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Mutations of complement lectin pathway genes *MBL2* and *MASP2* associated with placental malaria

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Abstract

Background: Innate immunity plays a crucial role in the host defense against malaria including *Plasmodium falciparum* malaria in pregnancy, but the roles of the various underlying genes and mechanisms predisposing to the disease are poorly understood.

Methods: 98 single-nucleotide polymorphisms were genotyped in a set of 17 functionally related genes of the complement system in 145 primiparous Ghanaian women with placental malaria, defined by placental parasitaemia or malaria pigment, and as a control, in 124 non-affected primiparae.

Results: Placental malaria was significantly associated with SNPs in the lectin pathway genes *MBL2, MASP2, FCN2* and in *properdin*. In particular, the main African mannose-binding lectin deficiency variant (*MBL2*G57E, rs1800451) increased the odds of placental malaria (OR 1.6; permuted p-value 0.014). In contrast, a common *MASP2* mutation (R439H, rs12085877), which reduces the activity of MBL-MASP2 complexes occurred in 33% of non-affected women and in 22% primiparae with placental malaria (OR 0.55, permuted *p*-value 0.020).

Conclusions: Excessive complement activation is of importance in the pathogenesis of placental malaria by mediating inflammation, coagulation, and endothelial dysfunction. Mutated MBL and MASP2 proteins could have direct intrinsic effects on the susceptibility to placental malaria, in addition to their roles in regulation of downstream complement activation.

Keywords: Lectin pathway, Mannose-binding lectin, MBL2, MASP2, Ficolin, Complement, Innate immunity, Malaria, Placenta, Pregnancy

Background

In sub-Saharan Africa, *Plasmodium falciparum* infection during pregnancy is a major cause of maternal anaemia, preterm delivery (PD), low birth weight (LBW) and infant mortality. In areas endemic for *P. falciparum*, 85 million pregnancies occur annually, and malaria-associated LBW in Africa results in an estimated 100,000 indirect infant deaths each year [1-3]. Pregnant, particularly primiparous, women are at increased risk. In pregnant women, parasites expressing specific variants

of the *P. falciparum* Erythrocyte Membrane Protein-1 (PfEMP1) adhere to the placental endothelium lining the intervillous space which results in placental sequestration of infected red blood cells. The local accumulation of infected red blood cells and of malaria pigment (haemozoin), i.e. placental malaria, triggers the infiltration of inflammatory cells and a profound pro-inflammatory response [4,5]. This is confronted by an insufficient production of specific antibodies against the parasites and their PfEMP1 binding domain. Only with successive pregnancies, protective acquired immune mechanisms gradually develop and expand, and the disease manifestation declines [6-8].

In conditions of lacking or weak acquired immunity, e.g. early childhood and pregnancy, innate immune

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responses may play a predominant role in host defense against malaria [9]. Toll-like receptors (TLRs), for instance, are crucial mediators of innate immunity to *P. falciparum* [10], and variant signalling as deduced from functionally relevant single nucleotide polymorphisms (SNPs) has been associated with an increased risk and/ or manifestation of malaria in pregnancy [11,12]. Overall, one third of the variability in susceptibility to placental malaria is thought to be due to host genetic factors [13]. These may also include the complement system, composed of three pathways, the classical, the lectin and the alternative pathway.

Mannose-binding lectin (MBL, encoded by *mbl2*) is a serum protein involved in the initiation of innate immune responses by binding to microbial surface oligosaccharides, and activating the lectin pathway. Upon binding, MBL forms a complex with mannan-binding lectin serine peptidase 2 (MASP2) that cleaves C4 and C2 to form the C3 convertase (C4b2a). Subsequent complement activation leads to opsonization and phagocytosis of the target microbes, as well as formation of membrane attack complexes [14]. The role of MASP1 and MASP3 (encoded by *MASP1*) are still debated [15]. An important function of MASP1 is its ability to activate the alternative pathway proenzyme pro-D to active factor D [16]. MASP2 can also form active complexes with ficolins which bind to acetylated carbohydrates or, e.g., to acetylated LDL. Ficolins also recognize deposited C-reactive protein (CRP), and may thus collaborate with CRP in the initiation and control of inflammatory responses [15].

