

SHORT REPORT

Open Access

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

Jae in Lee^{1†}, Gyu-Cheol Lee^{2†}, Young hee Oh¹, Young ki Lee³, Min young Kim¹, Chan Hee Lee^{4*}

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 mamastroviruses 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

* Correspondence: chlee@cbu.ac.kr

† Contributed equally

⁴Department of Microbiology, College of Natural Sciences, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea
Full list of author information is available at the end of the article

glass beads were added. The mixture was vigorously shaken via vortexing and was centrifuged at 4°C and 3000 rpm for 30 min. The 200 µL of 10% stool suspension was used for extracting RNA via the Tri-reagent method [24] and the extracted viral RNA was used for RT-PCR. The Mon340 and Mon348 primers were used for the amplification of the ORF1a region, and the Mon269 and Mon270 primers for the amplification of ORF2 (Table 1). For the synthesis of cDNA, 8 µL dNTP, 5 µL 5X buffer, 2.5 µL 10 pmole Mon348 or Mon270, 0.5 µL RNase inhibitor (Promega, Madison, WI), 0.5 µL MMLV reverse transcriptase (Promega), and 3.5 µL diethyl pyrocarbonate (DEPC) treated water and 5 µL RNA extract were added. The reaction conditions for the synthesis of cDNA were 42°C/60 min, 95°C/5 min, and 4°C soaking. For PCR, synthesized 5 µL cDNA was added to 6 µL dNTP, 5 µL 10× PCR buffer, each of the 2.5 µL 10 pmole primers, 0.5 µL exTaq polymerase (TaKaRa, Otsu, Shiga, Japan), and 28.5 µL DEPC treated water. The PCR conditions for ORF1a were 94°C/3 min, 94°C/30 sec, 50°C/20 sec, and 72°C/30 sec, 30 cycles, 72°C/5 min, and for ORF2, 94°C/3 min, 94°C/30 sec, 50°C/30 sec, and 72°C/1 min, 35 cycles, and 72°C/5 min. The amplified gene products were observed in 1.2% agarose gel. The PCR products were purified using a PCR purification kit (SolGent Co., Daejeon, South Korea) and were sequenced using ABI 3730XL DNA Analyzer (Applied Biosystems, Carlsbad, CA).

Multiple alignment and phylogenetic analysis were conducted using the ClustalX program and the PHYLIP package. For the distance matrix between the DNA sequences, the Dnadist program was used, and a phylogenetic tree was constructed using the neighbor-joining (NJ) method in the Neighbor program.

In 89 of the 120 AstV specimens isolated from 2004 to 2005, the nucleotide sequence of the partial ORF1a amplicon amplified. The phylogenetic analysis results showed the nucleotide sequence of most of the partial ORF1as to be HAstV-1, and three isolates (SE0512016, SE0410092, and SE0512003) were grouped with the HAstV-1 Dresden strain (Fig. 1). The 73 HAstV-1 isolates were diverged earlier from sheep AstV, an out-group, and were distant from the group to which the

