

HEPATITIS C VIRUS GENOTYPING IN CHRONIC HEPATITIS C PATIENTS

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Abstract

Chronic hepatitis C virus infection is a massive worldwide healthcare burden with estimated costs in the USA alone of over \$5 billion per annum. The virus has a 9.5kb positive sense single-stranded RNA genome with striking heterogeneity between isolates, which has led to it being divided into 6 genotypes and more than 50 subtypes and many quasispecies that has been arisen due to the infidelity of the viral polymerase, which lacks of a proofreading function. The virus exists as a range of related but not identical species at the quasispecies. In each infected individual, HCV circulates as a quasispecies in which the population consists of a number of closely related but distinct genetic species. The distribution of the genotype might be influenced by the mode of transmission and racial group. The only current effective treatment is combination therapy with pegylated interferon plus ribavirin (peg-IFN α + RBV) for 24–48 weeks based for genotypes 1 and 4 is 48 weeks, whereas the treatment for genotypes 2 and 3 is completed in 24 weeks. It has proved effective in up to 50% of those infected with HCV genotype 1 and 4 and it varies with other genotypes. HCV genotype is consider to be a clinically important parameter for determining both; the potential response and the duration of treatment.

Keywords : HCV, IFN, RBV, genotype

Introduction

There is considerable sequence heterogeneity in Hepatitis C virus (HCV). There are currently six major genotypes, designated by numerals 1 to 6 with more than 50 subtypes. The most widely used method for determining the HCV genotype is the commercially available line probe assay (INNO-LiPA), which is based on genotype-specific

oligonucleotides from the 5' untranslated region (UTR) that are immobilised on a nitrocellulose strip. However, this methodology does not always distinguish between subtypes 1a and 1b. Currently, nucleic acid sequencing technology is the optimal means of defining virus genotypes. For example, a TRUGENE HCV 5' noncoding region (NCR) genotyping assay uses the DNA amplicons that are produced in the 5' NCR of the HCV genome. This amplification is followed by DNA sequencing of the amplicon, and the subtype is then verified by comparing the sequence with a series of prototypic HCV genomes. Various studies have shown that the TRUGENE assay is the most accurate assay for HCV genotyping; however, samples with low viral loads can fail to produce a satisfactory result [1].

HCV Genotypes

Based on genomic sequence similarity, HCV has been subdivided into six genotypes (1–6), and each genotype includes several subtypes (Figure 1). While some regions are better conserved than others, the genetic difference between genotypes is approximately 33% over the whole genome. Within genotypes, a variable number of more closely related distinct subtypes differ by 15% to 20% in their nucleotide sequence [2]. In each infected individual, HCV circulates as a quasispecies: the population consists of a number of closely related but distinct genetic species. The quasispecies exist because of the infidelity of the viral polymerase, which lacks a proofreading function. HCV genotypes are generally identified by nucleic acid-based assays, such as direct sequencing, line probe assay, and real-time polymerase chain reaction (PCR). Most commercial assays target the highly conserved 5' NCR. The genotypes are distributed in many countries around the world; genotypes 1 and 2 show an especially broad distribution. In North America, genotype 1a predominates, followed by genotypes 1b, 2a, 2b, and 3a. In Europe, genotype 1b is predominant, followed by genotypes 2a, 2b, 2c, and 3a. Genotypes 4 and 5 are found almost exclusively in Africa. Subtype 1a is prevalent in North and South America, Europe, and Australia, and subtype 1b is common in North America and Europe and is found in parts of Asia. Genotype 2 is present in most developed countries but is less common than genotype 1. Genotype 3a appears to be prevalent among injected drug users and may have entered North America and the United Kingdom with the widespread use of heroin in the 1960s [3]. In Egypt, genotype 4a is the most prevalent genotype, and up to 20% of the population is infected with this subtype. Other genotype 4 subtypes are found in the Middle East as well as North and Central Africa. Genotype 6 is common in several distinct locations in South-east Asia, such as Hong Kong and Vietnam. Genotype 5a is responsible for at least 30% of cases in South Africa but is

seldom found elsewhere. The distribution of genotypes is also influenced by the mode of transmission and racial group distribution. In Europe and the United States, individuals who acquire HCV from a blood transfusion are likely to be infected with subtype 1b; however, with the introduction of blood donation screening, the number of individuals with this subtype is likely to decrease. Genotypes 1a and 3a are the most prevalent genotypes among intravenous drug users; with the decline in blood transfusions, acquired HCV infection may become the predominant genotype in Europe and the United States.

