Evolution, classification and genotypes of HCV: the clinical significance of determining HCV genotypes

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Abstract
Chronic hepatitis C, attributed to infection with hepatitis C virus (HCV), is a global health problem. The overall prevalence of viral hepatitis worldwide is estimated to be 3.8% with over 185 million people infected with HCV. Clinically, HCV can establish a persistent, chronic infection contributing to progressive liver disease, including cirrhosis and hepatocellular carcinoma (HCC), requiring intensive treatment regimens, possible liver transplantation and long-term care. Due to the chronic nature of HCV infection and the tremendous burden on healthcare resources, clinicians and laboratorians have looked for key epidemiological, pathological and viral characteristics that may provide insight into disease progression, severity and response to therapy to permit the administration of effective therapeutic regimens as well as long-term management of infected individuals. Determination of viral genotype has been identified as one parameter that could provide direction in the clinical management of patients with chronic HCV infections. The following review provides background on determination of HCV genotypes and the relevance of viral genome characterization in the current clinical setting.

Keyword *- Hepatitis C virus, Evolution, Classifications, Genotypes*

I. HCV Evolution

There is no historical, archeological and paleontological record of HCV, like many other viruses. The analysis of the current distribution and genetic relatedness provide the limited evidence to construct the HCV evolutionary history. Genetic relatedness analysis involves the calculation of the time of divergence of HCV and its variants by using the rate of sequence change with time (1). Several studies have described the rapid sequence drift of HCV over time, a process of diversification that leads ultimately to the existence of identifiably separate strains or isolates within human populations (2). Presence of HCV patients infected with same, homogenous source batch of antirhesus D immunoglobulin used in 1977 in Ireland provided a good opportunity to calculate rate of sequence change by evolutionary distance. Sequence comparisons in the NS5 and E1 regions of HCV isolates from the recipients after 17-20 years later revealed the rates of sequence change as 0.4 and 0.7×10^{-3} per site per year respectively (3) with no evidence for variation in rate between individuals.

Large numbers of NS5 and E1 sequences are available for different HCV subtypes and types and different levels of diversity within these different groupings presumably reflect their time of diversification from each other. Assuming the rate of sequence change is constant over time, time of divergence for the variants of same type can be calculated. The average time of divergence of variants of subtype 1b was about 70-80 years ago, and that subtypes 1a and 1b diverged from each other about 300 or more years ago. Estimation of the time of origin of the major HCV genotypes (types 1-6) is problematic, but study data and analogy with other viruses suggest that divergence occurred at least 500-2000 years ago (3).

Different molecular mechanisms including mutation, genetic drift, recombination and natural selection shape the molecular evolution of HCV. Analogous to the other viruses, HCV circulates as heterogenous but related genomes, called quasispecies. The processes of neutral and adaptive evolution of HCV operate during the course of chronic infection within an individual, leading to both continued fixation of nucleotide changes over time and the development of variable degrees of sequence diversity within the replicating population at a given time point (2). This

viral population is composed of a dominant sequence called master sequence and a number of different sequences. Master sequence generally represents the concensus sequence of the population. This sequence might not represent an actual genome in the quasispecies, but it is useful to identify the adaptive (Drawinian) changes that affect a representative proportion (>50%) of total quasispecies population (2).

The heterogeneity of HCV within an individual can be attributed to error prone RNA-dependent RNA polymerase (RdRp) of HCV, NS5B. The absence of proof reading activity of NS5B creates the de novo mutation. Insertion of point mutations by the RdRp is the primary element contributing to the high genetic variability of HCV. The HCV mutation rate *in vivo* is about 2.5×10^{-5} per nucleotide per genome replication during HCV infection (4). It was hypothesized that the existence of HCV as quasispecies may be the basis of the mechanism for viral persistence. With high number of diversified RNA produced per day and NS5B without proofreading activity would leads to production of escape mutants, therefore, HCV establishes chronic infection.

Genetic drift is also a contributor to the molecular evolution of HCV. There are alternative mechanisms to explain persistent hepatitis C virus infection. The hypervariable region (HVR1) was a major site for genetic mutations in HCV after the onset of hepatitis. The frequent mutations in HRV1 are involved in HCV persistent infection. During chronic infection, clearance of HCV by anti-HRV1 antibodies is slower than the rate of escape from the antibodies by amino acid-substitution (5).

