

Genetic evolution of human enteroviruses circulating in Thailand during 2006 to 2014

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Introduction

Hand, foot and mouth disease (HFMD) is an important epidemic disease in childhood in Asia-Pacific region, including China, Japan, Malaysia, Taiwan, Singapore and Thailand. The disease is caused by many enteroviruses, in particular enterovirus 71 (EV71) and coxsackievirus A16 (CA16). Emergence of new genotypes of EV71 have been reported from this region in the past decade including inter- and intratypic recombination between EV71 and CA16 genotypes in China. Based on VP1 nucleotide sequences, this study aimed to investigate the prevalence of EV71 and CA16 genotypes circulating in Thailand during 2006 to 2014. Full genome sequencing was also performed in 32 EV strains to explore for genetic recombination that might explain the larger areas and disease severity of some HFMD outbreaks.

Materials and Methods

RNA was extracted from virus suspensions and subjected to sequencing. Phylogenetic trees of VP1 and complete genome were constructed by neighbor-joining method with MEGA software (version 4.0). The reliability of the neighbor-joining tree was estimated by bootstrap analysis with 1000 pseudo-replicate datasets, and the bootstrap values of over 90% supporting each cluster are shown at the nodes. Homologous recombination was analyzed using Simplot software (version 3.5.1; Stuart Ray, Johns Hopkins University, Baltimore, MD, USA). Similarity plots and bootscan analyses were employed using the oldest EV71 strains (A, B5, C2, C4), CA16 strain G-10 and prototype CA16 as the reference strains.

Acknowledgement

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Results:

Phylogenetic analysis of 107 VP1 sequences of EV strains deposited in GenBank database showed that the strains circulating in Thailand from 2000 to 2014 belonged to EV71 genotypes B4, B5, C1, C2, C4a, C4b and C5; while almost of the CA16 strains belong to subgenotype B1a (Figure 1). Genotyping based on full genome sequences of 32 EV isolates yielded concordant result (Figure 2). An analysis for genetic recombination on the entire genome of 25 EV71 and 7 CA16 strains showed 6 recombinant viruses. One of 25 EV71-B5 recombined with 3C region of EV71-A, the only one C2 strain recombined with 2A region of EV71-C4, and 2 C4a strains recombined with 3D region of CA16. Interestingly, the two C4b strains were found to emerge from multiple recombination such that few positions in their P1 regions recombined with EV71-C2, and few positions in their P2 and P3 regions recombined with CA16.