As mentioned, there is considerable sequence heterogeneity among isolates of HCV, the most widely used method for determining the genotype is the commercially available line probe assay (INNO-LiPA), which is based on genotype-specific oligonucleotides from the 5' UTR that are immobilised on a nitrocellulose strip. The oligonucleotides are then probed with a biotin-labelled 5' UTR amplicon, which binds to the bands in a genotype-specific manner; the oligonucleotides are then visualised using a streptavidin-peroxidase reaction. The genotype is then identified with a pattern algorithm. However, this methodology does not always differentiate between subtypes 1a and 1b. Currently, nucleic acid sequencing technology is the optimal means of defining virus genotypes. For example, a TRUGENE HCV 5' NCR genotyping assay uses the DNA amplicons that are produced in the 5' NCR of the HCV genome. In 2002, Trabaud and his group used the TRUGENE HCV 5' NCR assay as a direct sequence to analyse HCV genotyping data [4]. Most laboratories currently use the TRUGENE assay to determine the genotype from the product of an initial reverse transcription PCR reaction, such as the product that is generated by the Roche Amplicor. Moreover, in a 2002 study by Roque-Afonso et al., TRUGENE HCV 5' NCR was assessed in comparison with the reverse hybridisation-based assay INNO-LiPA. Beginning with the amplification products that were generated by the diagnostic Roche AMPLICOR HCV test, both assays were used to define the genotype [5]. A total of 205 patients were used in the study, and 34 patients were tested prospectively with both methods. A total of 171 samples were stored at -20°C for up to 2 years after the LiPA genotyping. The TRUGENE procedure failed to determine a genotype in six low-titre samples. In 2003, Germer et al. compared the TRUGENE HCV 5' NCR genotyping kit, with Gene Librarian modules 3.1.1 and 3.1.2, and the VERSANT HCV genotyping assay to test 96 HCV RNA-positive patient specimens, including HCV genotypes 1, 2, 3, 4, 5 and 6. The authors concluded that TRUGENE HCV 5' NCR provided the most accurate genotyping results [1].

TRUGENE HCV 5' NCR genotyping relies on the cross-linking and immunoprecipitation (CLIP) method, which uses the PCR amplicons that are generated by

the Roche AmpliCor kit to cause a combined PCR and sequencing reaction. The reaction is then compared to other known sequences using Gene Librarian software. For each reaction, the fragments are simultaneously sequenced in both directions in a single tube with two dyes that are attached to the primers (Figure 2).

The accuracies of the TRUGENE and INNO-LiPA assays were 74% and 76%, respectively in our study. The 95% and 98% confidence intervals are determined for the 5' NCR with the sequencing software. In a 1999 study by Germer, approximately 89.4% of the specimens were successfully classified after a computer-assisted analysis of the sequence data. The limitations of the TRUGENE 5' NCR assay are related to the low discriminating power of 5' NCR for determining particular types and subtypes [6].

