A meta-analysis of the endothelial protein C receptor rs867186 genotype in malaria

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Abstract

BACKGROUND : During P. falciparum infection, the binding of P. falciparum erythrocyte membrane protein 1 (PfEMP1) to endothelial cells (EC) results in the sequestration of pRBC. Several receptors located on the endothelial cells, including intercellular adhesion molecule 1 (ICAM-1), CD36, and endothelial protein C receptor (EPCR), contribute to PfEMP1 adhesion to the microvasculature. PfEMP1, expressed on the surface of parasitized red blood cells (pRBC), is composed of cysteine-rich interdomain regions (CIDR) and Duffy binding-like (DBL) domains. CIDRα1 competitively binds to EPCR with activated protein C (APC) and impairs cytoprotective and anticoagulant effects by APC, which plays important roles in severe malaria (SM) pathogenesis such as cerebral malaria (CM) and severe malaria anemia (SMA). The strategy to inhibit EPCR binding to pRBC while concomitantly strengthen its binding to APC may be crucial in restoring disrupted protein C (PC) system's function. The purpose of this study is to evaluate the association between malaria severity and the EPCR genotypes as well as with soluble EPCR (sEPCR), and the study also addresses the physiological relevance of EPCR genetic polymorphism. RESULTS : In this study, we conducted a meta-analysis on the eligible studies by comparing the frequency of EPCR rs867186-GG versus rs867186-GA and -AA genotype in SM, mild malaria (MM) or uncomplicated malaria (UM) patients and healthy individuals from Thailand, Uganda, Benin, Tanzania, and Ghana. We also determined the relationship between rs867186 genotype and sEPCR levels. Our results showed that the genotype rs867186-GG is higher in MM/UM than in SM patients. SM patients carrying the rs867186-GG genotype have higher plasma soluble EPCR (sEPCR) levels than in rs867186-AG and rs867186-AA carriers. MM/UM patients carrying the rs867186-AG genotype have significantly higher level of sEPCR compared to those carrying rs867186-AA. Similarly, the rs867186-GG is associated with high sEPCR level in healthy individuals. CONCLUSIONS : This meta-analysis demonstrates that pRBCs and EPCR interactions are associated with malaria severity, and treatments that block their binding via PfEMP1 CIDRα1 could be a potential therapy for SM.

Background

We define severe malaria (SM) as cerebral malaria (CM) or severe malarial anemia (SMA). SM also include malaria patients with high levels of parasitemia, hypoglycemia, and increased creatinine, which is indicative of high mortality among patients with *P. falciparum* infections [1, 2]. The pathological feature of SM is the sequestration of pRBC to host microvasculature beds in a variety of organs and tissues. The sequestration occurs when *P. falciparum* binds to endothelial cells (known as cytoadherence) via the *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) [3-9]. PfEMP1 protein is encoded by approximate 60 *var* genes. However, each parasite only expresses one *var* gene at a time [10]. The extracellular part of PfEMP1 is composed of cysteine-rich interdomain regions (CIDR) and Duffy binding-like (DBL) domains [11-13]. DBL-CIDR in tandem within N-terminus is the most conserved extracellular region [14]. The parasite sequestration, which is associated with disease severity, is determined by the binding preference and affinity of PfEMP1 to host endothelial cell receptors [15-17]. The most widely studied receptors are CD36 and intercellular adhesion molecule 1 (ICAM-1). CIDRα2-6 domains of PfEMP1 bind to CD36 [11, 18], and DBLβ5 domains bind to ICAM-1 [13, 18]. pRBCs usually bind to CD36 on endothelial cells (or platelets and

monocytes) in uncomplicated or mild malaria (UM/MM), and bind to ICAM-1 in SM [15-17]. Recently, CIDRα1 domains of PfEMP1 was found to bind endothelial protein C receptor (EPCR) [11, 18]. Turner et al. discovered that EPCR interacts with PfEMP1 domain cassette 8 (DC8) and DC13 (both of which contain CIDRα1 domain) and correlated with SM [19]. The binding of CIDRα1 to EPCR inhibits activation of protein C (APC)'s, induces apoptosis, inflammation, and coagulation. As a result, pRBCs-EPCR binding suppresses cytoprotection and barrier functions in hosts [20-22], which are part of mechanisms of SM of CM and SMA [23]. By examining different subsets of PfEMP1 *var* genes in CM samples from Ugandan children by qRT-PCR, Shabani et al. found that children expressed the highest number of EPCR-PfEMP1 binding transcripts during the onset of CM and SMA, as they recovered from the disease, the number of EPCR-binding PfEMP1 transcripts decreased [12]. Their findings that increased EPCR-PfEMP1 binding (v*ar* CIDRα1 domain) transcript is associated with severity of malaria indicate that EPCR-binding PfEMP1 transcript could be a novel therapeutic target for SM. One interesting thing is that although the abundance of total transcripts increased with malaria severity, there is no individual transcript significantly increased, which suggests that all EPCR-binding CIDRα1 subtypes are required to be targeted to deplete *P. falciparum* sequestration in host blood circulation, in particular for sequestration in brain blood circulation [11]. Several lines of evidence support a correlation between the plasma EPCR (soluble EPCR, sEPCR) levels and the genetic polymorphisms of the *EPCR* gene, particularly the EPCR rs867186-A/G genotype [1, 2, 24-26]. sEPCR can competitively bind to the CIDRα1 domain and therefore displaces EPCR binding sites for APC, which restores cellular EPCR function. Due to lack of systemic analysis of EPCR genotypes, sEPCR levels, and their relationship with malaria disease severity, in the present study, we performed a meta-analysis based on related literature to determine the clinical significance of the EPCR gene in SM pathogenesis.

