The identification of novel transmission blocking vaccine targets in the *Plasmodium falciparum* gametocyte life stage using a systems-wide approach through comparative proteomics and bioinformatics

Author: Danny Radford
Acknowledgement: Edwin Lasonder

UNIVERSITY OF PLYMOUTH
School of Biomedical Sciences

Introduction

- *Plasmodium falciparum* is a unicellular protozoan responsible for causing the most severe form of malaria out of the *plasmodium spp.* It is estimated that 219 million cases of malaria occurred worldwide in 2017 (World Health Organisation (WHO), 2018).
- The mature gametocyte is the 2nd least metabolic active life cycle stage (Delves *et al*., 2013), therefore analysis of membrane targets is central to developing a successful transmission blocking vaccine.
- In this study, we provide a comprehensive overview of significantly enriched proteins in the gametocyte life cycle stage of *P.falciparum*. We also provide evidence of proteins which are transmission blocking vaccine targets from literature mining, protein interaction network and protein properties.

Aim: To investigate *P.falciparum* gametocytogenesis during blood stage development and identify novel transmission blocking vaccine targets in gametocytes by using a systems-wide approach through comparative proteomics and bioinformatics.

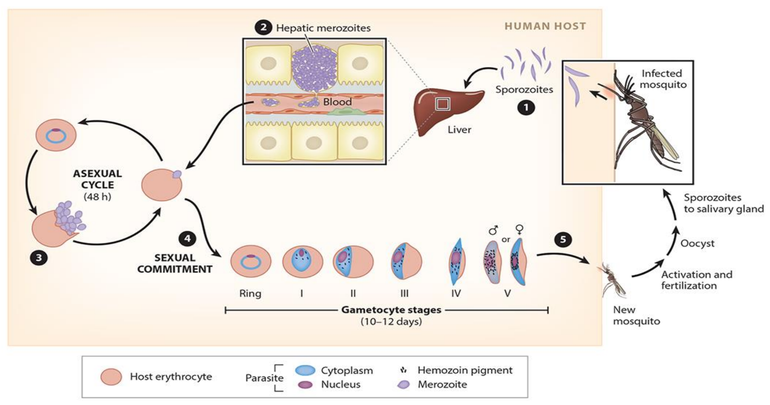

Figure 1 (Josling, Williamson and Llins, 2018) – The *Plasmodium falciparum* life cycle: 1) Sporozoites invade liver cells. **2)** Merozoites develop in the liver and released into the blood stream where they invade erythrocytes and replicate. **3)** New merozoites can continue undergoing asexual development. **4)** Alternatively, can also become committed to sexual differentiation into gametocytes. **5)** Mature stage V gametocytes are taken up in mosquito blood producing oocysts.

Methods

Liquid chromatography tandem mass spectrometry data from the blood life cycle stages of *P.falciparum* was analysed using MaxQuant. Proteins were quantified using a label-free approach (Cox *et al*., 2014). Protein expression from the four blood life cycle stages were compared using Venny 2.1.0 and Perseus 1.0. Gene ontology (GO) enrichment analysis and KEGG pathway analysis were performed on the LFQ data using David 6.8, with a statistical cut off of 0.05. We then performed protein-protein interaction analysis using STRING.