In addition to its possible importance in viral persistence, quasispecies may have a role in response to DAA therapy. Emergence of drug-resistant mutations is a problem challenging the development of direct-acting antiviral drugs. Like most RNA viruses, HCV evolves rapidly because of high-level viral replication through an error-prone RNA polymerase that lacks associated proofreading capacity. As a consequence, the viral population exists as a complex mixture of genetically distinct, but closely related, variants commonly referred to as a quasispecies, whose composition is subject to continuous change due to the competition between newly generated mutants and existing variants with different phenotypes and fitness. The efficacy of DAA is limited by presence of these mutations, resulting in amino acid substitutions within the targeted proteins which affect viral sensitivity to these compounds (6).

In overall, with current knowledge it is not possible to determine a geographical region or temporal origin for the common ancestor of HCV genotypes. Molecular evolutions as well as antigenic and biological differences between HCV types are correlated with the long term endemic infection with no significant exchange of types between different geographical regions (7).

II. Classification and genotypes of HCV

A. Classification system of HCV

A standard system for HCV classification is of importance in studies of the epidemiology, evolution, and pathogenesis of HCV. Soon after HCV was discovered, it was apparent that genetically distinct strains were prevalent in different geographic areas. HCV has been classified in different genotypes and subtypes. Within genotypes, phylogenetically distinct clusters may be found that are called subtypes. Clinically genotypes and subtypes are very similar, though they vary in responsiveness to direct-acting antiviral agents. The pairwise distance between genotypes ranges from 29%to 34% for genomic nucleotide sequences and 24% to 33% for amino acid sequences spanning the polyprotein (1) and were primarily divided into11 genotype. However, phylogenetic analyses indicated that genotype 10 is closely related to genotype 3 and genotypes 7, 8, 9 and 11 to genotype 6 (8). Therefore, a new consensus nomenclature system was proposed to be used for HCV classification 6 (1). According to this system, HCV is classified into genotypes on the basis of <70% similarity of nucleotide sequence and phylogenetic relationship. The more closely related HCV strains (70% -80% sequence similarity) within genotypes are designated as subtypes. Genotypes are numbered in order of discovery and subtypes are assigned by lowercase letters (Table no 1). International standards for nomenclature established six major genotypes that are phylogenetically distinct, and subsequent reports have resulted in the proposal of a seventh genotype. Nowadays, HCV is classified into 7 genotypes and 67 subtypes (9). To perform HCV sequence classification, annotation, and analysis, several sequence databases are dedicated specifically to HCV, such as (Los Alamos HCV Sequence Database, euHCVdb, Hepatitis Virus Database: http://s2as02.genes.nig.ac.jp/)

Table 1: Genotypes/subtypes that are presently defined in the Los Alamos Database

Genotype	Assigned subtypes

1	**a, b, c, d, e, f, g, h, i, j, k, l, m**
2	**a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r**
3	**a, b, c, d, e, f, g, h, i, k, l**
4	**a, b, c, d, e, f, g, h, k, l, m, n, o, p, q, r, s, t, v, w**
5	**a**
6	**a, b, c, d, e, f, g, h, i, j, k, l, m, n, o, p, q, r, s, t, u, v, w, xa**
7	**a**

B. Clinical Significance of HCV genotypes

Although the impact of HCV heterogeneity and genotypes on the day-to-day clinical management of chronic HCV infection has not been established, its role as an epidemiologic marker has been clearly shown. Furthermore, the sensitivity and specificity of serological and virological assays for the detection of HCV may be influenced by the heterogeneity of HCV. However, the exact role of genotypes in the progression of liver disease, the outcome of HCV infection, and the response to antiviral drugs are much less well understood than their role as an epidemiologic marker. The study of these issues has been hampered by the long natural history of HCV infection and the lack of information about the exact time of exposure to the infection. However, the heterogeneity of HCV might have biological importance (10). Unavailability of cell culture system to propagate of HCV and to study differences in cytopathic properties as well as neutralization of HCV led to use of nucleotide sequence comparisons and genotyping as the main techniques for characterizing different variants of HCV. Several studies have been performed to investigate the clinical significance of HCV genotypes. The relationship of HCV genotype with molecular epidemiology and transmission has been suggested and summarized below.

