論文の内容の要旨

論文題目　A study of norovirus in Japanese pediatric patients: epidemiology, genetic variation, and structural analysis of histo-blood group antigen binding specificity
（日本の小児患者におけるノロウイルスの研究：疫学、遺伝的多様性、組織血液型抗原との結合特異性の構造解析）

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Acute gastroenteritis is still a health burden in both developed and developing countries including Japan. Diarrheal viruses are considered to be the most significant enteropathogens of acute gastroenteritis. Accumulated surveillance data have shown that the predominant genotypes of diarrheal viruses in each epidemic season changes over time. Therefore, the molecular surveillance of diarrheal viruses circulating in Japanese population is needed.

In this study, 2,908 fecal specimens collected from pediatric outpatients presented with acute gastroenteritis in Hokkaido, Tokyo, Shizuoka, Kyoto, Osaka, and Saga from 2009 to 2014 were screened for several kinds of diarrheal viruses by using multiplex RT-PCR. The genotypes of diarrheal viruses were further classified by semi-nested PCR and sequence analysis. The surveillance revealed diarrheal viruses currently circulating in 72.1% of the patients. Norovirus was detected in 43.0% of patients whereas the prevalence of rotavirus, human parechovirus, enterovirus, adenovirus, sapovirus, astrovirus, and Aichi virus were 17.2%, 7.3%, 7.0%, 5.8%, 5.3%, 2.9%, and 0.1%, respectively. Norovirus was the most dominant agent causing gastroenteritis in Japan and detection rate was dramatically increased year by year. Co-infections of diarrheal viruses were observed with 14.4% of double infection and 1.1% of triple infection. Mixed viral infections were commonly found in Japanese outpatients and norovirus seems to play a major role in co-infections. Most of the children with viral diarrhea were younger than 3 years of age accounting for 72.2%. Norovirus and rotavirus were

detected throughout the year with a peak in cold and dry seasons, while other common diarrheal viruses were detected throughout the year without any specific season.

Interestingly, during the epidemiologic study of norovirus, a GII.4 new variant 2012 was found in Japanese population. From literature search, GII.4 has been responsible for the majority of outbreaks. It has been reported that epidemic outbreak occurred every 2 to 3 years, punctuated by the emergence of new variants with different antigenicity. From a total of 2,908 fecal specimens, 43.0% of norovirus positive samples with several genotypes were detected in this study. Norovirus GII.4 dominated over other genotypes (64.5%). The Den_Haag_2006b (36.9%) was detected as the predominant variant in co-circulation with New_Orleans_2009 (14.9%) until March 2012. Subsequently, they were displaced by Sydney_2012. The Sydney_2012 variant has been responsible for the majority of norovirus infections from 2012 until now. Although Sydney_2012 variant has a common ancestor with New_Orleans_2009 variant, analysis of P2 subdomain showed a high level of diversity compared with other predominant variants in four amino acid changes at the antigenic sites; epitope A (position 294 and 368), epitope D (position 393), and epitope E (position 413).

Analysis of norovirus circulating in 5-year surveillance in Japanese patients revealed a change of predominant variant of norovirus GII.4 in each epidemic season over time. It was found that most of sequence variations among GII.4 circulating variants mostly occur in P2 subdomain, including HBGA binding site. The change of amino acid residues in putative epitopes may affect the binding capacity with HBGA of the new variants. Therefore, HBGA recognition of new variants is needed to be elucidated. Within norovirus GII.4 predominant variants circulating in Japanese population, a panel of saliva and human gastric mucosa was used for determination of norovirus binding patterns. The Sydney_2012 showed the strong binding to human saliva samples and preparations of gastric mucosa from secretor individuals irrespective of thier ABO and Lewis phenotypes, but absence of clear binding to the same samples from Le^a and Le-negative non-secretor individuals. Along with previous studies on changes of binding specifities occured in GII.4 variants, it was suggested that Sydney_2012 acquired elevalted levels of binding to blood groups substances in secretor individuals but deleted levels of binding to those in non-secretor individuals, indicating the occurence of sequence substitutions in the HBGA binding site which was predicted to participate in the recognition of both α1,2- and α1,4-fucosylated glycan structures. The expression of α1,2-fucosylated glycan is crucial for susceptibility to infect with GII.4 Sydney_2012.

In conclusion, the surveillance revealed a wide variety of diarrheal viruses currently circulating in Japanese pediatric outpatients in a very high detection rate; norovirus was the most important pathogens detected

throughout Japan. Interestingly, a new variant Sydney_2012 emerged and showed a high diversity in either antibody-blockade epitope or HBGA binding site. The change of amino acid may lead to norovirus escape from the existing herd immunity and also change their target population through HBGA binding alteration. The data obtained from my studies are valuable to analyze the relationships among viruses that cause acute gastroenteritis in Japanese population, and to compile the overall picture of the diarrheal virus groups that associated with acute gastroenteritis in pediatric patients in Japan.