[Faculty Non-Competitive]

Homologs of Human Dengue-Resistance Genes, confer antiviral resistance in mosquitoes

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Abstract

Dengue virus (DENV) is transmitted by mosquitoes and is a major public health concern. Our understanding of the molecular interaction of DENV with mosquito host cells is limited and we propose to expand upon the recent results from a genome-scale, small interfering RNA (siRNA)-based study that identified mammalian host proteins associated with resistance to dengue/West Nile virus (DENV/WNV) infection. The study identified 22 human DENV/WNV resistance genes (DVR) and we hypothesized that a subset would be functionally conserved in Aedes aegypti mosquitoes, imparting cellular defense against flaviviruses in this species. We identified 12 homologs of 22 human DVR genes in the *Ae. aegypti* genome and identified that silencing of the two candidates, AeFKBP1 and AeATCAY, homologs of human FKBP1B and ATCAY were associated with viral increase. Depletion of AeFKBP1 or AeATCAY in vivo increased viral dissemination through the mosquito at 14 days post-infection. Our results demonstrated that AeFKBP1 and AeATCAY mediate resistance to DENV akin to what has been described for their homologs in humans. AeFKBP1 and AeATCAY provide a rare opportunity to elucidate a DENV-resistance mechanism that may be evolutionarily conserved between humans and *Ae. aegypti.*

Methodology/Project Design

- *In silico* identification of mosquito homologs
- *In vitro* RNAi silencing assay in Aag2 (*Ae. aegypti*) cells
- Plaque assay to determine viral titer and infection status of experimental Aag2 cells
- *In vivo* RNAi silencing assay in adult Ae. aegypti mosquitoes involving intrathoracic injection of dsRNA targeting AeATCAY & AeFKBP1 (see Fig 3A)
- Real-time PCR to estimate viral titers
- Analysis of gene expression during infection for AeATCAY & AeFKBP1 using real-time PCR

Figure 1. siRNA-mediated silencing assay during DENV2 infection in Aag2 cells.