a). Molecular epidemiology and transmission

Because of geographic clustering of distinct HCV genotypes, genotyping may be a useful tool for tracing the source of an HCV outbreak in a given population. Examples include tracing the source of HCV infection in a group of Irish women to contaminated anti-D immunoglobulins (11). All of these women were infected with HCV genotype 1b, a genotype identical to the isolate obtained from the implicated batch of anti-D immunoglobulin. Hohne et al. used genotyping to trace the sources of outbreaks in Germany (12). More recently, genotyping and molecular characterization of HCV isolates provided evidence for a patient-to-patient transmission of HCV during colonoscopy (13). The index case as well as the two other infected patients had HCV genotype 1b. Nucleotide sequencing of the NS3 region showed that the three patients had the same isolate (100% homology), strongly suggesting a common source of infection. Although Zein et al. found no association between HCV genotypes and the mode of HCV acquisition in their population (14), others have provided evidence for such an association (15; 16; 17). It has been suggested that genotypes 3a and 1a are closely associated with intravenous drug use and that genotype 1b is seen more often in patients who acquired HCV through blood transfusion. This information may be useful in tracing sources of HCV epidemics.

Suspected nonconventional routes of HCV transmission could also be investigated by molecular analysis of HCV strains from different persons. These include the vertical and sexual routes. Weiner et al. showed that a single predominant HCV variant was transmitted to an infant born to a mother infected with multiple variants (18).

Reports on the sexual transmission of HCV infection are conflicting. The detection of anti-HCV positivity ranged from 0% in partners of transfusion-associated hepatitis patients to 8% in male homosexuals and 5% in household contacts (19). A possible explanation is that sexual transmission occurs only in association with specific HCV genotypes or in the presence of specific mutations along the HCV genome.

b). Outcome of acute HCV infection

After initial exposure to HCV, the infection fails to resolve in the majority of patients (80%) who become chronically infected. The ability to evolve into chronic disease associated with liver damage is by far the most striking feature of HCV.

The spontaneous clearance of HCV following acute infection in a small proportion of patients has been the focus of intense investigations. It has been proposed that differences in the host cellular (20) or humoral immune responses to HCV are important in spontaneous clearance, but these proposals remain unproved. Amoroso et al. specifically investigated the role of HCV genotypes in persistence of HCV infection following an acute exposure. The rate of evolution to chronicity after acute exposure to HCV was 92% in patients exposed to HCV genotype 1b infection, compared with 33% to 50% in patients exposed to other genotypes (21). These data provided evidence that viral factors, including the HCV genotype, may potentially play an important role in the development of chronic infection following acute exposure to HCV.

c). Diagnosis

One major concern related with the hepatitis C infection is to prevent the new cases of infection. Therefore, the diagnosis of HCV is very important.

ELISA is the routeinly used technique for diagnosis and most ELISA techniques detect the presence of anti-HCV antibodies in individuals by utilizing recombinant proteins derived from only one genotype (mainly subtypes 1a). The reported antigenic variation between between genotypes has raised a question about the effectiveness of these tests in determination hepatitis C patients infected with other variants of HCV. First and second generation HCV antibody tests were found to less sensitive for detection
of type 2 and type 3 than type 1 infections. Antibody reactivity to the c100-3 protein was also reduced in patients infected with HCV types 2b and 3a (22). Dhaliwal *et al* examined the sensitivity of third generation ELISA assay and found approximately 5 fold reduction in reactivity of type 2 and type 3 samples compared to that of type 1 samples (23). These third-generation assay have higher sensitivities and specificities than second generation assay and are much less strongly influence by the infecting genotype.

d). Disease progression

The role of HCV genotypes in the progression of liver disease is one of the most controversial areas of HCV research. Most of the studies have the form of cross-sectional studies in which the frequency of infection due to different genotypes are in comparison with different stages or outcomes of the disease, such as cirrhosis and hepatocellular carcinoma (HCC). Several studies showed that subtype 1b infections proceed much faster to sever forms of chronic hepatitis (24), cirrhosis and HCC (25). Howerver, there are also reports failed to show the association between genotype 1b infections and faster progression into cirrhosis (26). In Middle East and Europe, type 4 infection is associated with high frequency of cirrhosis and HCC. But in Central Africa it has a benign course of disease. Unfortunately, there are no studies characterizing infections of other subtypes.

e). Direct-acting antiviral (DAA) treatment for HCV

A greater understanding of the hepatitis C virus (HCV) genome and proteins has enabled efforts to improve efficacy and tolerability of HCV treatment. Notably, this has led to the development of multiple direct-acting antivirals (DAAs), which are medications targeted at specific steps within the HCV life cycle. DAAs are molecules that target specific nonstructural proteins of the virus and results in disruption of viral replication and infection. There are four classes of DAAs, which are defined by their mechanism of action and therapeutic target.

