the McCa/a genotype. There were, however, no significant associations between SI1/2 or SI2/2 genotype and CM or SA when compared with SI1/1 genotype.

Discussion

This study investigated known SNPs of the *SI* and *McC* blood group antigens located on the *CR1* gene to determine their distribution and association with severe malaria in Ghanaian children. We found that McCa/b genotype was associated with increased susceptibility to severe malaria and McCb/b was associated with reduced risk of severe malaria which was independent of age and sex. In contrast, there was no significant association between severe malaria and SI1/1, SI1/2, SI2/2 and McCa/a genotypes. Further, there was a trend towards decreased susceptibility to both CM and SA for McCb/b genotype when compared with the McCa/a genotype. There were however no significant

Table 1 Demographic and clinical characteristics of study participants

	Complicated malaria			<i>P</i> -value
	CM	SA	UM	
No. of subjects	50	50	50	
Age (years) mean $\pm$ SD	4.41 $\pm$ 2	3.23 $\pm$ 2	6.03 $\pm$ 3	<0.001
Haemoglobin concentration (g/dl) mean $\pm$ SD	7.53 $\pm$ 2	3.92 $\pm$ 1	9.81 $\pm$ 1	
GM parasite density ($\times 10^3$)/µl	46	51	62	0.285
No. of females (%)	26 (55)	20 (49)	21 (45)	
No. of males (%)	24 (45)	30 (51)	29 (55)	

Values reported as mean. *P*-values obtained after one-way analysis of variance (ANOVA).SD: standard deviation; GM: geometric mean; CM: cerebral malaria; SA: severe malarial anaemia; UM: uncomplicated malaria.

Table 2 Genotypic and allelic frequencies of Swain–Langley (SI) and McCoy (McC) genotypes

Malaria phenotype	SI1/1 (%)	SI1/2 (%)	SI2/2 (%)	Allele freq (SI2)
<i>SI (R1601G): rs17047661</i>				
UM (<i>n</i> =50)	4 (8)	15 (30)	31 (62)	0.77
CM (<i>n</i> =50)	1 (2)	14 (28)	35 (70)	0.84
SA (<i>n</i> =50)	6 (12)	17 (17)	27 (54)	0.71
<i>McC (K1590E): rs17047660</i>				
	McCa/a (%)	McCa/b (%)	McCb/b (%)	Allele freq (McCb)
UM (<i>n</i> =50)	29 (58)	13 (26)	8 (16)	0.29
CM (<i>n</i> =50)	27 (54)	22 (44)	1 (2)	0.24
SA (<i>n</i> =50)	26 (52)	23 (46)	1 (2)	0.25

Swain–Langley alleles (SI1/1: homozygous wild type; SI1/2: heterozygous; SI2/2= homozygous); McCoy alleles (McCa/a: homozygous wild type; McCa/b: heterozygous; McCb/b: homozygous mutant; amino acids substituted at CR1 CCP 25 (R: arginine; G: glycine; K: lysine; E: glutamic acid; freq: frequency; UM: uncomplicated malaria; CM: cerebral malaria; SA: severe anaemia malaria).

Table 3 Association between SI, McC and severe malaria

Genotypes	Cases (100)/controls (50)	Unadjusted OR (95% CI)	P-value	Adjusted OR (95% CI)	P-value
SI1/1	7/4	0.87 (0.24–3.11)	0.83	0.73 (0.18–2.94)	0.66
SI1/2	31/15	1.05 (0.50–2.19)	0.90	1.29 (0.57–2.90)	0.41
SI2/2	62/31	1.00 (0.50–2.01)	1.00	0.86 (0.40–0.84)	0.69
McCa/a	53/29	0.82 (0.41–1.62)	0.56	0.82 (0.39–1.74)	0.61
McCa/b	45/13	2.33 (1.11–4.90)	0.026	2.31 (1.03–5.20)	0.043
McCb/b	2/8	0.10 (0.02–0.51)	0.005	0.12 (0.02–0.64)	0.013

OR: odds ratio; CI: confidence interval.

associations between SI1/2 or SI2/2 genotype and CM or SA when compared with SI1/1 genotype.