Methods

Search strategy

Pubmed, Medline, Web of Science, Scopus and Embase were searched until April 2019 using the following terms: "endothelial protein C receptor," "EPCR," "malaria," "severe malaria/complicated malaria/uncomplicated malaria," "cerebral malaria," "severe malaria anemia," "polymorphism," and "clinical studies." Articles screened were obtained by titles first and then by article abstracts. After excluding non-relevant publications, the remaining papers were evaluated in the full-text version based on inclusion/exclusion criteria. All searched data were retrieved, and the language of publication was restricted to English.

Selection criteria

In this meta-analysis, we collected all eligible publications regarding the correlation between EPCR and clinical outcomes and clinicopathological features in malaria patients. The inclusion criteria includes: (1) studies that evaluate and analyze rs867186-A/G genotype using DNA from peripheral blood mononuclear cells published during 2014 to 2016; (2) studies that determine the correlation between rs867186-AG genotype and malaria severity; (3) studies that define CM as the presence of one of the following signs: high *P. falciparum* parasitemia (>100,000 parasites/μl), hypoglycaemia (glucose level <22 nmol/l), severe

anemia (hematocrit <20% or hemoglobin <7g/dl), increased serum creatinine (>3 mg/dl), and unrousable coma (or a Blantyre Coma Score below 3) caused by malaria infection regardless of other signs (cerebral malaria); and (4) studies that have enough information to estimate and analyze hazard ratio (HR) and 95% confidence interval (CI). The exclusion criteria includes: (1) letters, case reports, reviews, editorials, conference abstracts, and expert opinion; (2) studies without information on the genotype of the AA and AG alleles of rs867186 (rs867186-AG genotype); and (3) all in vitro/ex vivo studies including cell culture and animal studies.

Data extraction

Two investigators independently extracted the data from the eligible studies. Disagreements were resolved through full discussion until consensus was reached. The following data was identified and recorded for each study: year of publication, the first author's name, number of cases, sample source, rs867186-AG genotype, and clinicopathological parameters. Data for study characteristics were collected and summarized, and the heterogeneity of each study was evaluated as well.

Statistical analysis

The data analysis was performed using the software Review Manager 5.3 (Cochrane Collaboration, Oxford, UK) and Stata 12.0 (Stata Corporation, TX, USA). Comparisons of dichotomous measures were conducted by pooled odds ratios (ORs). We determined heterogeneity by a Chi-square test with significance set at P <0.10; We determined the total variation among studies by I square. I square value is an estimate of variance due to between-study heterogeneity rather than chance (the Cochran Q statistics). P-value of <0.05 was considered to be statistically significant. If there were heterogeneity among studies (when I square exceeded 50%), we used a random-effect model to pool the ORs; otherwise, a fixed-effect model was selected.

Results

1. 1*. Identification of relevant studies and study characteristics*

Twenty-eight articles were identified, and twenty-three of those were excluded due to non-original articles (review), laboratory studies, and studies irrelevant to the current analysis. Finally, five studies from 2014 to 2016 [1, 2, 24-26] met the selection criteria and were eligible and included in the final meta-analysis (Fig. 1). A total of 2995 SM, 487 uncomplicated malaria, and 2105 healthy controls from Thailand, Uganda, Benin, Tanzania, and Ghana were enrolled. The characteristics are listed in Table 1. The definition criteria for the five manuscripts' SM are presented in Table 2.

2. *rs867186 A>G and the risk of malaria*

We compared the genotype frequencies of GG (rs867186-GG) versus AA (rs867186-AA) of rs867186 in malaria patients and healthy individuals. The prevalence of rs867186-GG is not significantly higher in

healthy controls than in malaria patients. The pooled OR from 3 studies, including 2532 malaria patients and 2105 healthy individuals, is shown in Fig. 2 (OR=0.92, 95% CI=0.74-1.15, P=0.49).

3. *Association of rs867186-G variant with higher plasma levels of soluble EPCR (sEPCR)*

Two studies were eligible to analyze the relationship among PROCR gene, rs867186-G (GG or AG) variant, and plasma soluble EPCR levels. In the Beninese studies conducted by Moussiliou et al. [24], no patient had the homozygous recessive genotype rs867186-GG. At convalescence, the overall analysis of genotype showed higher plasma sEPCR levels among CM and SM patients carrying the rs867186-AG genotype compared with those carrying rs867186-AA; data are shown in Table 3 (297ng/ml vs. 158ng/ml, respectively; P=0.03). At admission, among CM and SM patients, those having rs867186-AG genotypes showed an increase in plasma EPCR levels than those carrying rs867186-AA genotypes (P=0.17), but they did not reach significant difference [24]. The studies from Shabani [26] showed that CM and SMA patients carrying rs867186-GG or rs867186-AG genotype have higher sEPCR levels than rs867186-AA carriers (194ng/ml and 131ng/ml vs. 84.5ng/ml, P=0.007 and P<0.001, respectively, Table 3). Besides, a significant difference was observed with higher plasma sEPCR levels among uncomplicated malaria patients carrying the rs867186-AG genotype compared to those carrying rs867186-AA (161ng/ml vs. 86.5ng/ml, P<0.001, Table 3). Similarly, the rs867186-GG was associated with higher sEPCR levels in healthy individuals (350ng/ml vs. 241ng/ml, P<0.006, Table 3). Our results show the association between the rs867186-G variant and higher circulating sEPCR levels both in SM patients, including CM and SMA, in high *P. falciparum* parasitemia burden (>250,000 parasites/μl) [24, 26], and UM/MM and healthy individuals.

4. *Association of rs867186-GG genotype with the protection of SM*

Among SM and uncomplicated/mild malaria (UM/MM) patients, we analyzed genotype frequencies of the GG alleles (rs867186-GG) and compared with AA and AG alleles (rs867186-AA+AG) of rs867186. We observed that the prevalence of rs867186-GG is higher in mild malaria than in SM patients. The pooled OR from 2 studies, including 892 patients with SM and 437 mild malaria is shown in Fig. 3 (OR=0.36, 95% CI=0.14-0.91, P=0.03). The possible reason that the rs867186 genotypes distinguish between mild malaria and SM but not healthy controls and malaria patients (Fig. 3) may be due to healthy controls lacking the pressure exerted by parasites of the genus *Plasmodium*, which causes malaria infection.