MBL binds to *P. falciparum*-infected red blood cells [17], and may consequently be involved in innate immune responses and parasite clearance. MBL deficiency caused by common SNPs increases the risk of severe malaria although findings are partially inconsistent [18-22]. As for (asymptomatic) placental infection, one recent study failed to show an association with MBL levels or *MBL2* genotypes [23].

The aim of the present study was to analyse the influence of lectin pathway polymorphisms on the susceptibility to placental malaria and its manifestation. Following a pathway-oriented approach and using Sequenom's MassaARRAY® system, SNPs of *MBL2*, *MASPs* and *ficolins* (*FCN*) and other important complement system genes (*SERPING1, C3, CFB, CFH, CFP*) were included in a novel "complement-chip". Because the coagulation system may also be involved in adverse pregnancy outcomes [24], we also included respective regulatory genes (*thrombomodulin, thrombospondin, FLT1*).

Methods
Patients
A total of 893 women attending for delivery were recruited between January 2000 and January 2001 at the Presbyterian Mission Hospital in Agogo, located in the hyper-to holoendemic Ashanti Region of Ghana. The study protocol was reviewed and approved by the Committee on Human Research Publication and Ethics, School of Medical Sciences, University for Science and Technology, Kumasi, Ghana, and informed consent was obtained from all participants. Diagnostic procedures, malariologic indices and clinical characteristics have been described earlier [11,24]. For the present study, all 304 primiparous women with live singleton deliveries were included. In brief, women were clinically examined, socioeconomic data were documented, and samples of placental intervillous and peripheral venous blood were collected into EDTA. Malaria parasites in intervillous blood were counted microscopically on Giemsa-stained thick blood films per 100 high-power fields (magnification x1,000), and the presence of leukocyte-associated haemozoin was recorded. Placental infection was defined as the presence of *P. falciparum* parasites and/or haemozoin in placental thick blood films. In addition, *P. falciparum*-specific PCR assays were performed [25]. Haemoglobin (Hb) was measured by a HemoCue photometer (Angelholm, Sweden), and anaemia defined as Hb < 110 g/l. Preterm delivery was defined as a gestational age < 37 weeks, on the basis of the Finnström score [26], and low birth-weight as < 2,500 g.

SNP selection
Based on our previous finding of an association of *MBL2* polymorphisms with severe malaria in children [22], all lectin pathway genes as well as other relevant downstream complement and alternative pathway genes were chosen for genotyping [27]. Additionally, we included genes related to the coagulation system (*thrombomodulin, thrombospondin*) and pre-eclampsia (*FLT1*). In total, 17 genes were chosen for genotyping (Table 1). For each gene, first, we selected SNPs with assumed relevance based on published data on protein function, activity or disease association. Secondly, we included potentially functional nonsense and missense SNPs, and finally, we also included some intronic, promoter or 3' end SNPs as markers of association. We mainly focused on SNPs with minor allele frequencies > 0.05 in both African and European populations based on HapMap data.

Genotyping
DNA was extracted from peripheral blood (QIAmp; Qiagen, Germany). Twenty-seven of the samples from 304 primiparous women were excluded from genotyping based on low DNA quality or quantity. Among the 277 samples genotyped, 8 were removed because of a genotyping call rate < 0.9 leaving 269 samples for the final analyses.

Table 1 Genes selected for genotyping.

HUGO Gene Symbol	Gene name	SNPs genotyped	SNPs analysed for association
MBL2	mannose-binding lectin 2	5	3
MASP1	mannan-binding lectin serine peptidase 1	8	7
MASP2	mannan-binding lectin serine peptidase 2	11	10
FCN1	ficolin 1	9	7
FCN2	ficolin 2	9	9
FCN3	ficolin 3	3	1
SERPING1	serpin peptidase inhibitor (C1 inhibitor)	5	4
C3	complement component 3	7	5
CFB	complement factor B	5	5
CFH	complement factor H	8	6
CFP	complement factor properdin	6	3
THBD	thrombomodulin	5	1
THBS1	thrombospondin 1	4	3
THBS2	thrombospondin 2	4	4
THBS3	thrombospondin 3	2	2
THBS4	thrombospondin 4	2	2
FLT1	fms-related tyrosine kinase 1	5	4
	Total number of SNPs	**98**	**76**

