Genetic validation of the function of PfEMP1 in *Plasmodium falciparum* rosette formation

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1. Background

- Rosetting is a *P. falciparum* adhesion phenotype where infected erythrocytes (IEs) binds to two or more uninfected erythrocytes. It has been linked to severe forms of malaria [1].

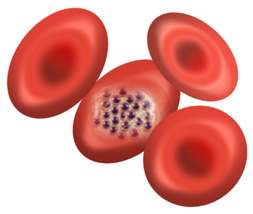

Fig 1: Rosetting adhesion phenotype

- *P. falciparum* erythrocyte membrane protein 1 (PfEMP1) mediates the binding of IEs to host receptors. They are encoded by ~60 distinct *var* genes, which are mutually expressed. Switches in expression of individual *var* gene accounts for antigenic variation.

- Rosette-specific PfEMP1 variants encoded by 'group A' *var* genes have been previously identified e.g. ITvar60.

- The DBLα domain of rosette specific variants is the functional erythrocyte-binding region. Key residues involved in RBC binding in ITvar60 have also been identified - Y73, K97 and K263 [2].

Fig 2: Structure of ITvar60 PfEMP1

- However, the role of PfEMP1 in adhesion has never been investigated using reverse genetic techniques.

2. Aim

- Test a strategy to generate homogenous parasite population which expresses a single var gene.
- Test whether specific residues (Y73, K97 and K263) within DBLα of IT4var60 are sufficient to mediate the rosetting phenotype, using CRISPR/Cas9.

3. Inducible expression of *var* genes

- The Rowe lab designed a strategy to generate parasites population expressing a single *var* gene, by co-expressing *var* gene with a drugR gene via a 2A peptide.

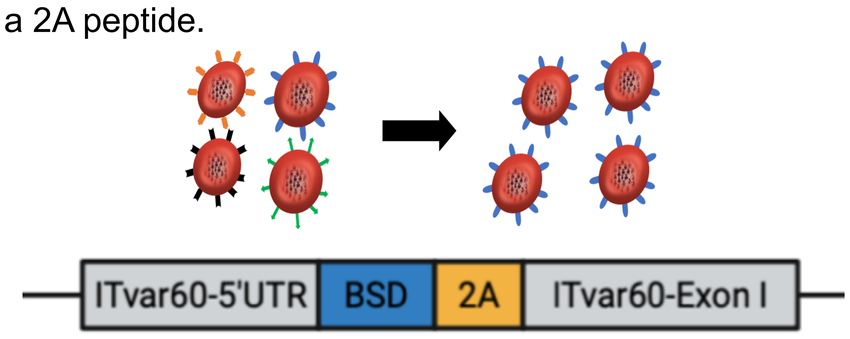

Fig 3: Strategy for exclusive transcriptional activation of *var60* and silence of other var genes

- When parasite is kept under drug pressure, the specific *var* gene is co-activated with drug R gene, while shutting off other *var* genes.

4. Expression of ITvar60 *var* gene

Var60 inducibly expressed under drug pressure (Blasticidin)

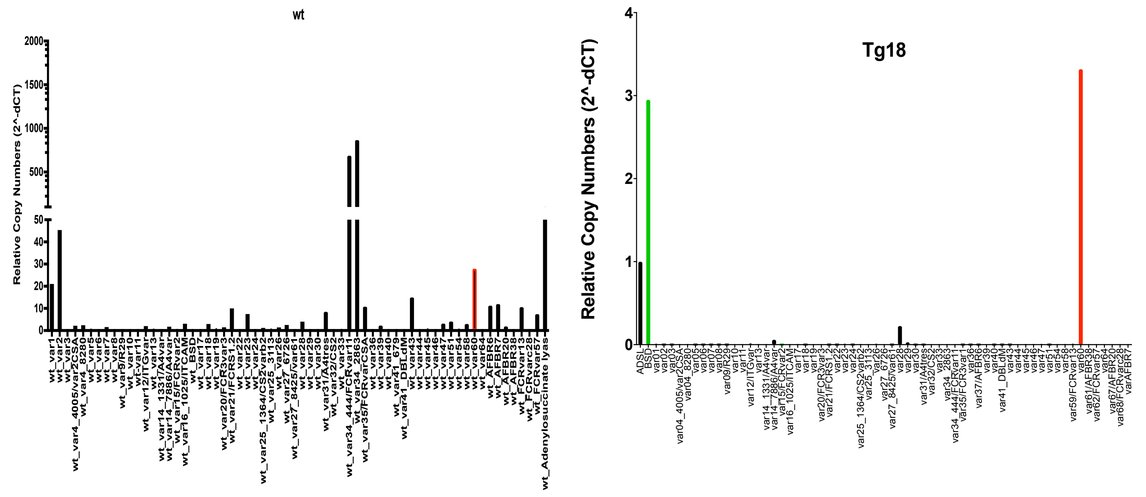

Fig 4: Quantification of transcription level of IT4 var genes in WT and Y73A mutant by qPCR

5. ITVar60 mutagenesis

- CRISPR/Cas9-mediated ITvar60 mutagenesis to generate Y73A transgenic mutant. Rosetting frequency (RF) of Y73A was 5%, compared to ~80% in WT.

