# Molecular mechanisms of PfEMP1-EPCR Adhesion in severe *P. falciparum* infection

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**Abstract**

A major global health burden is still posed by *P. falciparum* malaria, which causes significant morbidity and mortality in cases of severe illness. Recent findings pertaining to the expression of P. falciparum erythrocyte membrane protein 1 (PfEMP1) on infected erythrocytes and their close association with the onset of severe malaria syndromes, along with the fact that these PfEMP1 variants possess EPCR binding domains, offer novel avenues for enhancing our comprehension of the molecular mechanisms behind the pathogenesis of severe malaria. EPCR is well-known for its critical function in the PC system and for its capacity to bolster the cytoprotective effects of activated protein C (APC), which in turn provide vascular and tissue protective effects in a variety of organ systems, including the liver, kidney, brain, and lung. The novel concept that EPCR is fundamental to the pathophysiology of severe malaria is supported by observations that binding of PfEMP1 to EPCR causes an acquired functional deficit of the PC system. Therefore, focusing on the PfEMP1-EPCR connection and regaining the PC system's functionality may offer fresh approaches to the creation of cutting-edge adjuvant treatments for malaria. In conclusion, The PROCR gene, which has 20 SNPs in its coding region, was investigated. Using a molecular dynamics technique and other bioinformatics tools. Out of those 20 SNPs, 1 SNP is highly deleterious and 1 SNP is benign.

**Key Words: SNPs, EPCR, *P. falciparum***

## Introduction

Malaria remains one of the most common causes of death and morbidity in the world [1]. One of the largest obstacles to socioeconomic growth is widespread, especially in developing countries [2, 3]. The P. falciparum erythrocyte membrane protein 1 (PfEMP1) family of proteins is the primary parasite ligand expressed on the surface of infected erythrocytes. Var genes, which are expressed in a mutually exclusive manner, encode the PfEMP1 family [4]. The var gene family is divided into three main subgroups, A, B, and C. Certain genes in groups B and C var encode PfEMP1 variants that bind CD36, whereas other genes in groups B and C var encode PfEMP1 variants that bind CD36 [5, 6]. The adhesion of P. falciparum-infected erythrocytes to brain endothelial cells is mediated by PfEMP1 subtypes carrying domain cassettes (DCs) 8 (group B/A hybrid) and 13 (group A), as was recently shown [7, 8]. A clonal parasite line that expresses DC8 can also adhere to primary microvascular cells in the heart, lungs, and dermis [7]. Moreover, it has been noted that high transcription levels of the DC8 and DC13 genes are significantly associated with severe childhood malaria [9]. These results suggest that the cyto-adhesion of P. falciparum-infected erythrocytes to different endothelial cells depends on PfEMP1 variants DC8 and DC13. More recently, it has been demonstrated that the endothelial protein C receptor, or EPCR, can function as an endothelium receptor for DC8 and DC13 PfEMP1 variants [10]. It's interesting to note that, when compared to children with moderate and uncomplicated malaria, parasite isolates from patients with severe malaria show much higher binding to human brain microvascular endothelial cells by EPCR [10]. The endothelial protein C receptor gene (PROCR) (OMIM 600646) encodes the 46-kDa type 1 transmembrane glycoprotein known as EPCR (RefID), which is produced on endothelial cells and homologous to major histocompatibility complex class I/CD1 family proteins [11, 12]. EPCR attached to endothelial cells promotes the synthesis of protein C, which serves as a protein C receptor. Soluble EPCR (sEPCR), which is created via metalloprotease-mediated EPCR membrane shedding, is another form of EPCR that can be found in human plasma [13]. By competing with endothelial cell-bound EPCR for protein C, sEPCR stops protein C from being activated, unlike endothelial cell-bound EPCR. Remarkably, variations in plasma sEPCR levels are mostly determined by the genetic composition of rs150846093 (Ser88pro), a non-synonymous PROCR gene single-nucleotide polymorphism (SNP) [14–21]. The rs150846093-G allele has a strong correlation with elevated sEPCR levels. Codon 88 encodes glycine instead of serine in the transmembrane

domain of the EPCR. The allele rs150846093-G is the cause of this. Therefore, rs150846093 may result in conformational changes in EPCR that is attached to endothelial cells.