HCV genotypes and subtypes

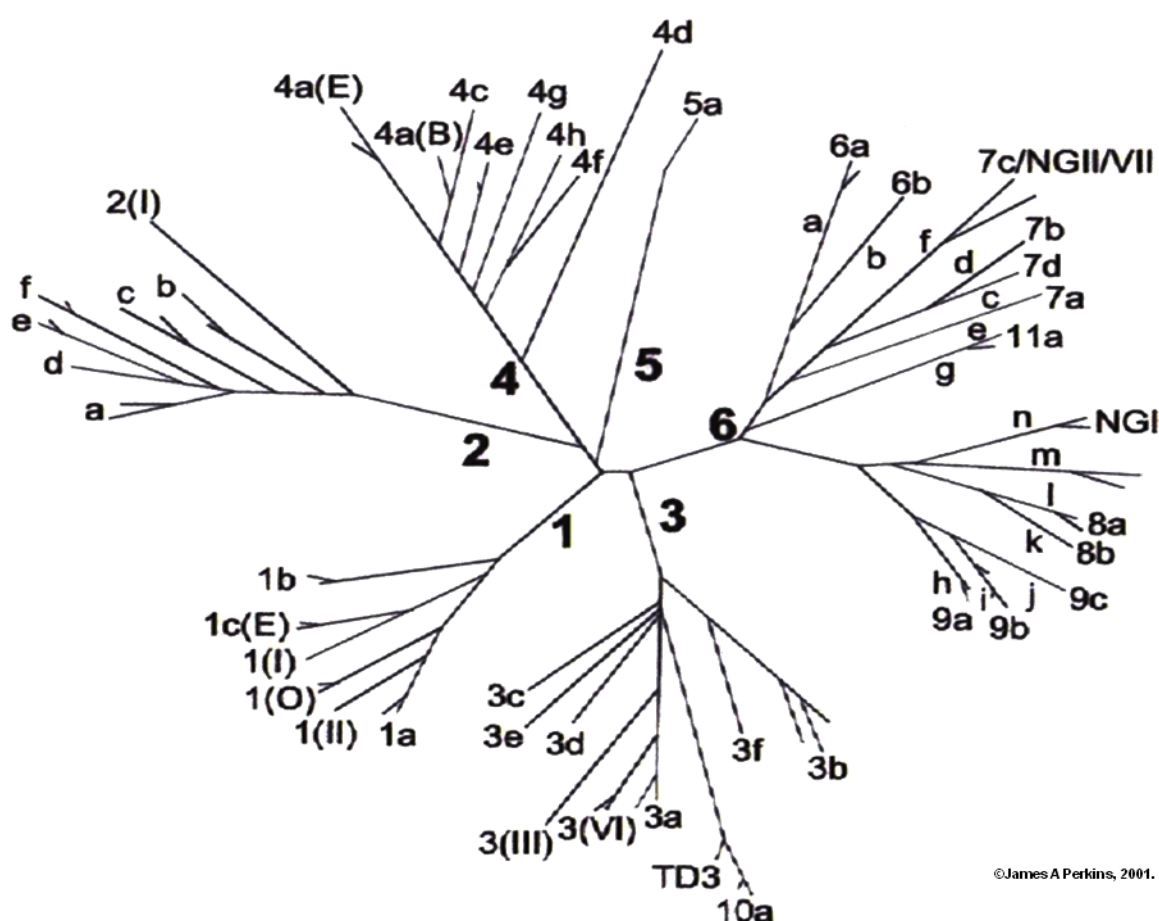


Figure 1 A phylogenetic tree of the relationship among HCV genotypes. These relationships are based on nucleotide sequences of the NS5B region. The differences among HCV genotypes can vary by up to 30%, whereas differences among subtypes vary by only 5–10%.

In Germer's 1999 study, 89.4% of the specimens were successfully classified after a computer-assisted analysis of the sequence data. The findings of the present study did not

encounter the limitations that were reported for the TRUGENE 5' NCR assay, which may include a low discriminating power of 5' NCR for determining particular types and subtypes [6]. The INNO-LiPA and TRUGENE HCV 5' NCR results showed discrepancies at the type level for 1.4% of the samples and at the subtype level for 14.2% of the samples. The TRUGENE 5' NCR and INNO-LiPA results also showed discrepancies at the type level for 2% of the samples and at the subtype level for 8.1% of the samples. Furthermore, two distinct strains of HCV that were classified as genotype 2 were correctly identified by TRUGENE HCV 5' NCR, and genotype 1 was incorrectly ascribed by INNO-LiPA. In conclusion, the performance characteristics of the 5' NCR methods were similar, and both methods produced accurate results at the genotype level. However, neither method should be used for subtyping genotypes because the sensitivity of HCV genotyping assays depends on the patient's viral loads, and the amount of the HCV that can be successfully genotyped depends on the method of genome amplification. However, methods that rely on nucleic acid amplification products that are generated directly from the HCV 5' NCR are typically efficient because amplification products are consistently generated from diverse HCV strains and are readily available from qualitative or quantitative HCV RNA testing [7].