5. *Sensitivity analyses and publication bias*

A sensitivity analysis is an important part of a meta-analysis as it aims to determine the robustness of the observed outcomes. A sensitivity analysis was performed to assess the results' stability. We perform the sensitivity analysis by excluding one study at a time to see whether or not the results remained consistent. The results showed that the pooled ORs are not significantly changed, indicating the stability of analytic

results. The funnel plots are largely symmetric, indicating that there are no publication biases in the meta-analysis of the role of *rs867186-GG* genotype in SM (Fig. 4).

Discussion

The cell surface protein of EPCR was originally cloned in 1994 [27]. Multiple ligands of EPCR have been found, including PfEMP1, protein C (PC)/activated protein C (APC), factor VIIa, tissue factor, and a specific variant of the T-cell receptor. Under physiological conditions, Protein C is activated by the thrombin-thrombomodulin complex, and activated protein C (APC) then cleaves the protease-activated receptor (PAR1) at Arg46, which triggers an anti-apoptotic and anti-inflammatory reaction to inhibit thrombin production and stabilizes endothelial barrier function [21, 28, 29]. In SM, PfEMP1 binding to EPCR, which subsequently blocks APC binding to EPCR. Once eliminating the APC/EPCR binding, thrombin upregulates of NFκB, tumor necrosis factor (TNF) and interleukin (IL)-6, downregulates Angiopoietin 1 (Ang1). Consequently, APC induces apoptosis, inflammation, and disrupts endothelial barrier integrity [11, 13, 22].

Each *Plasmodium falciparum* genome holds about 60 PfEMP1 encoding var genes. DC13-containing PfEMP1 variants can bind to both ICAM-1 and EPCR [22]. Both ICAM-1 [30-32] and EPCR [9, 33-36] are involved in the pathogenesis of CM, for instance, ICAM-1: PfEMP1 binding and resetting have been identified as one of the virulence factors [37], while EPCR: PfEMP1 binding enables parasites dominate host infections with limited anti-malaria immunity [9, 33-36]. sEPCR is released from surface EPCR on the endothelial cells, so two of them share the same binding affinity; therefore, the recombinant sEPCR' binding to PfEMP1-DC13 and -DC8 variants could displace cellular EPCR on the surface of endothelial cells [19], which probably increases the risk of venous thrombosis by way of increased activation of APC [19, 38]. Thus, if individual who is less likely to develop SM due to expressed EPCR would show a higher risk for thrombotic disease [19, 38].

To assess the effect of rs867186-GG polymorphism for the risk of SM, we analyzed genotyped patients with mild malaria and SM on rs867186-GG of previous publications. We found that the rs867186-GG genotype appears significantly more frequent in patients with mild malaria than those with SM (P=0.03 in Fig. 3). However, when we compared genotypes rs867186-GG versus rs867186-AA in malaria patients and healthy individuals, the frequency of rs867186-GG in malaria patients and healthy controls is very similar (p=0.90 in Fig. 2). This was not caused by studies bias, as the heterogeneity is calculated as I^2=0 (Fig. 2), and this implies that we can take a fixed-effect model to pool the ORs rather than a random effect model. The genotype difference of the GG and AA alleles of rs867186 is likely due to the pressure exerted by parasites of the genus *Plasmodium* that cause malaria. Thus, comparing healthy controls that lack these pressure with any disease status is unnecessary. This is a possible reason that these genotypes distinguish between mild and SM but not between healthy controls and malaria patients. The studies from Hansson et al. [1] and Schuldt et al. [25] reported that the genotype rs867186-GG does not have a protective role in malaria patients compared to healthy controls. However, they did not compare the difference in rs867186-GG genotype between SM and MM.

The metalloprotease cleaves the surface cellular EPCR, which releases a soluble EPCR (sEPCR) that circulates in the plasma [19]. The relationship between the polymorphisms in EPCR and plasma sEPCR levels has been conducted in patients with thrombosis to evaluate the risk of venous thrombosis by Medina et al. [39]. Their data indicate that individuals carrying some specific genotypes have high sEPCR and APC levels, thus having a lower risk of venous thromboembolism [39]. However, no systemic analysis has been made in malaria patients regarding the EPCR genotype and plasma EPCR levels. When we examined the relationship between rs867186 polymorphisms and plasma sEPCR evaluating risk of SM, we found that the carriers of the rs867186-GG genotype have significantly higher sEPCR levels than those with the AG and AA genotypes in SM; carriers of the rs867186-AG genotype have significantly higher sEPCR levels than those with AA genotype in uncomplicated malaria, and carriers of the rs867186-GG genotype have significantly higher sEPCR levels than those with AG genotype in healthy individuals (Table 3). These results support that the rs867186 GG genotype is associated with elevated sEPCR levels in SM (Table 3). The loss of endothelial protein C receptor link coagulation and inflammation to parasite sequestration in CM [26, 40], and rs867186-GG is associated with increased soluble EPCR and can potentially mediate protection against SM.

Two studies involving adult malaria patients [2, 12] revealed that the EPCR rs867186-G allele could mediate protection against SM, while the other three studies involving child malaria patients showed that EPCR gene variants are not associated with SM [1, 25] or increased mortality among children with CM [24]. Although they utilized similar criteria for SM, adult and child malaria may have different pathophysiology. If more studies in this field are available in the future, the EPCR polymorphism study should be conducted on adult and child malaria separately.