## Materials and Procedures

### SNP data acquisition

The National Center for Biotechnology Information (NCBI) SNP database (http://www.ncbi.nlm.nih.gov/snp) was searched using a variety of parameters to identify Homo sapiens non-synonymous, stop-gained, synonymous, mRNA UTR (5' and 3'), and intronic areas [22].

### SIFT

A sequence homology-based technique called SIFT (Sorting Intolerant From Tolerant) (http://sift.jcvi.org/www/SIFT_BLink_submit.html) is used to categorize variations in amino acids. We sent a question with the gene identification number and the amino acid changes obtained from the NCBI to use the improved SIFT version, called SIFT-Blink. The SIFT predictions are displayed in a normalized probability score chart for each of the 20 amino acids. SIFT values below 0.05 are predicted to be detrimental [23, 24].

### POLYPHEN 2

Polymorphism Phenotyping v2, a tool, can be accessed at http://genetics.bwh.harvard.edu/pph2/.predicts how amino acid replacement would affect the structure and function of proteins using structural homology. Using sequence, structural, and phylogenetic characteristics, PolyPhen-2 gathers data to define an amino acid substitution. [25, 26]. We used a protein FASTA sequence with two variants of each amino acid at each mutational location to submit the query. For information on amino acid contact, numerous alignments of homologous sequences, and three-dimensional protein structures, PolyPhen examines a variety of protein structure databases. A position-specific independent counts (PSIC) score and its difference are generated for each of the two variations. A PSIC score difference of 1.5 or greater is considered damage. One of four categories—potentially harmful (1.25–1.49), maybe damaging (1.50–1.99), probably damaging (2.00), or benign (0.00–0.99)—is formed from the PolyPhen values.

**I-MUTANT 2.0**

I-Mutant2.0 (http://folding.uib.es/cgi-bin/i-mutant2.0.cgi) is an automatic prediction tool that uses support vector machines (SVMs) to anticipate changes in protein stability brought on by single-point mutations. Protein structure and protein sequence are the two components of I-MUTANT 2.0, a predictor of changes in protein stability following a single point mutation at the site. The free energy change (ΔΔG), which is determined by deducting the mutant protein's free energy change (Kcal/mol) from the original protein's free energy change (Kcal/mol), is predicted by I-Mutant [27]. The free energy change (ΔΔG) calculation, amino acid and position substitutions, and the FASTA sequence of the query protein were given. The mutant protein is said to be extremely stable if its DDG value is zero, and less stable if it has a larger negative value.

**Results**

**Data mining**

We have chosen SNPs from the human EPCR gene based on several criteria, such as stop-gain, intronic areas, mRNA untranslated regions UTR (5' and 3'), non-synonymous (nsSNPs) coding area, and coding synonymous (sSNPs) regions. The stop-gain region contains eight SNPs (0.55%), the intronic area contains sixteen SNPs (93.14%), the mRNA UTR region contains eighty-two SNPs (6.59%), the 5' UTR contains twenty-five SNPs, and the 3' UTR contains fifty-seven SNPs. The coding area of the 860 SNPs consists of 15 sSNPs (0.86%) and sixty-two SNPs (nsSNPs; 1.96%). Since the vast majority of SNPs were found to be located in the intronic region, we decided to focus our investigation on the non-synonymous region coding (20 nsSNPs) and the regulatory domain of the EPCR gene. The functional significance of these sorted nsSNPs was subsequently evaluated using a range of bioinformatics methods (Fig. 1).