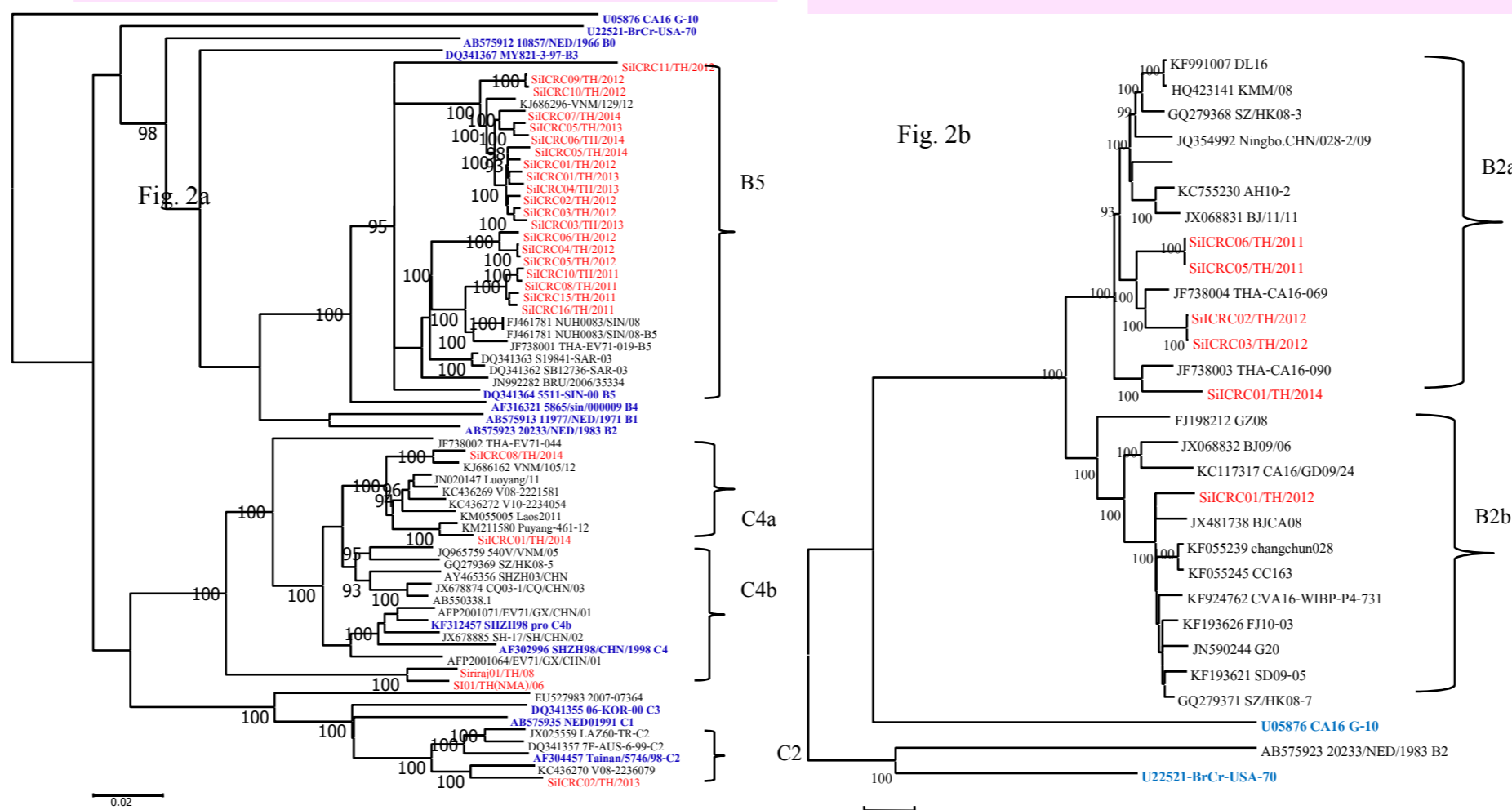


Figure 2. Phylogenetic analysis based on full genome sequences of: (a) EV71; and (b). The red colour denotes strains generated in this study; and blue colour denotes the reference strains.

