Genome Sequence of Genotype 1A Hepatovirus A Isolated from Plasma from a Haitian Child

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ABSTRACT Genotype 1A hepatovirus A was identified by quantitative reverse transcription-PCR and isolated from plasma from a Haitian child with acute undifferentiated febrile illness and malaise. The strain was most closely related to Brazilian strains, consistent with recognized patterns of virus movement in the Caribbean region.

HAV genomic RNA was detected by quantitative reverse transcription-PCR (qRT-PCR) in 2 (0.3%) of 677 plasma samples collected from febrile children as part of this study, by using the primer system described by Jothikumar et al. (5) and a modified HAV probe, namely, 5’-6-carboxyfluorescein (FAM)-CTTARGCTARTACTTCTATGAAGAGATGC-black hole quencher 1 (BHQ1)-3’, in which two R degeneracies (underlined) were inserted to replace a G at position 417 and an A at position 422. Primers and probe were combined and freeze-dried in a single glass vial (6). The quantification cycle (Cq) values for sample 15-1-1251, which was collected in January 2015, and sample 18-1-2097, which was collected in November 2016, were 33.37 and 40.27, respectively. Attempts were made to isolate the virus in MRC-5 cells (7) and were successful only for sample 18-1-2097. Sample 18-1-2097 was from a 6-year-old child who presented with a temperature of 38.1°C and complaints of malaise and mouth sores; no jaundice was noted.

The genomic sequence of sample 18-1-2097 was obtained from virus RNA that had been extracted from plasma (8) and Sanger sequenced with a gene-walking approach (9, 10) using nonoverlapping primers. Briefly, viral RNA was extracted from 140 μL of plasma using a QIAamp viral RNA extraction kit (Qiagen, Valencia, CA), RT was performed using the AccuScript high fidelity first strand cDNA kit (Agilent Technologies, Santa Clara, CA) in the presence of SUPERase-In RNase inhibitor (Ambion, Austin, TX), and PCR was sequentially performed using QS high fidelity DNA polymerase (New England Biolabs, Inc., Ipswich, MA) and the primers identified in Table 1. To determine the sequence of the 5’ end of the virus genome, 20 μL of

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purified RNA was treated with DNase- and RNase-free proteinase K (New England Biolabs) to remove the 5’ VPg (11–13), followed by 5’ rapid amplification of cDNA ends (RACE) using a FirstChoice RLM-RACE kit (Thermo Fischer Scientific) following the manufacturer’s instructions. Quality scores for the Sanger sequences ranged from 52 to 67 (11–13).

Excluding the poly(A) tail, the virus genome length is 7,477 ribonucleotides (nt) (A, 2,181 nt; U, 2,449 nt; G, 1,638 nt; C, 1,209 nt), with a G+C content of 38.1%.

The maximum likelihood phylogeny was constructed by using IQ-TREE (14–19) with all available complete genomes from humans available in GenBank; genotyping followed the methods described by Ramachandran et al. (20). The phylogenetic analysis shows that the genome sequence of the Haitian HAV isolate belongs to a well-supported monophyletic clade in genotype 1A that includes HAV genomes from the Americas, including Brazil, Mexico, and the United States, between 2009 and 2018 (Fig. 1). In particular, the genome sequence of the Haitian isolate clusters near a Brazilian HAV genome from 2017; the phylogenetic proximity and the short branches separating the Haitian genome sequence from the Brazilian sequence, which was from an HAV case that occurred 1 year after the collection of the virus in Haiti, are consistent with a recent common source or exchange of viruses between these two countries.

Data availability. The virus was designated hepatovirus A/0789/Haiti/2016, and its sequence was deposited in GenBank under accession number OK625565.1.

