Mistyping of Two Slovenian Hepatitis C Virus Subtype 2c Isolates as Subtype 2b by Two 5' Noncoding Region Genotyping Methods

During our recent comparison of the reliabilities of four different hepatitis C virus (HCV) genotyping methods (4, 5), two Slovenian HCV subtype 2c isolates which were misclassified as subtype 2b by the two most widely used genotyping methods, second-generation line probe assay (Inno LiPA; Innogenetics, Ghent, Belgium) (7) and restriction fragment length polymorphism (RFLP) (1) analysis, were identified. Namely, although these two 5' noncoding region (5'NCR)-based genotyping methods clearly identified both isolates as HCV subtype 2b, this subtype could not be confirmed by two core-based genotyping methods and by sequence analysis of the 270-bp fragment of the NS-5 region. Thus, a commercial core-based nested PCR coupled with DNA enzyme immunoassay (GEN-ETI-K-DEIA; Sorin Biomedica, Saluggia, Italy) (8) assigned genotype 2 and excluded subtypes 2a and 2b for both Slovenian isolates, since the genotype 2-specific probe included in the assay hybridized with PCR products and both subtype 2a- and 2b-specific probes did not. By the second core-based genotyping method, Okamoto's genotype-specific PCR (3), HCV subtype could not be exactly determined in either isolate due to multiple PCR products obtained. Finally, sequence and phylogenetic analysis of the 270-bp fragment of the NS-5 regions, performed as described previously (6), clearly classified both Slovenian isolates as subtype 2c.

Discrimination of HCV subtypes 2a/2c and 2b by currently used 5'NCR-based genotyping methods relies on a single nucleotide variation observed at position –161 of the HCV genome (1, 7). Previous studies have shown that guanine and adenine at this position should be specific for HCV subtypes 2a/2c and 2b, respectively (7). Thus, for discrimination of HCV genotype 2 subtypes, subtype-specific probes designed based on the variation at position –161 and restriction endonuclease ScrFI, which recognizes and cuts the sequence CC↓NGG (in which the arrow represents the cut), are used in the current versions of the line probe assay (Inno LiPA) and the RFLP method, respectively. However, when we sequenced 115-bp fragments of the 5’NCR (from nucleotide –180 to –66) of both Slovenian misclassified HCV isolates, we recognized that in some rare cases, the previously described specificity of nucleotide position –161 is not mandatory. Our sequence analysis clearly showed adenine instead of guanine at position –161 in both Slovenian subtype 2c isolates. This finding suggests that our isolates were probably mistyped as HCV subtype 2b by both of the 5’NCR-based genotyping methods, line probe assay and RFLP, due to a single point mutation at position –161. A guanine-to-adenine mutation at this position probably produced a mishybridization of the 2b subtype-specific probe, the loss of an ScrFI restriction site, and the consequent misclassification of both isolates as subtype 2b.

To the best of our knowledge, we describe here the first HCV subtype 2c isolates which were wrongly classified as subtype 2b by the two currently most widely used nonsequencing 5’NCR-based HCV genotyping methods. Our results support the recent finding that current nonsequencing HCV 5’NCR-based genotyping methods require careful redesign and that until then, genotyping in HCV regions other than the 5’NCR is strongly recommended for typing of European HCV genotype 2 isolates (2).

REFERENCES


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