Conclusions

This meta-analysis summarizes previous observations and indicates the EPCR rs867186-GG genotypes are associated with increased soluble EPCR and may have an important role in SM. pRBCs bind to EPCR on endothelial cells and block APC's access to EPCR, inducing pro-apoptotic, pro-inflammatory, and loss of local vascular barrier integrity. Targeting EPCR-PfEMP1 binding can potentially reverse *P. falciparum* sequestration in host blood circulation, and in particular for sequestration in brain blood circulation, which could be an effective intervention to prevent SM. In the future, more studies are needed to assess the adhesive abilities of PfEMP1 expressed in isolation of pRBC from SM, CM patients, and even pregnant women with malaria. Furthermore, the mechanism of EPCR genotype protecting individuals against SM leads to an increased risk of thrombotic disease is also worthy of exploration.

Although we only found five publications with a total of 2995 SM and 487 uncomplicated malaria, performing a meta-analysis is justified since we are working with a fixed-effect. A summary based on two or more studies yields a more precise estimate of the true effect than either study alone [41].

Abbreviations

Ang1 Angiopoietin 1

APC	Activated protein C
CIDR	Cysteine-rich interdomain regions
CM	Cerebral malaria
DBL	Duffy binding-like
DC8	Domain cassette 8
EC	Endothelial cells
EPCR	Endothelial protein C receptor
ICAM-1	Intercellular adhesion molecule 1
MM	Mild malaria
ORs	Odds ratios
PC	Protein C
PfEMP1	*P. falciparum* erythrocyte membrane protein 1
pRBC	Parasitized red blood cells
sEPCR	Soluble endothelial protein C receptor
SM	Severe malaria
SMA	Ssevere malaria anemia
UM	Uncomplicated malaria

Declarations

Authors' contribution

LY and ML contributed substantially to the study design, data acquisition, analysis, and interpretation of data. RX and SG contributed substantially to the acquisition, analysis, interpretation of data, and performed the statistical analysis. LY and ML have been involved in the drafting process and critical revision of the article for important intellectual content. The corresponding authors had full access to all data and the final responsibility for the decision to submit the article for publication. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

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Supplementary material

PRISMA Checklist

Competing interest

The authors have no financial relationships and conflicts of interest in the manuscript.

References

1. Hansson HH, Turner L, Moller L, Wang CW, Minja DT, Gesase S, Mmbando B, Bygbjerg IC, Theander TG, Lusingu JP et al: Haplotypes of the endothelial protein C receptor (EPCR) gene are not associated with severe malaria in Tanzania. Malaria journal 2015, 14:474.
2. Naka I, Patarapotikul J, Hananantachai H, Imai H, Ohashi J: Association of the endothelial protein C receptor (PROCR) rs867186-G allele with protection from severe malaria. Malaria journal 2014, 13:105.
3. Moxon CA, Grau GE, Craig AG: Malaria: modification of the red blood cell and consequences in the human host. British journal of haematology 2011, 154(6):670-679.
4. Nielsen MA, Staalsoe T, Kurtzhals JA, Goka BQ, Dodoo D, Alifrangis M, Theander TG, Akanmori BD, Hviid L: Plasmodium falciparum variant surface antigen expression varies between isolates causing severe and nonsevere malaria and is modified by acquired immunity. Journal of immunology (Baltimore, Md : 1950) 2002, 168(7):3444-3450.
5. Jensen AT, Magistrado P, Sharp S, Joergensen L, Lavstsen T, Chiucchiuini A, Salanti A, Vestergaard LS, Lusingu JP, Hermsen R et al: Plasmodium falciparum associated with severe childhood malaria preferentially expresses PfEMP1 encoded by group A var genes. The Journal of experimental medicine 2004, 199(9):1179-1190.

6. Bull PC, Pain A, Ndungu FM, Kinyanjui SM, Roberts DJ, Newbold CI, Marsh K: Plasmodium falciparum antigenic variation: relationships between in vivo selection, acquired antibody response, and disease severity. The Journal of infectious diseases 2005, 192(6):1119-1126.
7. Rottmann M, Lavstsen T, Mugasa JP, Kaestli M, Jensen AT, Muller D, Theander T, Beck HP: Differential expression of var gene groups is associated with morbidity caused by Plasmodium falciparum infection in Tanzanian children. Infection and immunity 2006, 74(7):3904-3911.
8. Kyriacou HM, Stone GN, Challis RJ, Raza A, Lyke KE, Thera MA, Kone AK, Doumbo OK, Plowe CV, Rowe JA: Differential var gene transcription in Plasmodium falciparum isolates from patients with cerebral malaria compared to hyperparasitaemia. Molecular and biochemical parasitology 2006, 150(2):211-218.
9. Lavstsen T, Turner L, Saguti F, Magistrado P, Rask TS, Jespersen JS, Wang CW, Berger SS, Baraka V, Marquard AM et al: Plasmodium falciparum erythrocyte membrane protein 1 domain cassettes 8 and 13 are associated with severe malaria in children. Proceedings of the National Academy of Sciences of the United States of America 2012, 109(26):E1791-1800.
10. Scherf A, Lopez-Rubio JJ, Riviere L: Antigenic variation in Plasmodium falciparum. Annual review of microbiology 2008, 62:445-470.
11. Mkumbaye SI, Wang CW, Lyimo E, Jespersen JS, Manjurano A, Mosha J, Kavishe RA, Mwakalinga SB, Minja DT, Lusingu JP et al: The Severity of Plasmodium falciparum Infection Is Associated with Transcript Levels of var Genes Encoding Endothelial Protein C Receptor-Binding P. falciparum Erythrocyte Membrane Protein 1. Infection and immunity 2017, 85(4).
12. Shabani E, Hanisch B, Opoka RO, Lavstsen T, John CC: Plasmodium falciparum EPCR-binding PfEMP1 expression increases with malaria disease severity and is elevated in retinopathy negative cerebral malaria. BMC medicine 2017, 15(1):183.
13. Tuikue Ndam N, Moussiliou A, Lavstsen T, Kamaliddin C, Jensen ATR, Mama A, Tahar R, Wang CW, Jespersen JS, Alao JM et al: Parasites Causing Cerebral Falciparum Malaria Bind Multiple Endothelial Receptors and Express EPCR and ICAM-1-Binding PfEMP1. The Journal of infectious diseases 2017, 215(12):1918-1925.
14. Su XZ, Heatwole VM, Wertheimer SP, Guinet F, Herrfeldt JA, Peterson DS, Ravetch JA, Wellems TE: The large diverse gene family var encodes proteins involved in cytoadherence and antigenic variation of Plasmodium falciparum-infected erythrocytes. Cell 1995, 82(1):89-100.
15. Newbold C, Warn P, Black G, Berendt A, Craig A, Snow B, Msobo M, Peshu N, Marsh K: Receptor-specific adhesion and clinical disease in Plasmodium falciparum. The American journal of tropical medicine and hygiene 1997, 57(4):389-398.
16. Ochola LB, Siddondo BR, Ocholla H, Nkya S, Kimani EN, Williams TN, Makale JO, Liljander A, Urban BC, Bull PC et al: Specific receptor usage in Plasmodium falciparum cytoadherence is associated with disease outcome. PloS one 2011, 6(3):e14741.
17. Wassmer SC, Lepolard C, Traore B, Pouvelle B, Gysin J, Grau GE: Platelets reorient Plasmodium falciparum-infected erythrocyte cytoadhesion to activated endothelial cells. The Journal of infectious diseases 2004, 189(2):180-189.

