Hepatitis C Virus: Genomics, Clinical Features and the Changing Landscape of Treatment by Directly Acting Antivirals (DAA)

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ABSTRACT

The Hepatitis C Virus (HCV) is estimated to infect about 3% of the world population with chronic infections resulting in liver fibrosis, cirrhosis and hepatocellular carcinoma. Though culturing of the virus was a big challenge; the whole genome could be deciphered through many molecular biology techniques. The treatment of HCV through antivirals and later in combination with interferons produced considerable toxicity. However, with the development of directly acting antivirals (DAA), in recent years, has shown good control of HCV. The use of DAAs in the treatment regimen of HCV has been a game changer, as vaccine is not yet available.

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INTRODUCTION

The attention of scientists and the public health community was drawn to the seriousness of hepatitis in the 1940's after World War II when blood banking had just begun in earnest and when alarming rates of post-transfusion hepatitis (PTH) infections among recipients of blood transfusion was noted [1]. It was however not until the mid 1970s that researchers using specific serological assays demonstrated that the cause of PTH was not due to hepatitis A or hepatitis B viruses [1,2]. However, at that time, the cause and name of the virus had not yet been discovered. Thus, this unknown virus was temporarily named non-A, non-B hepatitis (NANBH) [1]. It was not until 1989 that the putative agent that caused NANBH was isolated and identified by Michael Houghton and coworkers at Chiron Corporation in Emeryville, CA, in collaboration with Daniel Bradley of the Centers for Disease Control in Atlanta, GA; employing exclusively molecular biology techniques using the blood of an experimentally infected chimpanzee [3,4]. In his landmark publication on April 21,1989, Michael Houghton said," Thus, our data indicate that clones 5-1-1 and 81 are derived from the genome of a blood-borne NANBH virus that we now term hepatitis C virus (HCV). The cDNA clones reported here were obtained in the absence of prior knowledge concerning the virus, the viral genome, and the presence of circulating antibodies. As such, this represents cloning without characterization of the infectious agent. This approach should be relevant to studies of other diseases in which an unknown infectious agent (viral or other) might be involved". Following the discovery of HCV, research on the molecular biology of the nucleotide sequence of the entire viral genome was determined, and the agent was characterized as a single-stranded positive-sense RNA virus of about 9600 nucleotides in length [5,6].

CLASSIFICATION & GENOTYPES OF HCV

Hepatitis C virus (HCV) belongs to the *Flaviviridae* family under the Hepacivirus genus. (Fig. 1) The virus is transmitted through contaminated blood or blood products and evolves into a chronic infection in 60-80% of those infected. With approximately 200 million carriers worldwide, HCV is responsible for more than 50% of adult liver transplantations in the western world. As with most RNA viruses, the lack of proofreading polymerase causes the genome to exhibits high genetic diversity. Importantly, HCV seems to lack many of the constraints on variability that are seen for most of its close relatives, which do not display a great heterogeneity in nature. HCV variability is so great that numerous quasi-species appear within single infected individuals[3]. Thus, HCV is classified into 7 phylogenetic clades which are designated as genotypes numbered 1 to 7, with more than 30% nucleotide sequence divergence, and over 70 variants or subtypes (1a, 1b, 2a, etc.) within each genotype [7,8]. The HCV genotypes generally show differences in worldwide distribution (Fig. 2)for the global distribution of HCV genotypes), transmission, disease progression, and response to treatment [9,10]. In fact, HCV genotypes are important predictors of treatment outcome [11]. While genotype 1, 2, and 3 viruses are prevalent almost worldwide, HCV genotypes 4 – 6 are to a large extent restricted to distinct geographical regions, including Africa and Middle East (genotype 4), South Africa (genotype 5), and Southeast Asia (genotype 6).

