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# Evidence of a Recombinant Wild-Type Human Astrovirus Strain from a Kenyan Child with Gastroenteritis▽

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**A human astrovirus (HAstV) strain from Kenya was characterized by nucleotide sequence analysis. Sequences from open reading frame 1a (ORF1a) clustered with genotype 6/7, those from ORF1b clustered with genotype 3, and those from ORF2 clustered with genotype 2. A recombination point in the ORF1b-ORF2 junction was identified, with a second possible recombination point within the ORF1a region.**

Human astroviruses (HAstVs), in the genus *Mamastrovirus* of the family *Astroviridae*, have a single-stranded positive-sense RNA genome of approximately 6.4 kb in length comprised of three open reading frames (ORFs) (16, 19). ORF1a and ORF1b, located at the 5′ end of the genome, are conserved among astroviruses (AstVs) and encode the nonstructural proteins serine protease and RNA-dependent RNA polymerase, respectively. ORF2, located at the 3′ end of the genome, is highly variable and encodes the capsid proteins (16, 24). Eight HAstV serotypes have been defined by immunology-based assays (16, 19, 21). Based on nucleotide sequence analysis of a partial region of the 3′ or 5′ end of ORF2, eight HAstV genotypes have been described, with correlation between antigenic serotypes and genotypes (1, 18, 21, 27). An investigation to genotype HAstVs detected in diarrheal stool specimens, by commercial immunoassay and/or reverse transcriptase PCR (RT-PCR), from Kenyan pediatric patients revealed discordant genotyping results for one of the strains, NK180. The aim of this investigation was to analyze, by nucleotide sequence analysis, this HAstV strain and to compare its genetic relationships to known HAstV genotypes.

Viral nucleic acid was extracted from 120 μl of the HAstV-positive stool suspension using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) and amplified using published primers (Table 1). The RT-PCR conditions used were identical to those described for the detection of HAstVs using type-common primers (1), except that an annealing temperature of 45°C was used to improve amplification. The purified RT-PCR products were cloned using a CloneJet PCR cloning kit (Fermentas Inc., Glen Burnie, MD). At least 10 clones each from the different regions were sequenced using the BigDye Terminator kit (Applied Biosystems, Foster City, CA). The sequence data were assigned the following GenBank accession numbers (in parentheses): ORF1a (FJ842149), ORF1b-ORF2 junction (FJ842147), and ORF2 (FJ842148). Published HAstV gene sequences used in the phylogenetic analysis included the following (GenBank accession numbers are in parentheses): HAstV-1 (L23513), HAstV-2 (L13745), HAstV-3 (AF117209), HAstV-4 (DQ070852 and Z33883), HAstV-5 (DQ028633 and U15136), HAstV-6 (Z46658), HAstV-7 (AF248738), HAstV-8 (AF260508), and newly described KS106210 (AF361035) and KS106209 (AF361034) from Korea (11) and MLB1 (FJ222451) from Australia (4). Sequences from ORF1b/ORF2 for the HAstV-3 to HAstV-6 Oxford strains (AF292074-8), HAstV-7 (AF248738), and an HAstV-8 South African strain (AF292073) were used in further analysis. Nucleotide sequences were aligned and compared using Sequencher 4.7. Multiple sequence alignments were generated by using MAFFT version 6 (http://mafft.cbrc.jp/alignment/server/) and edited using BioEdit version 7.0.9.0 (27 June 2007) (9). Phylogenetic trees were constructed using MEGA software version 4 (25) by both neighbor-joining and maximum parsimony methods. A Recombination Detection Program (RDP version 3.34) and SimPlot (version 3.2) (24) were used to investigate recombination events in the aligned sequences.

The presence of HAstV in the diarrheal stool specimen was confirmed by RT-PCR. Based on a pairwise comparison of the 266-bp nucleotide sequence from ORF1a, strain NK180 could be assigned to genogroup B (Fig. 1A), while analysis of the 3′ end of ORF2 assigned the same strain to genotype 2 (Fig. 1B), which resorts within genogroup A. Pairwise comparison of the ORF1b region assigned NK180 to genotype 3 (Fig. 1C). The ORF1a region of strain NK180 showed high sequence identity to the HAstV-7 Oxford ref-