22 SNPs were excluded from genetic association analyses because of low minor allele frequency, poor genotyping quality, or lack of accordance with Hardy-Weinberg equilibrium. Gene symbols and names are given as defined in the HUGO Gene Nomenclature Committee's database

SNP genotyping was performed using Sequenom's MassARRAY MALDI-TOF Mass Spectrometry Compact platform and iPLEX Gold chemistry (Sequenom Inc, San Diego, CA) with standard protocols. A total of 98 SNPs were assayed in four multiplexes of 15 to 34 markers each. Eight SNPs that failed to meet a call rate of > 0.8 were removed. 14 SNPs (for which no previous frequency data were available) were excluded due to a minor allele frequency < 0.05, leaving 76 SNPs for the final analyses (Table 1). Genotypes were analysed using Sequenom's MassARRAY Typer version 4.0 software. All data was checked twice manually and all outlying data or low intensity results were removed. The integrity of control and duplicate sample results, as well as negative control samples was checked during the evaluation process.

Statistical analysis

Analyses were performed with PLINK v1.06 [28], SPSS (SPSS Inc., Chicago, Illinois; release 15.0, 2006) statistical software, and with R language. Hardy-Weinberg equilibrium was tested in infected and non-infected women separately, with the standard Chi-square test, to identify possible genotyping problems. Associations of placental malaria and the SNPs were tested with basic allelic association as well as with several genotypic models (dominant, recessive, and trend included in PLINK). In addition to the main affection status of placental malaria, also other phenotypes were analysed, including low birth weight, preterm birth, maternal anaemia as

well as *P. falciparum* positivity by microscopy or PCR from placental or peripheral blood samples. Permutation tests were used to adjust for multiple testing, as well as due to the correlatedness of the tests because of linkage disequilibrium (LD) between markers. Haplotypes were constructed and tested with PLINK using 2-to 6-marker sliding windows over all areas where adjacent SNPs were located less than 10 Mb from each other.

Odds ratios (OR) along with their confidence intervals (CIs) were estimated with PLINK and R scripts [29]. Joint ORs for two loci were evaluated by collapsing genotypes to susceptibility allele carriers and non-carriers, and combining these over both loci, resulting in just four susceptibility classes. This was done in order to avoid sparse class frequencies and thus overly wide confidence intervals.

Finally, interactions were screened with PLINK procedure *epistasis*, which tests all pairs of SNPs separated by at least 1 Mb and in different chromosomes. A *p*-value threshold of 0.001 was used to avoid most false positives. We acknowledge that our data is small for making robust inference on potential interactions, therefore these results should be considered preliminary.

Results

The characteristics of the 269 primiparous Ghanaian women are shown in Table 2. Fifty-four percent of the women had placental malaria, defined as the presence of parasites or hemozoin in the intervillous placental blood (placental parasitemia, 48%, placental hemozoin, 43%).

Table 2 Characteristics of 269 primiparous Ghanaian women admitted for delivery

	All	Placental malaria	No placental malaria	*P-value*
Number of subjects	269	145	124	
Mean age, years (± SD)	21.45 (± 3.51)	21.00 (± 3.17)	21.98 (± 3.82)	0.051[1]
Mean maternal hemoglobin, g/l (± SD)	112.6 (± 1.82)	106.3 (± 1.76)	120.0 (± 1.61)	< 0.001[1]
Mean birth weight, grams (± SD)	2760 (± 485)	2698 (± 485)	2833 (± 478)	0.023[2]
Mean gestational age, weeks (± SD)	38.11 (± 2.50)	38.07 (± 2.77)	38.16 (± 2.17)	0.81[1]
Proportion with				
> 3 antenatal care visits	0.49	0.44	0.55	0.083[3]
maternal anaemia (Hb < 11 g/dL)	0.41	0.55	0.24	< 0.001[3]
low birth weight (< 2500 g)	0.25	0.30	0.20	0.091[3]
preterm delivery (< 37 weeks)	0.25	0.27	0.23	0.48[3]
Placental blood samples				
PCR positive (*P. falciparum*)	0.66	0.95	0.32	< 0.001[3]
Microscopy positive (*P. falciparum*)	0.48	0.88	0.00	< 0.001[3]
Haemozin pigments present	0.43	0.80	0.00	< 0.001[3]

[1] Mann-Whitney U-test

[2] Student's t-test

[3] Chi-square test

Placental *P. falciparum*-PCR was positive in 66% and maternal peripheral blood *P. falciparum*-PCR positive in 59% of cases. Maternal hemoglobin and child birth weights were lower in cases with placental malaria than in uninfected mothers ($p < 0.001$ and $p = 0.023$). Gestational age did not differ between the groups.

