論文の内容の要旨

論文題目 **MOLECULAR EPIDEMIOLOGY OF VIRUSES CAUSING ACUTE GASTROENTERITIS IN JAPANESE CHILDREN**

和訳 **日本の急性胃腸炎患児における下痢症ウイルスの分子疫学的研究**

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ABSTRACT

Acute gastroenteritis remains one of the most common illnesses of children worldwide. Viral pathogens, such as rotavirus, norovirus and sapovirus, are the most frequent causative agent for the most severe disease in children. The accumulated data revealed that the predominant genotype of diarrhea viruses in each epidemic season changed over time. Thus, the aim of this study was to follow up the molecular epidemiology of diarrhea viruses in Japanese children with acute gastroenteritis.

The study was carried out on 1,195 fecal specimens collected from children of acute gastroenteritis in six different locations of Japan: Sapporo, Saga, Tokyo, Osaka, Maizuru, and Shizuoka. The specimens were divided into two groups: (1) 1,018 collected from Sapporo, Saga, Tokyo, Osaka, and Maizuru during 2007-2009 and (2) 187 from Shizuoka during 2008-2009. The presence of diarrhea viruses was examined by multiplex PCR, semi-nested PCR, and DNA sequencing.

Diarrhea viruses were detected in 54.5% (652 of 1,195). Norovirus was recognized as the causative agent of illness in 26.6% of the children with acute gastroenteritis in five areas (Sapporo, Saga, Tokyo, Osaka, Maizuru) during 2007-2009. GII.4/2006b variant accounted for 100% of the detected GII.4 strains. However,

other genotypes, such as GII.1, GII.2, GII.3, GII.6, GII.7, GII.12, and GII.14, were also detected. Moreover, GII.6/GII.14 recombinant strains emerged, for the first time in Japan, and became the second leading strain (11.9%) after the GII.4/2006b variant in 2007-2008. Homology modeling of the GII.14 capsid protein showed several amino acid changes in the surface exposed P domain, suggesting that these changes may be induced by selective pressure, driving virus evolution.

Group A rotavirus was detected in 15.5% (156 out of 1,008). The infection in 2007-2008 (19.3%) was higher than those in 2008-2009 (12.1%). G1P[8] was the most prevalent (62.8%), followed by G3P[8] (21.8%), G9P[8] (14.7%), and G2P[4] (0.7%). The number of G3P[8] strains increased threefold from the former season (2006-2007) from 7.3% to 21.8%, whereas G2P[4] decreased from 11.4% to 0.7%. In the phylogenetic analysis, G3 rotaviruses were closely related to “the new variant G3” 5091 strain, which previously emerged in Japan and China. Furthermore, nucleotide sequence analysis of 33 P[8]-nontypeable strains revealed 5 nucleotide mismatches at the primer binding site. Based on previously reported (2003-2007) and current (2007-2009) data of rotavirus surveillance in the five areas of Japan, in Sapporo, Osaka, and Maizuru, G1P[8] and G3P[8] were detected at high frequencies, ranging from 47.2-57.7% and 31.7-47.4%, respectively. In Tokyo, G1P[8] (47.4%) was the predominant strain, followed by G9P[8] (20.6%), whereas in Saga, G3P[8] (38.9%) and G9P[8] (36.1%) were identified as the most dominant types. None of G9P[8] was detected in Sapporo. This study highlights the genetic diversity and the significance of rotavirus diarrhea in Japan.

Sapovirus was detected in 3.4% (37 of 1,008). GIV genogroup emerged as the predominant strain in 2007-2008. The change of sapovirus distribution continued in 2008-2009 with the appearance of GI/1 and the disappearance of the GIV strains. The

study also showed that the GIV strains had their polymerase gene of genogroup II, suggesting that the GIV strains isolated in 2007-2008 were intergenogroup recombinants (GII/GIV). This study is the first to report the emergence of this GII/GIV recombination in Japan.

In Shizuoka, norovirus accounted for 55.6% of all 187 samples tested, showing a relatively high detection rate. Of note, norovirus GII.6 infection in the present study was identified with a high prevalence rate, accounting for 40.4%, being the second most common genotype following GII.4 infection (53.8%). The GII.6 infection rapidly emerged as a leading genotype during a short period of two months (November and December). At least three distinct GII.6 subclusters (a-c) appeared in different part of the world during the past 19 years. The GII.6 strains in this study clustered together in a single branch in subcluster c. Alignment of the full-length capsid genes revealed that GII.6 strains identified here had amino acid differences, particular in the P2 subdomain up to 10.9-17.5% compared with that of subcluster a and b viruses. These results indicate that the GII.6 that emerged in Shizuoka is a new variant. Homology modeling of the GII.6 capsid domain demonstrates that significant amino acid variations are positioned on loops of P2 and P1 subdomains. This also supports the idea that a new variant may possibly emerge from the accumulation of mutations in the P2 subdomain through immune pressure.