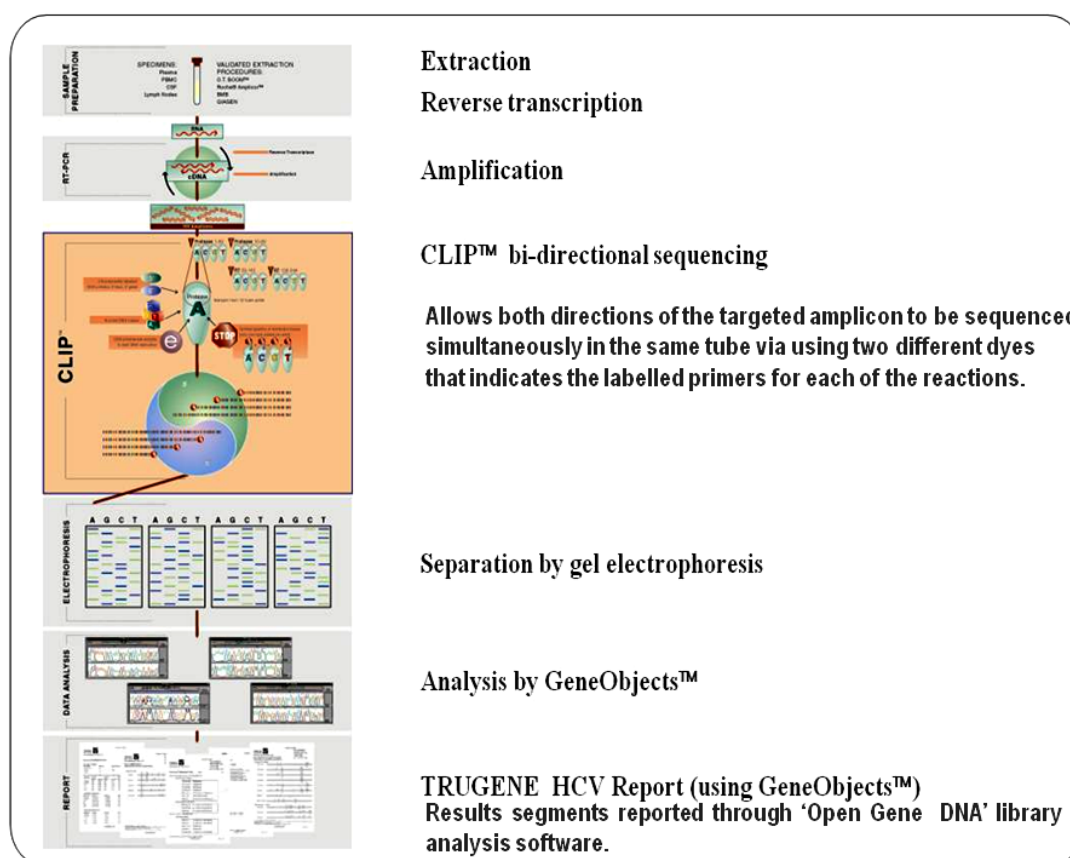


Figure 2 Diagram of the TRUGENE HCV assay.

The method is based on the CLIP approach, which uses the PCR amplicons that are generated by the Roche Amplicor kit to cause a combined PCR and sequencing reaction. The output sequence is then compared with other known sequences using Gene Librarian software. For each reaction, the fragments are simultaneously sequenced in both directions in a single tube with two dyes that are attached to the primers (VisbGene; www.visgene.com).

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