A total of 76 SNPs of 17 candidate genes (Table 1) could be subjected to association analyzses. SNPs with at least suggestive association with placental malaria were found on the *MBL2, MASP2, Ficolin 2 (FCN2)* and *Complement factor properdin* (*CFP*) genes (Table 3).

Among these SNPs, *MBL2*C* (G57E, rs1800451), the main African MBL variant, which is associated with malaria in children [22], was present with an allele frequency of 36% in women with placental malaria, and 26% in controls ($p = 0.01$). The results suggested a dominant effect for an increased susceptibility to placental malaria (Table 3): homozygous or heterozygous *MBL2*C* was more frequently observed in women with placental malaria (61%, 88 out of 145) than in non-affected women (45%, 56 out of 123) resulting in an OR of 1.8 (95% CI, 1.1-3.0) for the susceptibility allele in our data. *MBL*C* is significantly associated to microscopy positivity of placental blood samples, whereas other diagnostic tests show non-significant trends in the same direction (Table 4). *MBL2*C* is not associated to low birth weight, maternal anaemia or preterm birth.

With regard to *MASP2*, two markers, rs12085877 (R439H, $P = 0.02$) and rs1033638 (3' untranslated region, $P = 0.03$), remained significantly associated with placental malaria after permutation tests. These exon 11 variants are located only 930 bp apart (Figure 1a). In

this area, one major haplotype including both markers and spanning from rs2273347-rs2273346 conferred increased odds of placental malaria, with an overall $p = 0.05$, and for the haplotype AAGTA specifically, $p = 0.021$ (Table 5). However, the LD between the two major SNPs was surprisingly weak (rs12085877-rs1033638: $r^2 = 0.037$); and most of the risk increase could be attributed to R439H. This amino acid residue is located in the activation peptide of MASP2 (Figure 1b), between the second CCP domain and the serine protease domain, and rhe R439H mutation inhibits the normal function of the MBL-MASP2 complex [30]. When analysed with respect to different diagnostic tests, R439H showed strongest negative association to the presence of malaria pigment in the placenta ($p = 0.008$, OR 0.47, 95% CI 0.26-0.83), but was not associated to microscopy or PCR positivity of the placenta (Table 4). This would suggest a role of the association especially for chronic placental infection. Also, functionality of this mutation was supported by a non-significant trend towards low birth weight in individuals with the 439H variant ($p = 0.057$, OR = 0.51, 95% CI 0.24-0.99).

At the 5' end of the *MASP2* gene, the region rs3765900-rs56392418-R99Q as a whole was associated with placental malaria at $p = 0.05$. Specifically, haplotype TGG was at present in 53% and 21% of women with and without placental malaria, respectively ($p = 0.0051$, Table 5; Figure 1a). None of the markers forming the haplotype were individually associated with placental malaria in permutation tests.

In the *FCN2* gene, two SNPs showed suggestive associations with placental malaria (Table 3). Located in

Table 3 Single marker results of allelic and genotypic association with placental malaria

Gene	SNP	Mutation	Allelic associations						Genotypic association	
			All. freq cases	All. freq controls	P-value	Permuted P-value	Odds ratio	95% CI	Best genotypic model	Empirical p-value
MBL2	rs1800451	G57E	0.36	0.26	0.014	0.014	1.60	1.10-2.32	Dominant	0.021
MASP2	rs12085877	H439R	0.11	0.19	0.014	0.020	0.55	0.34-0.89	Allelic	0.015
MASP2	rs1033638	3'UTR	0.36	0.27	0.04	0.022	1.52	1.01-2.26	Trend	0.064
FCN2	rs3128624	Intron 2	0.67	0.62	0.198	0.204	1.27	0.88-1.81	Dominant	0.053
FCN2	rs7037264	Intron 3	0.68	0.64	0.271	0.261	1.23	0.85-1.76	Dominant	0.042
CFP	rs909523	Intron 6	0.57	0.47	0.019	0.029	1.51	1.07-2.13	Trend	0.043

intron 2 (rs3128624) and intron 3 (rs7037264), they are separated by only 73 bp. These SNPs were not associated to other phenotypes.