The findings of our study conflicts the results of two similar studies carried out in Kenya and the Gambia. In contrast to the Kenyan study where CM and SA cases were matched with uncomplicated controls by age and sex and reported a significant association between SI2/2 genotype and decreased susceptibility to CM when compared with SI1/1 genotype, there was no significant association between SI2/2 genotype and decreased susceptibility to CM when compared with SI1/1 genotype in the authors' study.⁸ The authors' study rather reported a trend towards decreased susceptibility to both CM and SA for McCb/b genotype when compared with the McCa/a genotype. The disparity between our study and this study may be because of the fact that the children in our study were much older than the children in the Kenyan study. For instance, the mean age of children with CM in our study was ~4.4 years, whereas

in the Kenyan study the mean age was ~2.4 years. Likewise, the mean age of children with SA in our study was ~3.4 years, whereas in the Kenyan study the mean age was ~1.2 years.

Secondly, while the Gambian study reported that neither of the SI and McC alleles separately or combined conferred protection against CM nor SA when malaria-infected children were compared to children classified as 'non-malaria controls',⁹ the authors reported significant association between McC alleles and clinical outcome of malaria.

In a similar Ghanaian study conducted by Hansson *et al.*,²² with the aim to determine the genotype frequencies of the SNPs defining the Knops blood group antigens and determine their possible associations with the severity of malaria, the authors concluded that the SI and McC alleles or their combinations do not seem to confer any protective advantage against malaria infection or resulting disease severity. This conclusion contrasts our finding of positive association between

Table 4 Association between SI and McC genotypes (R1601G and K1590E) with SA and CM

CM						SA					
Genotypes	No. of cases (%)	No. of controls (%)	OR	95% CI	P-value	Genotypes	No. of cases (%)	No. of controls (%)	OR	95% CI	P-value
<i>R1601G</i>						<i>R1601G</i>					
SI1/1	1 (20.00)	4 (80.00)	Ref.	–	–	SI1/1	6 (60.00)	4 (40.00)	Ref.	–	–
SI1/2	14 (48.28)	15 (51.72)	3.73	0.37–37.58	0.26	SI1/2	17 (53.13)	15 (46.55)	0.76	0.18–3.20	0.70
SI2/2	35 (53.03)	31 (46.97)	4.52	0.48–42.59	0.19	SI2/2	27 (46.55)	31 (53.45)	0.58	0.15–2.28	0.44
<i>K1590E</i>						<i>K1590E</i>					
McCa/a	27 (48.21)	29 (51.79)	Ref.	–	–	McCa/a	26 (47.27)	29 (52.73)	Ref.	–	–
McCa/b	22 (62.86)	13 (37.14)	1.82	0.77–4.31	0.18	McCa/b	23 (63.89)	13 (36.11)	1.97	0.83–4.67	0.12
McCb/b	1 (11.11)	8 (88.89)	0.13	0.02–1.15	0.07	McCb/b	1 (11.11)	8 (88.89)	0.14	0.02–1.19	0.07

CM: cerebral malaria; SA: severe malarial anaemia; Ref.: reference category.

McCa/b genotype and severe malaria and a negative association between McCb/b and severe malaria. The differences in the results may be because of the type of control subjects used between the two studies. Hansson *et al.*²² used healthy children between the ages of 0 and 15 years with or without detectable *P. falciparum* in a thick blood smear as controls while the authors used children with mean age of 6.03 years with UM.

While it is worrying that the findings of these studies have not produced a consistent body of evidence, it is important to note that the mechanism by which *SI* and *McC* alleles could bring about resistance or susceptibility to severe malaria is not yet clear. While it is tempting to speculate that amino acids substitutions in *SI* and *McC* alleles may influence the binding of *P. falciparum* to erythrocytes and thereby affect susceptibility and clinical outcome of severe malaria, it is also appropriate to note that the location of these polymorphisms and the binding site of *PfEMP1* are different. Knops blood group polymorphisms have been mapped to CCP 25 located at the C-terminal domain of the CR1 molecule while *PfEMP1* interacts with the C3b binding site in LHR-B and LHR-C involving SCRs 8, 9, 10 and 15, 16, 17, respectively.^{23,24} Furthermore, it has been demonstrated so far in literature that the first three CCPs of the four long homologous repeats of CR1 are usually the active sites.^{10,25} The LHR-D of CR1 has the ability to bind mannan-binding lectin (MBL) and C1q,^{26,27} but the exact location where the binding takes place is not known¹⁰ and could be the subject for further studies.

