A subset of group A-like var genes encodes the malaria parasite ligands for binding to human brain endothelial cells

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AUTHOR SUMMARY

Cerebral malaria is the most deadly outcome of infection with the protozoan parasite Plasmodium falciparum (1). The pathology of cerebral malaria is characterized by the accumulation of parasitized red blood cells in small blood vessels in the brain. This accumulation occurs because of the binding of parasite adhesion molecules (ligands) on the surface of infected red blood cells to human receptors on brain microvascular endothelial cells; however, the parasite and host molecules involved in this interaction are unknown. We investigated the parasite ligands used for adhesion to human brain endothelial cells to gain fundamental insights into the host–parasite interactions that lead to cerebral malaria. We identified the parasite adhesion ligands as a restricted subset of variant surface antigen molecules encoded by genes called “Group A var genes.” These findings represent an advance in our understanding of cerebral malaria and identify potential targets for therapies and disease prevention.

To investigate host–parasite interactions involved in cerebral malaria, we used Plasmodium falciparum–culture-adapted parasite strains and a human brain endothelial cell line, HBEC-5i (2). Initial parasite populations bound poorly to HBEC-5i, showing that these parasites did not show the adhesion phenotype involved in cerebral malaria. However, repeated rounds of selection for HBEC-5i–binding cells yielded highly adherent parasite populations showing “cerebral malaria-type” adhesion properties. We then were able to analyze gene transcription in the highly adherent (selected, cerebral malaria-type) parasites compared with the nonadherent (unselected, “non–cerebral malaria-type”) parasites. We looked for genes that showed markedly increased transcription in the cerebral malaria-type parasites obtained after selection to identify candidate adhesion ligands. Only one or two genes out of the whole genome showed a striking increase in transcription after selection for cerebral malaria-type adhesion properties, allowing straightforward identification of the parasite adhesion ligands.

We successfully selected three P. falciparum strains—HB3, 3D7, and IT/FCR3—for HBEC-5i binding and analyzed global gene expression throughout the 48-h blood-stage life cycle of selected and unselected pairs from each strain using microarray technology (Fig. P1) (3). The only genes that were up-regulated markedly and consistently after selection for HBEC-5i binding were one or two var genes encoding specific members of the Group A-like var genes.

Fig. P1. Group A-like PfEMP1 variants mediate adhesion of infected red blood cells to HBEC-5i. P. falciparum-infected red blood cells (iRBC) were selected for binding to a layer of HBEC-5i, mimicking the sequestration that occurs in cerebral malaria cases. The entire transcriptomes of both unselected (nonbinding) and selected (binding) populations of parasites were analyzed by microarray. In the three P. falciparum strains tested, HB3, 3D7, and IT/FCR3, Group A-like var genes were the most up-regulated genes in the selected populations. The up-regulated genes encode a subset of Group A PfEMP1 variants containing the specific domains DCB and/or DC13. Antibodies raised against the up-regulated PfEMP1 variants blocked the adhesion of iRBC to HBEC-5i, indicating that the DCB and DC13 type of Group A PfEMP1 variants are the parasite ligands mediating adhesion to human brain endothelial cells. Pf VSA, P. falciparum variant surface antigen.


The authors declare no conflict of interest.

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Data deposition: The microarray data reported in this paper have been deposited in the Gene Expression Omnibus (GEO) database, www.ncbi.nlm.nih.gov/geo (accession no. GSE32211).

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*P. falciparum* erythrocyte membrane protein one (PfEMP1) family. PfEMP1 is a parasite-derived variant antigen expressed on the surface of infected red blood cells that plays a major role in malaria pathology and immune evasion. The genome of every *P. falciparum* isolate contains about 60 var genes that encode highly polymorphic PfEMP1 variants. The var gene repertoires of different *P. falciparum* isolates are largely nonoverlapping, resulting in huge diversity in the PfEMP1 family in natural *P. falciparum* populations. Only one var gene is expressed at a time in an infected red cell, and switching of expression to a different var gene results in antigenic variation of malaria parasites. The up-regulated var genes in the HBEC-5i-selected parasites fall into a subset called “Group A,” characterized by a conserved upstream sequence and location in the subtelomeric regions of the chromosomes. The up-regulated Group A-like PfEMP1 variants from the three different HBEC-5i-selected *P. falciparum* strains were variable in amino acid sequence, as expected for PfEMP1, but showed strong similarities in domain composition. PfEMP1 variants are composed of cysteine-rich extracellular domains of various types, and sets of domains that commonly occur together are called “domain cassettes” (DC). The up-regulated PfEMP1 variants in HBEC-5i-selected parasites contained either DC8 or DC13. We raised antibodies against the up-regulated PfEMP1 variants that were able to block adhesion of infected erythrocytes to HBEC-5i, confirming the adhesive function of the DC8 and DC13 PfEMP1 variants.

At present, there are no suitable animal models for human cerebral malaria; therefore, the in vivo significance of in vitro findings is difficult to test. We investigated the clinical significance of our data by examining antibody recognition of the selected and unselected parasite lines by plasma samples from African children recovering from cerebral malaria. This investigation revealed enhanced recognition of the HBEC-5i-selected parasites compared with unselected parasites, highlighting the significance of these strains and associated expressed proteins in cerebral malaria.

In summary, we have shown that the *P. falciparum* ligands for adhesion to HBEC-5i are a subset of Group A-like PfEMP1 variants characterized by DC8 or DC13. Furthermore, we validated the interaction between *P. falciparum*-infected red cells and HBEC-5i as an in vitro model for cerebral malaria, which will serve as a useful tool for testing adhesion-blocking drug and vaccine candidates. Furthermore, the strong support provided in our study for the involvement of PfEMP1 in cell adhesion interactions associated with life-threatening *P. falciparum* infection suggests that the identified variants could be used as possible targets for preventative therapies and treatments for cerebral malaria.