Results

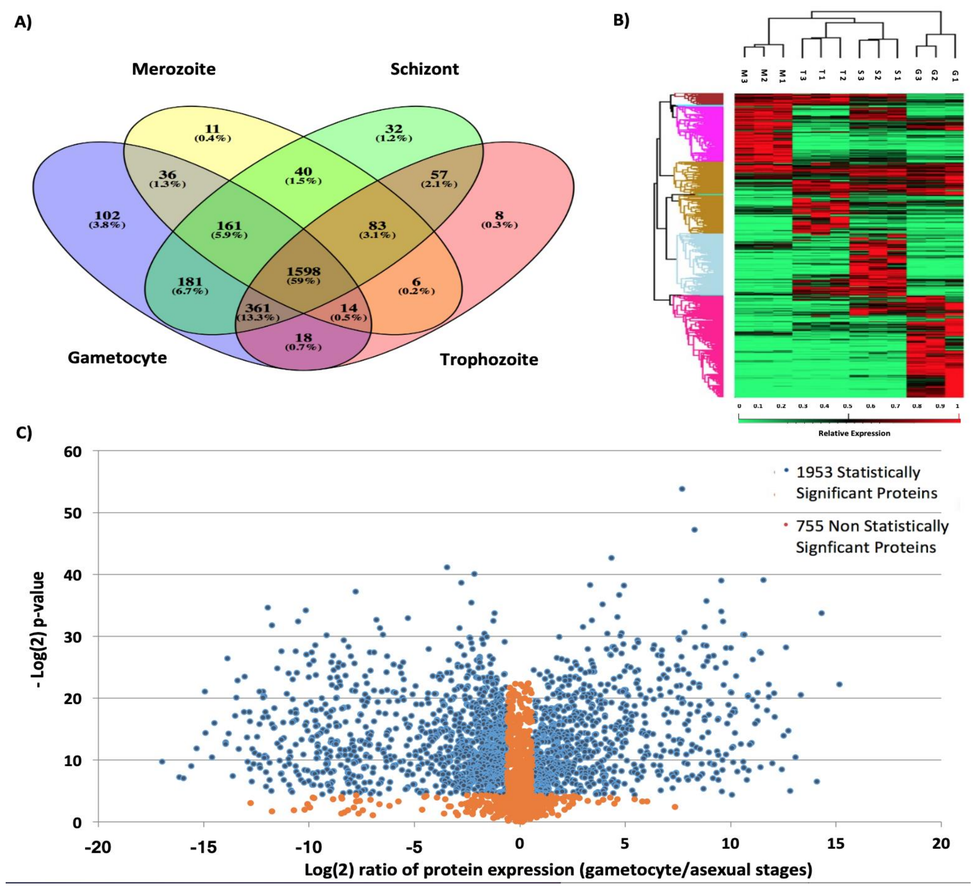

Figure 2 – Protein Expression Analysis: 2a) Venn diagram showing the number of proteins expressed in blood life cycle stages and their overlap. **2b)** Hierarchical Analysis of the relative LFQ values of the four life stages and their three replicates. Gametocyte replicates are labelled G; schizont replicates are labelled S; trophozoite replicates are labelled T, and merozoite replicates are labelled M. Relative expression is shown ranging 0-1, this is shown by a colour gradient from green to red with green representing 0 and red representing 1. **2c)** Volcano plot displaying *P. falciparum* proteins' fold change against their p-value to show most differentially expressed and significant.

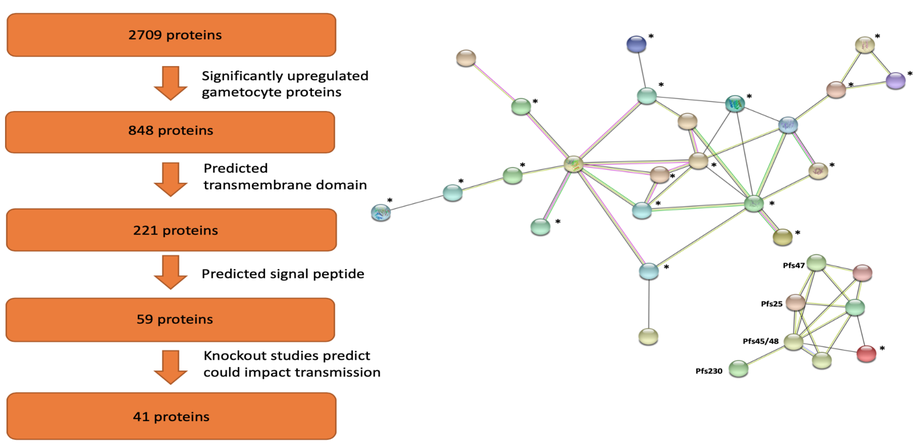

FIgure 3 – Vaccine candidate identification: 3a) Workflow, starting with all proteins in *P.falciparum* LFQ data to vaccine candidate proteins. **3b)** STRING analysis performed on the 59 proteins with a predicted transmembrane domain and signal peptide. Established targets in the literature are labelled with their name whereas novel vaccine candidates are asterisked; no asterisks means no affect on transmission in *P.berghei* knockout study.