Apart from the presumable effect of these amino acid substitutions, the potential association between *McC* polymorphisms and clinical outcome of severe malaria in this study could be because of the fact that polymorphisms in the *CR1* gene may be acting in concert with other parasite, host, geographic and social factors which modify the clinical outcome of *P. falciparum* infection.⁴

This study has some strengths and limitations. The diagnoses and ascertainment of the various phenotypes of malaria was rigorous. However, the study was statistically underpowered to observe significant associations because of logistical constraints. This study nevertheless adds valuable data to the dearth of literature in this area of research in Africa.

Conclusion

Here, the authors have demonstrated that McCa/b and McCb/b genotypes of the McC blood group antigens may be associated with increased susceptibility to severe malaria and significant protection from severe malaria, respectively. There was a trend towards decreased susceptibility to both CM and SA for McCb/b genotype when compared with the McCa/a

genotype. Our study adds to the limited evidence of the relationship between the polymorphisms of the Knops blood group antigens and the clinical outcome of severe malaria in West Africa. Further studies with large sample size are recommended in West Africa and Africa at-large to provide a more uniform body of evidence on the relationship between these SNPs and severe malaria.

Acknowledgements

We would like to thank the children who participated in our studies as well as their parents for their willingness to contribute to this research project. We are grateful to the clinicians and nurses who made this research work feasible. We are also beholden to the staff of the Immunology Department of the Noguchi Memorial Institute for Medical Research who contributed to the success of this research project in diverse ways.

Disclaimer Statements

Contributors

We hereby declare that except for references to other people's work, which have been duly acknowledged, this article is the result of our research conducted at the Departments of Microbiology (UGMS, Korle-Bu) and Immunology of Noguchi Memorial Institute for Medical Research (NMIMR, Legon).

Funding

This project was supported by EDCTP Senior Fellowship Grant-TA.2007.40200.012; 2008 and Afro-Immunoassay (AIA) Phase 2 Project Grant-EC101/2008.

Conflicts of interest

Funding agencies were neither a part in the design of the study, the data collection, data analysis, data interpretation, writing of the manuscript, nor in the resolution to submit the manuscript for publication.

Ethics approval

This research obtained ethical approval from the Ethical and Protocol Review Committee of the Noguchi Memorial Institute for Medical Research, Ghana, and the University of Ghana Medical School.

References

- 1 Aregawi M, Cibulskis RE, Otten M, Williams R. *World malaria report 2009*. Geneva: World Health Organization; 2009.
- 2 World Health Organization. Severe falciparum malaria. World Health Organization, Communicable Diseases Cluster. Trans R Soc Trop Med Hyg. 2000;94(Suppl 1):S1–90.
- 3 Waitumbi JN, Opollo MO, Muga RO, Misore AO, Stoute JA. Red cell surface changes and erythrophagocytosis in children with severe *Plasmodium falciparum* anemia. Blood. 2000;95(4):1481–6.