Complement factor properdin (*CFP*) is a positive regulator of the alternative complement pathway. It had one SNP associated with placental malaria (rs909523; allele C; $p = 0.029$ after permutation; Table 3). This variant, located in intron 6 of the gene, was not associated with any other assessed malaria phenotype. The region rs1048118-rs8177079-rs909523 was associated at $p = 0.028$, and specifically, the haplotype ATA was protective with $p = 0.007$ (present in 8% and 15% of women with and without placental malaria, respectively).

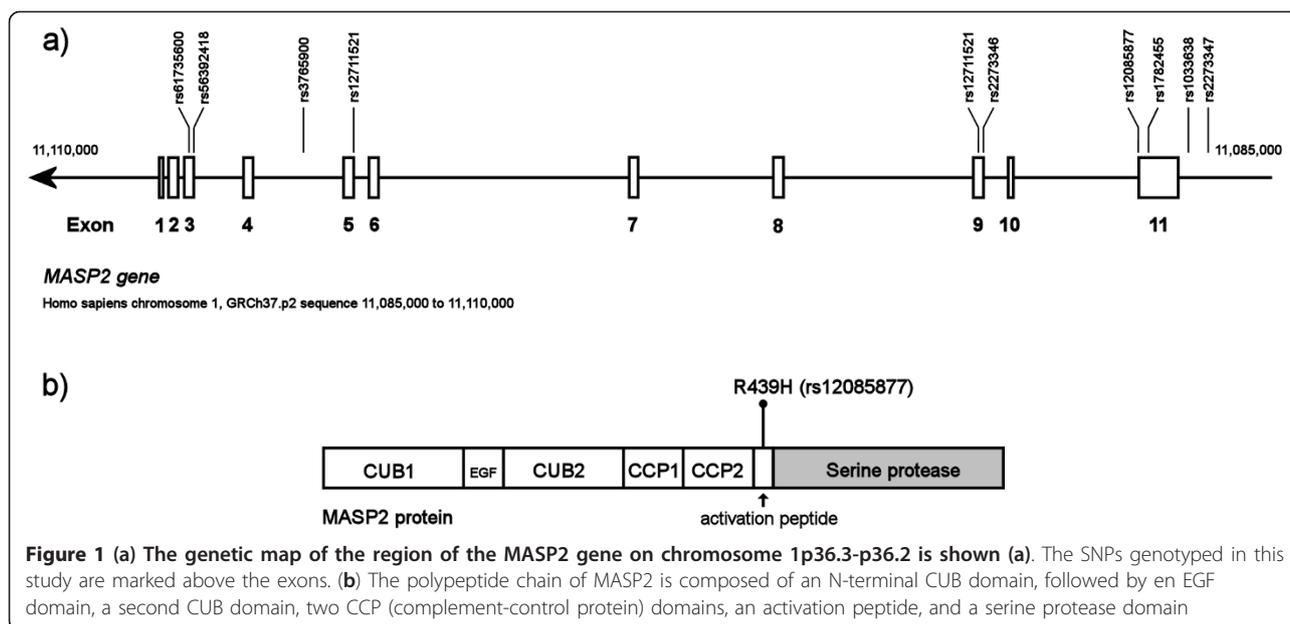As MBL forms an active complex with MASP2, the potential interaction between the *MBL2* and *MASP2* genotypes was also analysed, to evaluate whether specific allele combinations of the two could render the individual susceptible to malaria. Placental malaria was detected in 39% of the women carrying no risk alleles for *MBL2* (G57E) or *MASP2* (R439 wildtype), compared to 65% of women with risk alleles at both loci ($p = 0.007$; OR 2.90, 95% CI 1.34-6.48. The increased risk for those with risk alleles at both loci compared to all other individuals gave an OR of 2.13 (95% CI 1.33-3.65, $p = 0.003$). These results suggest an additive effect of both risk alleles, but an excess risk due to interaction could not be shown.