TABLE 1. Primers used to genotype HAstV strains in this study

| Primer | Region amplified | Amplicon size (bp) | Reference(s) |
|---|---|---|---|
| Mon2/prBeg | ORF2 | 296–342 | 15, 17 |
| Mon348/Mon340 | ORF1a | 289 | 1 |
| Mon270/Mon344 | ORF1b/ORF2 | ~1,200 | 1, 21 |
| DM17/Mon344 | ORF1b/ORF2 | ~887 | 20 |

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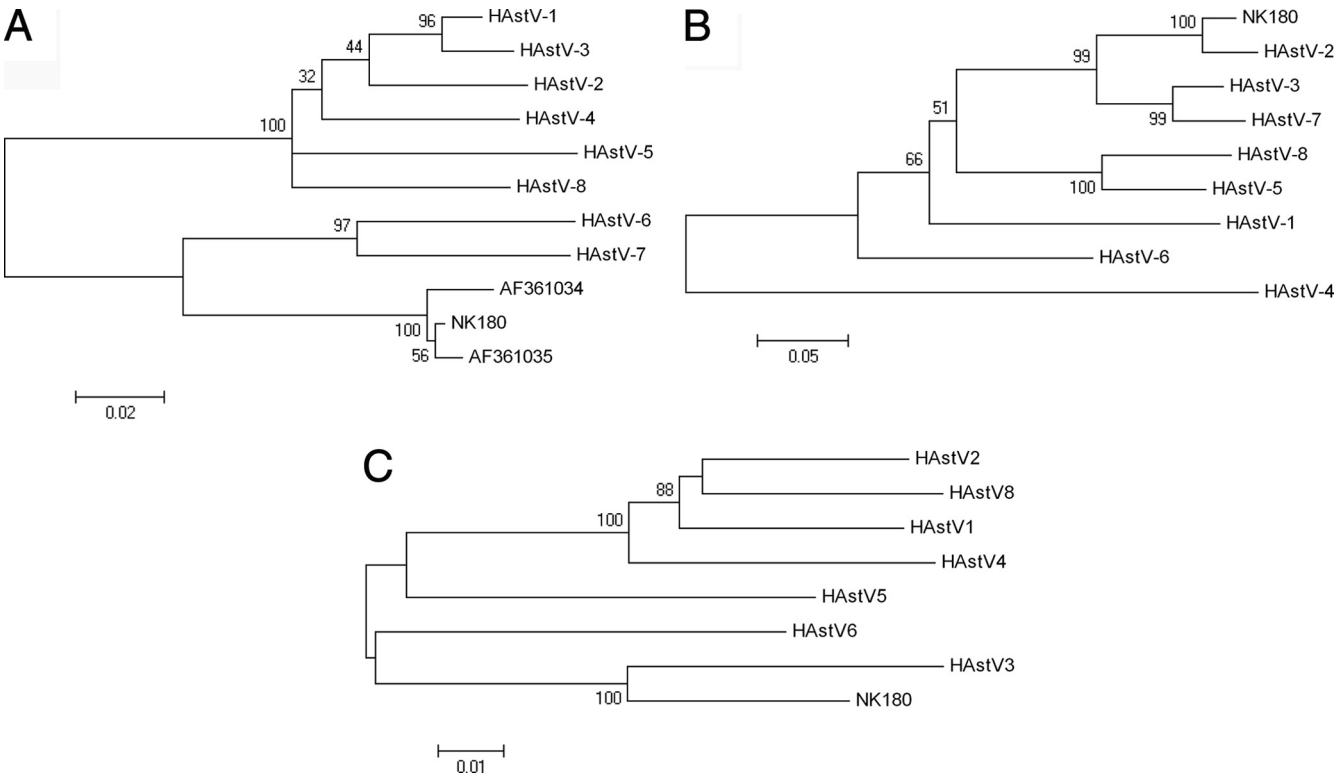


FIG. 1. Phylogenetic analysis of different genetic regions of HAstV strain NK180. Neighbor-joining phylogenetic trees of the ORF1a protease region (nt 1182 to 1448) (A), ORF2 capsid region (nt 6459 to 6777) (B), and ORF1b (nt 3676 to 4278) (C) were determined using MEGA version 4. Bars indicate the number of changes per site, and bootstrap percentages are indicated.

