

There are eight out of 17 highly divergent outlier genes concentrated in a 920-kbp genomic island of differentiation on chromosome 2. The first four outliers in this region, *AaFAS3–6*, encode fatty acid synthases (FASs) involved in lipid biosynthesis. Together, these four genes accounted for 78 iSNPs (mean of 2.7 iSNPs/kb), 10 of which were nonsynonymous. Upon host-cell infection by DENV (all serotypes), the positive-sense single-stranded RNA genome is translated into a polyprotein and processed to produce three structural and seven non-structural (NS) proteins. The latter reorganize host-cell membranes of the endoplasmic reticulum (ER) to form replication complexes (77). To meet lipid requirements, DENV-2 is known to hijack lipid metabolic machinery, including the NS3-mediated recruitment of host-derived FAS proteins (78,79). In mosquito-derived C6/36 cells, a FAS inhibitor not only changed the cellular lipid profile but inhibited DENV-2 ~1,000 fold when administered 4 and 8 hours post-infection (79). *Aedes aegypti* reportedly has seven FAS paralogs (*AaFAS1–6* and *AaFAS-like*) (80). Of these, only *AaFAS1* has been investigated experimentally and confirmed as a DENV-2 host factor (80). The other *AaFAS* genes demonstrated greater expression in males than females (80), but in RNA-seq experiments with *Ae. aegypti* females, *AaFAS3* and *AaFAS5* typically show expression levels ranked between the 50th and 90th percentiles following exposure to DENV and ZIKV (80–85). Thus, it is plausible that DENV-2 takes advantage of multiple *AaFAS* paralogs and recruits their gene products to the ER.

Little is known about the next outlier gene in the island, AAEL002234 located 52 kb downstream, which encodes a protein with an ankyrin-repeat domain often implicated in protein-protein interactions (44). It was second only to *AaCHD7* in iSNP number ($n = 87$), with a density of 13.7 iSNPs/kbp and 28 yielding an amino acid change. A different ankyrin-repeat protein was shown to have antagonistic properties against DENV-2 in mosquito salivary glands (121), but gene AAEL002234 has yet to be investigated. Located 132 kbp downstream of AAEL002234, the outlier gene *AeOSTC* (AAEL002198) is relatively small (transcript length = 699 bp) but

accounted for 8 iSNPs (5 synonymous, 3 in the 5' UTR). It is a non-catalytic subunit of the oligosaccharyltransferase (OST) complex, which catalyzes a key step of *N*-linked glycosylation (122). Additionally, the OST complex, along with the Sec61 complex and the translocon-associated protein (TRAP) complex, form the translocon, a multi-protein assembly associated with ribosomes responsible for co-translational insertion of proteins into the ER (123). This process is conserved among most eukaryotes and all metazoans (124), and both CRISPR/Cas9 mutagenesis screens and protein-interaction mapping identified human OSTC (alias DC2) and/or additional OST subunits as host factors for DENV-2 replication (92,94–95). Experiments with inactive mutants of OST catalytic subunits STT3A and STT3B indicated that although OST was essential for DENV-2 replication, its enzymatic activity was not, pointing to structural properties of the OST complex in the ER (95). The lone study of OST as a DENV host factor in *Ae. aegypti* showed that knock down of *AaSTT3A*, *AaSTT3B*, and subunit *AaMAGT1* in Aa20 cells had no effect on DENV-2 replication, but neither *AaOSTC* nor the seven additional OST subunits were investigated (125).

The outlier gene *AaCk1ε* (AAEL019753) starts 6.5 kb downstream of *AaOSTC*, and produces a serine/threonine kinase best known for its role in circadian rhythms (37). It had 27 iSNPs (9.1 iSNPs/kbp), 10 of which caused amino acid substitutions. The gene *AaCk1ε* is the ortholog of *Ck1ε* (*casein kinase 1 epsilon*), synonymous with *discs overgrown/doubletime* (*dco/dbt*) in *Drosophila*, which phosphorylates serine/threonine residues to regulate proteins in a variety of molecular pathways (37,126–128). Although *AaCk1ε* is not known to affect DENV replication or infection, *AaCk1ε* has regulatory roles in pathways that do, including Wnt signaling (129) and apoptosis (130,131). Further, in humans, serine/threonine kinases, including isoforms of Ck1, phosphorylate residues of flavivirus NS5 proteins (132–135). Studies of phosphorylated NS5, which localizes to the nucleus in DENV-infected host cells, suggest it promotes virus replication through multiple mechanisms, including interfering with immunity (94,136),