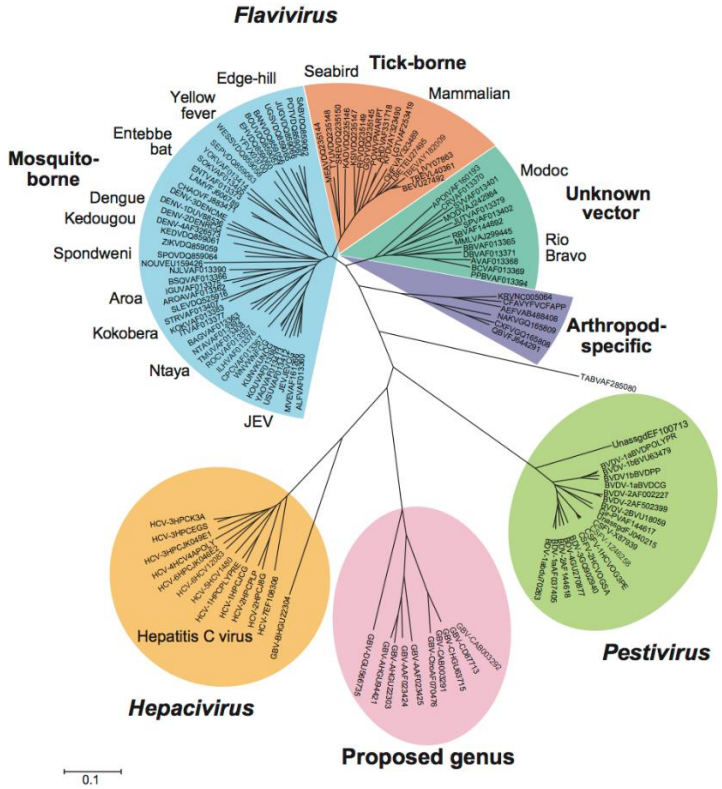

Fig 1 *Different viral genera of the family Flaviviridae.* (Source: ICTV).

