

THE MOLECULAR EPIDEMIOLOGY OF FOUR SEROTYPES OF DENGUE VIRUSES CIRCULATING IN THAILAND DURING 1974 TO 2001

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Dengue represents a major public health problem in Thailand, with all four viral serotypes co-circulating. This provides an optimal setting to investigate the molecular epidemiological history and evolutionary trends of these co-circulating dengue virus (DENV) serotypes in a highly endemic country; to characterize intra-serotypic variations of DENV in a locality; to determine the evolutionary forces shaping viral genetic diversity; to determine whether the changing prevalence of DENV could be attributed to instances of adaptive evolution in the viral genome. Furthermore, it permits the investigations of whether molecular determinants of the envelope (E) gene of DENV correlate with disease severity. To this end, we undertook a large-scale molecular epidemiological analysis of 324 E gene sequences of DENV representing each of the 4 serotypes (98 DENV-1, 105 DENV-2, 68 DENV-3 and 53 DENV-4) and 32 complete genome sequences of DEN viruses (10 DENV-1, 10 DENV-2, 6 DENV-3 and 6 DENV-4) isolated from children admitted from 1974 to 2001 in Thailand (Bangkok and Kamphaeng Phet) with varying degrees of dengue severity [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)]. Our results indicated that there was no obvious molecular correlate between disease severity and the phylogenetic position of their associated E genes. These analyses revealed extensive genetic diversity within a single geographic locality at a single time, including the discovery of a new and divergent genotype of DENV-4. However, despite this abundant genetic variation, there was no evidence of adaptive evolution in any gene, codon, or lineage of DENV-3 and DENV-4 (DENV-1 and DENV-2 analyses are ongoing.) The phylogenetic trees of DENV showed a strong temporal structure, ladder-like structure for all 4 serotypes; viral strains isolated at the earliest time-points tended to fall near the root of the trees, while those sampled more recently were located more remote from the root of the trees. These temporal orderings are caused by the continual birth and death of viral lineages; new lineages are regularly produced by mutation, but most go extinct relatively rapidly and few progress to circulate in subsequent years. Consequently, the rapid turnover of DENV lineages observed is, at most, the consequence of high rates of deleterious mutations in the viral genome coupled with seasonal fluctuations in the size of the vector population.

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MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DEN –4 VIRUSES IN BANGKOK, THAILAND

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Fifty-three dengue-4 (DEN-4) virus isolates from children in Bangkok, admitted with varying severity of DEN [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)]

from 1976-2002 were selected to sequence the envelope (E) gene for identifying specific sequence patterns which may correlate with disease severity and for assessing potential trends in molecular evolution and epidemiology of circulating DEN-4 viruses. Phylogenetic analysis revealed that the majority collected in the past 27 years comprised genotype I (47 cases), 5 of the 6 more recent isolates comprised a genotype never previously described. This newly discovered genotype was associated with DF (2 cases) and DHF/DSS (3 cases). No specific sequence differences were identified between DF and DHF/DSS isolates, suggesting that the E gene alone did not determine disease severity. The phylogenetic tree revealed that genotype I of DEN-4 virus appears to have become extinct beyond 1999 and has been replaced by new lineages that evolved locally, rather than having been introduced. However, it remains unclear whether this replacement represents a selection event, so that strains differ in fitness, or a random population bottleneck. One case of the genotype IIA appears to have been introduced in 2000 from neighboring Malaysia/Indonesia. However, it appears that this genotype could not be sustained. Sequencing of the entire virus genome of selected specimens is underway to further ascertain the molecular basis to any differences in fitness among strains.

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MOLECULAR EVOLUTION AND EPIDEMIOLOGY OF DENGUE-3 AND -4 VIRUSES IN BANGKOK, THAILAND

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Sixty dengue-3 (DEN-3) and fifty-three dengue-4 (DEN-4) virus isolates from children in Bangkok, Thailand, admitted with varying degree of dengue severity [dengue fever/dengue hemorrhagic fever/dengue shock syndrome (DF/DHF/DSS)] from 1974 to 2002 were selected to sequence the envelope (E) gene for identifying specific sequence patterns which may correlate with disease severity and for assessing potential trends in molecular evolution and epidemiology of circulating DEN-3 and -4 viruses within Bangkok. No specific sequence differences distinguished the DF and DHF/DSS for both DEN-3 and -4 was observed, suggesting that the E gene alone was not a determinant of disease severity. The phylogenetic trees showed that only genotype II of DEN-3 virus circulated in Bangkok during near past three decades and there is a clear separation within the large Thai group of viruses sampled from 1996 onwards being separated from the rest by a branch with 100% bootstrap support; and that the majority DEN-4 collected in the past 27 years comprised genotype I (47 cases), 5 of the 6 more recent isolates comprised a genotype never previously described. This newly discovered genotype was associated with DF (2 cases) and DHF/DSS (3 cases). The phylogenetic analysis revealed that the strains of DEN-3 virus prior to 1996 and DEN-4 viruses of genotype I beyond 1999 circulating in Bangkok appears to have become extinct and been replaced by new lineages that evolved locally, rather than having been introduced. These suggest that there have been a selective replacement of strain through time. One case of the genotype IIA for DEN-4 appears to have been introduced in 2000 from neighboring Malaysia or Indonesia where this genotype is known to circulate, but this genotype could not be sustained. It remains unclear and requires further study as to whether this replacement represents a selection event, so that strains differ