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Chapter 2

Genomic Heterogeneity of Hepatitis Viruses (A-E): Role in Clinical Implications and Treatment

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Additional information is available at the end of the chapter

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1. Introduction

Hepatitis is an inflammation of the liver. There are at least five different viruses causing hepatitis. Each of the five major hepatitis viruses, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), delta hepatitis virus (HDV) and hepatitis E virus (HEV) belong to a separate family. Currently, all these viral hepatitis (A-E) have been classified into different genotypes and subgenotypes. Several factors, including viral genotypes have been reported to be associated with disease progression and treatment response. Consequently, documentation of genotype recently have been proved to be a valuable tool not only for epidemiological reasons but also for clinical implications and treatment.

2. Hepatitis A virus

HAV is a member of the *Hepatovirus* genus of *Picornaviridae* family. HAV is a non-enveloped (naked), linear, single stranded RNA virus of an icosahedral symmetry measuring 27-32 nm in diameter [1]. HAV infection is hyper-endemic in vast areas of the world, with approximately 1.5 million clinical cases per year [2]. The worldwide distribution is uneven and is based on determinants such as socioeconomic conditions and geographic factors [3-5]. In developing countries, the incidence of disease in adults is relatively low because of exposure to the virus in childhood. Most adults in these areas show prevalence of antibodies against hepatitis A. In developed world endemicity is usually very low and clinical cases occur almost exclusively in adults [6,7]. The variable age distribution among hepatitis A patients in developing and developed countries is a consequence of differing standards of hygiene and sanitation. In many developing countries, improved hygiene standards and socio-economic conditions have led

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to a reduction in exposure to HAV in childhood and hence large non-immune adult population in the community. This leads to a shift or transition from asymptomatic childhood infections to an increased incidence of symptomatic or clinical disease in adults [8]. The persistence of circulating HAV may lead to hepatitis A outbreaks in susceptible non-immune adult population [8,9].

2.1. Disease severity

HAV causes an acute self-limited illness. The vast majority of hepatitis A patients make a full recovery and fatality rate is low. The estimated mortality rate is 0.1% for children less than 15 years old, 0.3% for adults ages 15 to 39, and 2.1% for adults ages 40 and old [10,11]. HAV does not lead to chronic hepatitis or a carrier state and only rarely leads to fulminant hepatic failure (FHF) [12]. FHF occurs during the first 4-6 weeks of illness which is characterized by sudden onset of high fever, marked abdominal pain, vomiting and jaundice followed by development of hepatic encephalopathy associated with deep coma and seizures [13,14]. Mortality is highly correlated with increasing age, survival being rare over the age 45 years [15]. The acute HAV super infection with chronic liver disease is also associated with severity and high mortality [16,17].

2.2. Genomic organization

Like all picornaviral genomes, HAV is divided into three parts: (i) 5′ non-coding region (NCR) that comprises approximately 10% of the genome (ii) single open reading frame (ORF) of 2227 amino acids, that encode all the viral proteins, with regions designated as P1 for capsid proteins, P2 and P3 for non-structural proteins and (iii) short 3′ non-coding region (Fig. 1).

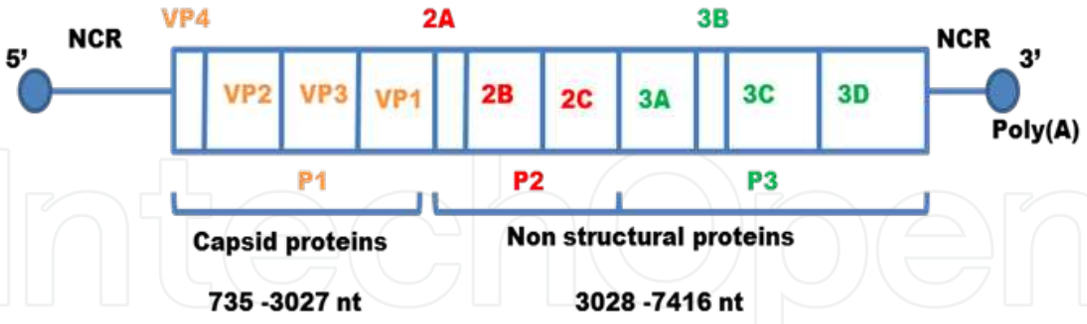


Figure 1. Genomic organization of hepatitis A virus: HAV genome is divided into a 5′ non-coding region (5′ NCR), a giant open reading frame, and a 3' non-coding region (3′ NCR). The coding region is subdivided into regions P1, P2 and P3. (Adapted from: Ref. 20)