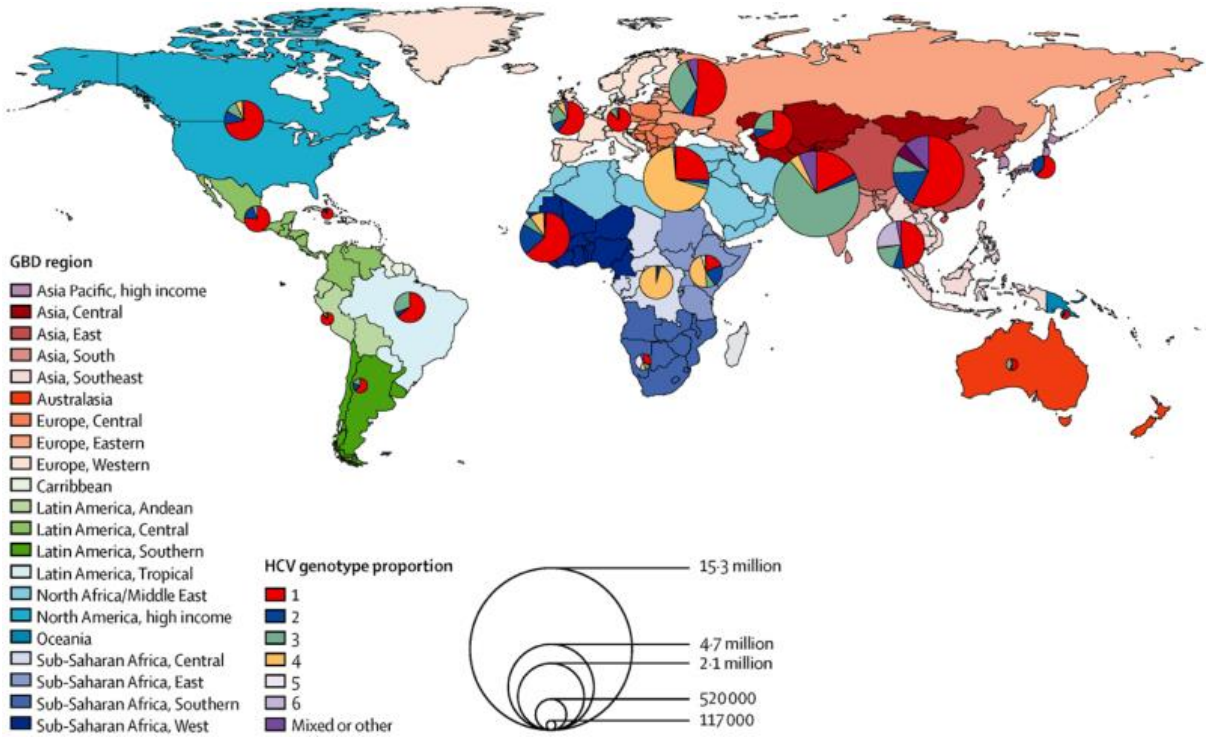

Fig 2 *Incidence of HCV around the world along with the distribution of different genotypes.* (Source: WHO)

EPIDEMIOLOGY AND NATURAL HISTORY OF HCV

Chronic hepatitis C virus (HCV) infection is a major and growing public health problem throughout the world. However, the global scale of the infection is not well known, due to the asymptomatic nature of the acute phase of infection. The World Health Organization (WHO) estimates that approximately 130 –170 million individuals representing 2.2 - 3% of the world population are chronically infected with HCV [12]. In addition, 3 – 4 million people are newly infected with HCV each year and 350,000 patients die from HCV-related disease [13,14]. There are also geographic and regional differences in the epidemiology and burden of HCV infection [15] with high rates of chronic infection in places such as Egypt (15%), Pakistan (4.8%), and China (3.2%) [14]. (Refer to Fig 2 for the geographic distribution and prevalence of HCV along with the distribution of its genotype). HCV infection has been referred to as the 'silent epidemic' as majority (75- 80%) of the infected individuals are hardly diagnosed because most of the acutely infected individuals are asymptomatic [16]. Moreover, most infected persons are unaware of their exposure to HCV, and do not get diagnosed until many years later [17]. Thus, the rate of chronic HCV infection is affected by the person's age at the time of infection, gender, race, and viral immune response [18]. A large proportion (about 75%) of HCV-infected persons will go on to develop chronic HCV infection, and are at risk for advanced liver complications including cirrhosis, liver failure, and hepatocellular carcinoma [18], a fact attributed to the genetic diversity of the virus and its tendency towards rapid mutation, thus allowing it to escape immune recognition. However, a good proportion of the infected patients (about 20 - 40%) will spontaneously clear the virus, becoming HCV RNA negative [19,20]. Similarly, an estimated 10 - 20% of chronic HCV infections advance to end-stage liver disease over one or two decades [19].

MODES OF TRANSMISSION

Percutaneous by shared drug injections, transfusion of blood or clotting factors (prior to screening that began in 1990s), reuse of contaminated personal care materials, tattooing and piercing equipment, risky sexual activities, etc. [13]. Vertical transmission from infected mother to fetus may occur at a very low rate (6%). For prevention, screening of individuals at increased risk is highly recommended [14].

