Reassessment of Genotype 1 Hepatitis C Virus Subtype Misclassification by LiPA 2.0: Implications for Direct-Acting Antiviral Treatment

Javier R. Guelfo, Juan Macias, Karin Neukam, Federico A. Di Lello, Jose A. Mira, Nicolas Merchant, María Mancebo, Rocío Nuñez-Torres, Juan A. Pineda, Luis M. Real

Unidad de Enfermedades Infecciosas y Microbiología, Hospital Universitario de Valme, Seville, Spain; Instituto de Biomedicina de Seville (IBIS), Seville, Spain; Cátedra de Virología, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Buenos Aires, Argentina

The effect of the hepatitis C virus (HCV) genotype 1 subtype on the response to treatment can be dramatic for some direct antiviral agent (DAA)-containing regimens (1–9). This is the case for simprevir, a DAA recently approved for clinical use, that in combination with pegylated interferon and ribavirin (peg-IFN/RBV) has a lower sustained virological response (SVR) rate for subtype 1a than for 1b (2). Simprevir plus peg-IFN/RBV treatment achieves rates of SVR similar to those for peg-IFN/RBV treatment in HCV-1a-infected patients who present the viral variation Q80K. This variation is frequent in the HCV-1a subtype, but it is not found in HCV-1b (2, 5). Similarly, daclatasvir plus peg-IFN/RBV treatment also achieves higher SVR rates among HCV-1b-infected patients than among HCV-1a-infected individuals (4). For interferon-free regimens, DAA combinations such as asunaprevir plus daclatasvir may only be indicated for subtype 1b, because of high relapse rates observed among patients with subtype 1a (3, 4). Because of these, accurate subtyping of HCV genotype 1-infected patients is needed for those who are candidates for DAs.

Sequencing of specific HCV genome regions with subsequent phylogenetic analysis is considered the gold standard of HCV genotyping (10). However, it includes several complex techniques and is time consuming. For these reasons, LiPA 2.0 is currently one of the most widely used genotyping assays. However, several studies have reported HCV genotyping errors using this commercial method (11–13). Those studies were not designed to evaluate the frequency of HCV-1 subtyping misclassifications, and they included few HCV-1-infected patients. Therefore, due to the high prevalence of HCV-1 infections in western countries and the importance of correct subtyping in clinical decisions, the accuracy of this method for HCV-1 subtyping needs to be evaluated in larger samples. For these reasons, we aimed to evaluate the accuracy of LiPA 2.0 for classifying HCV-1 by subtypes.

This study was performed according to the ethical guidelines of the 1975 Declaration of Helsinki, and it was approved by the ethics committee of the Hospital Universitario de Valme (Seville, Spain).

The first 110 consecutive patients starting from October 2001, who received a course of peg-IFN/RBV treatment in our hospital and who were infected with HCV-1, according to the LiPA 2.0 determination, were selected. All of them were Caucasian with a median age (quartile 1 to quartile 3) of 42 years (range, 39 to 46 years). Among the patients, 82 (81%) were male, and 48 (47.5%) were infected with HIV.

To determine the HCV-1 subtype, we analyzed the entire core genetic sequence. This region is more conserved than those traditionally used for HCV genotyping (NS5B or core/E1) and allows higher amplification rates (13, 14). The core region was amplified by nested PCRs. The first PCR was performed with 5 μl of cDNA using primers 1 and 2 (Table 1). For the second PCR, 4 μl of the first PCR product and primers 3 and 4 (Table 1) were used. For those samples that were not amplified with this protocol, primers 1 and 5 for the LiPA 2.0-identified subtype 1a or primers 1 and 4 for subtype 1b were used to perform the external PCR and then primers 6 and 7 (1a) or 8 (1b) were used in the internal PCR (Table 1). The nested PCR products were bidirectionally sequenced using standard capillary electrophoresis techniques. ClustalW2 software (www.ebi.ac.uk/Tools/msa/clustalw2/) was used for alignment of the forward and reverse sequenced strains to obtain consensus sequences.

As the genotyping method, we analyzed the evolutionary history, which was inferred by using the maximum likelihood method based on the general time reversible model. Evolutionary analyses were conducted in MEGA5 software (15). The initial tree(s) for the heuristic search was obtained by applying the neighbor-joining method to a matrix of pairwise distances estimated using the maximum composite likelihood (MCL) approach (15). A discrete gamma distribution was used to model the evolutionary rate differences among sites (4 categories [+G, parameter = 0.3742]). The analysis involved 110 sequences, including the sequences from patient samples and those selected as references. In the final data set, there were a total of 573 base pair positions corresponding to the complete core region for each sample.
Successful sequencing and genotyping were obtained in 101 (91.8%) patients. The 9 (8.2%) samples that were not amplified had a viral load of <3 log IU/ml.

According to LiPA 2.0, 58 (57.4%) samples were identified as HCV-1a, and the remaining 43 (42.6%) were identified as HCV-1b. By the sequence-based method, 67 (66.3%) samples were classified as HCV-1a, and the remaining 43 (42.6%) were identified as HCV-1b. In a similar study, HCV-1a-infected patients were not included because of the HCV genotype distribution (13).

In conclusion, our results show that the LiPA 2.0 assay is not an adequate tool for subtyping HCV genotype 1. This genotyping method has subtyping failures, and it is prone to errors with HCV genotype 1b. In a similar study, HCV-1a-infected patients were not included because of the HC1 genotype distribution (13).

Nucleotide sequence accession numbers. The sequences obtained in this study were submitted to GenBank under accession numbers KF060663 to KF060714 and KJ739737 to KJ739786.

ACKNOWLEDGMENTS

This work was partially funded by the RD12/0017/0012 project as part of the Plan Nacional R+D+i and cofinanced by ISCIII-Subdirección General de Evaluación y el Fondo Europeo de Desarrollo Regional (FEDER), the Ministerio de Sanidad y Servicios Sociales (EC11-304), and the Fundación para la Investigación y la Prevención del Sida en España (121004/10). J.M. is the recipient of a grant from the Servicio Andaluz de Salud de la Junta de Andalucía (B-0037). I.A.P. is the recipient of an intensification grant from the Instituto de Salud Carlos III (grant Programa-I3NS). K.N. is the recipient of a Miguel Servet research grant (CP13-00187).

These funding institutions did not have any role in the study design or collection, analysis, or interpretation of the data. They did not participate in the writing of the report or in the decision to submit the paper for publication.

We declare no conflicts of interest.

REFERENCES