HAV RNA genomes lack the cap assembly found at the 5′ end of mRNA species that normally guides the ribosomal complex to the translation start site [18]. Instead, an internal ribosome entry site (IRES) formed by the 5′NCR functions to initiate translations in HAV including other picornaviruses [19, 20]. However, unlike other picornavirus IRESes, the HAV IRES requires an intact eukaryotic initiation factor 4G for its optimal activity [20]. Several other host proteins

are found to be associated with synthetic RNAs representing segments of the 5′ NCR [21]. The viral capsid protein (P1) is further divided into VP4, VP2, VP3 and VP1 regions. The non-structural P2 and P3 polyproteins are divided into 2A, 2B, 2C and 3A, 3B, 3C, 3D respectively (Fig. 1). HAV polyprotein is processed into precursor intermediates and mature proteins by the proteolytic activities of encoded viral proteins. HAV 2A, 2B, 2C protein encodes 45, 251 and 335 amino acids respectively. The 2A and 3C are identified as processing enzyme in hepatitis A virus. The translated 2A regions function as intermediary, partially located on the surface (VP1) and some are assembled inside the virion [20]. Both 2B and 2C proteins play an important role in the replication of the viral RNA. P3 polyproteins encodes 3A, 3B, 3C and 3D proteins with 74, 23, 219 and 489 amino acids respectively. 3C protein acts as sole protease for HAV protein processing, while 3D is the RNA dependent RNA polymerase [22].

2.3. HAV genotypes and geographic distribution

Genetic heterogeneity of hepatitis A has been revealed by sequencing different genome regions, including VP3 carboxyl terminus, the VP1 amino terminus and the VP1/2A junction [23-25] (Fig. 2). The VP3 C-terminal region is relatively conserved, the VP1 amino acid terminus presents an intermediate variability, while VP1/2A junction is more variable and is used to distinguish one strain from another [25]. The genetic variability observed within the putative VP1/2A junction (168 nucleotides) initially defined seven (I-VII) genotypes [26-29]. However, recently new classification of HAV has been done based on the complete sequences of the 900 nucleotides of VP1 region [30] (Fig. 2).

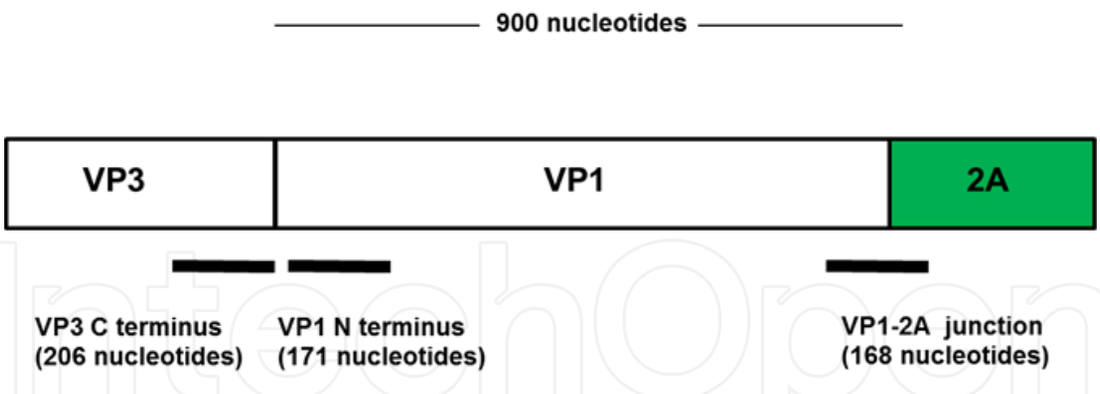


Figure 2. The genomic organization of VP3 C-terminal, the VP1 amino acid terminal and VP1/2A junction region of hepatitis A virus. The complete sequence of the 900 nucleotides of the VP1 gene has been used for new classification of HAV. (Adapted from: Ref. 30)

The phylogenetic analyses of VP1 sequences identified six genotypes (I-VI) that differ among themselves 15-25%. Three isolated from humans (I-III) and three from a simian origin (IV-VI). The genotypes I, II and III were further subdivided into sub-genotypes A and B, which differ in approximately 7.5% of base positions. The worldwide genotype distribution showed genotype I and III comprise the vast majority of human strains within the studied population (Fig. 3). Sub-genotype IA comprises the majority of the human strains studied and constitutes

major virus population in North and South America, China, Japan, Russia and Thailand. The sub-genotype IB contains strains from Jordan, North Africa, Australia, Europe, Japan and South America. Most of the remaining human HAV strains segregate into genotype III that is further divided into two sub-genotypes, IIIA, and IIIB [23,27,29]. The sub-genotype IIIA have been subsequently identified in specimens collected from humans with hepatitis A in India, Sri Lanka, Nepal, Malaysia, Sweden and the U.S.A [26,31].The IIIB sub-genotype is responsible for cases of HAV infection in Japan and Denmark.