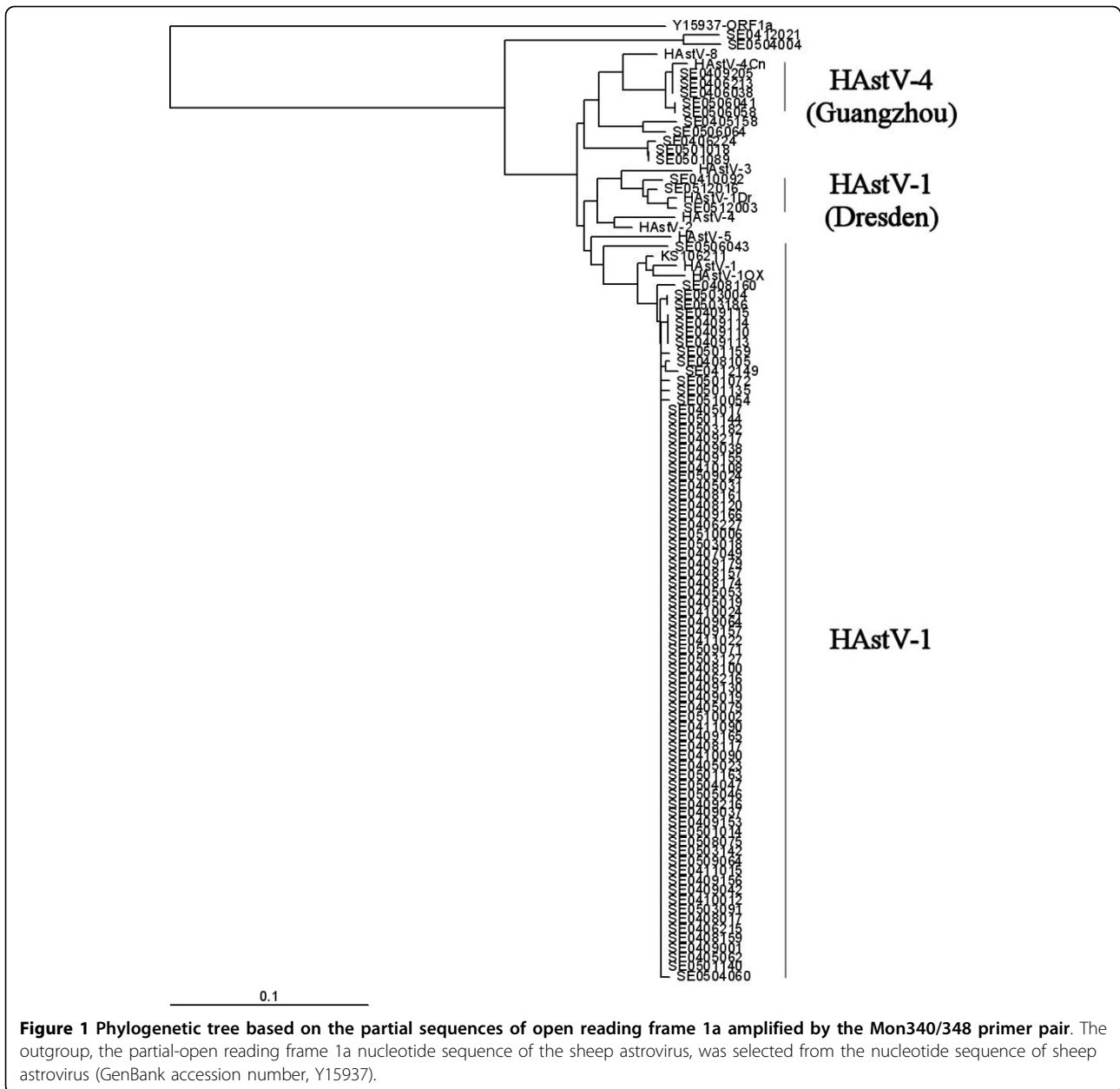
HAstV-1 prototype belonged, whereas the HAstV-1 prototype and the Oxford and KS106211 strains that were isolated in South Korea were grouped together (AF361036) [25] (Fig. 1). SE0506043 was placed between HAstV-1 and HAstV-5, and the phylogenetic branch diverged from HAstV-5 to the phylogenies of HAstV-2,4 (Goiania strain) and 3,1 (Dresden strain) and to the phylogeny of HAstV-8,4 (Guangzhou strain). SE0406224, SE0501018, SE0501089, SE0405158, and SE0506064 isolates diverged earlier and grouped together, keeping a distance from all the ten references. SE0412021 and SE0504004 were distant from all the references for which the nucleotide sequence of ORF1a was available (Fig. 1). SE0406038, SE0406213, SE0409205, SE0506041, and SE0506058 grouped with the HAstV-4 Guangzhou strain (Fig. 1). In case of ORF2, the 88 nucleotide sequences were analyzed and the phylogenetic tree was constructed. The HAstV-1 prototype, the Oxford strain, and the Dresden strain clustered, unlike in the case of ORF1, and the HAstV-4 Goiania, Dresden, and Guangzhou strains clustered in the same group (Fig. 2). In the nucleotide sequence of the ORF2 of the sheep AstV, which was closest to HAstV among the animal AstVs, HAstV-4 and 8 diverged earliest, followed by HAstV-3, 5, 7, 2, and 6 (Fig. 2). 75 partial ORF2 sequences were grouped in the place that diverged earlier than the HAstV-1 prototype, and the SE0405158 and SE0506064 isolates were in between the HAstV-1 Dresden isolates and the Oxford isolate whereas SE0512003, SE0512016, and SE0410092 belonged to the HAstV-1 Dresden isolate (Fig. 2). SE0501018, SE0501089, and SE0406224 grouped in the HAstV-8 reference, and SE0406038 and SE0406213 grouped in the HAstV-4 Guangzhou strain (Fig. 2). The SE0504004, SE0412021, and SE0501110 isolates grouped in HAstV-6, and no isolates grouped in HAstV-2, 3, 5, and 7.

