

RESEARCH NOTE

Genomic Classification and Genetic Relationships of a New Variant of Hepatitis A Virus Isolated in Cuba

Belkis I Díaz*^{+/}, Carlos A Sariol*, Raiza Martínez, Licel Rodríguez

Departamento de Virología, Instituto de Medicina Tropical "Pedro Kouri", Apart. Post. 601, Mnao 13, Havana, Cuba

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From the antigenic point of view, the hepatitis A virus (HAV) presents a unique serotype (SM Lemon 1983 *Infect Immunol* 42: 418-420). Knowing the nucleotide sequence of the genomic region located at the putative VP1/2A junction (JL Cohen 1987 *J Virol* 61: 50-59) has enabled a differentiation of strains and their classification into seven genotypes in some of which sub-genotypes A and B can be defined (BH Robertson 1992 *J Gen Virol* 73: 1365-1377). This region has proved to have a relatively high degree of heterogeneity as compared to other genome regions (EA Brown 1989 *J Virol* 63: 4932-4937) and, at the same time it turns out to be very stable as a result of the serial passage in animals (non human primates) and cell culture. That is why it has been selected for the strain classification.

According to parameters previously established by other authors (R Rico-Hesse 1987 *Virology* 160: 311-322, Robertson *loc. cit.*) defined strains whose divergence in the nucleotides position is approximately 15-20%, belong to the same genotype, and those that differ only 7.5% belong to the same sub-genotype. Thus, if the amino-acid sequence corresponding to the VP1/2A region is considered, it

can be seen that within the same genotype there is a limited number of differences in the amino-acid (one or two substitutions of the possible 56) while between different genotypes the number of substitutions can reach up to 10 (Robertson *loc. cit.*).

The genomic classification of the strains of a specific geographic area allows to relate them according to molecular epidemiology, suggesting the geographical origin and the transmission pattern in certain place. That is, if the strain circulates endemically or if it has been imported from another region.

Cuban strain M2 was isolated in 1991 from a child's faeces during an outbreak of acute viral hepatitis (P Más 1991 *Rev Cubana Med Trop* 43: 206-207). It has been subjected to studies of protein characterization, electronic microscopy and immunomicroscopy (B Díaz 1996 *Rev Cubana Med Trop* 48: 123-129) and is currently used for diagnosis at the National Reference Laboratory for Viral Hepatitis, Pedro Kourí Tropical Medicine Institute (IPK). This study aims at classifying the Cuban strain from a genomic point of view and comparing it with other strains, specially from America. For this purpose, it was cultured in FRhK4 cells from which RNA was extracted (V Deubel 1990 *J Virol Methods* 30: 41-54) followed by reverse transcription - polymerase chain reaction (RT-PCR) and nested PCR of the VP1/2A zone, by using sets of primers designed by Robertson (*loc. cit.*). The product of PCR was sequenced according to the Sanger Method (F Sanger 1977 *Proc Natl Acad Sci USA* 74: 5463-5467) using a sequencing kit for PCR products (Sequenase version 2.0, USB, Amersham). The nucleotide sequence of the strain was aligned with the sequences of HAV strains reported to EMBL by means of the program CLUSTAL W (JD Thompson 1994 *Nucleic Acid Res* 22: 4673-4680). Homology higher than 92% was found with the strains of sub-genotype IA, between 86% and 91% with those of IB and lower than 83% with the strains of other genotypes. The lesser homology (71% to 73%) was shown with strains from sub-genotype IIIB, specifically with those isolated in Japan (data not shown).

The data gathered from the alignment of M2 with the American strains made possible the elaboration of a dendrogram allowing to see the genetic relationship between them (Fig. 1). The Unweighted Pairwise Group Method of Arithmetics Averages (UPGMA) included in the NEIGHBOR program of the phylogenetic inference package PHYLIP version 3.5c (J Felsenstein 1993 *PHYLIP*, Department of Genetics, University of Washington, Seattle) was used as previously reported by other authors (Rico-Hesse 1987 *loc. cit.*, Deubel 1990 *loc. cit.*).

*Corresponding author. Fax: +53-7-336051 / 246051. Present address (until April/99): Eberhard Karls Universität Tübingen, Abteilung für Medizinische Virologie und Epidemiologie der Viruskraheiten, Hygiene Institut der Universität, Silcherstraße 7, D-72076 Tübingen, Germany Fax: +49-7071-293435. E-mail: belkis-ivette.diaz-mendiondo@student.uni-tuebingen.de Received 6 July 1998 Accepted 11 September 1998