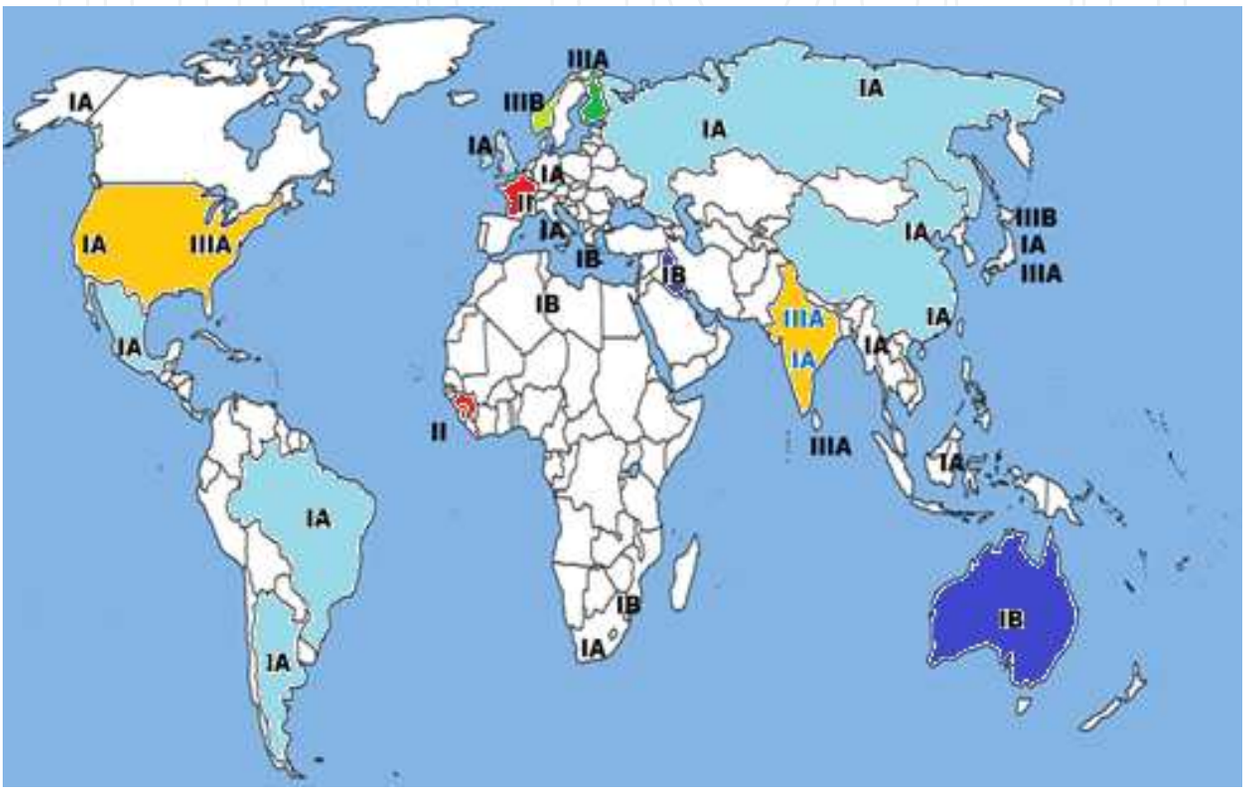


Figure 3. Worldwide distribution of hepatitis A virus genotype(s) according to the VP3 carboxyl terminus, the VP1 amino terminus and the VP1/P2A junction. (Adapted from: Ref. 4, 23, 26, 27, 29, 30 & 31)

2.4. Effect of HAV genotype on disease severity

Although, HAV causes an acute self-limited illness but rarely, it also involved in severe course such as fulminant hepatitis, relapsing hepatitis, prolonged and cholestatic hepatitis. The question arises, if genetic heterogeneity/ genotypes play any role in self-limiting or severe course of the disease. There are contradictory reports regarding the role of HAV genotype in disease severity. Fujiwara *et al.,* [32], reported an association between the severity of hepatitis A and nucleotide variations in the central portion of the 5' NCR of Japanese HAV RNA. Similar study from South Korea reported no association between 5'NCR sequence variations and disease severity [33]. Several other studies also reported no association between genotype or nucleotide changes with disease severity [34-37]. Recently, a comparative analysis of disease severity between genotype 1A and IIIA revealed that the patients with genotype IIIA were

older and had high alanine aminotransferase (ALT) levels, prolonged prothrombin times and lower serum albumin level [38]. Another study indicated that co-infection of 2 sub-genotypes (1a and 1b) in a patient with acute hepatitis accounted for the prolonged and severe course of illness [39]. Therefore, additional studies are needed to define the precise role of viral genotypes in the severity of hepatitis A.

2.5. HAV genotype and antiviral treatment response

Since most of the cases are self-limiting, acute HAV does not require antiviral therapy in immunocompetent patients related to any genotype. The immunosuppressed patients should be recommended ribavirin, so that these patients should not progressed to cirrhosis. Liver transplantation is the only treatment option for patients with fulminant hepatic failure due to HAV. Since hepatitis A exists as a single serotype and human is the only host, it is possible to eradicate by selective vaccination against individuals who are susceptible and sero-negative for HAV-IgM.