18. Mustaffa KMF, Storm J, Whittaker M, Szestak T, Craig AG: In vitro inhibition and reversal of Plasmodium falciparum cytoadherence to endothelium by monoclonal antibodies to ICAM-1 and CD36. Malaria journal 2017, 16(1):279.
19. Turner L, Lavstsen T, Berger SS, Wang CW, Petersen JE, Avril M, Brazier AJ, Freeth J, Jespersen JS, Nielsen MA et al: Severe malaria is associated with parasite binding to endothelial protein C receptor. Nature 2013, 498(7455):502-505.
20. Pendurthi UR, Rao LVM: Endothelial cell protein C receptor-dependent signaling. Current opinion in hematology 2018, 25(3):219-226.
21. Bernabeu M, Smith JD: EPCR and Malaria Severity: The Center of a Perfect Storm. Trends in parasitology 2017, 33(4):295-308.
22. Lennartz F, Adams Y, Bengtsson A, Olsen RW, Turner L, Ndam NT, Ecklu-Mensah G, Moussiliou A, Ofori MF, Gamain B et al: Structure-Guided Identification of a Family of Dual Receptor-Binding PfEMP1 that Is Associated with Cerebral Malaria. Cell host & microbe 2017, 21(3):403-414.
23. Mosnier LO, Lavstsen T: The role of EPCR in the pathogenesis of severe malaria. Thrombosis research 2016, 141 Suppl 2:S46-49.
24. Moussiliou A, Alao MJ, Denoeud-Ndam L, Tahar R, Ezimegnon S, Sagbo G, Amoussou A, Luty AJ, Deloron P, Tuikue Ndam N: High plasma levels of soluble endothelial protein C receptor are associated with increased mortality among children with cerebral malaria in Benin. The Journal of infectious diseases 2015, 211(9):1484-1488.
25. Schuldt K, Ehmen C, Evans J, May J, Ansong D, Sievertsen J, Muntau B, Ruge G, Agbenyega T, Horstmann RD: Endothelial protein C receptor gene variants not associated with severe malaria in ghanaian children. PloS one 2014, 9(12):e115770.
26. Shabani E, Opoka RO, Bangirana P, Park GS, Vercellotti GM, Guan W, Hodges JS, Lavstsen T, John CC: The endothelial protein C receptor rs867186-GG genotype is associated with increased soluble EPCR and could mediate protection against severe malaria. Scientific reports 2016, 6:27084.
27. Fukudome K, Esmon CT: Identification, cloning, and regulation of a novel endothelial cell protein C/activated protein C receptor. The Journal of biological chemistry 1994, 269(42):26486-26491.
28. Mohan Rao LV, Esmon CT, Pendurthi UR: Endothelial cell protein C receptor: a multiliganded and multifunctional receptor. Blood 2014, 124(10):1553-1562.
29. Deane R, LaRue B, Sagare AP, Castellino FJ, Zhong Z, Zlokovic BV: Endothelial protein C receptor-assisted transport of activated protein C across the mouse blood-brain barrier. J Cereb Blood Flow Metab 2009, 29(1):25-33.
30. Howell DP, Levin EA, Springer AL, Kraemer SM, Phippard DJ, Schief WR, Smith JD: Mapping a common interaction site used by Plasmodium falciparum Duffy binding-like domains to bind diverse host receptors. Mol Microbiol 2008, 67(1):78-87.
31. Bengtsson A, Joergensen L, Rask TS, Olsen RW, Andersen MA, Turner L, Theander TG, Hviid L, Higgins MK, Craig A et al: A novel domain cassette identifies Plasmodium falciparum PfEMP1 proteins binding ICAM-1 and is a target of cross-reactive, adhesion-inhibitory antibodies. J Immunol 2013, 190(1):240-249.

