Viral Diagnosis of Unknown Pathogens by Using Next-generation Sequencing: A Study of Human Parechovirus

Kuo-Chien Tsao[1*], Yu-Nong Gong[1], Guang-Wu Chen[2], Shu-Li Yang[1], Ching-Ju Lee[1], Shin-Ru Shih[2]

[1.] Chang Gung Memorial Hospital, [2.] Chang Gung University

Background/Objective

It is difficult to detect emerging viruses by traditional assay. Next-generation sequencing (NGS) technology is a promising strategy to identify emerging viruses in clinical samples. By using NGS, this study focused on viral detection and genetic characterization for cytopathic effect (CPE) positive isolates, which could not be identified by routine culture assay.

Method

16 CPE (+) clinical isolates with unidentified pathogens were collected from 2008 to 2012 by CGMH. All isolates were extracted for nucleic acid and analyzed for full genomic sequence by NGS with Illumina MiSeq. NGS reads were de novo assembled into contigs and further cataloged by BLASTN. We also conducted the genomic sequence using Sanger sequencing method to verify NGS data and mapped NGS reads to the PCR products to evaluate single-nucleotide polymorphisms (SNPs).

Result

We identified and confirmed 15 newly identified viruses from 16 isolates. The majority of viruses are human parechoviruses (HPeVs), which were genotyped as HPeV-1 (n=5), HPeV-3 (n=1), and HPeV-4 (n=1). The other cases are 6 human rhinoviruses, 1 adenovirus, and 1 rotavirus. We found 7 SNPs of HPeV occur in ORF region. Phylogenetic analysis of 6540 bp in ORF region for 7 HPeV strains showed from 79% to 96% of identities with published strains. Moreover, we found an unusual HPeV-3 strain has recombination with HPeV-4 at positions 2701 to 4821 (ORF region) by SimPlot software.

Conclusion

We demonstrated that NGS is capable of identifying unknown pathogens in clinical samples. The majority of the unknown pathogens in this study were identified as HPeVs. This emerging virus of HPeV-1 or -3 genotype has been reported to cause severe illness or meningitis. We also firstly reported two phenomena of genetic diversity including SNPs and recombination of HPeV strains in Taiwan. It is evident that epidemiology of HPeV in Taiwan needs to be further studied.