3. Hepatitis B virus

HBV is a member of the *Hepadnaviridae* family and is known to be one of the smallest human DNA virus [40,41]. HBV infection is very common worldwide with more than 350 million (5%) of the world's population is chronic carriers [42-44]. Most acute infections with hepatitis B virus are self-limited with clearance of virus and development of immunity [45,46]. However, an estimated 5% to 10% of adults and 85% to 95% of children develop chronic hepatitis B virus infection [47].The prevalence of HBV infection varies throughout the world [44,48]. The prevalence of HBV infection in Asia and Africa is high (8%), with perinatal and early childhood transmission resulting in a high rate of chronicity [44,46,48]. The Mediterranean and Eastern Europe have an intermediate (3-5%) endemicity and maintained mainly through sexual, household, nosocomial and perinatal transmission [44,46,48]. The prevalence of chronic infection in some areas of Europe, North America and Australia is less than 1% and disease is mostly transmitted via sexual contact or through intravenous drug use [46].

3.1. Disease severity

Chronic hepatitis B (CHB) is responsible for 1 million deaths per year globally [49]. It is a major cause of cirrhosis of the liver and hepatocellular carcinoma (HCC) worldwide [50,51].The integrity of the host immunological system, viral replication and probably the genetic heterogeneity (genotypes and mutations) of the (HBV) play an important role in the determination of the natural history [52,53]. However, the high morbidity and mortality associated with chronic HBV results in a substantial medical and economic burden on healthcare systems, marking HBV as an international health problem [52,53].

3.2. Genomic organization

HBV is an enveloped virus (42-47 nm in diameter) with an inner icosahedral nucleocapsid containing DNA genome [54]. The viral genome is approximately about 3.2 kb long, partially double-stranded relaxed circular (rc-dsDNA) structure and covalently bond to the viral encoded DNA polymerase [50,55-57]. The HBV genome has a highly compact coding structure consisting of four overlapping reading frames (ORF) designated as P (polymerase), S (surface/envelope), Pre-C/C (core) and X (HBx protein) (Fig. 4) [55,56,58].The polymerase ORF is the largest and it overlaps the X, the core and the pre-S/S. The pre-S/S ORF has 3 initiation codons encoding the small hepatitis B surface protein (SHBs), middle hepatitis B surface protein (preS2 + S) and large hepatitis B surface protein(pre-S1 + pre-S2 + S) proteins. C ORF encodes the core protein (HBcAg) and pre-core or e' antigen (HBeAg) and it contains an N-terminal extension of 29 amino acids [55,56,58,59].

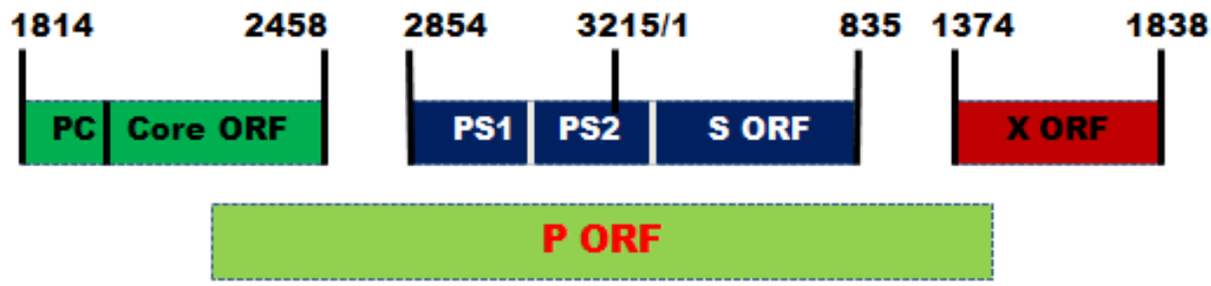


Figure 4. The schematic representation of HBV genome. The circular HBV genome is presented as a linear form. The coding regions for e/core, surface, polymerase and X proteins are designated as Pc/C, S, P and X respectively. (Adapted from: Ref. 59, 55 & 58)

3.3. HBV genotypes and geographic distribution

HBV can be classified into 10 genotypes A to J, based on the comparison of complete HBV genomes [60-64]. These genotypes are based on a divergence of 8% or more in the complete nucleotide sequence of the entire viral genome [61-64]. There are numerous subgenotypes (currently approximately 24) has been identified based on genetic diversity of HBV [65,66]. These subgenotypes were differentiated by a sequence divergence by at least 4% [65,66]. Since genotypic variation of HBV is reflected in a partial sequence of HBV, therefore genotyping of HBV is possible without determining the entire genomic sequence [60,67,68]. As shown in Fig. 5, the S gene is more conserved than the pre-S region, therefore the analysis of the S gene is much more suitable for genotyping. Recently, a fragment of 1306 bp partially comprising HBsAg and polymerase coding regions(S/POL) has also been used for genotyping (Fig. 5) [69]. Several methods have been employed to genotype hepatitis B virus; (a) Direct sequencing [70] (b) Restriction fragment length polymorphism (RFLP) [71] (c) Line Probe Assay (LiPA) [72] (d) Genotype specific PCR [73].