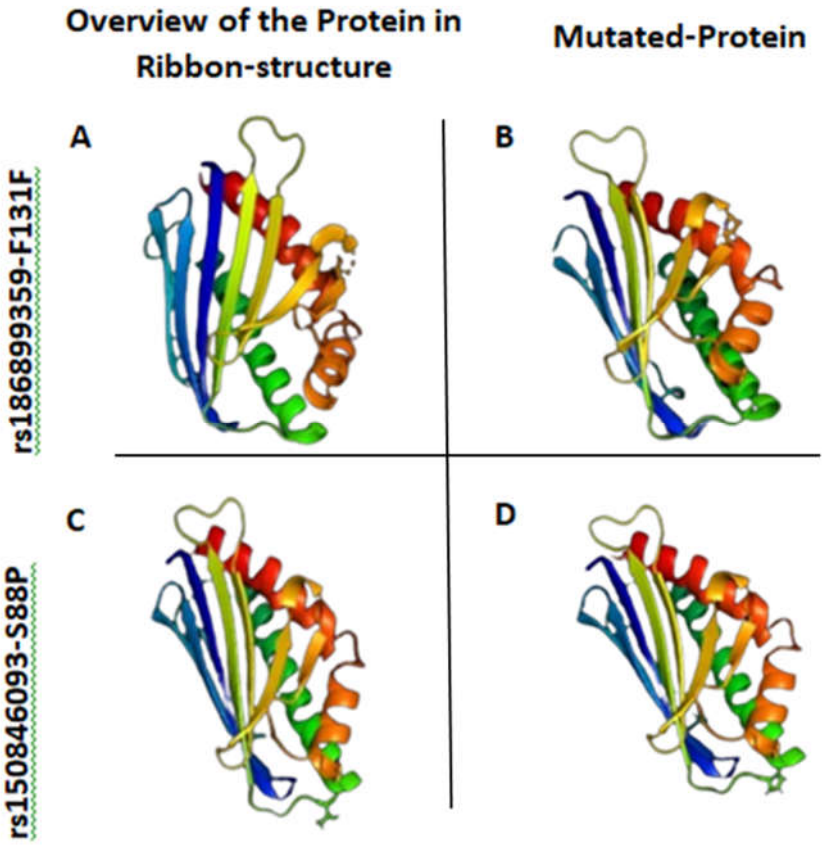


Fig 1. **Wild type and mutated EPCR structure**

**Utilizing SIFT Blink, analysis of harmful nsSNPs in the EPCR gene**

SIFT makes predictions about whether the substitution of an amino acid will modify the protein's functionality based on sequence homology and the physical characteristics of amino acids. The protein sequence's mutational locations and amino acid residues were supplied as input to the SIFT Blink service. A score of ≤0.05 indicates that the nsSNP is more harmful or destructive to protein function, whereas a score of >0.05 predicts the tolerated range. Six nsSNPs (25.46%) had scores between 0.01 and 0.05, five nsSNPs (22.08%) had scores between 0.06-0.10, and nine of the twenty nsSNPs (52.42%) had 0.00 values, indicating that they were predicted to be intolerant.

**Using PolyPhen-2 to analyze harmful nsSNPs in the EPCR gene**

Phylogenetic characteristics, the number of sequences, and structural elements that define the amino acid substitution are the basis for the PolyPhen prediction. Polyphen 2 was given the same protein sequence for SIFT (20 nsSNPs total), complete with mutational sites and amino acid alterations. An influence on amino acid substitution is zero, whereas a negative impact

on protein function is indicated by a larger positive score. The range of PolyPhen scores is 0 to 1. Two nsSNPs (8.9%) were determined to be possibly detrimental, with score values ranging from 0.6 to 0.8; four nsSNPs (28.2%) were determined to be benign (neutral), with score values of zero; and thirteen nsSNPs (62.9%) were predicted to be probably damaging. In order to quantitatively describe the negative impacts, the PolyPhen score is helpful.

**I-MUTANT 2.0 is used to analyze harmful nsSNPs in the EPCR gene**

I-Mutant 2.0 was sent the query protein sequence, mutational sites, and amino acid substitutions, which were then sent to SIFT, Blink, and PolyPhen 2.0. I Mutant 2.0 forecasts single-point mutations that change a protein's stability. The free energy changes (DDG) value has a greater negative correlation with I-Mutant 2.0. A decrease in the protein's stability will occur. The two nsSNP variants that were shown to be the least stable and most harmful were S88P and F131G, based on these ratings. Each of them had a DDG value of -0.72 and -2.48.

**Table 1. : SIFT Blink, PolyPhen 2.0, I-Mutant 2.0, and PANTHER combined prediction for potentially harmful nsSNPs of the PROCR gene**

| | **rs150846093** | **rs186899359** |
|---|---|---|
| **Location** | 20:35174893 | 20:35176238 |
| **Allele** | C | T |
| **Position** | 88 | 131 |
| **Chromosome** | 20 | 20 |
| **Position** | 35174893 | 35176238 |
| **Amino Acid** | S/P | F/F |
| **SIFT prediction** | Tolerated (0.5- 1.0) | Tolerated (0.5- 1.0) |
| **PolyPhen prediction** | 1.000 | 0.046 |
| **Clinical Significance** | Benign | Deleterious |

