Investigating the conservation, function, and vaccine potential of PfEMP1 DBLepsilon and DBLzeta domains

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Abstract

Background: *Plasmodium falciparum* erythrocyte membrane protein 1 (PfEMP1) is a parasite-derived infected erythrocyte surface antigen that facilitates adhesion and immune evasion. The PfEMP1-encoding *var* gene family is unique to the subgenus *Laverania*, the closest relatives of *P. falciparum*. Modular in structure, PfEMP1 comprises of duffy-binding like (DBL) domains and cysteine-rich interdomain regions (CIDR). Of interest, PfEMP1 DBLε and DBLζ domains bind to serum proteins such as non-immune immunoglobulin M (IgM) and α_2-macroglobulin (α_2M). These interactions have been linked to both severe and pregnancy-associated malaria. We hypothesise that the serum binding PfEMP1 DBLε and DBLζ domains are potential targets for disease intervention and thus there is need to examine their conservation, function, and vaccine potential.

Methods: We have analysed the Pf3k Normalised varDB (714 single clones) and PlasmoDB databases to understand the patterns of PfEMP1 domain conservation across the global *P. falciparum* population, and the *Laverania*, respectively. We have also selected some African parasite isolates, recently culture-adapted for rosetting and IgM binding, and characterised their predominant PfEMP1 variants.

Results: With 319 hits (BLAST alignments) at ≥80% amino acid (aa) identity, the IgM and α_2M-binding TM284VAR1 DBLζ2 domain was conserved in approximately 45% of the Pf3k Normalised varDB *P. falciparum* isolates. This supersedes the conservation of the chondroitin sulphate A (CSA)-binding 3D7VAR2CSA DBLpam2 domain (210 hits at ≥80% aa identity, 29% of isolates) which is currently under vaccine development to protect against pregnancy-associated malaria. Other IgM-binding domains such as 3D7VAR2CSA DBLεpam5 (692 hits, 95% of isolates), IT4VAR1CSA DBLε5 (400 hits, 55% of isolates) and HB3VAR06 DBLζ2 (87 hits, 12% of isolates) also had good levels of conservation. Domains involved in other adhesion phenotypes (rosetting, CSA, endothelial protein C receptor (EPCR), intercellular cell

adhesion molecule 1 (ICAM1) or cluster of differentiation 36 (CD36)) did not rival this level of conservation. Moreover, the majority of the domains within the hypervariable PfEMP1 family are conserved in less than 5% of the parasites at a similar cut off. Analysis of other species of *Laverania* (PlasmoDB) revealed a homologue of an IgM-binding domain, IT4VAR60 DBLε12, in the gorilla parasite, *P. praefalciparum*, from which *P. falciparum* originated (PPRFG01_1151300, 95% aa identity), while a homologue of TM284VAR1 DBLζ2 was found in the chimpanzee parasite *P. reichenowi* (PRG01_0043100, 83% aa identity). There was also evidence of full-length conservation of IT4VAR60, TM284VAR1 and VAR2CSA within the *Laverania*. Taken together, these analyses show that the PfEMP1 serum-binding phenotype is well-conserved and has an ancient origin. It is plausible that structural and/or functional constraints have limited the recombination of the domains involved, thereby making them an interesting target for disease intervention. Profiling of two new IgM-binding *P. falciparum* lines has shown that the predicted architecture of the IgM-binding PfEMP1 variants includes a DBLε and/or DBLζ domain, which could potentially be involved in serum-binding.

Conclusions: Future work will characterise the function of DBLε and DBLζ domains by expressing recombinant proteins and examining their binding phenotype and their potential for generating strain-transcending antibodies. This study will provide insight into *P. falciparum* virulence mechanisms and the viability of the serum binding DBLε and DBLζ domains as potential vaccine candidates to protect against lethal malaria.