Expression of conserved *Plasmodium falciparum* surface antigens is associated with severe malaria in Malian children

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Background

- ***Plasmodium falciparum* malaria** kills hundreds of thousands of children in sub-Saharan Africa annually[1]. Most deaths are due to severe malaria (SM), including cerebral malaria (CM) and severe malarial anemia (SMA).
- **RIFINs and STEVORs** are large families of parasite proteins expressed on the surface of infected red blood cells and may mediate severe malaria pathogenesis through multiple mechanisms (Figure 1).
- These families are extremely diverse, with unique variants encoded by ~150 *rif* genes and ~30 *stevor* genes, respectively.
- Our group recently identified a subset of **"strain-transcendent"** RIFINs/STEVORs that are unusually conserved across *P. falciparum* strains.
- The expression of specific RIFINs/STEVORs in clinical infections remains poorly understood. Identifying conserved variants that are preferentially expressed in severe disease may yield promising vaccine targets.
- Here, we used RNA sequencing to compare expression of strain-transcendent RIFINs/STEVORs in Malian children experiencing severe or uncomplicated malaria episodes. We hypothesized that a subset of antigens would be preferentially expressed in severe malaria cases.

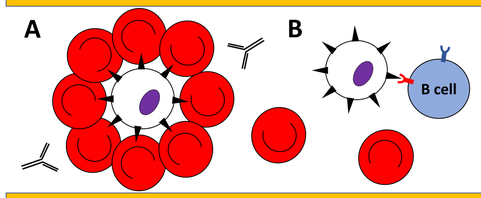

Figure 1: Involvement of RIFINs/STEVORs in severe malaria pathogenesis. **A)** RIFINs and STEVORs mediate binding to uninfected erythrocytes, occluding blood flow and preventing immune recognition. **B)** An infected erythrocyte binds to a B cell inhibitory receptor via RIFINs.

Objective

To identify strain-transcendent RIFINs and STEVORs that are differentially expressed in severe malaria cases versus uncomplicated malaria controls.

Methods

CASE-CONTROL STUDY OF SEVERE MALARIA

- Children ages six months to five years in Bamako and Bandiagara, Mali, 2014 – 2018.
- Cases: Children with severe malaria seeking hospital care, including cases of CM (Blantyre score ≤ 2), SMA (hemoglobin ≤ 5 g/dl), and concurrent syndromes (CM+SMA).
- Controls: Uncomplicated malaria (UM) cases with and without a history of severe malaria. Matched to cases on age, sex, ethnicity, residence, and time.

SAMPLE PROCESSING

- RNA extracted from whole-blood samples using PreAnalytiX PAXgene kits.
- Sequencing libraries enriched with the Roche SeqCap system.
- RNA sequenced using the NovaSeq6000 platform.

ANALYSIS

- Aligned sequenced reads to the 3D7 reference genome to obtain raw gene counts.
- Estimated normalized gene expression for each strain-transcendent RIFIN/STEVOR as transcripts per million (TPM) (Figure 3).
- Conducted differential expression analysis for 1) all SM cases and 2) CM cases (alone or concurrent with SMA) versus UM controls using the edgeR[5] platform (Figure 3). `

Figure 2: Overview of sample processing and analysis pipeline.