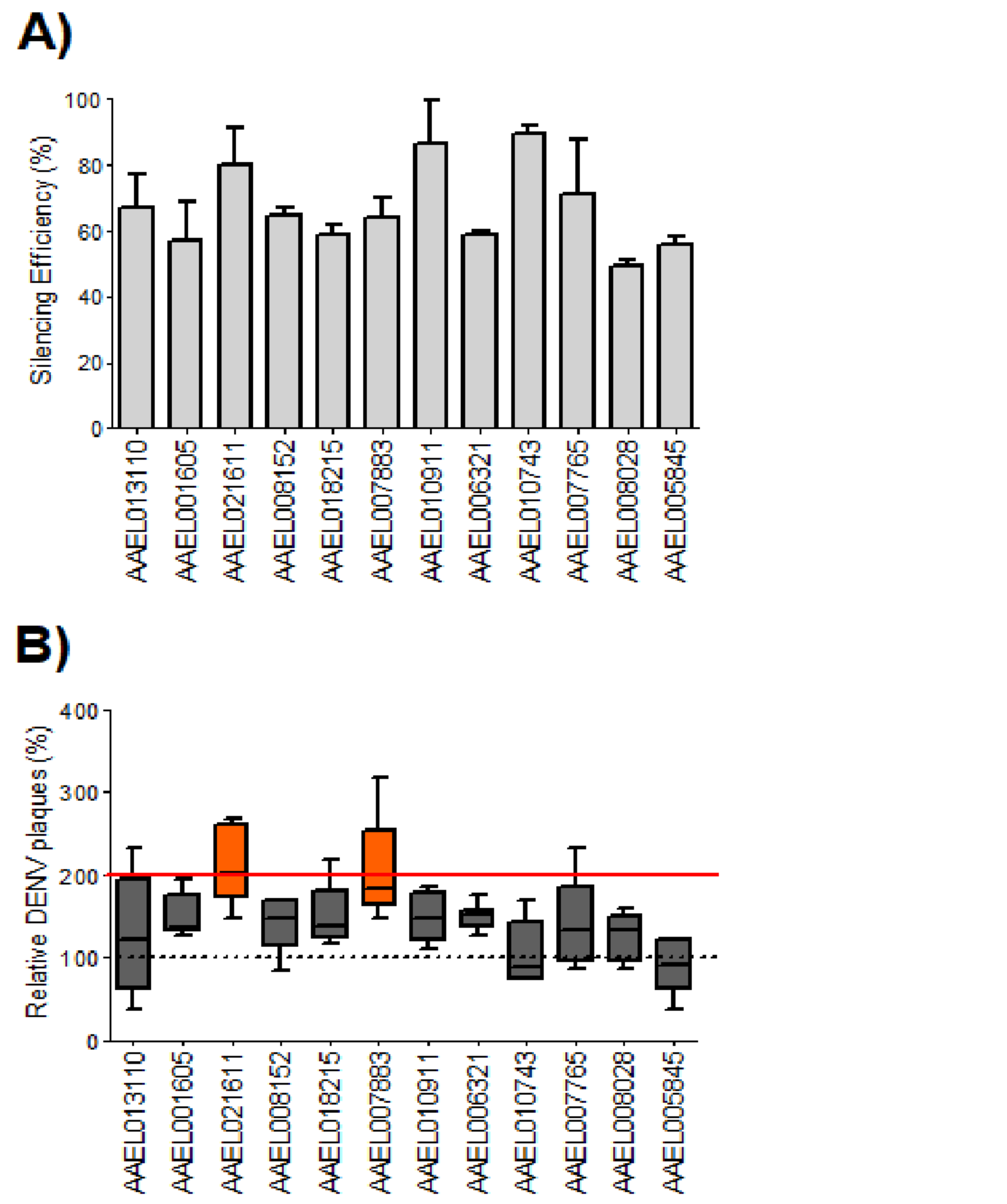


Figure 2. Sequence analysis of AeFKBP1 and AeATCAY.

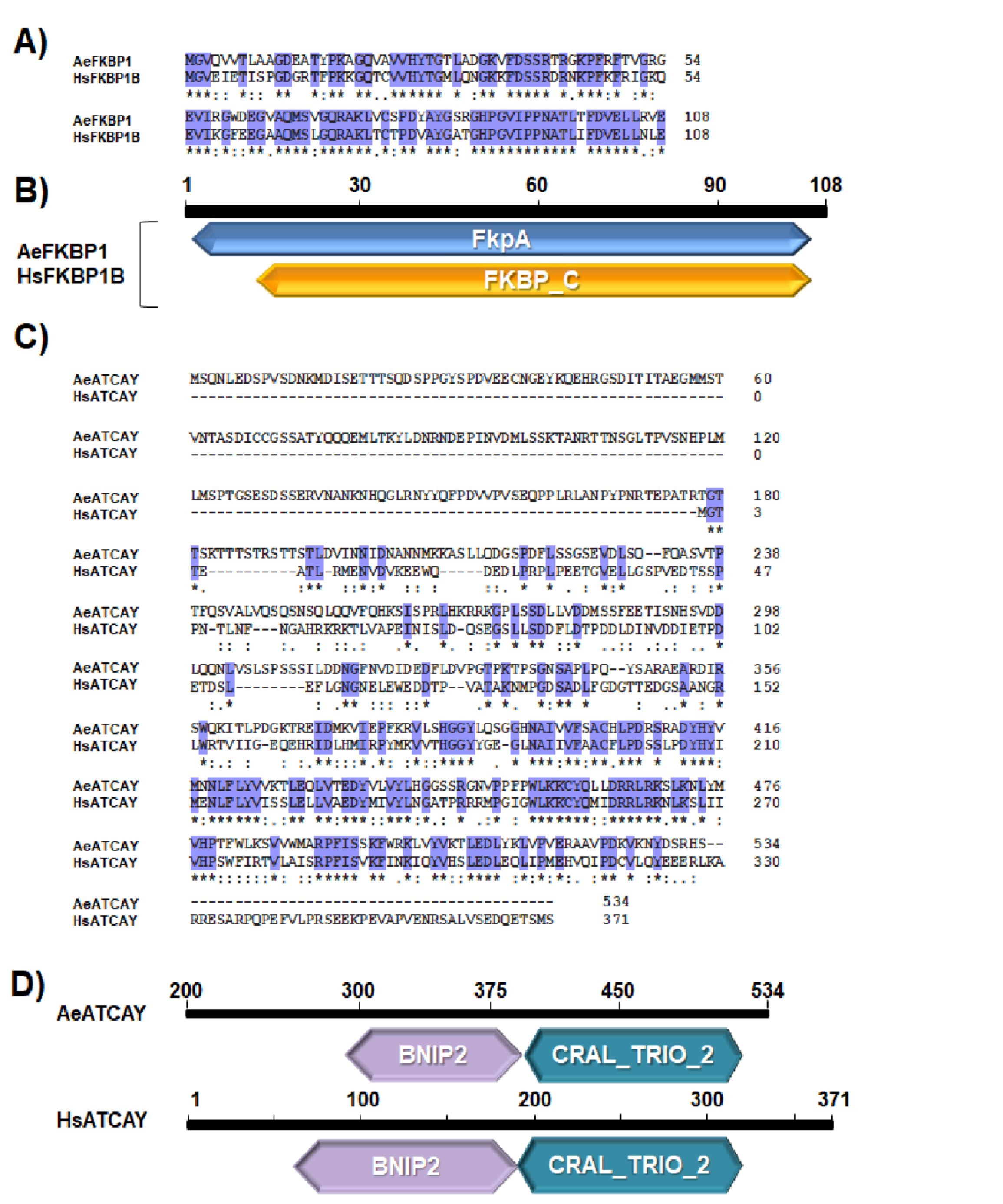
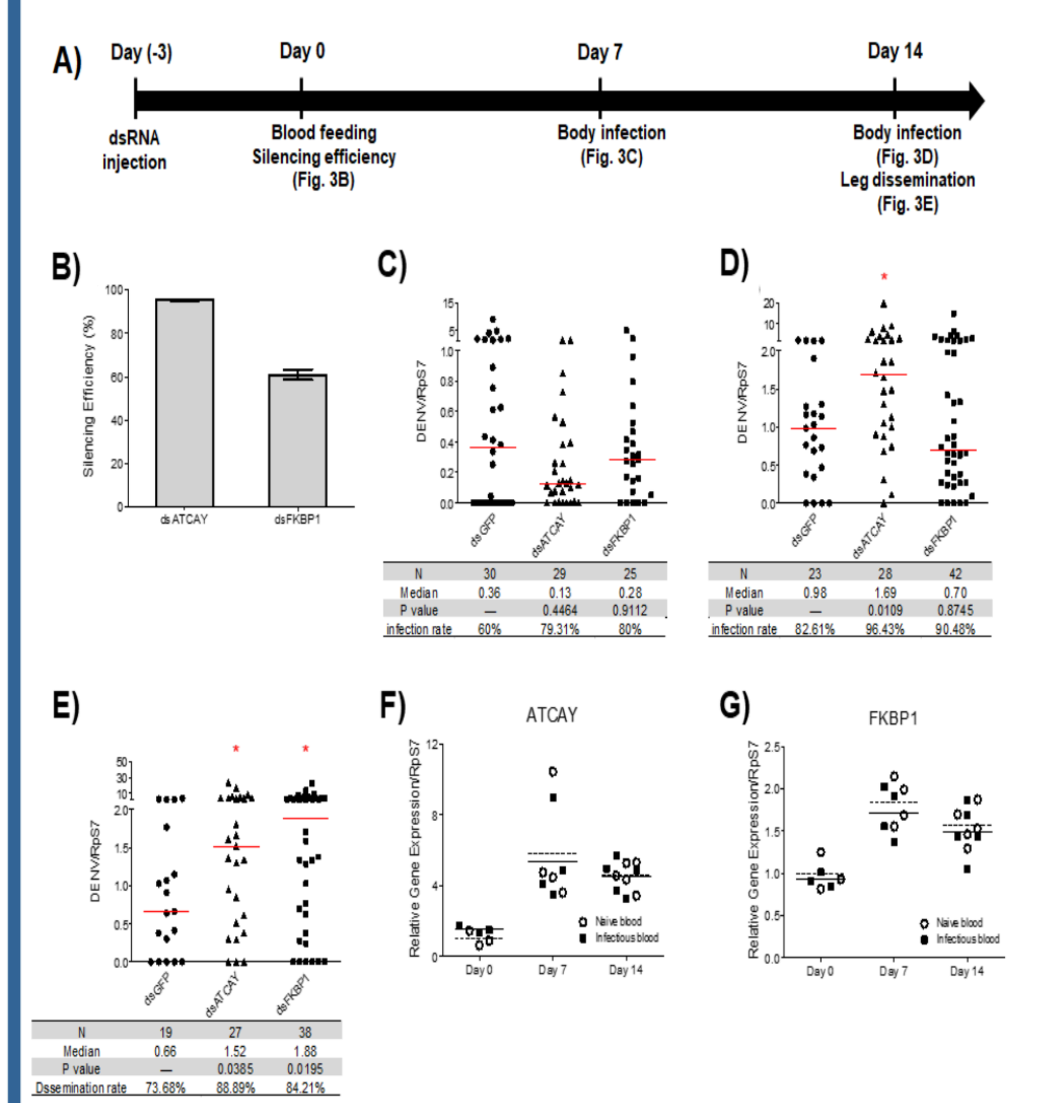


