Molecular and Epidemiological Trend of Sapovirus, and Astrovirus Infection in Japan

Human sapoviruses (SVs), formerly known as Sapporo-like viruses, are single-stranded positive-sense RNA viruses in the family Caliciviridae, which have been found to cause outbreaks of gastroenteritis in children’s hospitals and mental health care facilities [1, 2]. SVs can be divided into five genogroups (GI to GV), among which, GI, GII, GIV and GV are identified within humans [1]. Human astroviruses (HAstVs) are non-enveloped, single-stranded positive -sense RNA viruses in the family Astroviridae, which may be the second most common cause of viral gastroenteritis in young children [3, 4].

A total of 628 fecal specimens were collected from infants and children with acute gastroenteritis in five different places (Maizuru, Tokyo, Sapporo, Saga and Osaka) in Japan during July 2006 to June 2007. The viral genome was extracted applying a QIAGEN® kit (QIAamp Viral RNA kit; QIAGEN®, Hilden, Germany). Using Polymerase chain reaction (PCR) with specific primers resulted in the identification of AstV and SV [5]. The nucleotide sequences of PCR products positive for SV and HAstV were determined with the Big-Dye terminator cycle sequencing kit and an ABI Prism 310 Genetic Analyzer (Applied Biosystems Inc., Foster City, CA, USA). Phylogenetic and molecular evolutionary analyses were conducted using the MEGA version 3.2 software package, AZ, USA [6].

From 628 fecal specimens tested, 3.8% (24 of 628) were positive for SV, while HAstV was detected in 2.4% (15 of 628). In the present study, SV infection was apparently confined within a period of 6 months (November 2006 to April 2007) and HAstV infection apparently lasted 5 months (January 2007 to May 2007). Most of SV- and HAstV-infected patients were in the 1- to 24-month age group. The distribution of gender in positive cases was 55% in males and 45% in females.

All the SV and HAstV amplicons were characterized for serotypes and genetic relationship with the reference strains based on their capsid regions. Their partial nucleotide sequences were compared with each other as well as with reference SV and HAstV strains available in the DDBJ DNA/GenBank database by BLAST.

SVs detected in this study were clustered into only one distinct genogroup I (Fig. 1A). SV genogroup I clustered into two genotypes (GI/1 and GI/2). SV GI/1 was predominant, followed by GI/2 and accounted for 92% (22 of 24) and 8% (2 of 24), respectively. Analysis of nucleotide and amino acid sequences revealed that SVGI/1 was closely related to Lyon30388/98, Plymouth/92 and Manchester/93 strains. HAstV detected in this study belonged to only genotype (HAstV-1d) (Fig. 1B). CLUSTAL X indicated that these SVs and HAstVs were very similar at the amino acid level as well as at the nucleotide level.

The sequences of SVs and HAstVs detected in the study were submitted to GenBank and have been assigned accession numbers FJ823057-FJ823096, respectively.

The results of this study highlight the impact of SVs and HAstVs in diarrheal diseases among children in five different cities in Japan. In conclusion, our findings suggest that SVs and HAstVs are important enteric viruses coexisting in Japanese infants and children. The epidemiology of HAstV serotypes is only beginning to be addressed in Japan.

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Fig. 1. Phylogenetic tree of nucleotide sequences of Japanese SV and AstV. (A) Neighbor-joining phylogenetic tree based on nucleotide sequences of capsid region of SV. (B) Neighbor-joining phylogenetic tree based on nucleotide sequences of capsid region of HAstV. The numbers in the branches indicate the bootstrap values. Reference strains of SV and HAstV were selected from DDBJ/GenBank under the accession number indicated in bold. Japanese SVs and HAstVs are highlighted in italics. The scale indicates nucleotide substitutions per position. Reference SV and HAstV strains and their accession numbers used in this study are as follows: SV strains: Mexl14917/00 (AF435813), Plymouth/92 (X86559), Lyon30388/98 (AJ251991), Lyon/598/97/F (AJ271056), Chiba-000766F/2000 (AJ412808), Chiba-000764F/2000 (AJ412807), 4408/Maizuru/Jp (AB180209), PEC (AF182760), London/92 (U95645), Mex340/90(AF435812), Cruiseship/00 (AY289804), Hou7-1181/90 (AF435814), Arg39 (AY289803), Stockholm/97 (AF194182), Houston/90 (U95644), Parkville/94 (U73124), Houston/86(U95643), Sapporo/82(U65427), Manchester/93 (X86560) and Bristol98 (AJ249939). HAstV strains: Porcine AstV (AB037272), Dhaka-ast-173 (EU333900), Dhaka-ast-317 (EU333901), AF211956, AY324858, AY720892, EU022556, EU022555, HAstV1 (L23513), HAstV2 (L13745), HAstV6 (L38507) and HAstV4 (L38506).
Fig. 1. Continued.

References

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