Table 1 – Vaccine candidate table: transmission blocking vaccine candidates have been ranked in order of priority with 1 being top, displayed with results from *P. berghei* knockout database (www.pberghei.eu) cellular component ontology.

Accession Number	Predicted Product	Priority	P.berghei knockout database	Cellular Component Ontology
PF3D7_0828800	GPI-anchored micronemal antigen	1	Disrupts fertilization	Cell surface, Microneme, Cytoplasm
PF3D7_1028700	Merozoite TRAP-like protein	1	Disrupts fertilization	Cell surface, Microneme
PF3D7_1136900	Subtilisin-like protease 2	1	Disrupts fertilization	Membrane, Osmiophilic body, Microneme
PF3D7_1234400	Microgamete surface protein	1	Disrupts fertilization	Cell surface, Osmiophilic body
N/A	Conserved plasmodium protein (11 in total)	2	Not tested	Membrane
PF3D7_1354400	V-type proton ATPase 21 kDa proteolipid subunit	3	Modification failed	Membrane, Vacuole
PF3D7_1128700	GPI-anchor transamidase	3	Modification failed	Membrane
PF3D7_1127500	Protein disulfide-isomerase	3	Not tested	Membrane, Endoplasmic reticulum
PF3D7_1122100	GPI transamidase component GPI16	3	Not tested	Membrane, Endoplasmic reticulum
PF3D7_0508200	Longevity-assurance (LAG1) protein	3	Not tested	Membrane, Cell surface
PF3D7_0829600	Early transcribed membrane protein 8	3	No homolog	Membrane, Vacuole
PF3D7_0904400	Signal peptidase complex subunit 3	3	No homolog	Membrane
PF3D7_0212000	GDP-fructose:GMP antiporter	3	No homolog	Membrane
PF3D7_0912400	Alkaline phosphatase	3	No homolog	Membrane, Vacuole, Apicoplast
PF3D7_0624300	CPW-WPC	3	No homolog	Membrane
PF3D7_1248300	Conserved plasma protein	3	No homolog	Membrane, Apicoplast
PF3D7_0800600	Plasmodium exported protein	3	No homolog	Membrane
PF3D7_0108700	Secreted ookinete protein	3	Modification failed	Membrane, Nucleus, Apicoplast
PF3D7_0919100	DNAJ-like molecular chaperone protein	3	Modification failed	Membrane
PF3D7_1021400	Endomembrane protein 70	3	No homolog	Membrane
PF3D7_1462300	GTP-binding protein	3	No homolog	Membrane
PF3D7_0731800	Gametocyte exported protein 8	3	No homolog	Membrane
PF3D7_1024800	Exported protein 3	3	No homolog	Membrane, Cytoplasm, Vacuole

Conclusion

- In this study, we identified DNA replication to be significantly enriched in the gametocyte life stage. This supports the theory that male gametocytes accumulate proteins for DNA replication (Khan *et al*., 2005) whilst in the human host preparing to undergo rapid mitosis on activation in the mosquito midgut.
- Both CITH and DOZI were upregulated in gametocytes supporting the theory of transcriptional repression supported by Miao *et al*. (2013).
- Forty-one novel transmission blocking vaccine targets have been identified. Further research should utilise immunofluroescent analysis, *Streptococcus pyogenes* CRISPR/Cas9 system (conditional KO) and microarray technology for validation.

Contact Info

Danny Radford – Kent and Medway Medical School, University of Kent Pears Building, Parkwood Road, Canterbury. Kent. CT2 7FS.
Email: dannyradford98@outlook.com ORCID: 0000-0003-4499-5866

Dr Edwin Lasonder – Northumbria University Biomedical Sciences. Email: edwin.lasonder@northumbria.ac.uk

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