For most of the isolates, all the nucleotide sequences of ORF1a and ORF2 belonged to HAstV-1 and were slightly distant from the references (the prototype and the Dresden and Oxford strains). The isolates, however, grouped together, with a high similarity between them. This indicates that the AstVs circulating in the Seoul metropolitan area were HAstV-1 and had the difference evolutionary course from the HAstV-1 circulating abroad. In several isolates, the genotypes of ORF1a and ORF2 did not coincide with each other. SE0506041 and SE0506058, however, which grouped in the HAstV-4 Guangzhou isolates in the analysis of the partial ORF1a, grouped in HAstV-1 in the analysis of the partial ORF2. In addition, SE0506043, which was in between HAstV-1 and HAstV-5 in the analysis of ORF1a, was closer to the prototype than HAstV-1 was. SE0406224, SE050018, and SE0501089, which were in between HAstV-8 and HAstV-1 in the analysis of ORF1a, grouped in places

Table 1 Primers used for the detection of human astroviruses

Primers	Position*	Sequence (5'→3')	Size (bp)	References
Mon340	1182-1203	CGTCATTATTTGTTGCATACT	289	[26]
Mon348	1450-1470	ACATGTGCTGCTGTTACTATG		
Mon269	4526-4545	CAACTCAGGAAACAGGGTGT	449	[24]
Mon270	4955-4974	TCAGATGCATTGTCATTGGT		

The nucleotide numbering is based on the sequences of human astrovirus type 1 (GenBank accession number: Z25771).