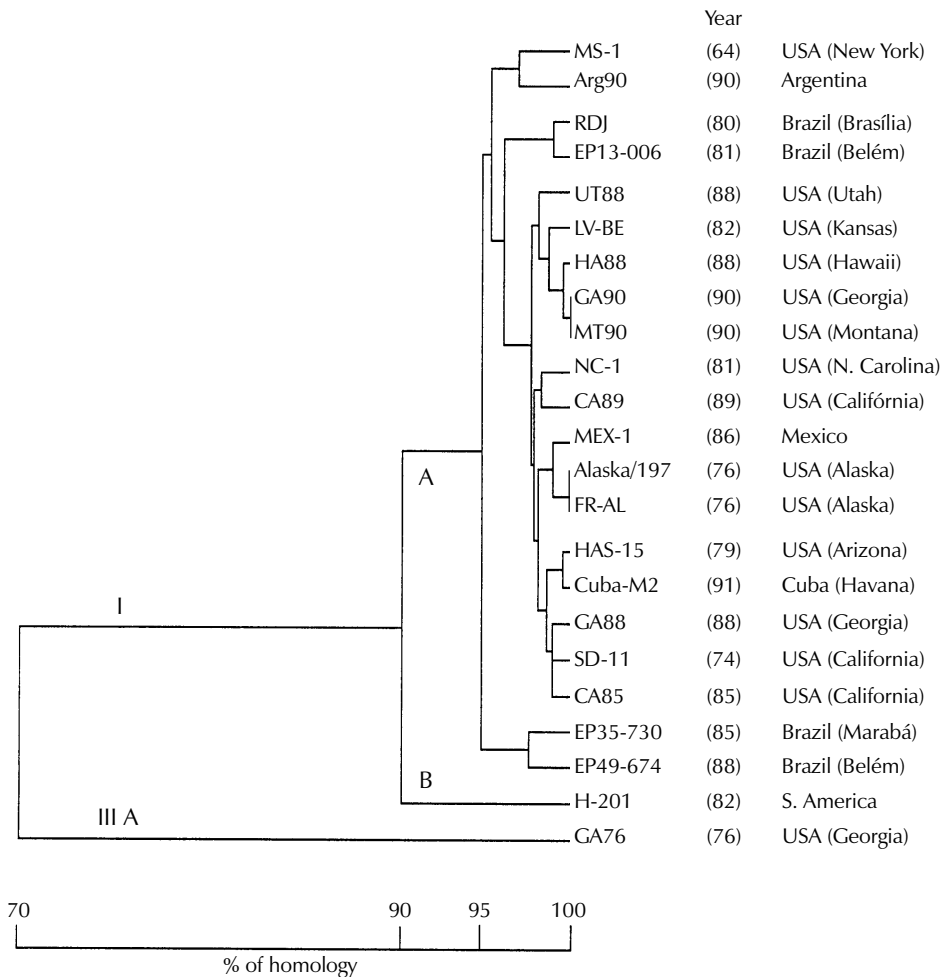


Fig. 1: genetic relatedness of M2 strain with other HAV strains. Most of the strain belongs to geographic cluster of USA, sub-genotype IA represented. As comparison two strains one from genotype IIIA (GA76) and other one from IB (H-201) are also represented. The dendrogram illustrates a comparison of sequences of the putative VP1/2A junction of those strains. The location of the node between strains or clusters indicates the approximate percentage nucleotide identity between sequences shown on the abscissa. The year and the location of isolation is indicated.

The deduced amino acid sequence was compared with the consensus sequences established for human genotypes and sub-genotypes IA, IB, II, IIIA, IIIB and VII (Robertson *loc. cit.*), finding 1, 2, 3, 6, 7 and 3 changes respectively.

The above-mentioned results suggest that M2 strain could classify within sub-genotype IA which groups 67% of the human strains isolated between 1960 and 1992 and most of the American strains.

Within this sub-genotype, almost every strain maintains its amino acid sequence well preserved. However, a few strains with some changes, when compared with the consensus, have been reported. At codon 49 of the studied zone of M2 strain, a Ile substitution at Leu 2A-21 was observed and is de-

termined by a nucleotide change (C ® A) on the first basis of that codon (Fig. 2).

From these results, taking into account the nucleotide sequence reported above, it is possible to postulate that once new genotype variant had been described, the previous percent of homology used as cut-off to establish genotypes and sub-genotypes are amenable of change. However, in this study the variation in the percents of homology did not modify the relations neither with all strains used by Robertson (*loc. cit.*) in her study (data not shown) nor with the strains in the sub-genotype IA.

Within the sub-genotype IA, the strain M2 classified as member of one of three geographically

	1	10	20	30	40	50
consenso	ESMMSRIAAGDLESSVDDPRSEEDRRFE			SHIECRKPYKELRLEVGVKQRLKYAQEEL		
EP35.730	-----			-----M-----		
TKM 002	-----			---Y-----		
PRC16-(7)	-----			-----D-----		
S23-1(3)	---N-----			---G-----		
M2	-----			-----I-----		

Fig. 2: comparison of the deduced amino acid sequences of VP1/2A junction of strains belong to sub-genotype IA which differ from the consensus sequence. The consensus is shown on the top line.

related clusters of viruses former described (Robertson *loc. cit.*) (Fig. 1), together with the strains from USA.

The amino acid sequences in this cluster has shown to be highly conserved. Although this is a homologous amino acid change it does not correspond to any of the reported changes for other strains. Presently we can not say if this change has effect on the function of the protein.

In spite of the regular composition in amino acid sequence and considering that the year of isolation of HAV strain M2 was 1991, our results confirm the circulation of a different variant belonging to this geographical cluster in the sub-genotype IA.

The punctual modification (L ® I) could be in

connection with the geographical condition of our country, which is an island, being a niche allowing the development of new variants.

Considering that the Cuban strain classifies within the same sub-genotype as most of the strains from the region it could be inferred that is not an imported one from other geographical region. Taking into account the epidemiological behavior of hepatitis A in Cuba, it could be postulated that this strain could circulate with an endemic transmission pattern. This can be differentiated from other setups (ex. Japan and Western Europe) where infections are imported due to travel. However it could only be demonstrated by extending the study to the rest of the Cuban isolated strains.

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