HCV STRUCTURE & GENOME ORGANIZATION

For a long time, the structural analyses of HCV virions have been limited due to the difficulty in cultivating the virus in cell culture systems, a prerequisite for yielding sufficient viral particles (Fig. 3A) for electron microscopy [18]. Moreover, the composition of the HCV virion tends to vary depending on where it is produced, i.e., in patients, animals, or the infectious tissue culture system [21]. Despite these challenges, a couple of studies have shown that HCV virions isolated from cell culture have a spherical envelope containing tetramers (or dimer of heterodimers) of the HCV E1 and E2 glycoproteins [22,23]. Similarly, inside the virions a spherical structure has been observed [23] representing the nucleocapsid (core) that harbors the viral genome (Fig. 3B). Thus, the virion is believed to adopt a classical icosahedral scaffold in which glycoproteins E1 and E2 (Fig. 4A) are anchored to the host cell-derived double-layer lipid envelope [24]. The HCV genome of 9.6 kb is made up of a single stranded RNA molecule of positive polarity that encodes a unique open reading frame (ORF) that is translated into a precursor polyprotein of about 3000 residues. The ORF is flanked by two regulatory untranslated regions (UTR) (Fig. 4B), 5'UTR and 3'UTR, respectively. Both UTR bear highly conserved RNA structures essential for polyprotein translation and genome replication. The 5'UTR contain an internal ribosomal entry site (IRES) that binds the 40S ribosomal subunit and initiates polyprotein translation in a cap-independent manner [25]. The HCV polyprotein precursor is co-translationally and post-translationally processed by both cellular and viral proteases to yield at least three structural (core, E1, and E2), six non-structural (NS2, NS3, NS4A, NS4B, NS5A, and NS5B) viral proteins [26], and a small ion channel protein, p7, located between the structural and non-structural proteins. (Fig. 4B). These proteins have important roles at different steps of the virus life cycle and are involved in viral-viral as well as viral-host interactions [27].

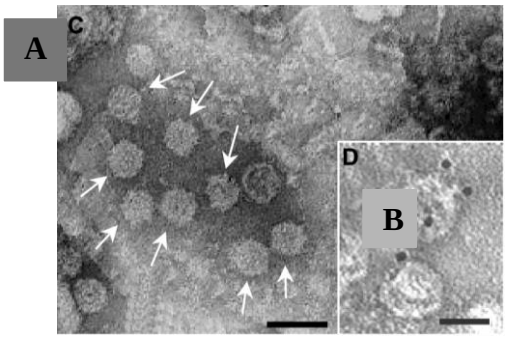

Fig 3A. *Particles of HCV as observed by electron microscopy.* **B.** *Stained genomes of HCV (*Source CDC)

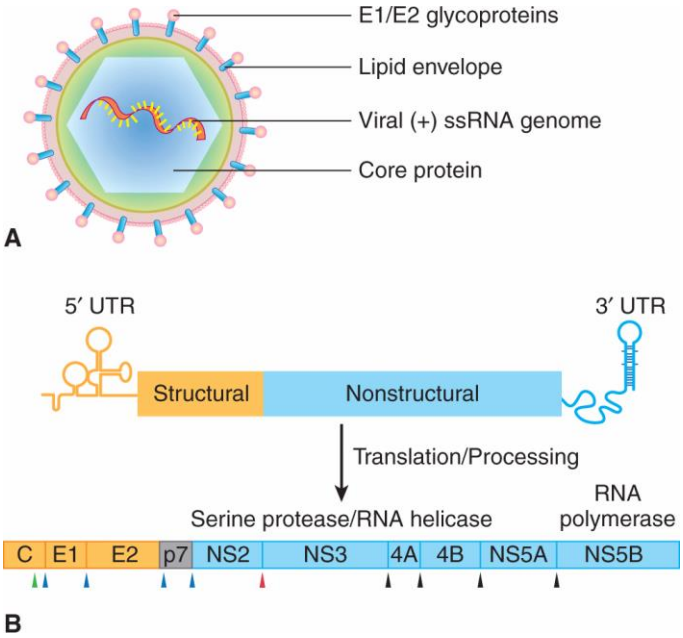

Fig 4A. *Structural features of HCV particles.* **B.** *Genomic features of HCV* (Source Swiss Institute of Bioinformatics)