- 4 Miller LH, Baruch DI, Marsh K, Doumbo OK. The pathogenic basis of malaria. *Nature*. 2002;415(6872):673–9.
- 5 Weatherall DJ, Miller LH, Baruch DI, Marsh K, Doumbo OK, Casals-Pascual C, et al. Malaria and the red cell. *Hematol Am Soc Hematol Educ Program*. 2002;35–57.
- 6 Rowe JA, Moulds JM, Newbold CI, Miller LH. *P. falciparum* rosetting mediated by a parasite-variant erythrocyte membrane protein and complement-receptor 1. *Nature*. 1997;388(6639):292–5.
- 7 Stoute JA, Odindo AO, Owuor BO, Mibei EK, Opollo MO, Waitumbi JN. Loss of red blood cell-complement regulatory proteins and increased levels of circulating immune complexes are associated with severe malarial anemia. *J Infect Dis*. 2003;187(3):522–5.
- 8 Thathy V, Moulds JM, Guyah B, Otieno W, Stoute JA. Complement receptor 1 polymorphisms associated with resistance to severe malaria in Kenya. *Malar J*. 2005;4:54.
- 9 Zimmerman PA, Fitness J, Moulds JM, McNamara DT, Wasehagen LJ, Rowe JA, et al. CR1 Knops blood group alleles are not associated with severe malaria in the Gambia. *Genes Immun*. 2003;4(5):368–73.
- 10 Krych-Goldberg M, Atkinson JP. Structure-function relationships of complement receptor type 1. *Immunol Rev*. 2001;180:112–22.
- 11 Roozendaal R, Carroll MC. Complement receptors CD21 and CD35 in humoral immunity. *Immunol Rev*. 2007;219:157–66.
- 12 Cserti CM, Dzik WH. The ABO blood group system and *Plasmodium falciparum* malaria. *Blood*. 2007;110(7):2250–8.
- 13 Tham WH, Wilson DW, Lopaticki S, Schmidt CQ, Tetteh-Quarcoop PB, Barlow PN, et al. Complement receptor 1 is the host erythrocyte receptor for *Plasmodium falciparum* PfRh4 invasion ligand. *Proc Natl Acad Sci USA*. 2010;107(40):17327–32.
- 14 Carlson J, Helmby H, Hill AV, Brewster D, Greenwood BM, Wahlgren M. Human cerebral malaria: association with erythrocyte rosetting and lack of anti-rosetting antibodies. *Lancet*. 1990;336(8729):1457–60.
- 15 Rowe A, Obeiro J, Newbold CI, Marsh K. *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. *Infect Immun*. 1995;63(6):2323–6.
- 16 Treutiger CJ, Hedlund I, Helmby H, Carlson J, Jepson A, Twumasi P, et al. Rosette formation in *Plasmodium falciparum* isolates and anti-rosette activity of sera from Gambians with cerebral or uncomplicated malaria. *Am J Trop Med Hyg*. 1992;46(5):503–10.
- 17 Moulds JM, Zimmerman PA, Doumbo OK, Kassambara L, Sagara I, Diallo DA, et al. Molecular identification of Knops blood group polymorphisms found in long homologous region D of complement receptor 1. *Blood*. 2001;97(9):2879–85.
- 18 Moulds JM, Kassambara L, Middleton JJ, Baby M, Sagara I, Guindo A, et al. Identification of complement receptor one (CR1) polymorphisms in West Africa. *Genes Immun*. 2000;1(5):325–9.
- 19 Kusi KA, Gyan BA, Goka BQ, Dodoo D, Obeng-Adjei G, Troye-Blomberg M, et al. Levels of soluble CD163 and severity of malaria in children in Ghana. *Clin Vaccine Immunol*. 2008;15(9):1456–60.
- 20 Moulds JM, Thomas BJ, Doumbo O, Diallo DA, Lyke KE, Plowe CV, et al. Identification of the Kna/Knb polymorphism and a method for Knops genotyping. *Transfusion*. 2004;44(2):164–9.
- 21 Hochberg Y. A sharper Bonferroni procedure for multiple tests of significance. *Biometrika*. 1988;75(4):800–2.
- 22 Hansson HH, Kurtzhals JA, Goka BQ, Rodriques OP, Nkrumah FN, Theander TG, et al. Human genetic polymorphisms in the Knops blood group are not associated with a protective advantage against *Plasmodium falciparum* malaria in Southern Ghana. *Malar J*. 2013;12:400.
- 23 Rowe JA, Rogerson SJ, Raza A, Moulds JM, Kazatchkine MD, Marsh K, et al. Mapping of the region of complement receptor (CR) 1 required for *Plasmodium falciparum* rosetting and demonstration of the importance of CR1 in rosetting in field isolates. *J Immunol*. 2000;165(11):6341–6.
- 24 Smith BO, Mallin RL, Krych-Goldberg M, Wang X, Hauhart RE, Bromek K, et al. Structure of the C3b binding site of CR1 (CD35), the immune adherence receptor. *Cell*. 2002;108(6):769–80.
- 25 Krych M, Clemenza L, Howdeshell D, Hauhart R, Hourcade D, Atkinson JP. Analysis of the functional domains of complement receptor type 1 (C3b/C4b receptor; CD35) by substitution mutagenesis. *J Biol Chem*. 1994;269(18):13273–8.
- 26 Ghiran I, Klickstein L, Nicholson-Weller A. Mannan binding lectin binds complement receptor type 1 and augments IgG-stimulated phagocytosis. *Immunopharmacology*. 2000;49(1):3.
- 27 Klickstein LB, Barbashov SF, Liu T, Jack RM, Nicholson-Weller A. Complement receptor type 1 (CR1, CD35) is a receptor for C1q. *Immunity*. 1997;7(3):345–55.