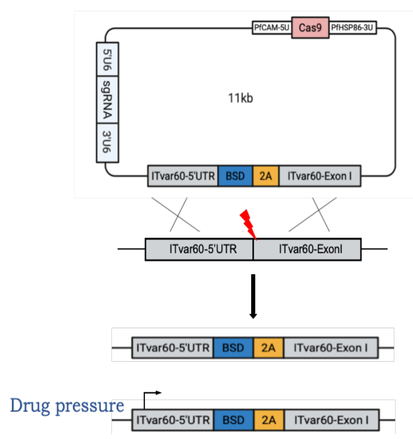

Fig 5: Strategy for generation of transgenic parasites

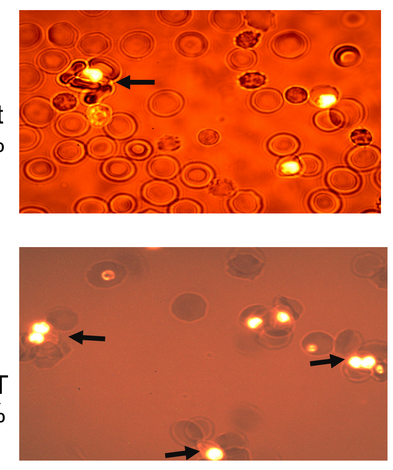

Fig 6: Rosette frequency of WT ITvar60 and Y73A mutant

6. Surface expression of PfEMP1

Y73A mutants express ITvar60 PfEMP1 on their surface

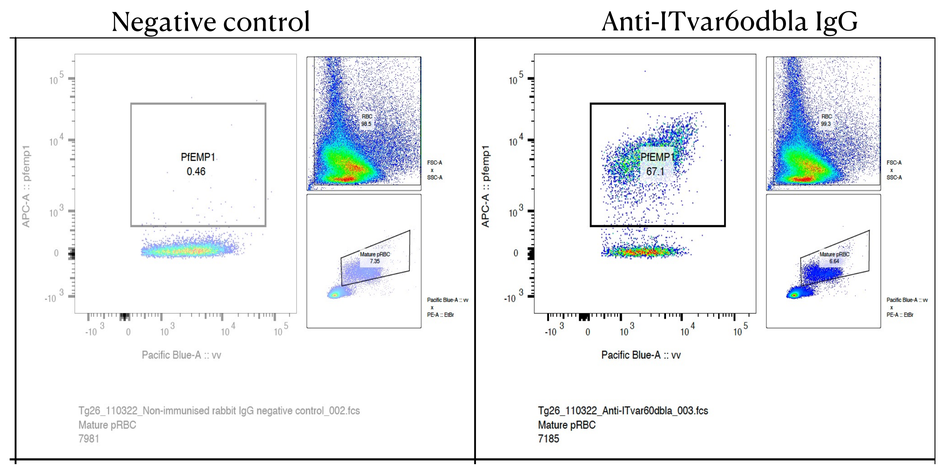

Fig 7: Flow cytometry analysis of Y73A DBLα domain

7. Summary

- A *var* gene co-expressed with a drug resistance gene (BSD) via 2A peptide can be inducibly and exclusively expressed under drug pressure.
- Successfully edited a var gene using CRISPR/Cas9.
- Y73 residue of ITvar60 DBLα is important for rosette formation: ~5% RF in Y73A mutants.

Reference

1. Rowe, A., Obeiro, J., Newbold, C. and Marsh, K. 1995. *Plasmodium falciparum* rosetting is associated with malaria severity in Kenya. *Infection and immunity*, *63*(6), pp.2323-2326.
2. Angeletti, D., Sandalova, T., Wahlgren, M. and Achour, A. 2015. Binding of Subdomains 1/2 of PfEMP1-DBL1α to Heparan Sulfate or Heparin Mediates *Plasmodium falciparum* Rosetting. *PLOS ONE*, 10(3), p.e0118898.

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