Title: Genetic validation of the function of PfEMP1 in *Plasmodium falciparum* rosette formation using CRISPR-Cas9 genome editing.

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Rosetting, the binding of *P. falciparum* infected erythrocytes to uninfected erythrocytes to form clusters (rosettes), is thought to contribute to severe malaria pathology. This adhesion phenotype is mediated through the adhesive properties of *P. falciparum* erythrocyte membrane protein 1 (PfEMP1), encoded by the *var* gene family (~60 distinct copies per parasite genome, expressed in a mutually exclusive manner). The N-terminal domain (NTS-DBLα) of PfEMP1 has been implicated as the functional erythrocyte binding domain. However, the role of PfEMP1 in adhesion in live infected erythrocytes has not been studied by reverse genetics, mainly due to the difficulty in genetically manipulating *P. falciparum*. Here, we use CRISPR-Cas9 genome editing to generate a population of parasites expressing a single *var* gene and to add epitope tags to PfEMP1. We also investigated whether the PfEMP1 variant "IT4var60" is sufficient to mediate the rosetting phenotype in *P. falciparum*. Our results reveal that a *var* gene co-expressed with a drug resistance gene via a 2A peptide can be inducibly and exclusively expressed under drug pressure. We have also shown that the IT4var60 PfEMP1 variant is sufficient to mediate rosetting in *P. falciparum*, as rosetting was completely abolished in IT4var60-knockout transgenic parasites. We further reveal that specific residues within the DBLα domain of IT4var60 PfEMP1 variant may be critical in *P. falciparum* rosette formation. Thus, our results suggest potential targets for the design and development of anti-rosetting interventions, and provide a general strategy for reverse genetic studies of PfEMP1.