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A PROPOSED RAPID METHOD FOR GENOMIC CHARACTERIZATION OF GBV-C/HEPATITIS G VIRUS (HGV)

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Summary GBV-C/Hepatitis G virus (HGV) has been identified as an infectious agent for humans although its potential involvement as a pathogenic virus is still controversial. Hitherto, 3 genotypes have been identified worldwide by c-DNA sequencing. This method allows genomic viral RNA clustering according to the geographical source of the strains, but its potential value in type- (or even strain-) specific pathogenesis has only started to be explored. Since this method requires highly specialized laboratories and is rather expensive, we propose a rapid method based on differential restriction fragment length polymorphism (RFLP) of 5' NCR amplicons. Using *Hinf* I, *Dra* I and *Mae* II endonucleases, it is possible to obtain different restriction patterns to discriminate among 1a, 1b, 2a, 2b and 3 subtypes/types. This methodology could be useful for large scale molecular epidemiology as well as for studies on viral pathogenesis.

Key words: GBV-C/HGV genotyping, RFLP

GBV-C/HGV agent has been recently identified[1, 2] and is currently being investigated as a potential pathogen for the human being, although this is still a controversial issue[3-9].

GBV-C/HGV is a member of the *Flaviviridae* family, related to GBV-A virus and to a lesser extent to Hepatitis C virus (HCV). GB are the initials of a surgeon who developed acute hepatitis about 3 decades ago. His serum was serially passaged in South American marmosets, after which a virus not related to hepatitis A was postulated[10]. However, these findings were later challenged when it was suggested that the observed hepatitis induced in tamarins was indeed related to the reactivation of an endogenous marmoset hepatitis virus, rather than transmission of a human infection[11].

In 1995, the Virus Discovery Group from Abbott (Chicago, USA) demonstrated that a pool of infected tamarin plasma after the eleventh passage of the original GB inoculum contained viral sequences of two agents which were named GBV-A and GBV-B[12]. Subsequently, the same group documented in human sera the presence of a third related virus which they named GBV-C[1]. Six months later, another related nucleotide sequence of the same virus –called hepatitis G virus– was reported by investigators at the Centers for Disease Control and Prevention (Atlanta, USA) in collaboration with other groups[2].

Since then, several strains have been analyzed from USA, Asia, Africa and Europe[13, 15]. Nucleotide sequence alignment from different parts of the world allows GBV-C/HGV genomic clustering within three groups. –i.e. 1, 2 and 3– the first two of them including at least 2 subgroups. Importantly, this clustering seems to predominantly represent the geographical origin of the isolates, namely type 1 from West Africa, type 2 from USA and Europe, and type 3 from Asia.

It is still unknown whether nucleotide sequence diversity is also related to a different course of infection, as has been extensively reported for hepatits C virus. If so, it could provide an expla-

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nation to the observed discrepancy between the evolution of some patients[4].

Hitherto, GBV-C/HGV genomic characterization has been performed only by c-DNA sequencing. This method represents the gold standard, but is not applicable for large scale molecular epidemiological studies due to the need of highly specialized laboratories, as well as long time requirement and expensive costs.

Initially, a computer-based analysis of 44 GBV-C/HGV sequences deposited in GenBank from around the world (accession numbers U44402, U45966, U59518 - U59558) was carried out using the Clustal method with weighted residue weight table (Megalign 1.0 Program, DNAstar Package). This study allowed the recognition of 5' NCR conserved regions for all the isolates. These regions were chosen to design the primers for reverse transcription (RT) coupled to Nested PCR. Selected primers were: outer sense (GOS) 5' cgg cac tgg gtg caa gcc cca 3', outer antisense (GOA) 5' ccc ggc ccc cac tgg tcc ttg 3', inner sense (GIS) 5' agc ccc aga aac cga cgc cta 3' and inner antisense (GIA) 5' tat tgg tca aga gag aca ttg 3'. Theoretical amplicons of 326/327 bp were anticipated to be synthesized after RT-Nested PCR.

By computarized (MapDraw Program, DNAstar Package) and visual analysis of the above mentioned sequences it was established that the use of a set of 3 endonucleases could allow typing and subtyping of GBV-C/HGV isolates in agreement with the phylogenetic analysis of database sequences.

Accordingly, we propose a novel methodology for typing GBV-HGV isolates by RFLP of the amplicons obtained by RT-Nested PCR amplification of the 5'NCR region of the viral RNA.

Enzymatic cleavage is performed with *Dra* I, *Hinf* I, and *Mae* II endonucleases, following the manufacturer instructions. Restriction fragments obtained with each enzyme according to the genotype are shown in Table 1.

The proposed method has been successfully applied to detect and characterize GBV-C/HGV Argentinian isolates –i.e. among intravenous drug users– (unpublished data).

This methodology is intended to provide a useful tool for the simultaneous detection and typing of the GBV-C/HGV agent, and could be applied to both molecular epidemiological surveys as well as studies on viral pathogenesis.

TABLE 1.– RFLP patterns according to the GBV-C/HGV types and subtypes

Endonuclease	Type	Subtype	Fragment lengths (bp)
Dra I	1	a	327, 326
		b	326
	2	a	327
		b	327
	3		304, 23
Hinf I	1	a	327, 326
		b	326
	2	a	170, 108, 38, 11
		b	170, 119, 38
	3		327
Mae II	1	a	171, 170, 109, 47
		b	283, 32, 11
	2	a	216, 54, 31, 15, 11
		b	231, 54, 31, 11
	3		200, 54, 32, 30, 11

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Resumen

Propuesta de un método rápido para la caracterización genómica del virus GBV-C/hepatitis G (HGV)

El agente GBV-C/virus de Hepatitis G (HGV) ha sido identificado como infeccioso para el ser humano, aunque su potencial participación como virus patógeno es aún motivo de controversia. Hasta el presente, se han demostrado 3 genotipos mediante secuenciamiento del ADN-c, obtenido por retrotranscripción y amplificación por PCR del ARN viral. Este método seguido del análisis por computadoras de las secuencias nucleotídicas alineadas, permite el agrupamiento de las cepas en 3 tipos, cada uno de los cuales refleja un diferente origen geográfico. Recién ha comenzado a explorarse el potencial valor de la genotipificación en la comprensión de la patogénesis de la infección. Teniendo en cuenta que el secuenciamiento del ADN-c requiere de laboratorios de alta complejidad y que sus costos son elevados, se propone el uso del polimorfismo de la longitud de fragmentos de restricción sobre ampli-

cones correspondientes a la región 5' NCR del genoma viral. Utilizando las endonucleasas *Hinf* I, *Dra* I y *Mae* II es posible obtener diferentes perfiles de restricción que permiten discriminar entre los tipos/subtipos 1a, 1b, 2a, 2b y 3. Esta metodología podría ser útil para la realización de estudios de epidemiología molecular y patogénesis de la infección por GBV-C/HGV.

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Berganza. Que de cinco mil estudiantes que cursaban aquel año en la Universidad, los dos mil oían Medicina.

Ciprión. Pues ¿qué vienes a inferir deso?

Berganza. Infiero, o que estos dos mil médicos han de tener enfermos que curar (que sería harta plaga y mala ventura), o ellos se han de morir de hambre.

Miguel de Cervantes (1547-1616)

Novela y coloquio que pasó entre Ciprión y Berganza, perros del Hospital de la Resurrección.