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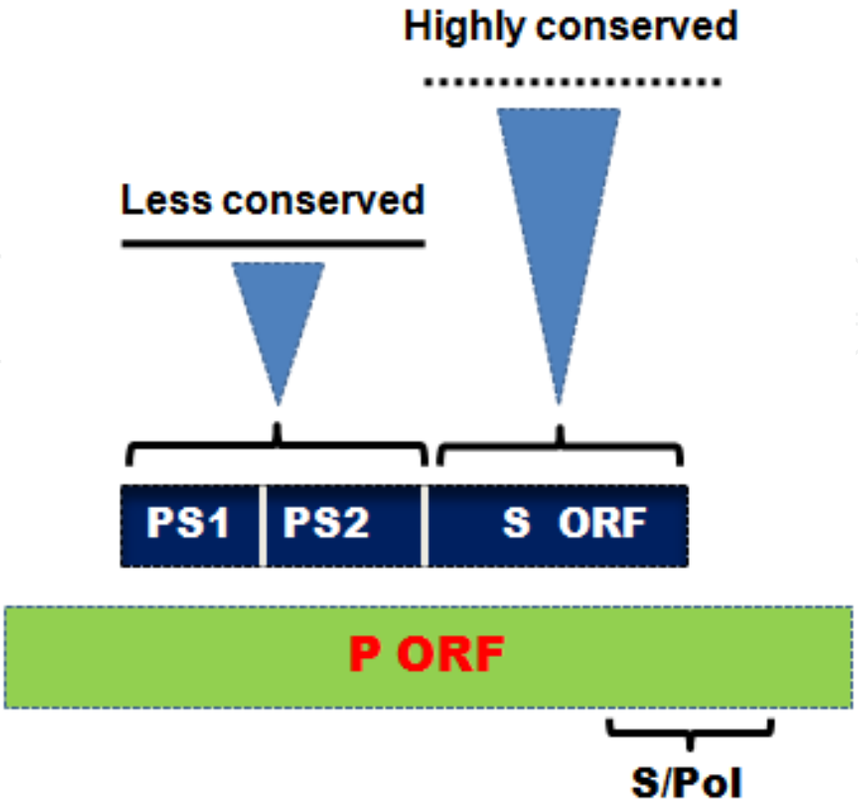


Figure 5. Common genotyping region of HBV genome. The most suitable region for genotyping is S and S/Pol indicated by dotted line (…). (Adapted from: Ref. 60, 67, 68 & 69)

The distribution of HBV genotypes varies across different geographical regions (Fig. 6). HBV Genotype A is most commonly found in the Americas, Africa, India and Western Europe [74, 75]. Genotype B is most common in Asia including Japan, Taiwan, Indonesia, China and Vietnam [76-79]. Genotype C is predominant in East Asia and countries of the Pacific Rim [68], while genotype D most commonly found in the Mediterranean, India, Middle East [75,80-82]. HBV genotype E is mainly identified in sub-Saharan Africa, genotype F in South and Central America as well as Alaska, and genotype G in Central and North America as well as Europe [83-85]. The most recent HBV genotype identified, genotype H, has been found in the United States, Mexico and Central America [86,87]. The genotype F is the most distantly related genetically of the other HBV genotypes (12.8-15.5%) [88]. Recently, genotype I, a novel inter-genotypic recombination among genotypes A, C and G was isolated in Vietnam and Laos [89]. The newest HBV genotype J, was identified in the Ryukyu island in Japan, and this genotype has a close relationship with gibbon, orangutan and human genotype C [90]. The epidemiology of HBV infection is still shifting and the apparent variations in geographical distribution of genotypes is effect of migration from countries with a high prevalence of HBV infection to countries with a lower prevalence.

3.4. Effect of HBV genotypes on disease severity

There are conflicting report about the role of HBV genotypes and severity of the disease. Recent study from Japan demonstrated that genotype B is associated with slower progression to cirrhosis of the liver compared to genotype C [91]. Another study suggested that acute infection with HBV genotype A increases the risk of progression to chronic infection [79,92]. In China, the patients contracted with HBV subtype C2 develops chronic infection more often than those infected with subtype B2 [78,93]. There are conflicting reports from India; one study found that patients infected with genotype D strains had severe chronic liver disease, while another study could not find genotype D strains to be involved in severity [75,94]. Study from East Asia indicated, genotype C as a predominant genotype in subjects with advanced chronic hepatitis (CH), liver cirrhosis (LC) and hepatocellular carcinoma (HCC) [95]. Taiwanese study demonstrated association of genotype C as a risk factor for HCC than genotype B [93,96]. A study from Hong-Kong found that patients infected with genotype B had a higher rate of hepatic decompensation compared to genotype C patients [97]. The HBV patients contracted fulminant hepatitis are more often associated with genotype B [98]. The characteristics of other genotypes (E-J) with disease severity has not been well documented.