modulating transcription by binding to components of the polymerase associated factor 1 complex (PAF1C) (93,94), and by binding to the spliceosome to alter pre-mRNA processing (137). In mosquito cells, some of the NS5 molecules also sublocalize to the nucleus (138), but relevance to the virus life cycle inside the vector is unknown. To our knowledge, *AaCk1ε* has not been investigated as an arbovirus host factor in *Ae. aegypti*, but since Ck1 isoforms prefer substrates primed by previous phosphorylation (139), a plausible hypothesis is that DENV exploits multiple host kinases, including Ck1ε, to regulate NS5 phosphorylation and its subcellular localization. Outside the genomic island, another outlier gene involved in circadian rhythmicity is *AaClk* (AAEL022593), which had 34 iSNPs (8.0 iSNPs/kbp), 3 of which caused amino acid changes. It is orthologous to the circadian gene *clock* (*clk*) in *Drosophila* (34), which encodes a transcription factor that drives expression of *period* (*per*) and *timeless* (*tim*) as part of a negative transcription/translation feedback loop (35,36). The PER and TIM proteins dimerize to form an inhibitory complex in which PER represses CLK activity following activation and stabilization by DBT/Ck1ε phosphorylation (37). Thus, two of our outlier genes, *AaClk* and *AaCk1ε*, are functionally connected through interactions with PER. Altogether, the high number of iSNPs for *AaCk1ε* and for *AaClk*, both circadian genes, suggest that adjustments to the circadian clock may have played a prominent role in *Ae. aegypti* divergence within SSA and between continents.

The final gene in the chromosome 2 island, *AaCHD7* (AAEL019793), is 19.5 kbp downstream from *AaCk1ε* and had the most iSNPs in our analysis (n = 95). Fifteen of the iSNPs were nonsynonymous, while 16 were concentrated in the 5' UTR. This gene encodes a chromodomain helicase DNA-binding protein (CHD) that when silenced in *Ae. aegypti* cells significantly decreased DENV-2 replication (86). All CHDs associate with chromatin and use ATP hydrolysis to disrupt histone-DNA contacts to alter chromatin structure or histone composition (90). In *Drosophila*, the *AaCHD7* ortholog (Kismet) repositions nucleosomes

proximal to the promoter of transcriptionally active genes and recruits histone-modifying enzymes that enable transcriptional elongation by RNA polymerase II (87–89). Kismet also localizes to enhancers of intestinal stem cells in adult flies and reportedly keeps cell proliferation in check (91). In mice, CHD7 also localizes to enhancer elements and modulates expression of specific genes (92). If these functions are conserved in *Ae. aegypti*, AaCHD7 proviral activity may stem from its regulation of other genes that influence DENV-2 production. Although human CHD7 has not been implicated in the flavivirus life cycle, other chromatin-associated proteins have, including PAF1C, a known binding partner of DENV-2 NS5 (93,94). Like CHD7, PAF1C is a key player in transcriptional elongation, suggesting that DENV may manipulate this process to its advantage in both mosquito and human hosts.

Outside of the chromosome 2 island, at least two other outlier genes influence vector-mediated transmission (Fig. 7). The first, *Or103* (AAEL017505) partly drives vector-host interactions. This gene had the highest iSNP density (20.6 iSNP/kbp) and 29 total, with nearly half causing an amino acid change. In comparisons of *Ae. aegypti* from Kenya, Uganda, and Thailand, *Or103* was one of two odorant receptors upregulated in antennae of human-preferring mosquitoes but downregulated in mosquitoes preferring a non-human host (38). The other was *Or4*, which occurs at the distal end of a region on chromosome 1 that was highly differentiated between West African populations varying in human preference (20). It is noteworthy that the signal for SNP-association with host preference was strongest 10 Mbp upstream of *Or4* in gene AAEL019513, a large gene encoding a Na/Ca-exchange protein that spans > 641 kbp. This gene is closely associated with two of our outlier genes, *CYP6P12* (AAEL014891), which occurs within one of its introns, and the unannotated outlier AAEL024267, located 36 kbp downstream of AAEL019513 toward *Or4*.

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