Results

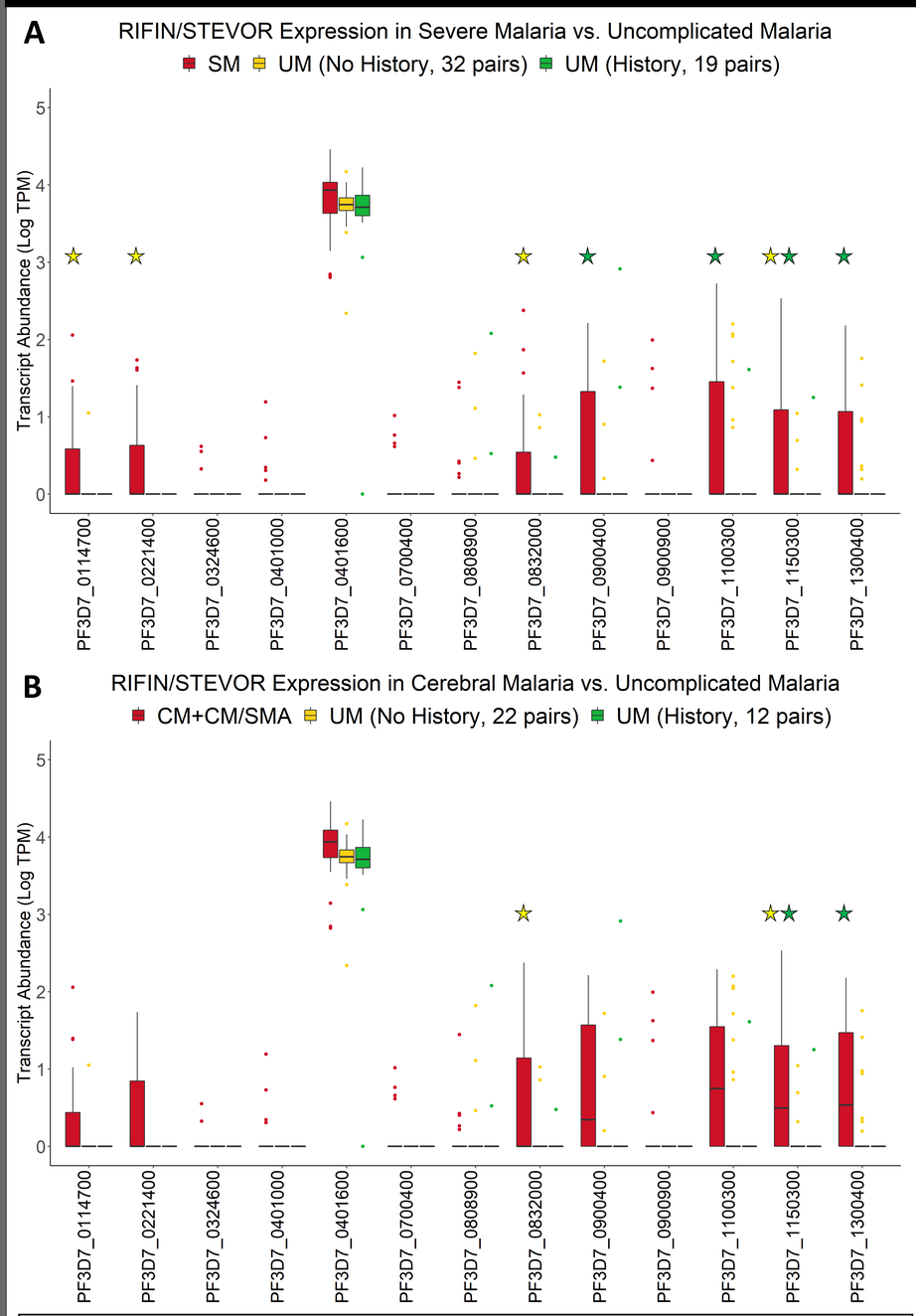

Figure 3: A subset of strain-transcendent RIFINs/STEVORs were upregulated in severe malaria syndromes. A) Severe malaria vs. uncomplicated malaria. **B)** Cerebral malaria (alone or concurrent with SMA) vs. uncomplicated malaria. Box plots show distributions of expression abundance, estimated as log transcripts per million. Legends include the number of matched pairs in each comparison. Stars indicate statistically significant differential expression between cases and individual control groups (α = 0.05).

Results

Variable		Severe Malaria Cases N = 33	Matched Controls Without History N = 32	Matched Controls With History N = 19
Age (years)		2.9 (1.3)	2.9 (1.2)	3.4 (1.0)
Sex				
	Male	17 (52%)	16 (50%)	11 (58%)
	Female	16 (48%)	16 (50%)	8 (42%)
Ethnicity				
	Bambara	5 (15%)	4 (13%)	1 (5%)
	Dogon	26 (79%)	26 (81%)	18 (95%)
	Malinke	0 (0%)	1 (3%)	0 (0%)
	Peulh	1 (3%)	1 (3%)	0 (0%)
	Sarakole	1 (3%)	0 (0%)	0 (0%)
Blood type				
	A	3 (9%)	9 (28%)	3 (16%)
	AB	4 (12%)	1 (3%)	1 (5%)
	B	15 (46%)	8 (25%)	6 (32%)
	O	11 (33%)	14 (44%)	9 (47%)
Hemoglobin (g/dL)		5.2 (2.7)	8.5 (2.0)	9.2 (1.7)
Blantyre coma score				
	1	7 (21%)	0 (0%)	0 (0%)
	2	15 (46%)	0 (0%)	0 (0%)
	3	3 (9%)	0 (0%)	0 (0%)
	4	3 (9%)	0 (0%)	0 (0%)
	5	5 (15%)	32 (100%)	19 (100%)

Table 1: Demographic and clinical characteristics. Cells display stratified frequencies (column %) or medians (IQR).

Conclusions

- Several strain-transcendent RIFINs/STEVORs were significantly upregulated in severe malaria versus uncomplicated malaria.
- One RIFIN was differentially expressed in severe/cerebral malaria cases compared to uncomplicated malaria controls *with or without* a history of severe disease. Another RIFIN was highly expressed regardless of disease severity.
- These conserved RIFINs/STEVORs may be important for severe malaria pathogenesis and are promising vaccine targets.

Future Directions

- Determine whether antibodies against strain-transcendent RIFINs/STEVORs are associated with protection from severe disease.
- Identify human receptor binding partners for promising targets.

Acknowledgments & References

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