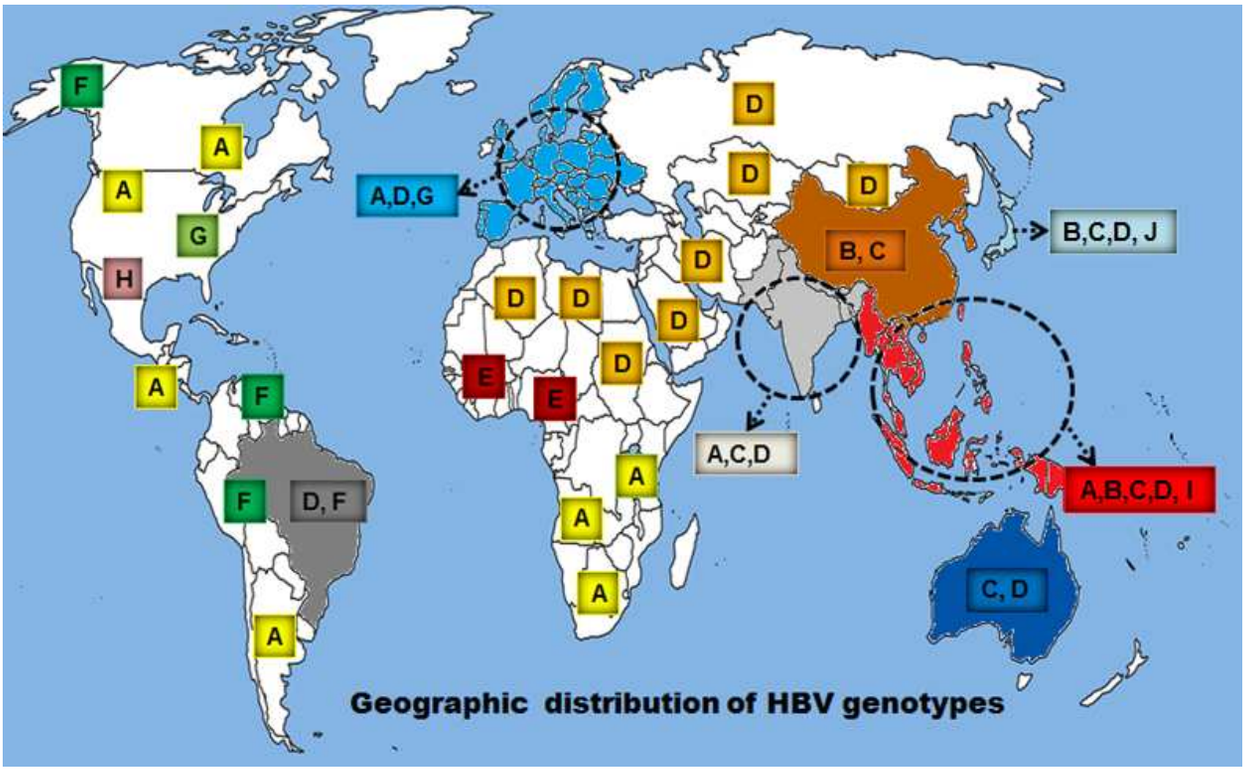


Figure 6. Worldwide geographic distribution of HBV genotypes. (Adapted from: Ref. 68, 74, 75, 77, 79, 81, 82, 83, 84, 86, 87, 88, 89 & 90)

3.4.1. Do genotypes influence seroconversion and seroclearance?

The seroconversion of e' antigen (HBeAg) and seroclearance of surface antigen (HBsAg) are important steps in the natural history of chronic HBV infection [99-102]. Early HBeAg seroconversion typically confers a favorable outcome, while late or absent HBeAg seroconversion after multiple hepatitis flares may accelerate the progression of chronic hepatitis to cirrhosis and hence a poor clinical outcome [99-102]. How these events are influenced by HBV genotypes are subject to a proper understanding and research? Taiwanese study demonstrated that genotype C infection was associated with lower rates of spontaneous HBeAg seroconversion than genotype B [101]. In case of HBsAg seroclearance, the HBV genotypes A and B patients had high frequency compared to genotypes C and D patients [83, 103].

3.4.2. Do genotypes influence frequency of viral mutations?

Several studies revealed that genotypes do influence the frequency of mutations in HBV genetic make-up. The HBV genotype C infections conferred a higher frequency of basal core promoter (BCP) A1762T/G1764A mutation than genotype B [104,105]. Similarly, patients with genotype D infection had a higher prevalence of BCP A1762T/G1764A mutation than those with genotype A infection [106]. The nucleotide(s) deletion within pre-S region is reported to be associated with genotypic variations. The pre-S deletion was higher in genotype C than genotype B patients [107]. In addition, the presence of pre-S deletion was an independent risk factor associated with disease progression as well as HCC development [108-110].