Discussion

Recent genome-wide association studies on malaria have had difficulties in identifying causal gene variants in

Table 4 Association of different phenotypes of *P.falciparum* placental malaria with mutations MBL2*G57E and MASP2*R439H

	MBL2*G57E (MBL2*C; rs1800451)				MASP2*R439H (rs12085877)			
	Wildtype (GG)	Mutation (AA or AG)	p-value	OR	Wildtype (CC)	Mutation (TT or TC)	p-value	OR
Placental malaria	45.5% (56/123)	60.7% (88/145)	0.014	1.84 (1.13-3.01)	57.7% (113/196)	43.8% (32/73)	0.054	0.58 (0.33-0.99)
Maternal peripheral blood								
PCR positive	53.7% (66/123)	63.4% (92/145)	0.108	1.50 (0.92-2.45)	62.2% (122/196)	49.3% (36/73)	0.070	0.59 (0.34-1.02)
Microscopy positive	24.8% (29/117)	29.0% (40/138)	0.482		28.9% (55/190)	22.7% (15/66)	0.423	
Placental blood								
PCR positive	60.2% (74/123)	70.3% (102/145)	0.094	1.57 (0.94-2.61)	68.4% (134/196)	58.9% (43/173)	0.151	
Microscopy positive	39.0% (48/123)	54.5% (79/145)	0.014	1.86 (1.15-3.05)	49.0% (96/196)	41.1% (30/73)	0.218	
Haemozin pigments present	36.9% (45/122)	48.3% (70/145)	0.064	1.59 (0.98-2.62)	48.0% (94/196)	29.2% (21/72)	0.008	0.47 (0.26-0.83)
Low birth weight	27.0% (33/122)	24.1% (35/145)	0.673		28.6% (56/196)	16.7% (12/72)	0.057	0.51 (0.24-0.99)
Maternal anaemia	35.0% (43/123)	40.7% (59/145)	0.378		38.8% (76/196)	35.6% (26/73)	0.673	
Preterm birth	27.0% (33/122)	23.4% (34/145)	0.571		23.5% (46/196)	29.2% (21/72)	0.344	

Figure 1 (a) The genetic map of the region of the MASP2 gene on chromosome 1p36.3-p36.2 is shown (a). The SNPs genotyped in this study are marked above the exons. (**b**) The polypeptide chain of MASP2 is composed of an N-terminal CUB domain, followed by en EGF domain, a second CUB domain, two CCP (complement-control protein) domains, an activation peptide, and a serine protease domain

African populations due to low LD [31,32]. In the present study, a pathway-oriented candidate gene approach to investigate the role of innate immunity and of the complement system in particular, deficiencies of which are known to increase susceptibility to a wide range of infections [33]. Genetic variants in the *MBL2, MASP2, FCN2* and *CFP* genes were shown to be potential risk factors for placental malaria in Ghana. These results thus suggest a relevant role for the lectin pathway of the complement system in the human defense against placental malaria.

Functional *MBL2* mutations interfere with the formation of oligomers and result in low serum levels of high molecular weight MBL and impaired MBL function [34]. Their role in malaria has been addressed in several studies [18-22,35], and a meta-analysis including four studies suggested an increased risk (odds ratio 1.29, 95% CI 1.08-1.53) for malaria in patients carrying the *MBL2*C* mutation [22]. The strongest association was found in young children [22] supporting the hypothesis that MBL is essential especially in individuals lacking

fully developed semi-immunity. In a study from Mozambique, none of ten *MBL2* SNPs examined were found to be associated with placental malaria [13], but *MBL2*C* was not included. The absence of association of the other SNPs can probably be explained by the low LD in Africans. These results suggest that the *MBL2*C* variant is a risk factor for placental malaria, in addition to infant malaria. It remains unclear, however, why such a disadvantageous disposition is maintained at a high frequency in malaria-endemic Africa; respective issues have been discussed elsewhere [21].