32. Joergensen L, Bengtsson DC, Bengtsson A, Ronander E, Berger SS, Turner L, Dalgaard MB, Cham GK, Victor ME, Lavstsen T et al: Surface co-expression of two different PfEMP1 antigens on single plasmodium falciparum-infected erythrocytes facilitates binding to ICAM1 and PECAM1. PLoS Pathog 2010, 6(9):e1001083.
33. Bernabeu M, Danziger SA, Avril M, Vaz M, Babar PH, Brazier AJ, Herricks T, Maki JN, Pereira L, Mascarenhas A et al: Severe adult malaria is associated with specific PfEMP1 adhesion types and high parasite biomass. Proc Natl Acad Sci U S A 2016, 113(23):E3270-3279.
34. Jespersen JS, Wang CW, Mkumbaye SI, Minja DT, Petersen B, Turner L, Petersen JE, Lusingu JP, Theander TG, Lavstsen T: Plasmodium falciparum var genes expressed in children with severe malaria encode CIDRalpha1 domains. EMBO Mol Med 2016, 8(8):839-850.
35. Cham GK, Turner L, Lusingu J, Vestergaard L, Mmbando BP, Kurtis JD, Jensen AT, Salanti A, Lavstsen T, Theander TG: Sequential, ordered acquisition of antibodies to Plasmodium falciparum erythrocyte membrane protein 1 domains. J Immunol 2009, 183(5):3356-3363.
36. Turner L, Lavstsen T, Mmbando BP, Wang CW, Magistrado PA, Vestergaard LS, Ishengoma DS, Minja DT, Lusingu JP, Theander TG: IgG antibodies to endothelial protein C receptor-binding cysteine-rich interdomain region domains of Plasmodium falciparum erythrocyte membrane protein 1 are acquired early in life in individuals exposed to malaria. Infect Immun 2015, 83(8):3096-3103.
37. Rowe JA, Claessens A, Corrigan RA, Arman M: Adhesion of Plasmodium falciparum-infected erythrocytes to human cells: molecular mechanisms and therapeutic implications. Expert Rev Mol Med 2009, 11:e16.
38. Angchaisuksiri P: Coagulopathy in malaria. Thrombosis research 2014, 133(1):5-9.
39. Medina P, Navarro S, Estelles A, Vaya A, Woodhams B, Mira Y, Villa P, Migaud-Fressart M, Ferrando F, Aznar J et al: Contribution of polymorphisms in the endothelial protein C receptor gene to soluble endothelial protein C receptor and circulating activated protein C levels, and thrombotic risk. Thromb Haemost 2004, 91(5):905-911.
40. Moxon CA, Wassmer SC, Milner DA, Jr., Chisala NV, Taylor TE, Seydel KB, Molyneux ME, Faragher B, Esmon CT, Downey C et al: Loss of endothelial protein C receptors links coagulation and inflammation to parasite sequestration in cerebral malaria in African children. Blood 2013, 122(5):842-851.
41. Yi JH, Wang D, Li ZY, Hu J, Niu XF, Liu XL: C-reactive protein as a prognostic factor for human osteosarcoma: a meta-analysis and literature review. PloS one 2014, 9(5):e94632.

Tables

Table 1. Basic characteristics of the included studies

Study	Year	Population	SM No. of cases			MM No. of cases			HC No. of cases			SNP	Conclusion Reported
			GG	GA	AA	GG	GA	AA	GG	GA	AA		
Naka	2014	Thailand	5	94	242	16	88	262				rs867186	Protective
Schuldt	2014	Ghana	108		1797				110		1756	rs867186	No association
Hansson	2015	Tanzania	8	68					7	60		rs867186	No association
Moussiliou	2015	Benin		19	103		9	41				rs867186	High mortality
Shabani	2016	Uganda	3	102	446	0	14	57	7	31	134	rs867186	Protective

Table 2. Definition criteria for severe malaria

Study	Severe malaria				
Naka 2014	High *Pf*	SMA	hypoglycemia	High serum creatinine	CM
Schuldt 2014	High *Pf*	SMA			CM
Hansson 2015	High *Pf*	SMA			CM
Moussiliou 2015	High *Pf*	SMA			CM
Shabani 2016		SMA			CM

High Pf: >100,000 parasites/µl
SMA: severe anemia: hematocrit (HCT)<20%, hemoglobin (Hb) <7g/dl
CM: cerebral malaria
High serum creatinine: serum creatinine >3mg/dl
Hypoglycaemia: glucose <22 nmol/l

Table 3: rs867186-G variant is associated with higher sEPCR levels in severe malaria

Study	Stages	Genotype (n=number)	Plasma levels of sEPCR		
			Median (ng/ml)	Interquartile range (IQR)	P value
Moussiliou 2015	#CM+###SM Convalescence	AA (n=103) AG (n=19)	158 297	131-211 174-383	 P=0.03
Shabani 2016	CM+ ##SMA	AA(n=390) AG (n=91) GG(n=3)	84.5 131 194	65.7-104 107-170 104-211	 *P<0.001 **P=0.007 ***P=0.71
	§UM	AA (n=25) AG (n=13)	86.5 161	75.4-113 142-164	 P<0.001
	§§Healthy CC	AA (n=79) AG (n=25) GG (n=6)	98.4 241 350	87.8-121 203-288 319-380	 P<0.006

*AG compared with AA, ** GG compared with AA, *** GG compared with AG

#CM (cerebral malaria), ##SMA (severe malaria anemia), ###SM: severe malaria: >250,000 parasites/µl, or SMA