3.5. HBV Genotype and antiviral treatment response

According to different investigators, the nucleotide sequence diversity among different isolates of the virus may play a significant role in response to therapy. The impact of HBV genotype on therapeutic response has been reported in several studies [111,112]. HBV genotype plays a vital role in sustained response (i.e. normalization of serum ALT level and HBeAg seroconversion post treatment) rate. The HBeAg positive patients treated with standard IFN-a, the sustained response rate is significantly better in genotype A and B patients than for genotype C and D [113-116]. Contrary to genotype A-D, patients infected with genotype E–J are rarer and their responses to IFN-based therapy remain largely unknown. In patients treated with nucleos (t) ide analogues, sustained response rate was contradictory in relation to genotype [117,118].

4. Hepatitis C virus

HCV is a small size (55-65 nm), enveloped virus, belong to a member of the family *Flaviviridae* [119,120]. It is mainly transmitted by exposure to contaminated blood or blood products [121,122]. This virus is target specific and replicates in the hepatocytes (liver cells). The initial phase of hepatitis C is called the acute infection [123]. The symptoms of acute HCV infection includes fatigue, jaundice, appetite loss, abdominal pain, nausea and vomiting, joint pain, dark

urine and clay-colored stool [124]. Sixty to seventy percent of people in the acute stage have no symptoms and hence most of HCV cases often goes undiagnosed [123,125]. On the other hand, patients who develop symptoms, the average time period between exposure and symptom onset is 4-12 weeks. Fifteen to twenty percent of acute cases spontaneously clear this virus within 2-12 weeks. However, up to 80% of people initially infected with HCV do not clear the virus from their bodies, and continue to have liver disease with 55% develops chronic liver disease [126,127]. Chronic hepatitis C is often silent, most of the times discovered only by routine serological, biochemical and radiological testing [128].

4.1. Disease severity

HCV is a major cause of liver associated disease all over the world with an estimated 3% of the world's populations are chronically infected [129,130]. In some endemic areas, such as the Middle East, North-East Asia and South Africa, the prevalence of HCV infection is as high as 20-30% [122]. Chronic infection often progresses to liver fibrosis, cirrhosis and hepatocellular carcinoma (HCC) in a substantial number of patients [126-128]. The rate of disease progression is variable and several factors have been identified as important in predicting the outcome of progression such as age at infection, gender, genotype/subtype, viral load, and mode of infection.

4.2. Genome organization

HCV viral genome is positive sense RNA with approximately 9.4 kb in length containing a poly (A) tail at the 3'end (Fig. 7). The sequence contained a 5'untranslated region (5'ÚTR) of 341 bases and 3'UTR of about 27 bases [120,131, 132]. The HCV genome has a single open reading frame coding for a polyprotein of 3010 amino acids. The poly-protein is cleaved after translation into structural and non-structural proteins necessary for viral replication and virion formation. The structural proteins situated towards the N-terminus of the genome and non-structural genes located near C-terminal. The 5'UTR is most conserved region of HCV and is pivotal for the initiation of translation and ribosomal binding [133-135]. The structural genes code for the capsid (core) protein (C) and envelope glycoproteins (E1, E2) [133,134]. The first 27 amino acids of the E2 gene constitute the hyper-variable region 1 (HVR1) of the genome and seems to be involved in virus evasion of the immune system and disease progression [136]. The non-structural genes code for a protease (NS2, NS3) and its cofactor (NS4A), a helicase (NS3), a protein of unknown function (NS4B), a phosphoprotein (NS5A), and an RNA-dependent RNA polymerase (NS5B) [137].

The virus replicates in the cytoplasm using an RNA-dependent RNA polymerase that lacks general proofreading ability [138]. This error-prone RNA polymerase is responsible for the genetic variability exhibited by HCV isolates and high spontaneous nucleotide substitution rate with a frequency of 10^{-2} to 10^{-3} substitutions per nucleotide site per year [139]. HCV circulates as a heterogeneous population or quasispecies which differ by 1-5%in nucleotide sequence diversity [140]. Quasispecies permits rapid adaptability of the virus in the event of environmental changes and a clever strategy utilized by the virus to escape selective forces such as nucleotide antiviral agents or the immune system [139,140].

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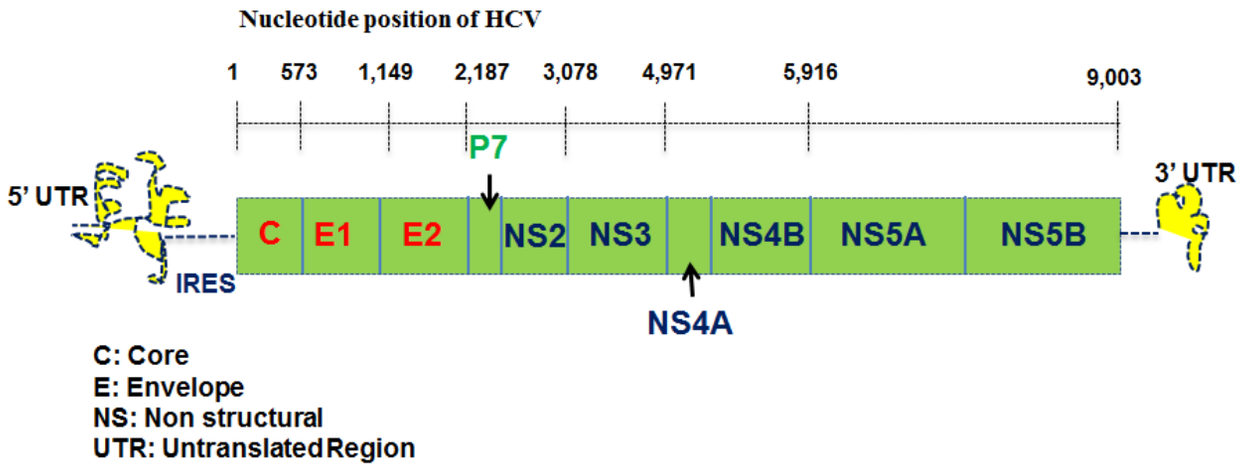


