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12th World Congress on Virology and Infectious Diseases, March 18-19, 2020, Amsterdam, Netherlands-Multiplex single nucleotide polymorphism assay to investigate multiple genes as host genetic risk factors for Enterovirus A71 infection severity

Shyam Sundar Nandi
National Institute of Virology, India

Enterovirus A71 (EV-A71) causes a variety of diseases like Hand Foot and Mouth Disease (HFMD), Acute Flaccid Paralysis (AFP), meningitis and encephalitis with severe complications and death. It has caused large scale outbreaks of HFMD with severe complications in Malaysia, Taiwan, Singapore and Chine since 1997. In 2008, EV-A71 caused nearly half millions HFMD cases and killed 126 people in 2008 and more than 1.6 million cases in China and 509 deaths in 2011 in China. About 140,000 HFMD cases have been recorded in China in first quarter of 2018. In India EV-A71 has been isolated sporadically from AFP, encephalitis patients and apparently healthy children. However, no EV-A71 caused outbreaks of HFMD or AFP have been reported in India. The reasons for this have not been explored. Recent research reports have identified point mutations (SNPs) in a small number of host genes leading to susceptibility to severe EV-A71 infections. EV-A71 genetic susceptibility markers are located on variety of various genes. Therefore multiple region sequencing or deep sequencing (NGS) methods are wont to identify the SNPs at the precise sites. The objectives of the study were to design a rapid and simple method to detect the EV-A71 genetic susceptibility markers and to determine prevalence of the EV-A71 genetic susceptibility markers in Indian populations. Multiplexed single nucleotide polymorphism assays (two) were designed and developed to probe 15 SNPs in 12 different genes (IPR Submitted). The assay is useful for large scale screening of populations for EV-A71 infection outcome.

Intoduction: Individual susceptibility to communicable disease seems to be variable. Tumor necrosis factor α (TNF-α) promoter polymorphism is said to higher mortality rates and morbidity rates of sepsis. In addition, allelic variants are related to increased or decreased susceptibility to human immunodeficiency virus, hepatitis B , meningococcus, tuberculosis then on. We have also demonstrated pronounced elevations in inflammatory cytokines like TNF-α, interleukin 1β (IL-1β), and IL-6 in fatal EV71 cases. In addition, HLA-A33 was found to be related to EV71 susceptibility in Taiwanese patients. Zhang et al. proved that gene polymorphisms in myxovirus resistance A, an antiviral protein induced by type I interferons α and β (IFN-α and IFN-β) which will inhibit virus replication, are associated with susceptibility to EV71 infection during a Chinese population. Recently, several gene polymorphisms in cytokines and chemokines, like IFN-γ, IL-8, IL-10, IL-17F, C-C motif chemokine ligand 2, and C-X-C motif chemokine 10, are demonstrated to be associated with susceptibility to EV71 infection in Chinese patients. All this evidence suggests that host genetic factors may play a crucial role in susceptibility to EV71 infection and its clinical severity.

Viral receptors have an important role within the early steps of virus infection and are a primary determinant of host range and tissue tropism. Recently, two human transmembrane proteins, scavenger receptor class B, member 2 (SCARB2, also known as lysosomal integral membrane protein II or CD36b like-2) and P-selectin glycoprotein ligand-1 (PSGL-1) , were identified as functional receptors for EV71. SCARB2 may be a type III double-transmembrane

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protein with N- and C-terminal cytoplasmic tails and is found primarily in lysosomes and endosomes. SCARB2 is expressed ubiquitously in human tissues; therefore, it might be involved in systemic EV71 infections. In contrast, P-selectin glycoprotein ligand-1 (PSGL-1; CD162), a sialomucin membrane protein expressed on leukocytes, features a major role in early stages of inflammation and is expressed on dendritic cells of lymph nodes and macrophages in the intestinal mucosa, which is thought to be the primary site of EV71 entry and replication. Stable PSGL-1 expression allows EV71 entry and replication and therefore the development of cytopathic effects. In addition, annexin II (ANXA2), a member of the annexin family on the surface of endothelial cells, was found to be a cellular factor involved in the early stages of EV71 infection. The interaction was specific to EV71, and therefore the binding of EV71 to ANXA2 on the cell surface enhanced viral entry and infectivity, especially at a coffee infective dose. Several lines of evidence suggest that cellular receptors and adhesion molecules play critical roles in efficient EV71 infection and therefore the development of disease in humans, but the associations of these genetic polymorphisms have rarely been investigated clinically. In 2014, Cai et al. tested four single-nucleotide polymorphisms (SNPs) [2'-5'-oligoadenylate synthetase 1 (OAS1) rs10774671, PSGL-1 rs2228315, SCARB2 rs41284767 and IL28B rs12979860] in 333 HFMD samples and 163 control samples. They demonstrated that the OAS1 rs10774671 SNP GG genotype contributed to coxsackievirus A16 susceptibility and was related to the event of mild HFMD. However, there has been no study investigating genetic polymorphisms in SCARB2, PSGL-1, and ANXA2 focused on children with EV71 infections so far . In this study, we evaluated genetic polymorphisms in EV71 receptors (SCARB2, PSGL-1) and therefore the adhesion molecule (ANXA2) and correlated the results with EV71 susceptibility and clinical severity.

Methods: We enrolled laboratory-confirmed EV71 cases and healthy age- and gender-matched controls in Taiwan from 2000 to 2012. We detected genetic polymorphisms in SCARB2, PSGL-1, and ANXA2 and correlated the results with EV71 susceptibility and severity.

Statistical analysis: Hardy-Weinberg equilibrium was tested for each SNP to detect any deviation in the control samples. Allelic and genotypic frequencies of SCARB2, PSGL-1 and Annexin II SNPs were compared between EV71 cases and therefore the control group to spot polymorphisms related to susceptibility and between the mild group and therefore the severe group to seek out polymorphisms related to severe CNS complications by chi-square and Fisher's exact tests. Odds ratios and 95% confidence intervals were calculated using multiple logistic regression after adjustment for gender and age. Bonferroni's adjustment and permutation testing with 5000 permutations were used to correct for multiple comparisons. These analyses were performed with SAS software version 9.3 (SAS Institute, Cary, NC), as appropriate. Pairwise linkage disequilibrium index (measured as r2) was estimated by using Haploview software, version 4.2.

Result: Demographics, clinical severity and outcomes

A total of 599 patients with laboratory-confirmed enterovirus 71 from northern, western, and southern Taiwan and 98 normal control cases were included within the study.

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