Identification of *fikk* gene expression specific to severe malarial syndromes in Malian children

Sarika Kapadia[1], Jonathan Lawton[2], Emily Stucke[2], Gabrielle Voithofer[2], Mark Travassos[2]

[1] University of Maryland, College Park, MD, USA
[2] Center for Vaccine Development and Global Health, University of Maryland School of Medicine, Baltimore MD, USA

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Background

- *Plasmodium falciparum* is responsible for the most severe form of human malaria, killing over half a million children every year and causing 80% of malarial deaths in children under 5.
- Unlike other *Plasmodium* species, *P. falciparum* actively exports approximately 18-22 kinases encoded by the *fikk* gene family
- FIKK (Phe-Ile-Lys-Lys) multi-gene family: Relatively understudied kinase gene family with phosphotransferase residues and export element (PEXEL)
- These novel *fikk* kinases are hypothesized to:
  - carry out the activation and subsequent trafficking of molecules in an infected erythrocyte (IE).
  - Influence stage-specific membrane remodeling
  - Regulate *var* expression

Question & Hypothesis

- **Question:** Do specific clinical syndromes of *P. falciparum* in Malian children show differential expression or overall increased expression of the *fikk* gene family?
- **Hypothesis:** Specific severe syndromes will express higher levels of overall *fikk* gene expression and specific *fikk* genes will predominate among the others.

Methods

Sample preparation and *de novo* assembly → FIKK Library preparation → RNAseq (Transcripts per Million) → TPM comparison and analysis

Results

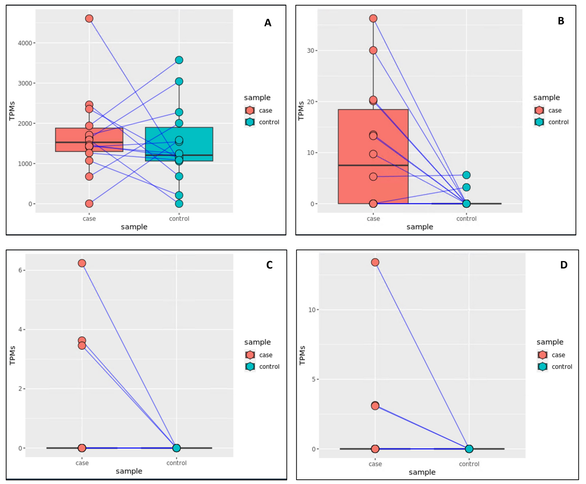

Fig 1. TPM Comparisons in Cerebral Malaria (CM) cases versus Uncomplicated Controls
A. There was no significant difference in overall *fikk* expression between cerebral malaria cases and controls (P = 0.6698; Wilcoxon signed-rank test). **B**. There was a significant difference in ***fikkA*** (fikk9.5) expression between cerebral malaria cases and controls (P = 0.01953; Wilcoxon signed-rank test). **C**. There was no significant difference in ***fikk0*** (new suggested *fikk*) expression between cerebral malaria cases and controls (P = 0.25; Wilcoxon signed-rank test, P = 0.22 Fisher's Exact test). **D**. There was no significant difference in ***fikkB*** (*fikk13*) expression between cerebral malaria cases and controls (P = 0.25; Wilcoxon signed-rank test, P = 0.22 Fisher's Exact test).

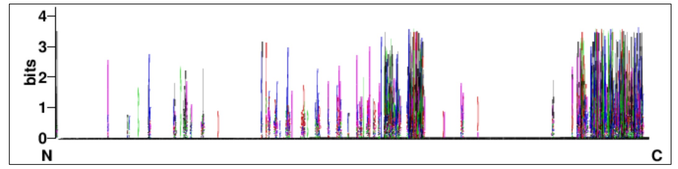

Fig 2.
Sequence Logo illustrating the level of sequence conservation in the 3D7 strain of the *fikk* multigene family. As predicted, the N-terminus remained highly variable, while the C-terminus remained more conserved. Overall, sequence conservation between the *fikk* genes is low compared to most *fikk* subgroup-specific sequence conservation (not shown).

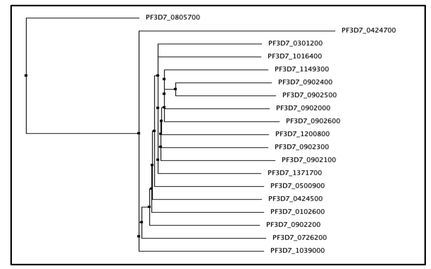

Fig 3.
Neighbor-joining pairwise tree of 3D7 *fikk* gene family F*ikk8*, the orthologous ancestral gene, is the outgroup. Phylogeny generated using Jalview and BLOSUM62 scoring.

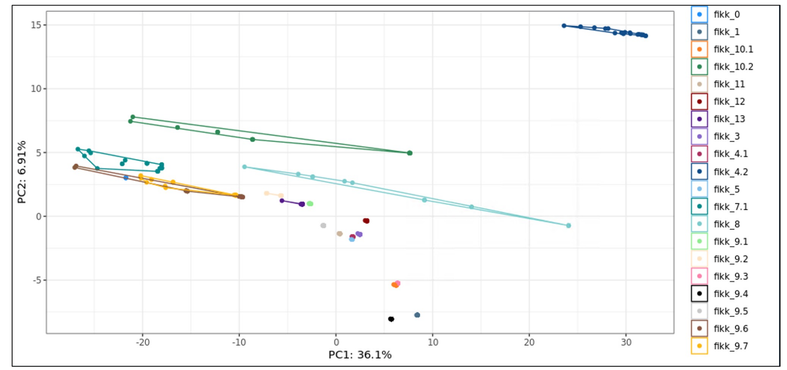

Fig 4. Multidimensional Scaling (MDS) of 20 *P. falciparum* strains
Clusters align closely with *fikk* subgroups once major subgroup outliers are removed (not shown). Hulls added to aid visualize subgroup conservation range and relative sequential similarity. A newly characterized *fikk* subgroup located in chromosome 7 falls between *fikk* hulls 9.6, 9.7, and 7.1.

Conclusions

1. A kinase family of proteins may play a role in cerebral malaria, as evidenced by a preliminary analysis of differential gene expression.

2. Distinctive sequence-similarity-based clustering suggests the existence of a new *fikk* subgroup in recent divergent malaria strains

Acknowledgments & References

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