Gene transcription in *Plasmodium falciparum*-infected red blood cells binding to blood group A antigen *in vitro*

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Background

Panning of *Plasmodium falciparum* infected red blood cells (iRBC) from malaria in vitro cultures on artificial blood group A oligosaccharides increases the ability of the *in vitro* cultured iRBC to bind endothelial cells (EC) and form ABO blood group specific rosettes[1]. iRBC binding to EC is essential for parasite survival in spleen intact hosts[2] and ABO blood group specific rosette formation is associated with disease severity and is believed to be an important factor underlying the increased risk of severe *P. falciparum* malaria seen in malaria patients with blood group A compared to blood group O[3].

Study-design

- *In vitro* selection[1] of 3D7 and FMG/IT parasites for binding to Blood group A (BGA) constructs and recombinant CD36.
- Adhesion assays[1] to compare the phenotypes of the isogenic selected parasite sublines (fig1 left).
- Sequencing of RNA obtained from synchronised ring and early trophozoite stages from the cultures, obtaining 50-100 mio reads per sample (fig 1 right).
- Data analysis using the Galaxy platform (https://usegalaxy.org). Sequence reads were assigned to coding sequences of the 3D7 and IT genomes obtained from PlasmoDB (https://plasmodb.org), respectively.

Figure 1. Adhesion and VSA-expression

Left: Adhesion of the parasite lines. Bars and errorbars show mean and standard deviation of results obtained in all assays (8 assays comparing 3D7 parasites and 5 assays comparing FMG parasites.
Right: Transcription of VSA-genes. Mean values of 3 sequencinfg experiments is shown. Proposed adhesion phenotype of var-genes was assigned according to their domain structure as described in ref 4.

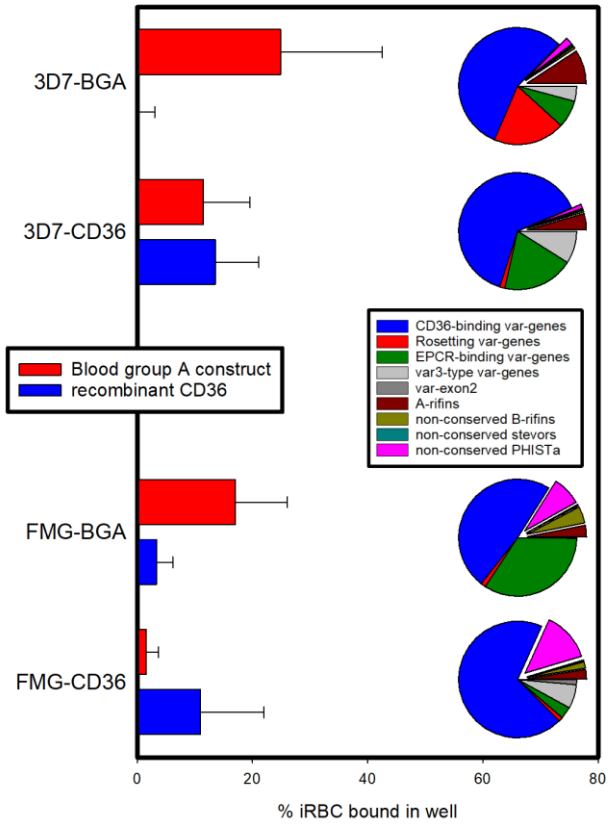

Table 1. 15 most over-expressed conserved genes in BGA selected parasites

Genes encoding proteins containing a Plasmodium Export Elemet (PEXEL)[5] or proteins shown to be exported to the iRBC by other means[6] are shown in **bold.**

3D7 Gene ID	IT gene ID	Function	p*
PF3D7_1009600	PfIT_100013600	protein phosphatase PPM10	0,000492
PF3D7_0810400	PfIT_080015300	aquaporin	0,000641
PF3D7_1476200	**PfIT_140082400**	**PHISTb**	0,00094
PF3D7_1039000	**PfIT_100042900**	**FIKK10.2**	0,00272
PF3D7_0601900	**PfIT_060005700**	**conserved Plasmodium protein, unknown function**	0,0034
PF3D7_0501200	**PfIT_050006600**	**PIESP2**	0,00428
PF3D7_0819500	PfIT_080024500	conserved protein, unknown function	0,00713
PF3D7_1403900	PfIT_140010500	serine/threonine protein phosphatase CPPED1	0,00751
PF3D7_1201100	**PfIT_120006700**	**RESA-like protein with PHIST and DnaJ domains**	0,00797
PF3D7_0220600	**PfIT_020025100**	**Hyp 9**	0,00808
PF3D7_0608800	PfIT_060012900	ornithine aminotransferase	0,00808
PF3D7_1014700	PfIT_100018700	prohibitin 2	0,0088
PF3D7_1342400	PfIT_130047700	casein kinase II beta chain	0,00985
PF3D7_0702400	**PfIT_070007300**	**SEMP1**	0,00991
PF3D7_0301400	**PfIT_030006200**	**Plasmodium exported protein, unknown function**	0,0108

*In each of 6 experimnets comparing tightly synchronised isogenic parasite pairs, log(transcription level in BGA selected parasite / trancription level in CD36 selected parasite) was calculated for each gene. This value was compared to a population mean of 0 in a one sample t-test.

Conclusions

Consistent with our previous results obtained with *var*- and *rif*-gene specific primers and real-time quantitative PCR[1], we were unable to show that iRBC binding to blood group A antigens is associated with expression of specific types of VSA as has previously been proposed[3,7].
Our data suggest that non-VSA genes showing limited sequence diversity among parasite strains might also be involved in binding to blood group A, rosette formation and EC-adhesion.

References

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