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

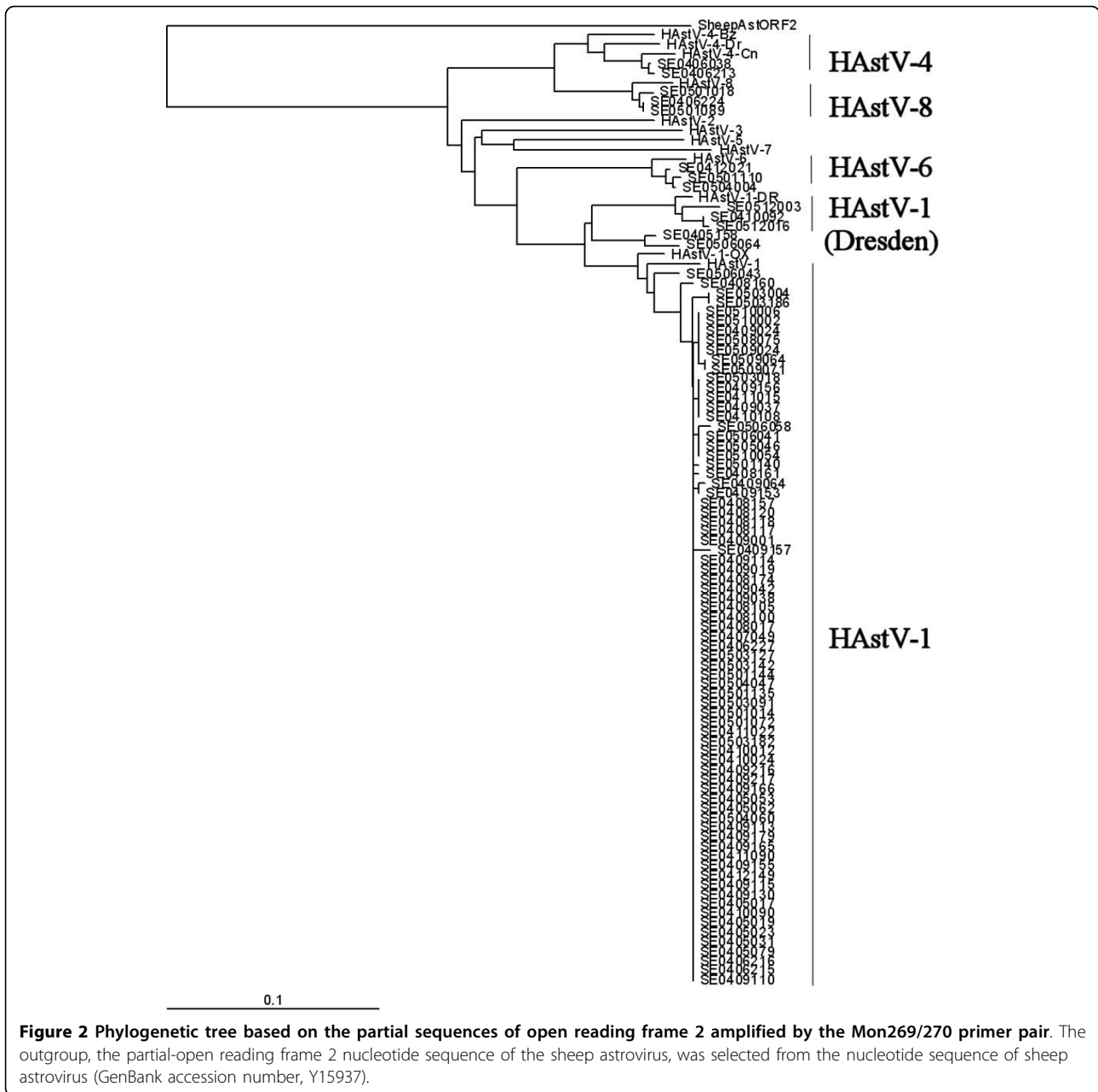


Figure 2 Phylogenetic tree based on the partial sequences of open reading frame 2 amplified by the Mon269/270 primer pair. The outgroup, the partial-open reading frame 2 nucleotide sequence of the sheep astrovirus, was selected from the nucleotide sequence of sheep astrovirus (GenBank accession number,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

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 HAstVs' types may evoke the appearance of new epidemic HAstVs, such as the influenza viruses, by antigenic drift.

Acknowledgements

This work was supported by the Korea Research Foundation Grant funded by the Korean Government (Ministry of Education, Science and Technology).

(The Regional Research Universities Program/Chungbuk BIT Research-Oriented University Consortium).

Author details

¹Seoul Metropolitan Research Institute of Public Health & Environment, Gwacheon, Gyeonggi 427-070, Republic of Korea. ²Water Analysis and Research Center, K-Water, Daejeon 306-711, Republic of Korea. ³Department of Public Health, Graduate School of Public Health & Social Welfare, Dankook University, Cheonan 330-716, Republic of Korea. ⁴Department of Microbiology, College of Natural Sciences, Chungbuk National University, Cheongju, Chungbuk 361-763, Republic of Korea.