erence strain (86% of nucleotides and 97.5% of amino acids), but the highest sequence identity was to two as-yet-untyped strains, KS106210 (99% of nucleotides and 100% of amino acids) and KS106209 (98% of nucleotides and 100% of amino acids) (Fig. 1A). Within genogroup B, the Kenyan strain and the two Korean strains clustered separately to the Oxford HAstV-6 and HAstV-7 reference strains (Fig. 1A). The phylogenetic relationship of strain NK180 to HAstV-6 and HAstV-7 was similar to that of Korean strains KA613 and KA-SUN to HAstV-6 and HAstV-7 (11) and is suggestive of a new distinct lineage in genogroup B. Through comparison of the nucleotide sequences from the conserved region of ORF1a, the 3′ end of ORF2, and the ORF1b-ORF2 transition region of strain NK180 with those of AstVs AstV-MLB1, AstV-MLB2, VA1-3, HMO-A, and HMO-B, it was evident that these viruses were unrelated (results not shown).

SimPlot analysis (24) of ORF1a showed a possible crossover site at nucleotide (nt) position 1377 (Fig. 2). Nucleotide sequences after the crossover point were highly similar to those of HAstV-3 (83%) and HAstV-7 (81%). However, before the putative crossover point, the homology was notably different, and the SimPlot analysis showed high nucleotide identity to HAstV-7 (86%) but not to HAstV-3 (81%). Nucleotide sequence and similarity plot analysis also identified a putative recombination site within ORF1b-ORF2. Pairwise sequence comparison of the first 1,074 nt at the 5′ end of the ORF1b-ORF2 amplicon (ORF1b) with the HAstV reference strains showed the highest sequence identity to HAstV-3 (92% of nucleotides and 98% of amino acids), while the 593 nucleotides at the 3′ end of the fragment (ORF2) showed the highest sequence identity to HAstV-2 (94% of nucleotides and 99% of amino acids) (results not shown). The recombination site was mapped to the highly conserved 52-nt junction region (bases 4274 to 4328) between ORF1b and ORF2 (Fig. 3). The possibility of a mixed infection was excluded, as all the clones from each genomic region produced identical sequence data. There is only one previously documented record of recombination in HAstVs in which intragenogroup recombination was identified in HAstV strains from Houston, TX, and Mexico City, Mexico (28). In these strains, the recombination point, also in the ORF1b-ORF2 transition region, was between HAstV-3 in ORF1b and HAstV-5 in ORF2. Coinfection with different HAstV antigenic types, as described in epidemiological studies in Japan (15), provides an opportunity for recombination to occur. Although coinfections were not identified in the specimen analyzed in this study, the patient was from a closed setting, i.e., an urban hospice (13), where different HAstV types were cocirculating (data not shown), thus providing the ideal setting for recombination to occur.

RNA viruses have a tendency toward recombination due to the nature of their polymerase, which naturally shifts frame at the ORF1a-ORF1b junction (15), and various immunological and intracellular constraints can allow a recombinant virus to adapt and rapidly emerge as the predominant