The four classes are nonstructural proteins 3/4A (NS3/4A) protease inhibitors (PIs), NS5B nucleoside polymerase inhibitors (NPIs), NS5B non-nucleoside polymerase inhibitors (NNPIs), and NS5A inhibitors. The HCV genotype, including genotype 1 subtype (1a or 1b), should be assessed prior to treatment initiation. Genotyping/subtyping should be performed with an assay that accurately discriminates subtype 1a from 1b, i.e. an assay using the sequence of the 5′ untranslated region plus a portion of another genomic region, generally the core-coding or the NS5B-coding regions (27). The results of the Phase III ASTRAL-1 trial in patients with HCV genotype 1 infection (22% with cirrhosis, 66% treatment-naïve, 34% treatment-experienced, 44% of whom were exposed to previous DAA) treated with the fixed-dose combination of sofosbuvir and velpatasvir for 12 weeks without ribavirin. An SVR12 was observed in 98% (323/328) of patients, including 98% (206/210) in those infected with genotype 1a and 99% (117/118) in those infected with genotype 1b (28).

The Phase III ASTRAL-2 trial in patients with HCV genotype 2 infection (14% with compensated cirrhosis, 86% treatment-naïve, 14% treatment-experienced) treated with the fixed-dose combination of sofosbuvir and velpatasvir for 12 weeks without ribavirin, showing SVR12 in 99% (133/134) of patients (29).

In 2016 and onwards, IFN-free regimens are the best options in treatment-naïve and treatment-experienced, DAA-naïve patients with compensated and decompensated liver disease, because of their virological efficacy, ease of use and tolerability. Indications depend on the HCV genotype/subtype, the severity of liver disease, and/or the results of prior therapy. The indications are the same in HCV-monoinfected and HIV coinfected patients (30).

f). Other clinical implications of the HCV

In addition to the lack of appropriate cell culture and small animal models, the existence of distinct HCV genotypes with antigenic variability complicates the development of an effective prophylactic or therapeutic vaccine (31). The factors that contribute to an effective immune response against HCV have not been clearly defined but it is likely that both humoral and cellular immunity will need to be generated through vaccination (32). However, neutralizing epitopes are among the most distantly related sequences across different HCV genotypes, probably contributing to the lack of protective immunity following multiple exposures to HCV, the number of patients with mixed genotypes and re-infection of individuals who have recovered from HCV infection in the past (31, 33, 8). These observations underline the quasispecies nature of HCV and the selection of strains to avoid immune pressure (19). Regions of the core protein, the NS3 protein and the hypervariable region (HVR1) located towards the N-terminus of the HCV E2 protein have been implicated as possible targets for HCV-specific cytotoxic T-lymphocyte (CTL) recognition (34, 35, 36, 37, 38). However, single amino acid changes within these epitopes can result in a failure of recognition by HCV-specific CTLs. Development of

an effective vaccine for HCV will need to account for the genetic differences between HCV clades and the variability observed within HCV quasispecies to escape immune surveillance (39).

Summary and future directions

Chronic viral hepatitis and the complications associated with progressive liver disease are a global health problem. One of the major causative agents, HCV, has been classified into distinct clades, genotypes and subtypes based upon sequence analysis of their respective genomes along with determination of phylogenetic relationships between the natural variants (Table 1). Genotypes of HCV have distinct geographical distributions. A clear understanding of regional genotypes as well as global migration patterns may help governments and healthcare systems prepare the resources required to treat the predominant HCV genotype infecting individuals in their regions. Currently, determination of HCV genotype has direct clinical implications for duration and dosage of combination therapy of DAA drug including ribavirin. However, the detection of drug-resistant mutations or natural polymorphisms in the HCV genome that affect response to therapy is not widespread as alternative treatment options available for patients with chronic hepatitis C are currently limited. It is clear that well designed studies are required to provide definitive insight into the role of HCV genotypes in disease progression for patients with chronic hepatitis.

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