Authors' contributions

JIL, MYK and CHL conceived this study. JIL, YHO and YKL designed and conducted the experiments. JIL and GCL analyzed the sequence data and carried out the molecular phylogenetic analysis. JIL, GCL and CHL wrote the manuscript. All authors read and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Received: 10 August 2010 Accepted: 10 September 2010

Published: 10 September 2010

References

- Mendez E, Arias CF: **Astroviruses**. In *Fields Virology*. Edited by: Knipe DM, Howley PM. Philadelphia: Lippincott Williams, 5 2007:981-1000.
- Finkbeiner SR, Kirkwood CD, Wang D: **Complete genome sequence of a highly divergent astrovirus isolated from a child with acute diarrhea**. *Virology* 2008, **5**:117.
- Chu DK, Poon LL, Guan Y, Peiris JS: **Novel astroviruses in insectivorous bats**. *J Virol* 2008, **82**:9107-9114.
- Koci MD, Schultz-Cherry S: **Avian astroviruses**. *Avian Pathol* 2002, **31**:213-227.
- Toffan A, Jonassen CM, De Battisti C, Schiavon E, Kofstad T, Capua I, Cattoli G: **Genetic characterization of a new astrovirus detected in dogs suffering from diarrhea**. *Vet Microbiol* 2009, **139**:147-152.
- Caracciolo S, Minini C, Colombrita D, Foresti I, Avolio M, Tosti G, Fiorentini S, Caruso A: **Detection of sporadic cases of Norovirus infection in hospitalized children in Italy**. *New Microbiol* 2007, **30**:49-52.
- Glass RI, Noel J, Mitchell D, Herrmann JE, Blacklow NR, Pickering LK, Dennehy P, Ruiz-Palacios G, de Guerrero ML, Monroe SS: **The changing epidemiology of astrovirus-associated gastroenteritis: a review**. *Arch Virol Suppl* 1996, **12**:287-300.
- Kirkwood CD, Clark R, Bogdanovic-Sakran N, Bishop RF: **A 5-year study of the prevalence and genetic diversity of human caliciviruses associated with sporadic cases of acute gastroenteritis in young children admitted to hospital in Melbourne, Australia (1998-2002)**. *J Med Virol* 2005, **77**:96-101.
- Klein EJ, Boster DR, Stapp JR, Wells JG, Qin X, Clausen CR, Swerdlow DL, Braden CR, Tarr PI: **Diarrhea Etiology in a Children's Hospital Emergency Department: A Prospective Cohort Study**. *Clin Infect Dis* 2006, **43**:807-813.
- Soares CC, Maciel de Albuquerque MC, Maranhao AG, Rocha LN, Ramirez ML, Benati FJ, Timenetsky Mdo C, Santos N: **Astrovirus detection in sporadic cases of diarrhea among hospitalized and non-hospitalized children in Rio De Janeiro, Brazil, from 1998 to 2004**. *J Med Virol* 2008, **80**:113-117.
- Akihara S, Phan TG, Nguyen TA, Hansman G, Okitsu S, Ushijima H: **Existence of multiple outbreaks of viral gastroenteritis among infants in a day care center in Japan**. *Arch Virol* 2005, **150**:2061-2075.
- Lyman WH, Walsh JF, Kotch JB, Weber DJ, Gunn E, Vinje J: **Prospective study of etiologic agents of acute gastroenteritis outbreaks in child care centers**. *J Pediatr* 2009, **154**:253-257.
- Svraka S, Duizer E, Vennema H, de Bruin E, van der Veer B, Dorresteyn B, Koopmans M: **Etiological role of viruses in outbreaks of acute gastroenteritis in The Netherlands from 1994 through 2005**. *J Clin Microbiol* 2007, **45**:1389-1394.
- Walter JE, Briggs J, Guerrero ML, Matson DO, Pickering LK, Ruiz-Palacios R, Berke T, Mitchell DK: **Molecular Characterization of a Novel Recombinant Strain of Human Astrovirus Associated with Gastroenteritis in Children**. *Arch Virol* 2001, **146**:2357-2367.
- Rivera R, Nollens HH, Venn-Watson S, Gulland FM, Wellehan JF Jr: **Characterization of phylogenetically diverse astroviruses of marine mammals**. *J Gen Virol* 2010, **91**:166-173.