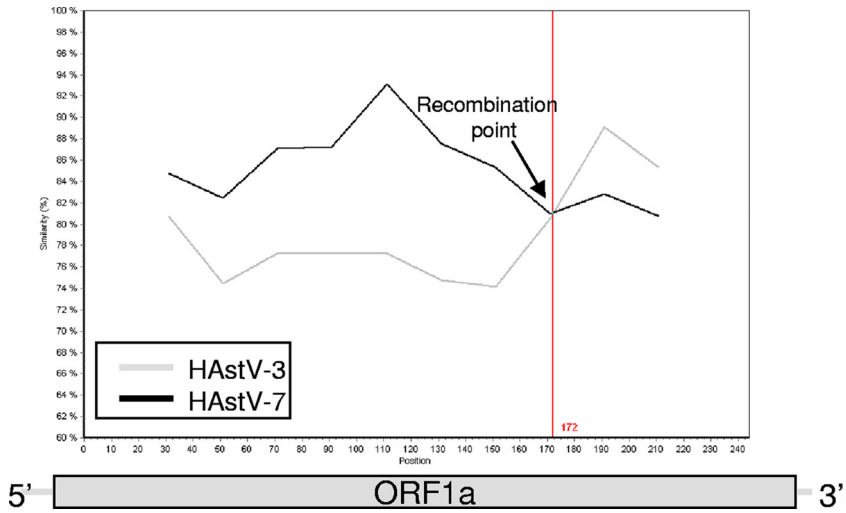


FIG. 2. Analysis of a possible recombination event occurring in the ORF1a region (244 bp) of HAstV strain NK180. The sequence was most similar to that of the HAstV-7 strain (GenBank accession number AF290508) before the recombination site at the 5′ end and that of the HAstV-3 strain (GenBank accession number AF141381.1) after the recombination site at the 3′ end, thereby depicting intergenogroup recombination. The window size was 50 bp, with a step size of 20 bp. The recombination breakpoint (indicated by an arrow) is located at nucleotide position 1377 with respect to the published sequence of HAstV-1 (GenBank accession number L23513).

population (31). Recombinant viruses have been demonstrated among other single-stranded positive-sense RNA viruses, including those in the families *Picornaviridae* and *Caliciviridae* (2, 3, 6, 10, 14, 23, 26). In the family *Caliciviridae*, novel recombination events in the norovirus (NoV) ORF1-ORF2 overlap (2, 22) and polymerase gene (30) have been described, and a double recombination event within the NoV polymerase region has been described (2).

As HAstVs are important pediatric diarrheal pathogens (7, 17, 20, 29), the application of reliable diagnostic assays and characterization methods are essential for burden of disease investigations. An alternate typing scheme was proposed to address viral virulence studies (8), and a new classification scheme was proposed (12) to accommodate highly divergent HAstVs (AstV-MLB1, AstV-MLB2, VA1-3, HMO-A, and HMO-B) (4, 5, 12). The recent identification of novel HAstVs (4) and human, mink, and ovine-like AstVs (12) and the occurrence of HAstV recombinants may have a significant impact on epidemiological studies. By ignoring

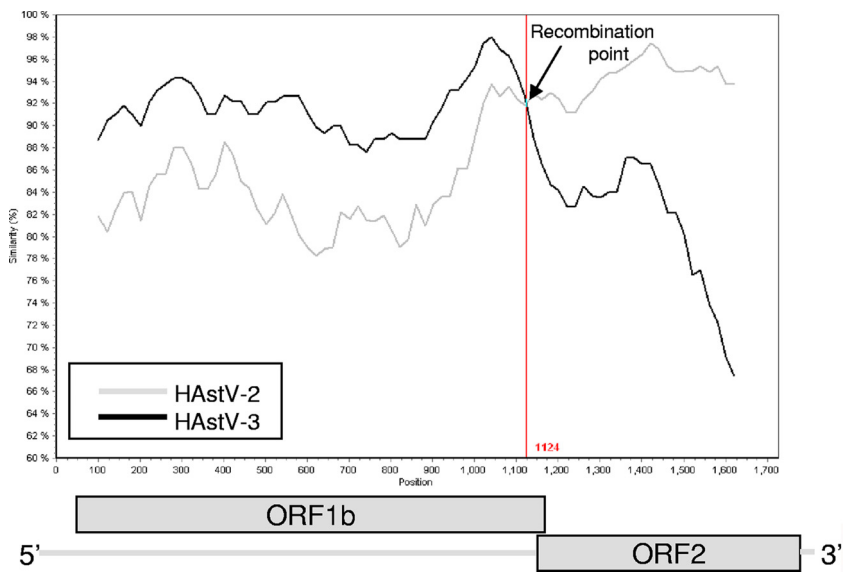


FIG. 3. Analysis of the recombination event occurring in the ORF1b-ORF2 junction (1,721 bp) of HAstV strain NK180. The sequence was most similar to that of the HAstV-3 strain (GenBank accession number AF141381.1) before the recombination site at the 5′ end and that of the HAstV-2 strain (GenBank accession number EU327561.1) after the recombination site at the 3′ end, thereby depicting intragenogroup recombination. The window size was 200 bp, with a step size of 20 bp. The recombination breakpoint (indicated by an arrow) is located at nucleotide position 4328 with respect to the published sequence of HAstV-1 (GenBank accession number L23513).

the presence of recombination and newly identified HAstVs, phylogenetic data may be skewed and misinterpreted.

**Nucleotide sequence accession numbers.** Nucleotide sequence data for ORF1a, the ORF1b-ORF2 junction, and ORF2 have been added to GenBank under accession numbers FJ842149, FJ842147, and FJ842148, respectively.

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