- UM: uncomplicated malaria, §§Healthy CC: healthy community controls

Figures

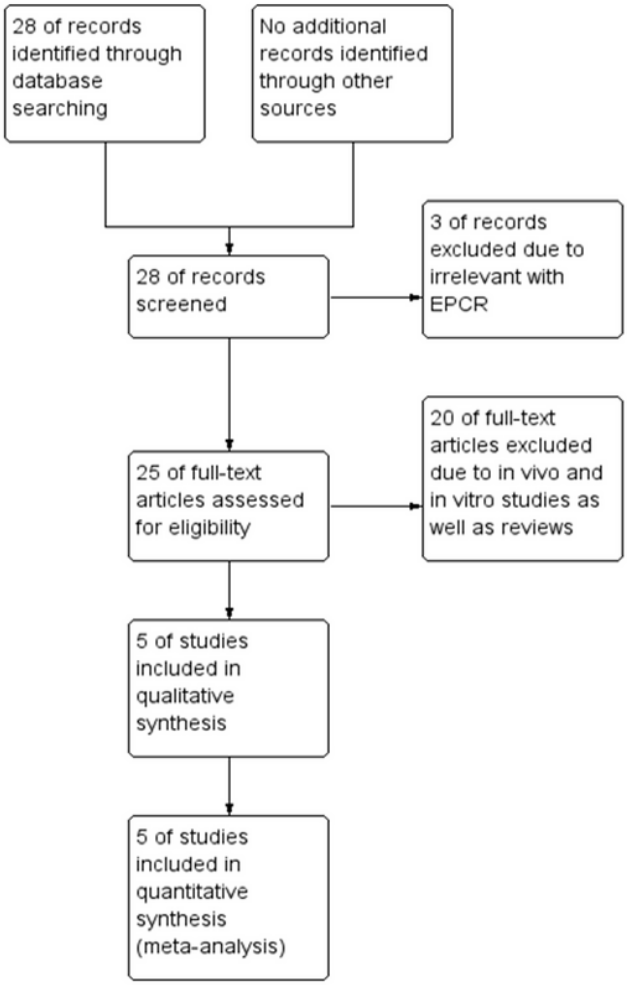

Fig.1

Figure 1

Flow chart of the study. Among twenty-eight publications identified by the search method, twenty-three of those were excluded due to exclusion criteria of laboratory studies, non-original articles, lack of matched controls, or studies irrelevant to the current analysis. Five studies were included in this meta-analysis.

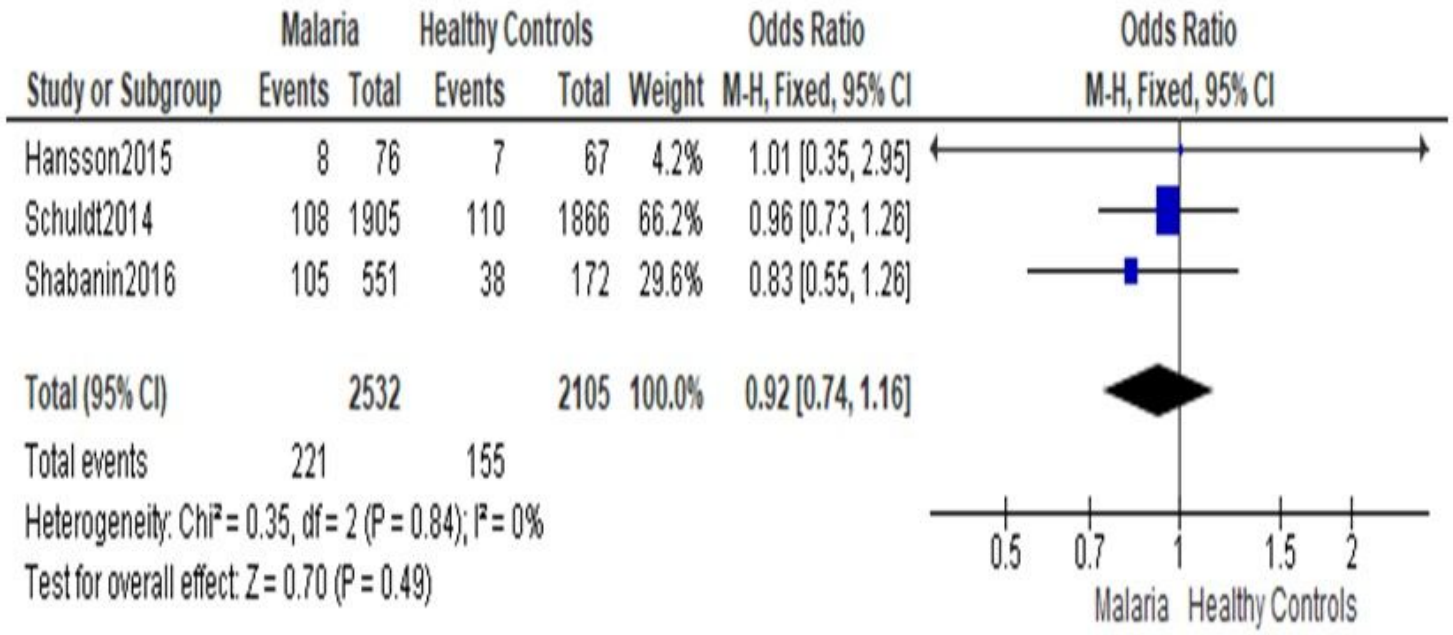

Figure 2

Forest plot for the risk of malaria and EPCR polymorphism in the genotype of rs867186 GG vs. AA. The frequency of rs867186-GG versus rs867186-AA in malaria patients and healthy individuals were compared. The prevalence of rs867186-GG does not increase in healthy controls than in SM patients. The pooled OR from 3 studies, including 2532 patients with malaria and 2105 healthy individuals, is shown (OR=0.92 95% CI=0.74-1.16, P=0.49).

