**Plasmodium falciparum** erythrocyte membrane protein 1 domain cassettes 8 and 13 are associated with severe malaria in children

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**AUTHOR SUMMARY**

During *Plasmodium falciparum* malaria infections, binding of infected erythrocytes to host endothelial receptors is crucial for parasite survival and clinical outcome. Members of the polymorphic protein family, *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), mediate this binding (1), and severe malaria syndromes are associated with expression of select subsets of PfEMP1 (2). These PfEMP1 subsets are also the first targets of antibody-mediated immunity, which has raised hopes for the development of PfEMP1-based vaccines. We used two methods to identify PfEMP1 sequences expressed in malaria patients and revealed that domain cassettes 8 and 13 were associated with severe malaria, highlighting their potential use as candidate antigens for PfEMP1 vaccine development.

Each *P. falciparum* genome harbors about 60 different PfEMP1-encoding *var* genes, each 3–10 kb long, which can be grouped into A, B, or C based on their upstream sequence (UPS). All PfEMP1 proteins are composed of different combinations of 3–10 Duffy binding-like (DBL) and cysteine-rich interdomain region domains. These domains can be divided into 147 subtypes, with intradomain type sequence similarities ranging from 38–98%. Some domain types exclusively appear in combination with each other, forming conserved “domain cassettes” found in most parasite genomes (3). Because of great sequence diversity, it has been difficult to identify and quantify *var* genes expressed in parasites from infected patients effectively. A number of approaches have been used to date, including *var* gene group-specific quantitative PCR (qPCR) (4) and semi-quantitative PCR based on the determination of the distribution of 350-bp-long DBLx transcript tags unique to each *var* gene (5). Based on these methods, the current consensus is that groups A and B are involved in severe childhood malaria, but more precise descriptions of the PfEMP1 domains and domain cassettes involved are needed to direct vaccine development to the most relevant PfEMP1/host interactions.

To identify PfEMP1 associated with severe malaria in children, 42 primer pairs were designed to target specific *var* loci corresponding to different PfEMP1 domains and domain cassettes (Fig. 1). Using qPCR, we quantified transcript levels of different *var* types in blood samples from 88 children hospitalized with severe malaria symptoms and 40 children admitted to the hospital with uncomplicated malaria. Our analyses unambiguously showed that domain cassette 8 type PfEMP1s are expressed more frequently and at higher levels in children with severe malaria compared with children with uncomplicated malaria. Among group A PfEMP1 types, it was also possible to associate the expression of domain cassette 13 with severe malaria; however, our findings do not exclude the possibility that other group A PfEMP1 types also play a role.

Near-full-length sequences of the predominant *var* transcripts were also obtained from three patients who had severe anemia...
and/or cerebral malaria. This was done by first sequencing DBLα sequence tags from cDNA to determine the predominant var transcripts. Next, near-full-length sequences of the most prevalent var transcripts were obtained by sequencing of PCR products generated by separate primers specific to each DBLα sequence and a primer targeting conserved loci found in the 3′ end of all var genes. These near-full-length var gene sequences were then annotated to study the domain composition of the encoded PfEMP1 proteins. This analysis confirmed results obtained from qPCR in that var genes encoding predicted domain cassettes, including domain cassettes 8 and 13, dominated the var transcript pool in these patients.

This sequence analysis also revealed that although PfEMP1 types with domain cassette 8 have a group B type UPS, the domains of the cassette carry sequence traits characteristic of group A PfEMP1. We have previously shown that antibodies to group A PfEMP1 domains are acquired first and early in life, supporting the theory that group A PfEMP1 types are associated with the first and most severe infections in life. To investigate the acquisition of antibodies to domain cassettes 8 and 13, we measured antibody levels against 99 different recombinant PfEMP1 domains from group A, B, and C PfEMP1 in 543 children from northern Tanzania aged 6 mo to 3 y. Our results indicated that antibodies against both domain cassette 8 and group A PfEMP1 domains are acquired earlier in life than antibodies to group B and C PfEMP1 domains.

In summary, using targeted qPCR and next-generation sequencing of *P. falciparum* PfEMP1-encoding var genes, we revealed important roles for PfEMP1 domain cassettes 8 and 13 in severe childhood malaria. In support of this notion, analysis of mounted antibody responses against PfEMP1 groups showed that antibodies to domain cassette 8 are acquired early in life by children living in malaria-endemic areas. These findings provide substantial insight into one of the world’s most important host/pathogen interactions and will aid in future efforts to prevent and treat severe malaria infections.