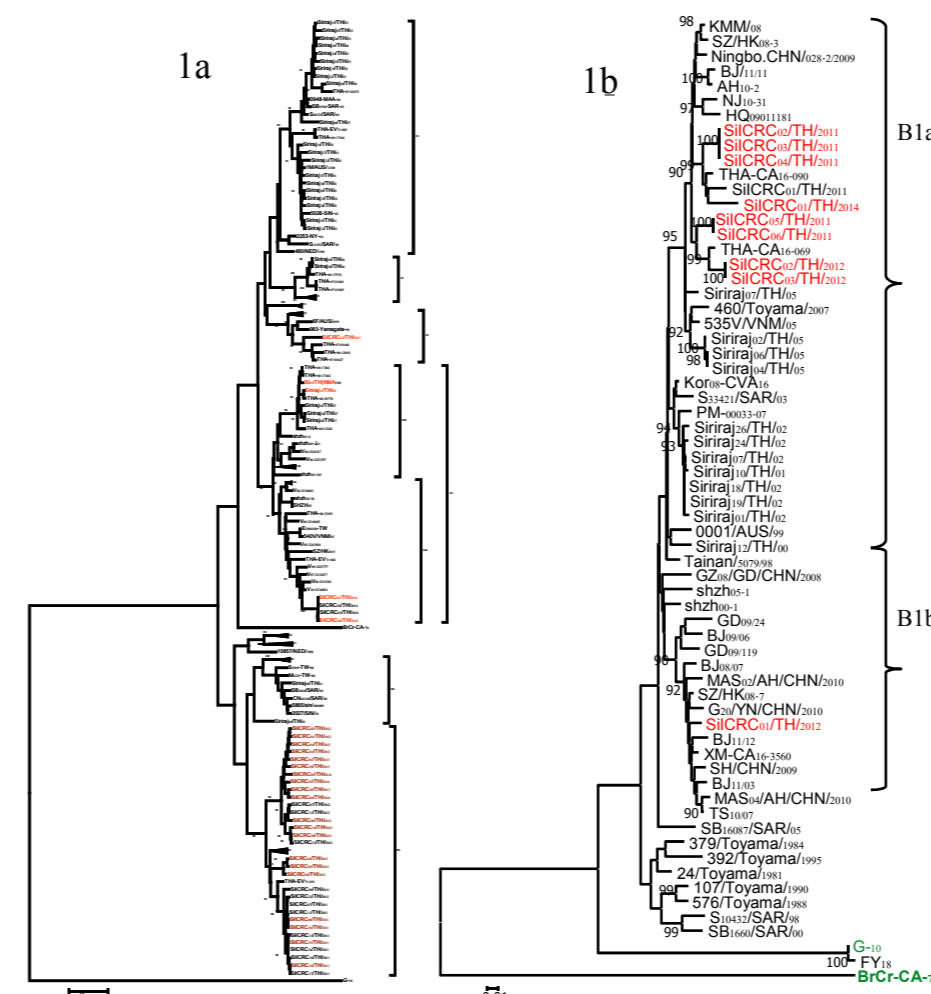


Figure 1. Phylogenetic analysis based on: (a) 839 bp of EV71-VP1; and (b) 891 bp of CA16 among 107 EV strains collected between 2000-2014. The red colour denoted strains with complete genome sequence.

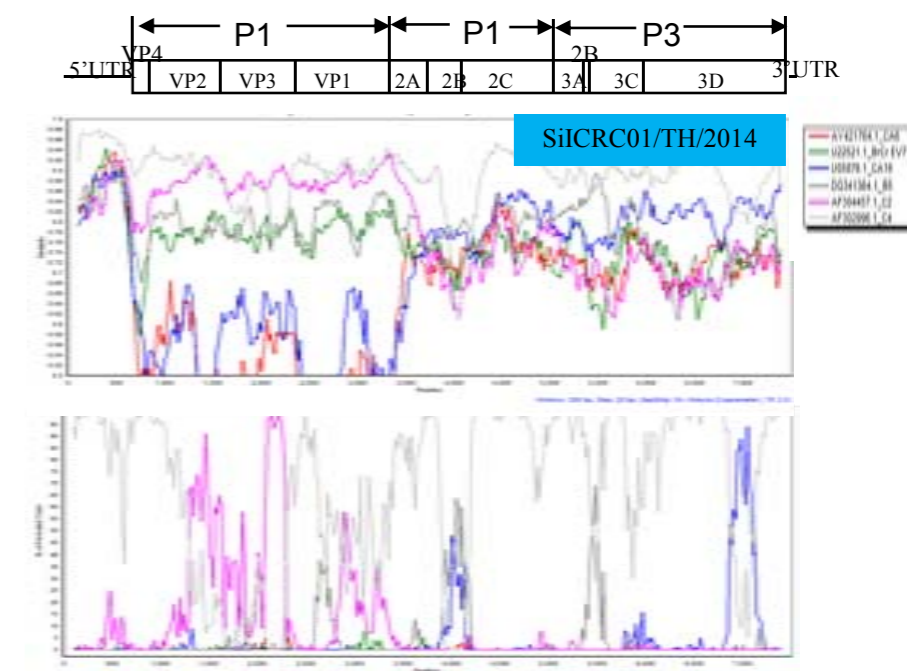


Figure 3. Simplot analyses shows recombination between a C4a genome and P1 region of EV71-C2 and 3D region of CA16.

Conclusion

Phylogenetic analysis demonstrated that EV71 subgenotypes B4, B5, C1, C2, C4a, C4b and C5 circulated in Thailand between 2000 and 2014. Nevertheless, almost of the genotype circulating after 2011 up to present belongs to subgenotype B5. The genotyping using either VP1 or full genome sequences yielded concordant results. Almost of CA16 strains belonged to subgenotype B1a. We demonstrated both inter-typic and intra-typic recombination in 6 of 25 EV71 strains: 1 B5, 1 C2 and 2 C4a and 2 C4b subgenotypes. It is noticeable that all 4 EV71 C4 strains in this study were recombinant viruses, in particular multiple recombination was observed in the two C4b strains. There was no genetic recombination observed in all 7 CA16 strains.