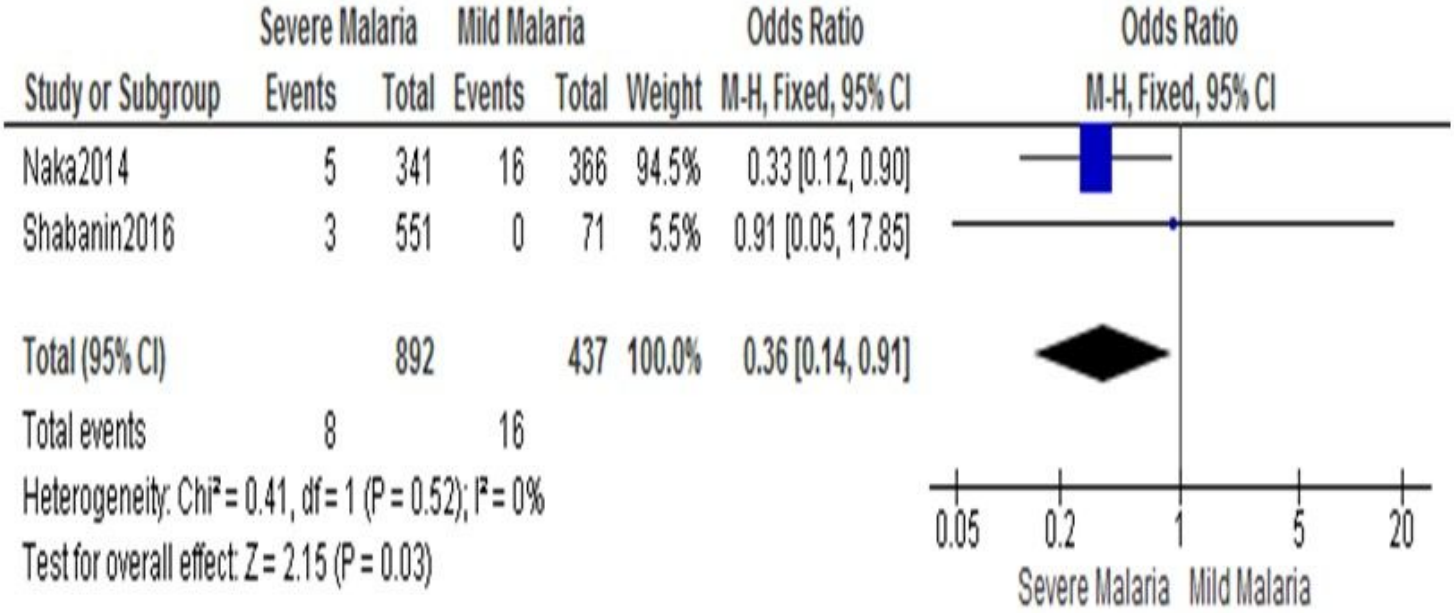

Figure 3

Forest plot for the protection of SM and EPCR polymorphism in the genotype of rs867186 GG vs. AG and AA. The frequency of rs867186-GG versus rs867186-AA and AG was analyzed in SM and

uncomplicated/mild malaria patients. The frequency of rs867186-GG is higher in mild malaria than in SM patients. The pooled OR from 2 studies, including 892 patients with SM and 437 mild malaria, is shown (OR=0.36, 95% CI=0.14-0.91, P=0.03).

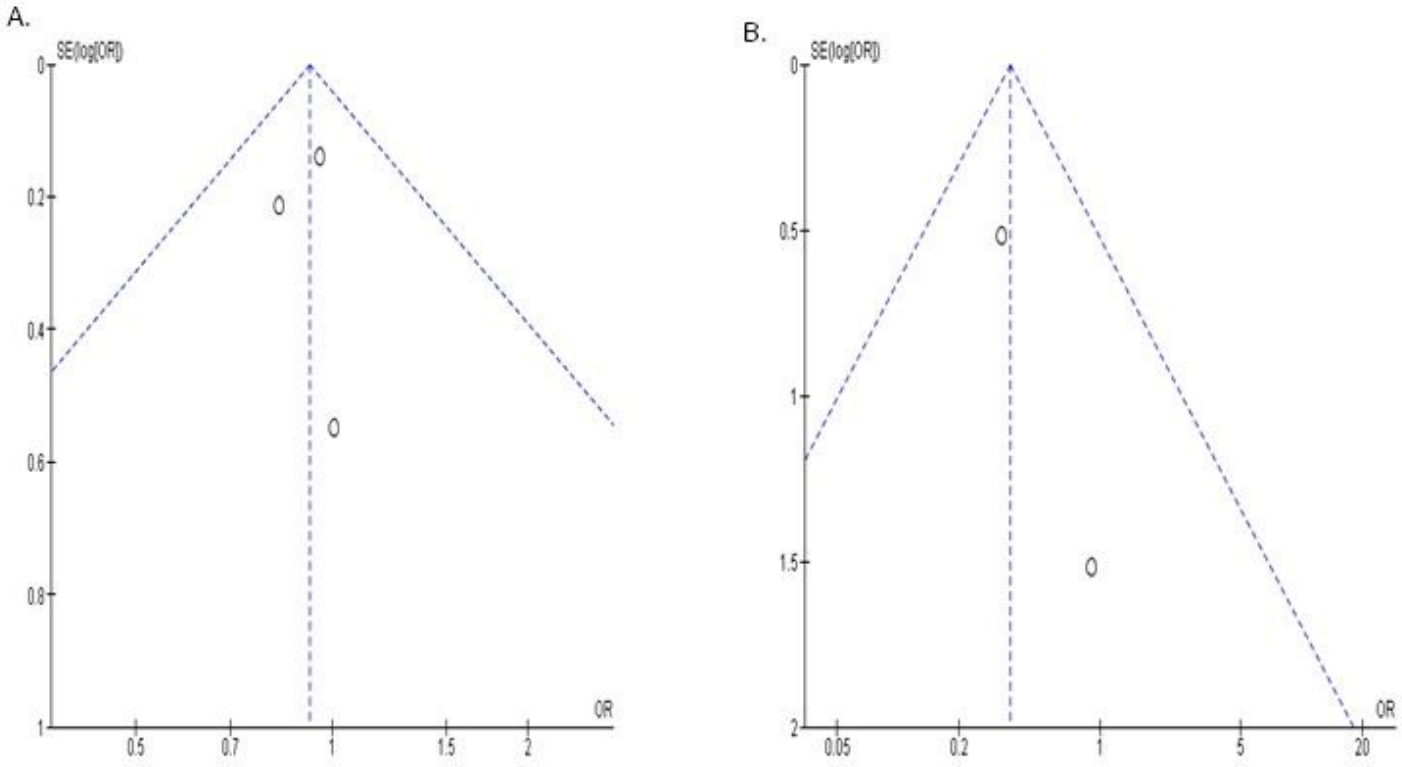

Figure 4

Funnel plot of publication bias in the meta-analysis of rs867186 genotype. The funnel plots are largely symmetrical, suggesting there are no publication biases in the meta-analysis of the role of rs867186-GG genotype in SM.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- PRISMAChecklist.pdf