**Discussion**

In silico analysis of amino acid substitution using growing bioinformatics techniques has advanced significantly in the modern era [28]. The main function of PfEMP1 is to attach and attach red blood cells to the blood vessel wall. Comprising CIDRs and DBL domains, the head structure of PfEMP1 mediates the most important binding properties of P. falciparum that are currently known. Significant associations were found between severe malaria and the PROCRs rs150846093 and rs186899359. The negative rs186899359 is present in both of these rs IDs. Two possible explanations for the association between rs150846093-SP and protection against severe malaria are shown in Table 1. The serine-to-proline transition at codon 88 in the transmembrane of EPCR may result in a conformational alteration in the molecule that prevents DC8 and DC13 PfEMP1 from binding EPCR. Increased sEPCR levels are associated with rs150846093-G [14–21]. Additionally, recombinant sEPCR inhibits the interaction of DC8-expressing parasites with human brain microvascular endothelial cells [10]. Therefore, the alternative theory that might be true is that higher levels of sEPCR in malaria patients with the rs186899359-FG genotype more severely impede the binding of the DC8 and DC13 PfEMP1s to EPCR on endothelial cells because these proteins preferentially bind to sEPCR over EPCR. The main goal of the current study is to profile the detrimental nsSNPs of EPCR associated with severe malaria. The SNP data were initially taken out of the NCBI database to have the nsSNPs ready for further computational analysis utilizing different bioinformatics tools. Numerous techniques, like SIFT Blink, PolyPhen-2, and I-Mutant 2.0, are available to predict the functional phenotypes of nsSNPs utilizing data on protein structure, cross-species conservation of protein sequences, and physicochemical properties [29, 30, 31, 32, 33, 34]. Previous studies have shown that prediction accuracy is increased by choosing functional mutations using a range of techniques and algorithms [35, 36]. The SIFT method assesses the relevance of evolutionary conservation to the suggested amino acid and forecasts if a specific modification is detrimental or acceptable to the protein's stability. To calculate the score, the method makes use of sequence homology. I-MUTANT is a tool for forecasting the impact of single-site mutations on protein stability. It can forecast variations between wild-type and mutant PROCR's Gibbs free energy ($\Delta\Delta G$). Potential ligands for PROCR (EPCR) include protein C or PfEMP1 from malaria parasites, and binding energy in I-MUTANT probably correlates with the degree of this connection. Changes in binding energy predicted by I-MUTANT for PROCR mutations (e.g., serine to proline) seem to be associated with the severity of malaria outcomes. The connection between the parasite and

host cells may be affected by a mutation that changes the binding site for protein C or P. falciparum erythrocyte membrane protein 1 (PfEMP1). Protein structures have been observed to kink in response to proline. This could alter the structure of the EPCR, which could affect its functionality or interactions. EPCR is involved in cytoprotective and anti-inflammatory signaling pathways. A mutation in one of these signaling pathways could alter the host's response to malaria infection. Certain PROCR mutations have been connected to protective traits against severe malaria. If the serine to proline mutation lessens the adhesion of infected erythrocytes to the EPCR, it might protect against severe malaria outcomes.