HCV LIFE CYCLE & REPLICATION

The exact mechanisms of the hepatitis C viral lifecycle are not fully known, since we until recently lacked an efficient infectious cell culture model. The known details have been worked out using predominantly molecular studies of recombinant expressed HCV proteins and by comparing with other members of Flaviviridae and positive stranded RNA viruses. HCV enters cells via receptor-mediated endocytosis involving the interaction of the viral particle with a series of cell surface receptors and/or factors [27]. Some of the factors or receptors that are involved in HCV entry into cells include the tetraspanin CD81, the cholesterol transporter scavenger receptor SR- BI/Cla1, the LDL receptor (LDL-R), the components of tight junctions claudins (CLDN) 1, 6, and 9 and occludin (OCLN), the receptor for epidermal growth factor (EGFR), ephrin receptor A2 (EphA2), and the cholesterol absorption receptor Niemann-Pick C1-like-1 (NPC1L1). In addition, glycosaminoglycans (GAG), such as heparin sulfate, and the lectins DC-specific intracellular adhesion molecule-3- grabbing non-integrin (SIGN) and liver-specific (L)-SIGN have been implicated in HCV cell attachment and entry [28]. HCV infection or entry into host cells is initiated by binding of the viral envelope proteins to cell surface receptors via a complex multistep process, followed by their internalization into endosomes in a clathrin-dependent manner [29,30]. Once inside the cell, the virus- containing endosome is uncoated to release the positive-strand viral genomic RNA into the cytoplasm; a process thought to occur after low pH-induced fusion of the viral and endosomal membranes, mediated by the viral envelop glycoproteins [29,31,32]. The released viral genomic RNA is then translated into a polyprotein within the membranous web. The polyprotein is subsequently co- and post- translationally modified into individual viral proteins, which leads to the establishment of replication complexes in ER-derived membranous compartments where viral RNA replication occurs via negative strand viral RNA synthesis [33]. The progeny viral genomes are either translated to produce additional viral proteins or packaged to assemble progeny infectious virions. These then bud into the lumen of the ER and leave the host cell through the secretory pathway, completing the viral lifecycle [28].

DIAGNOSIS

1.ELISA

This is a standard serological method for screening and primary testing for HCV diagnosis. It uses antigens from NS5 region in addition to core, NS3 and NS4 regions.

2. Q-RT PCR

This molecular technique makes Real Time Reverse Transcriptase detection of HCV RNA and has been the gold standard method for:

- Confirmation of active HCV infection
- Quantification of HCV RNA for monitoring the response to treatment
- For determining HCV genotype and subtype (also done by sequence-based method [18]

MODEL SYSTEMS

Research on HCV has been difficult due to the lack of *in vitro* cell culture systems and small animal models. The need for an infectious cell model is to be able to characterize in detail the steps of viral lifecycle such as entry, replication, assembly, and release. Not only is a model system needed for identifying viral components but also to understand disease progression and the host immune response against the viral infection. Currently, there is no single model, except for the chimpanzee, that can replicate all the above stages of HCV life cycle [27]. Instead, there are several models each simulating different stages of infection and for different viruses.

HCV TREATMENT

The hepatitis C virus induces chronic infection in 60-80% of infected persons. Approximately, 50% of these do not respond to therapy, depending on the infecting HCV genotype (gt) and those who clearly does not relapse even after strong immunosuppressive therapy, indicating that the virus is completely eradicated [34]. Since HCV is an RNA virus, it cannot integrate into host genome and has to keep replicating to persist in the liver. Therefore, HCV RNA levels in sera (IU/ml) are an accurate measurement of infection. Earlier, the standard therapy of chronic hepatitis C used to be a combination of pegylated (PEG)-IFN-2a or -2b subcutaneously, once a week, along with twice-daily oral administration of ribavirin [35].

Ribavirin with PEG-IFN is essential since ribavirin monotherapy does not lower HCV RNA in blood [35, 36]. HCV gt 1 and 4 has the lowest response rate to the combination therapy with up to 40- 50% cure rate, while gt 2 and 3 has a cure rate of up to 85% [34]. Ribavirin is a broad-spectrum antiviral agent, administered as a prodrug and is metabolized in the liver into a nucleoside analogue, but its mechanism of action remains unknown.

IFN therapy has several severe side effects and ribavirin adds to the problem with the additional side effects of hemolytic anemia. This makes IFN- ribavirin therapy difficult to adhere and non-responders should after consideration be taken off treatment, since no further benefits would be gained by adhering to this regimen.