Figure 3. dsRNA-mediated silencing assay during DENV2 infection in adult mosquitoes.



Results

- Silencing of two *Aedes aegypti* homologs of WNV/DENV2-resistance genes (ATCAY and FKBP1B) increased DENV2 infection in Aag2 cells
- Sequence analyses indicate a high degree of domain conservation for FKBP1 and ATCAY between humans and mosquitoes
- Viral dissemination in adult mosquitoes was increased following the silencing of AeATCAY and AeFKBP1 at 14 dpi
- Expression of selected DENV2 resistance genes increased during blood feeding but was unaffected by viral infection

Conclusions

Our data demonstrate that FKBP1 and ATCAY play anti-DENV roles in human cell lines and *Ae. aegypti*, revealing a potentially ancient antiviral defense mechanism. Silencing of AeFKBP1, and AeATCAY, homologs of human FKBP1B and ATCAY, both in an Ae. aegypti cell line and adult mosquitoes increases DENV2 viral titers. Human FKBP1B and AeFKBP1 have substantial conservation in their primary structures, and FKBP sequences are highly conserved among various organisms. Together, these data suggest that its anti-DENV function may have appeared during the early evolution of antiviral immunity. We hypothesize that these FKBP1- and ATCAY-mediated antiviral pathways represent ancient cellular defense mechanisms, but it remains to be seen if and how they are linked to the canonical pathways of innate immunity.