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Genetic Analysis of Outbreak of Hepatitis A Virus Infection among HIV-1 Seropositive Men

Tomohiko Koibuchi[1], Tateru Ishida[2], Tetsuya Nakamura[1], Atsushi Ajisawa[3], Masayoshi Negishi[3], Tetsuya Kashiyama[4], Akiko Takechi[4] and Aikichi Iwamoto[1,2]*

[1]*Department of Infectious Disease and Applied Immunology,*
[2]*Department Infectious Diseases, Institute of Medical Science, University of Tokyo, Shirokanedai 4-6-1, Minato-ku, Tokyo,*
[3]*Department Infectious Diseases, Tokyo Metropolitan Komagome Hospital, Honkomagome 3-18-22, Bunkyo-ku, Tokyo and*
[4]*Department of Internal Medicine, Tokyo Metropolitan Ohkubo Hospital, Kabukicho 2-44-1, Shinjuku-ku, Tokyo*

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We previously reported an outbreak of hepatitis A virus (HAV) infection among HIV-1 seropositive men who had sex with men (MSM) (1). HAV is classified into seven genotypes in terms of nucleotide sequence differences of the VP1/2A region (nucleotide 3024 to 3191) (2). We performed a genetic analysis of HAV to elucidate the chain of virus transmission.

Thirteen serum samples at the acute phase of HAV infec-

*Corresponding author: Fax: +81-3-5449-5427, E-mail: aikichi@ims.u-tokyo.ac.jp

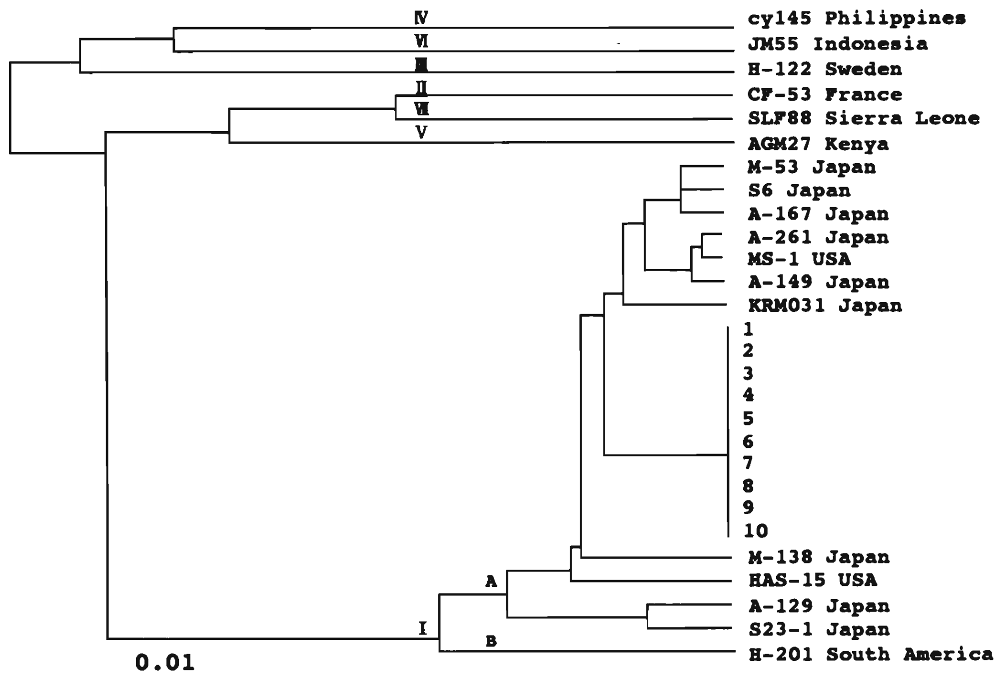

Figure. Phylogenetic tree based on the VP1/2A region of HAV genomes.
10 from the present study and 18 from references (2). Roman numerals I to VII designate the respective genotypes, whereas A and B designate sub-genotypes. The scale at the bottom indicates the percentage of divergence per length.

Table. Primers for nested PCR of VP2/1A region

Primers	Nucleotide Position	Sequence
HAV-1	2891-2914	5'-GGTTTCTATTCAGATTGCAAATTA-3'
HAV-2	3375-3398	5'-AGTAAAAACTCCAGCATCCATTTC-3'
HAV-3	2905-2925	5'-TTGCAAATTACAATCATTCTG-3'
HAV-4	3357-3377	5'-TTCAAGAGTCCACACACTTCT-3'

tion were collected. RNA from 140 μl of serum was extracted using the QIAamp viral RNA kit (Qiagen Inc., Valencia, Calif.) and subjected to reverse transcription using 5 μM random primer (Takara Co., Ltd., Kyoto). The VP1/2A region was amplified by polymerase chain reaction (PCR) using the primer pairs shown in Table (the external primer pairs were HAV-1 and HAV-2, and the internal primer pairs were HAV-3 and HAV-4). The first amplification, using 20 μl of cDNA, was performed in a volume of 50 μl with 1× Ex-Taq buffer, 0.2 mM dNTP, 0.5 μM primers, and 1.25U Ex-Taq (Takara Co., Ltd.), while amplification cycles were 35 of 30 sec at 94°C, 30 sec at 55°C, and 30 sec at 72°C with a final extension for 7 min at 72°C. Nested PCR was performed with 10 μl of the first reaction product in 50 μl under the same conditions at the first PCR. Amplified DNA fragments were sequenced by a direct sequencing method using a cycle sequence with dye termination chemistry (ABI Prism Big Dye terminator sequencing Ready Reaction Kit, Perkin-Elmer, Foster City, Calif.) on a Perkin-Elmer ABI-377 sequencer. A phylogenetic tree was constructed using the unweighted pair group method with an arithmetic mean (UPGMA) procedure using GENETYX-MAC Version 8.5 (Software Development Co., Tokyo).

The VP1/2A region could be amplified by the nested PCR in 10 of 13 patients. All 10 patients' VP1/2A regions had the same sequences. The figure shows a phylogenetic tree of these 10 sequences together with 18 HAV clones described in the literature (2). All 10 patients were grouped into genotype I A which is the most common HAV genotype in the world.

The striking homogeneity in the HAV isolates indicates that possibly one HAV clone spread among a high-risk population in a short time. These results reinforce the importance of educational and preventive measures for MSM against HAV.

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