Molecular characterization of partial-open reading frames 1a and 2 of the human astroviruses in South Korea

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Abstract

Human astroviruses (HAstVs) are among the major causes of gastroenteritis in South Korea. In this study, the partial regions of the open reading frame (ORF) 1a and ORF2 genes of HAstVs from gastroenteritis patients in nine hospitals were sequenced, and the molecular characterization of the viruses was revealed. 89 partial nucleotide sequences of ORF1a and 88 partial nucleotide sequences of ORF2 were amplified from 120 stool specimens. Phylogenetic analysis showed that most of the nucleotide sequences of ORF1a and ORF2 were grouped with HAstV type 1 but had evolutionary genetic distance compared with the reference sequences, such as the HAstV-1 prototype, Dresden strain, and Oxford strain. According to the phylogenetic analysis, some nucleotide sequences including SE0506041, SE0506043, and SE0506058, showed the discrepancy of the genotypes, but there was no proof of recombination among the HAstV types. In conclusion, this study showed that the dominant HAstV isolated from the Seoul metropolitan area in 2004-2005 was HAstV type 1, and that Korean HAstV-1 had the genetic distance in evolution compared with the reference sequences of HAstVs. Lots of nucleotide sequences of the ORF1a and ORF2 genes of HAstV will be useful for studying for the control and prevention of HAstV gastroenteritis in South Korea.

Findings

Astroviruses (AstVs), belong to the Astroviridae family, are non-enveloped, single-stranded, and positive-sense RNA viruses [1]. Their genomes have both 5’ and 3’ non-translated regions, and contain three open reading frames (ORFs), denoted as ORF1a, ORF1b, and ORF2, which encode a serine protease, an RNA-dependent RNA polymerase, and a structural protein, respectively [1,2]. AstVs are known to infect humans as well as a variety of mammalian and avian species [3-5]. In humans, eight serotypes have been described, which have been associated with up to ~10% sporadic cases of nonbacterial diarrhea in children [6-10] and 0.5-15% outbreaks [11-13].

Walter et al. (2001) analyzed the gene of AstVs and found that the ORF2 region belonged to human AstV (HAstv)-5 whereas the ORF1b region belonged to HAstV-3, and that recombination occurred between the HAstV types [14]. Besides, in some other studies, recombination was found to occur between mammastroviruses and HAstV [15]. Such recombination may result in a new epidemic HAstV because it is similar to antigen drift of influenza viruses [16-19]. Therefore, characterization of HAstVs genome is important to understand the recombination between human and mammalian AstVs, the origin of the viruses, and their molecular evolution, as well as the phylogenetic relationship among the HAstV genotypes. For this purpose, there is a need to obtain more complete genome sequences of HAstV. The complete genome sequences of seven genotypes (HAstV-1, 2, 3, 4, 5, 6, and 8) and the HAstV-7 ORF2 sequence are available [18,20-23]. In this study, the partial nucleotide sequences of ORF1a and ORF2 of HAstVs, responsible for sporadic gastroenteritis in South Korea, were obtained, and their molecular characteristics were investigated.

From 2004 to 2005, stool specimens of patients suspected to have acute gastroenteritis were provided by nine hospitals located in the Seoul metropolitan area. 1 g of a stool specimen was added into 9 mL phosphate-buffered saline solution, and three or four 3-mm
Table 1 Primers used for the detection of human astroviruses

| Primers   | Position* | Sequence (5′→3′) | Size (bp) | References |
|-----------|-----------|------------------|-----------|------------|
| Mon340    | 1182-1203 | CGTCAATATTGTGTTCATCACT | 289       | [26]       |
| Mon348    | 1450-1470 | ACATGTGCTGTGTTACTAG |           |            |
| Mon269    | 4526-4545 | CAACCTAGGAAACAGGGTTG | 449      | [24]       |
| Mon270    | 4955-4974 | TCATATGATCATGTCATG   |           |            |

*The nucleotide numbering is based on the sequences of human astrovirus type 1 (GenBank accession number: Z22771).
closer to HAstV-8 in the analysis of ORF2, and SE0405158 and SE0506064 were found to be HAstV-1, which was in between the HAstV-1 prototype and the Dresden strain.

Studies on the relation between the serotypes of HAstVs based on the base sequence of 300 nucleotides showed that there was a difference in genotypes between three ORFs [26]. Belliot et al. (1997) suggested that HAstV can be grouped into two genogroups, HAstV-1~-5 and HAstV-6~-7, based on ORF1a [25] and this was later supported by other studies [27,28]. In this study, all the references and isolates, excluding SE0504004, SE0510110, and SE0412021, also formed a large genogroup in the analysis of the partial ORF1a (Fig. 1). In contrast, Belliot et al. (1997) reported that such genotype was not found in their analysis of ORF1b and ORF2, and that HAstV could be classified into four clusters (HAstV-1, HAstV-6 and 2; HAstV-3, 4, and 8; and HAstV-5 and 7) in the analysis of the ORF2 partial sequence [26]. It has been reported, however, that in the analysis of a phylogenetic tree based on the full ORF2 amino acid sequence, three clusters (HastV-1, 7, and 3; HAstV-5 and 6; and HAstV-4 and 8) were found, and HAstV-2 was closer to the third cluster than to the other clusters [29]. In the analysis of the ORF2 partial sequences in this study, HAstV was classified into four clusters (HAstV-1, 2, 3, and 4).
clusters, as in the study by Belliot (1997) [26]. In the analysis of a phylogenetic tree based on the whole ORF2 sequence, however, HAstV could be classified into only three clusters, as in the study by Wang et al. (2001) [29]. Even if the genotype is well related with the serotype according to the partial sequence, a phylogenetic tree based on such relation may reflect a wrong phylogeny. Thus, it is considered that the evolutionary phylogeny of an AstV can be more accurately identified by a phylogenetic tree based on the whole base sequence of each gene. Although some studies asserted that the genotype discrepancy between the HAstV genes that occurred in their studies was due to the genetic recombinations between different serotypes [14,26], no proof of such recombination was found in any isolate that showed a discrepancy in genotypes. Although the mechanism of HAstVs’ variations is not yet clear, the genetic variations by recombinations among HAsVs’ types may evoke the appearance of new epidemic HAstVs, such as the influenza viruses, by antigenic drift.

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Figure 2 Phylogenetic tree based on the partial sequences of open reading frame 2 amplified by the Mon269/270 primer pair. The outgroup, the partial-open reading frame 2 nucleotide sequence of the sheep astrovirus, was selected from the nucleotide sequence of sheep astrovirus (GenBank accession number, Y15937).
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