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**TABLE 1** Primers for sequencing HAV

| Primer | Sequence (5’ to 3’) | Nucleotide positions in GenBank accession no. OK625565.1 | Reference | Nucleotide positions in GenBank accession no. MG049743.1 | Amplicon size (bp) |
|--------|---------------------|-------------------------------------------------------|-----------|-------------------------------------------------------|-------------------|
| 5’-RACE-R | AACAACTCACATAATCCGC | 480–461 | 2 | | |
| HAV for | GTAGGCTACGGGTAAGAAC | 392–410 | 2 | | 88 |
| HAV rev | AACAACTCACATAATCCGC | 480–461 | 2 | | |
| HAV For 4 | TACCTACACCGGTTGCTACAGG | 64–87 | 1 | | 417 |
| HAV rev | AACAACTCACATAATCCGC | 480–461 | 2 | | |
| HepA for 1 | CTAAAGTCTATTCTTCAAGAGATGC | 413–441 | This work | | 648 |
| HepA rev 1 | CAGCTCACCACATCAAACTTTGGAACACTTC | 1060–1031 | This work | | 1030–1001 |
| HepA for 2 | GGCCTCTACGATGCTCTTTTAT | 1005–1031 | This work | | 853 |
| HepA rev 2 | CTACCTGAAATATATGTTGGAAGAAAACC | 1857–1828 | This work | | 1827–1798 |
| HepA for 3 | GGCTTCTATCTGCAAATGTGG | 1771–1794 | This work | | 810 |
| HepA rev 3 | GATGTTAACACGGGAGGTGTGAAG | 2580–2553 | This work | | 2550–2523 |
| HepA for 4 | GGGAAAGGTCTCAGTTTGTTT | 2473–2493 | This work | | 847 |
| HepA rev 4 | CCTAGTATCAAGCTATCTATCCTCCTC | 3319–3294 | This work | | 3289–3264 |
| HepA for 5 | GTGCTTCCACTCCTTGAAGAAAGTAAAG | 3200–3226 | This work | | 952 |
| HepA rev 5 | GCTGGTTATCTTCTAAAGATTTAAG | 4151–4127 | This work | | 4121–4097 |
| HepA for 6 | GGTATATACCAAAATTGAGGAT | 4068–4090 | This work | | 816 |
| HepA rev 6 | CACAAAGAGTCACCTTCTGTACAT | 4883–4859 | This work | | 4853–4829 |
| HepA for 7 | GATCTGTAAGCTTCTTTAAGGTGGAG | 4763–4790 | This work | | 843 |
| HepA rev 7 | CTTGATAATGATGTTGATAATACCTC | 5605–5579 | This work | | 5575–5549 |
| HepA for 8 | GGGATTTCAAGGTTGTTGTGCTTAT | 5530–5554 | This work | | 844 |
| HepA rev 8 | GGCCATGTGCTTGAGATGACTCTCAAAGC | 6373–6347 | This work | | 6343–6317 |
| HepA for 9 | GGCTCAGGCTTGTGCTTATTATA | 6241–6266 | This work | | 773 |
| HepA rev 9 | GAACTACTCTGGAAAGACTAT | 7013–6992 | This work | | 6983–6962 |
| HepA for 10 | GAGGATTCTTTGTACTCGGAGATG | 6961–6983 | This work | | 542 |
| HepA rev 10 | TTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTTT | 7502–7477 | This work | | |
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FIG 1 Phylogenetic inference of the HAV strains from a human source. The maximum likelihood phylogenetic tree of HAV strains from humans is based on the complete genome and was inferred using IQ-TREE. Gray circles at internal nodes represent >90% bootstrap support. Colored circles at the tips show the collection locations based on the legend at the bottom. The red rectangle shows the magnification of the subtree based on the American subclade containing the isolates from Haiti, Brazil, Mexico, and the United States with GenBank accession numbers (rectangle in the full phylogenetic tree). The Haitian isolate is indicated with a red arrow. Genotype classification is shown to the right of the tree.

Countries

- Argentina
- Gabon
- Ireland
- Russia
- Thailand
- Brazil
- Haiti
- Japan
- Sierra Leone
- Uruguay
- Cameroon
- India
- Mexico
- Singapore
- USA
- China
- Indonesia
- Mongolia
- South Korea
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