Advances in our knowledge about the HCV life cycle and the generation of recombinant tissue culture infectious viruses, have enabled the development of Directly Acting Antiviral (DAA) compounds. Between May 2011 and August 2017, twelve therapies were approved by the FDA and another two in Japan. The first of these to become approved in combination with PEG-IFN and ribavirin were the first generation NS3/4A protease inhibitors, Telaprivir and Boceprivir, for the treatment of gt 1 infected individuals, with sustained virological response (SVR) of 65-75% [37,38]. However, both of these have been discontinued due to severe side effects. The second-generation protease inhibitors (Fig.. 5) however, showed comparable SVR rates with better tolerability [39]. A milestone in HCV therapy was the development of NS5B polymerase inhibitor-sofosbuvir, a nucleotide analogue that produces early chain termination after being incorporated into newly synthesized viral RNA [40]. Since then, many new drugs have been made targeting different nonstructural viral proteins. (Fig. 5).

Currently, we have a good single pill of combination drugs which is administered orally over a period of 8-12 weeks for effectively curing HCV infection (defined as SVR or absence of viral RNA after treatment) in more than 90% of patients.

HCV infected patients should be vaccinated against HAV and HBV, since co-infection is associated with a more severe liver disease progression.

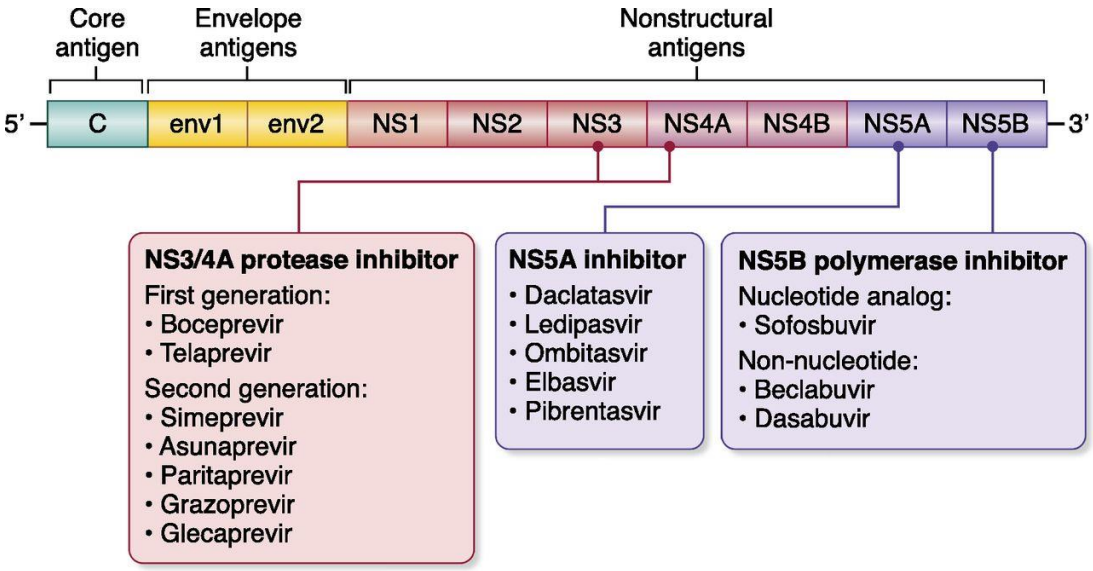

Fig 5 *Different generations of Directly Acting Antivirals and their targets* (Source American Society of Nephrology)

FUTURE PUBLIC HEALTH CHALLENGES:

Availability of highly effective DAAs that cure the vast majority of HCV infections is hailed as a major medical triumph, but still there are a few challenges. First, a large number of persistent HCV infections are clinically silent, often undiagnosed and will not be recognized by patients or physicians until liver damage has reached an advanced stage. So routine liver screening should become an important component of public health. Second, the newer DAAs are quite expensive and will remain out of reach of a majority of HCV patients living in low-

and middle-income countries for many years. Third, with the broader use of antiviral therapy, the problem of DAA resistance is on the rise.

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