Impact of Enterovirus 71 Evolution on Virulence and Antigenicity

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Enterovirus 71 (EV71), as a neurotropic virus, is the major etiology of hand-foot-and-mouth disease and occasionally causes neurological involvement. EV71 has increased viral activity in the Asia-Pacific region with sporadic cases or outbreaks in south Asia, Europe, and even Africa. Until now, seven genotypes A to G were identified, and predominant genotypes of EV71 continued to change in worldwide especially in Asia. To evaluate the effects of evolutional substitutions on viral antigenicity and virulence, reverse genetics system was applied for further examination. In antigenic evolution, substitutions at VP1 capsid protein co-operatively contributed to diverse antigenic properties as well as receptor binding ability among EV71 genotypes, which potentially increased viral fitness in evolution. Complete genome analysis revealed intra- and inter-serotypic recombination occurrence in re-emergent genotypes C2, B4 and C4. Occurrence of EV71 recombination increases viral fitness through the processing of EV71 evolution, such as predominant genotype C2 strain in Taiwan retrieving $3D^{pol}$ 251I substitution through recombination between EV71 and coxackievirus A8. *In vitro* and *in vivo* evidences demonstrate this substitution increases viral temperature resistance and virulence. Another substitution identified in 5' untranslated region determined the viral virulence *in vivo*. C158 substitution plays a pivotal role of virulence determinant on virus translation *in vitro* and EV71 virulence *in vivo*. In addition, substitutions at capsid proteins not only contribute to antigenicity changes in global evolution but also virulence in the host. Mouse adapted strain acquired various mutations among which $VP2_{149M}$ and $VP1_{145E}$ synergistically increase viral virulence in mice through increasing virus binding and RNA release. Furthermore, dynamic mutations were found in human isolates as adapting environment changes from human peripheral tissues to central nerve system. EV71 acquired several substitutions including those at capsid proteins, which might be benefited for viral replication and fitness under selective pressure in the human host. In conclusion, not only capsid protein region but also non-structural protein regions play roles in EV71 fitness intra- or inter-host evolution. These novel viral substitutions retrieved from global evolution or in the host co-contribute to re-emergent EV71 and viral virulence.