Protection from malaria provides an evolutionary benefit in Africa and respective SNPs can be expected to reach polymorphic frequencies. The *MASP2* mutation *R439H* (rs12085877) is absent in European and Asian populations but according to a previous study [30] and HapMap data, its allele frequency is 9-15% in Sub-Saharan Africa [36]. In the present study, *MASP2*R439H* occurred in 27% of the women. In contrast to *MBL2*C*, it was protective against placental malaria, especially chronic infection, and a trend towards protection from

Table 5 Haplotype associations of *MASP2* and *CFP2* genes for placental malaria

Gene area	Overall *p*-value[1]	Specific haplotype	Prevalence in cases	Prevalence in controls	*P*-value[2]
MASP2, exon 11					
rs2273347-rs2273346	0.05	AAGTA	0.12	0.19	0.021
MASP2, 5' end					
rs3765900-rs56392418-R99Q	0.05	TGG	0.53	0.21	0.0051
CFP					
rs1048118-rs8177079-rs909523	0.028	ATA	0.08	0.15	0.007

[1]Overall *p*-value for association of the haplotypes in the gene area
[2]*P*-value for association of the specific haplotype

low birth weight was discernible. MASP2 with the R439H mutation is able to bind to MBL, but the enzymatic activity of the complex is considerably reduced [30]. At present, conclusive arguments are lacking for the differential impact on placental malaria of two genetic variants which finally lead to reduced complement activation. Thus, it could be possible that these mutations alter the direct intrinsic functions of MBL and MASP2 in addition to the downstream complement activation. MBL has been shown to stimulate phagocytosis and opsonization also in absent of other complement components [37]. MASP2 on the other hand, is capable of promoting fibrinogen turnover directly by cleavage of fibrinogen and indirectly by cleavage of prothrombin, generating active thrombin [38]. MASP2 can, in addition to MBL, interact with ficolins and the balance between formation of ficolin-MASP2 complexes and MBL-MASP2 complex could influence the susceptibility of placental malaria.

Another explanation for *MASP2*R439H* protecting against placental malaria could involve a curbed production of C5a. Recent work has demonstrated that placental parasitaemia induces increased levels of this potent pro-inflammatory peptide in primiparae [39]. Data from adverse pregnancy outcomes in humans and from murine models of pathological pregnancies suggest that C5a could be an important regulator of placental angiogenesis, and excessive C5a could lead to functional placental insufficiency by impairing adequate vascularization of the placenta [40]. Elevated levels of C5a, however, are considered detrimental for host innate defense including defects in phagocyte and endothelial cell function [41]. Curbed activation in this regard could thus be beneficial.

Alternatively, these findings on a common MASP2 variant show some resemblance with deficiency in the closely related complement receptor 1 (CR1). CR1 is also involved in the activation and regulation of C4, and patients with CR1 deficiency show greatly reduced rosetting, i.e. binding and aggregation of infected and non-infected erythrocytes [42]. Cytoadherence is the key feature of placental malaria [2,8], and small alterations of this process could have substantial clinical consequences. Increased generation of C4b and C3b would thus assist adherence and rosetting of malaria infected parasites in the placenta.

As for the ficolins, two SNPs on the *FCN2* gene showed a suggestive association with placental malaria. These *FCN2* variants are located in introns and are thus not likely to be functional themselves. On the contrary, they might be involved in gene expression regulation or splicing, or in LD with the actual predisposing mutations.

Downstream complement amplification of the lectin pathway is normally activated when MASP2 cleaves C4 and C2 to form the C3 convertase (C4b2a). C2 bypass of the lectin pathway is also possible, and involves alternative pathway amplification where complement factor properdin (CFP) and complement factor D (CFD) are involved [43]. This connection of CFP to the lectin pathway could be an explanation of its association to placental malaria. However, the downstream amplification and regulation of the lectin pathway is obviously complex and yet not fully understood.

Excessive complement activation has been suggested to play a role in the pathogenesis of severe malaria, including cerebral malaria as well as severe malarial anaemia, and placental malaria [27]. Through recognition of parasites by MBL and ficolins, the lectin pathway might be able to abort infections and reduce the parasitemic load, but on the other hand be harmful by mediating excessive inflammation, coagulation, and endothelial dysfunction. The novel finding of the protective role of the functional *MASP2*R439H* mutation makes MASP2 an interesting target for drug and vaccine development. MASP2 inhibitors need to be studied as potential adjunctive therapy for the various manifestations of malaria [44].

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Authors' contributions
VH designed the study, participated in the statistical analyses and drafted the manuscript. PO carried out the statistical analyses. EL participated in the genotyping. GBA participated in the recruitment of study participants and sample collection in Ghana. FPM coordinated the sample collection and participated in drafting the manuscript. SM participated in the study design and drafting the manuscript. All authors read and approved the final manuscript.

Competing interests
The authors declare that they have no competing interests.

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