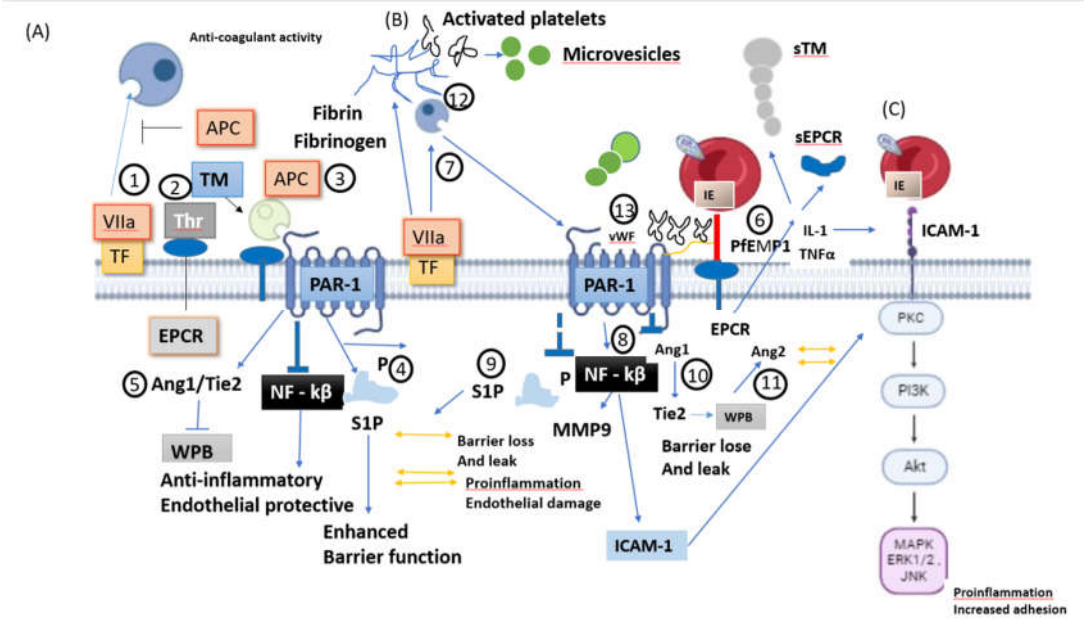


Fig 2: Interaction of EPCR- and ICAM-1-binding IEs in cerebral malaria. (A), EPCR effects in the absence of Plasmodium falciparum-IE. The combination of circulating activated factor VII (VIIa) and tissue factor (TF) results in the production of thrombin (Thr) (1). Thrombin starts the EPCR, and protein C (APC) is then activated by thrombomodulin (TM), which suppresses the generation of thrombin. 2. In order to cleave proteinase-activated receptor 1 (PAR-1), APC uses EPCR as a coreceptor. The EPCR-APC activation of PAR-1 has anti-inflammatory and anti-apoptotic effects while blocking the nuclear factor-κB pathway (3). As a consequence of S1P synthesis and S1P signaling, tight junctions are strengthened and the integrity of the endothelium barrier is preserved (4). Endothelial permeability is reduced Angioptoetin-1. The protein (Ang1) generated as a result of the APC-PAR-1 connection reduces the exocytosis of the Weibel-Palade body (WPB) by binding to TIP2. (5). (B), The effect of infected erythrocytes with surface-expressed EPCR-bound PfEMP1. When endothelial cells are activated by the IE-EPCR interaction, pro-inflammatory cytokines (IL-1, TNFα) are released, which causes EPCR and TM to shed off the endothelium surface and increases manifestation of ICAM-1 (6). With fibrin deposition, the EPCR-IE interaction leads to decreased APC levels and enhanced thrombin production. (7). Elevated thrombin levels cause the PAR-1 response to change, leading to the activation of RhoA and NFκB along with increased ICAM-1 surface expression on endothelial cells (8). The change in the PAR-1 response impairs endothelial barrier function by producing localized vascular leaks and suppresses S1P release, which results in the loss of tight junctions (9). Decreases in Ang-1 levels promote WPB exocytosis through Tie2 and creation of Ang2 and the von Willebrand Factor (vWF) 10). Raised Ang-2 levels also lead to a decrease in the integrity of the endothelium barrier and leakage, and they further enhance WPB exocytosis (11). Platelet microvesicles are produced when thrombin and cytokines activate platelets (12). Activated platelets and thrombi are created when they mix. (13). Similar to thrombi, complexes made of strings of vWF and activated platelets impede brain circulation. (C), The rise in ICAM-1 (in panel B) facilitates the adhesion of IE expressing PfEMP1 to the brain endothelium through a common DBLβ ICAM-1 motif. A significant fraction of ICAM-1-adhering IEs may first bind EPCR via their domains CIDRα1.

## Conclusion

In conclusion, research was done on the PROCR gene, which contains 20 SNPs in its coding region. A molecular dynamics approach and additional bioinformatics tools were used to profile the highly harmful SNP rs186899359. A researcher may not be able to conduct wet laboratory tests on each and every SNP to ascertain their biological impact on the structural and functional stability of the protein produced due to the abundance of SNP data available for the PROCR gene. As shown in fig. 2, the processes required to organize experiments to ascertain how each nsSNP impacts protein function will be difficult, expensive, and time-consuming. Thus, this analysis establishes the foundation for an alternative, faster, and less costly approach to rating amino acid alterations and locating putative PROCR gene SNP candidates for further wet lab testing.

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## Conflicts of interest

Conflicts of interest are not disclosed by the writers.

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