- Blackburne BP, Hay AJ, Goldstein RA: **Changing selective pressure during antigenic changes in human influenza H3**. *PLoS Pathog* 2008, **4**:e1000058.
- Guo L, Gonzalez R, Wang W, Li Y, Paranhos-Baccalà G, Vernet G, Wang J: **Complete genome sequence of human astrovirus genotype 6**. *Virology* 2010, **7**:29.
- Shen J, Ma J, Wang Q: **Evolutionary trends of A(H1N1) influenza virus hemagglutinin since 1918**. *PLoS One* 2009, **4**:e7789.
- Tu ET, Bull RA, Greening GE, Hewitt J, Lyon MJ, Marshall JA, McIver CJ, Rawlinson WD, White PA: **Epidemics of gastroenteritis during 2006 were associated with the spread of norovirus GII.4 variants 2006a and 2006b**. *Clin Infect Dis* 2008, **46**:413-420.
- Jiang B, Monroe SS, Koonin EV, Stine SE, Glass RI: **RNA sequence of astrovirus: distinctive genomic organization and a putative retrovirus-like ribosomal frameshifting signal that directs the viral replicase synthesis**. *Proc Natl Acad Sci USA* 1993, **90**:10539-10543.
- Lewis TL, Greenberg HB, Herrmann JE, Smith LS, Matsui SM: **Analysis of astrovirus serotype 1 RNA, identification of the viral RNA-dependent RNA polymerase motif, and expression of a viral structural protein**. *J Virol* 1994, **68**:77-83.
- Oh D, Schreiber E: **Molecular characterization of human astroviruses in Germany**. *Arch Virol* 2001, **146**:443-455.
- Silva PA, Cardoso DD, Schreiber E: **Molecular characterization of human astroviruses isolated in Brazil, including the complete sequences of astrovirus genotypes 4 and 5**. *Arch Virol* 2006, **151**:1405-1417.
- Noel JS, Lee TW, Kurtz JB, Glass RI, Monroe SS: **Typing of human astroviruses from clinical isolates by enzyme immunoassay and nucleotide sequencing**. *J Clin Microbiol* 1995, **33**:797-801.
- Kang YH, Park YK, Ahn JB, Yeun JD, Jee YM: **Identification of human astrovirus infections from stool samples with diarrhea in Korea**. *Arch Virol* 2002, **147**:1821-1827.
- Belliot G, Laveran H, Monroe SS: **Detection and genetic differentiation of human astroviruses, phylogenetic grouping varies by coding region**. *Arch Virol* 1997, **142**:1323-1334.
- Gabbay YB, Linhares AC, Cavalcante-Pepino EL, Nakamura LS, Oliveira DS, da Silva LD, Mascarenhas JD, Oliveira CS, Monteiro TA, Leite JP: **Prevalence of human astrovirus genotypes associated with acute gastroenteritis among children in Belém, Brazil**. *J Med Virol* 2007, **79**:530-538.
- Méndez-Toss M, Griffin DD, Calva J, Contreras JF, Puerto FI, Mota F, Guiscafré H, Cedillo R, Muñoz O, Herrera I, López S, Arias CF: **Prevalence and genetic diversity of human astroviruses in Mexican children with symptomatic and asymptomatic infections**. *J Clin Microbiol* 2004, **42**:151-157.
- Wang QH, Kakizawa J, Wen LY, Shimizu M, Nishio O, Fang ZY, Ushijima H: **Genetic analysis of the capsid region of astroviruses**. *J Med Virol* 2001, **64**:245-255.

doi:10.1186/1743-422X-7-221

Cite this article as: Lee *et al.*: Molecular characterization of partial-open reading frames 1a and 2 of the human astroviruses in South Korea. *Virology Journal* 2010 **7**:221.

Submit your next manuscript to BioMed Central and take full advantage of:

- Convenient online submission
- Thorough peer review
- No space constraints or color figure charges
- Immediate publication on acceptance
- Inclusion in PubMed, CAS, Scopus and Google Scholar
- Research which is freely available for redistribution

Submit your manuscript at
www.biomedcentral.com/submit