Figure 7. Genomic organization of HCV: The HCV genome contains a single open reading frame (ORF). The genes for structural proteins (C, E1, E2, P7) are situated towards the N-terminus of the ORF. Genes coding for proteins necessary for viral replication are found towards the C-terminus of the ORF. (Adapted from: Ref. 120, 131 & 133)

4.3. HCV genotypes and geographic distribution

The HCV genotype has been determined primarily based on analysis of partial genome sequences. Investigators have typically used sequence analysis of NS5B, core, E1, and 5'UTRs for HCV genotyping (Fig. 8). The most widely accepted classification system of HCV genotyping is that of Simmonds *et al.* 1993 [137]. This system is based on the sequence variability of the 222 base pairs of the NS5B region and it divides HCV isolates into six phylogenetically distinct groups, and more than 80 subtypes. However, following the description of the new genotypes 7-11, Robertson et al., 1998, measured that it was more accurate for the genotypes 7, 8, 9 and 11 to be assigned as subtypes of genotype 6 and the genotype 10 to be a subtype of the genotype 3 [141]. Several methods have been employed to genotype hepatitis C virus; (a) Direct sequencing [140] (b) Restriction fragment length polymorphism (RFLP) [142] (c) Line Probe Assay (LiPA) [143] (d) Genotype specific PCR [144].

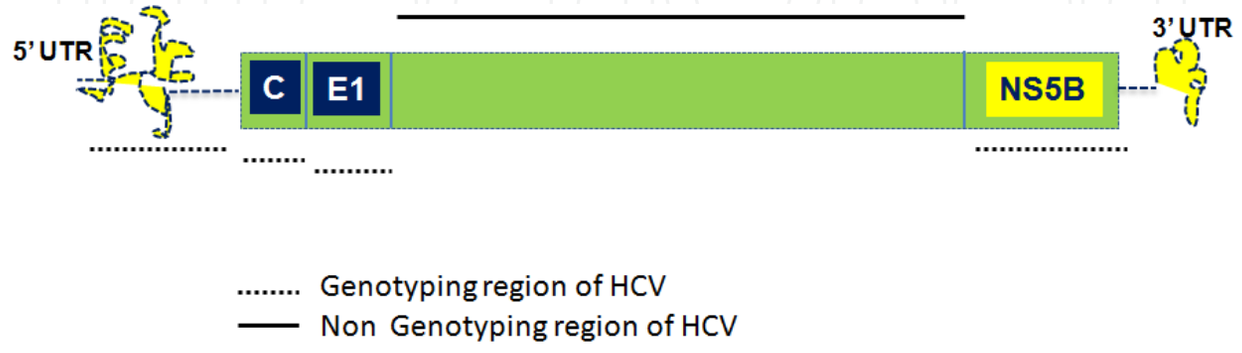


Figure 8. Genotyping region of HCV: The most suitable region for genotyping are 5' UTR, Core (C), E1 and NS5B indicated by dotted (…) line. (Adapted from: Ref. 138, 140, 141, 146 & 147)

At least 6 major genotypes of HCV, each comprising multiple subtypes have been identified worldwide [138,145-147]. Each genotype is separated by sequence divergence in the entire genome exceeding 30%. Substantial regional differences appear to exist in the distribution of HCV genotypes (Fig. 9). Although HCV genotypes 1, 2, and 3 appear to have a worldwide distribution, their relative prevalence varies from one geographic area to another [145]. HCV subtypes 1a and 1b are the most common genotypes in the United States [145]. These subtypes also are predominant in Europe [148]. In Japan, subtype 1b is responsible for up to 73% of cases of HCV infection [149]. Although HCV subtypes 2a and 2b are relatively common in North America, Europe, and Japan, subtype 2c commonly found in northern Italy [146]. HCV genotype 3a is particularly prevalent in intravenous drug abusers in Europe and the United States [150]. HCV genotype 4 appears to be prevalent in North Africa and the Middle East [151, 152], type 5 mainly in South Africa and type 6 principally in Hong Kong and Southeast Asia [137,153]. Mixed genotype infections from a major and a minor HCV population accounts for 4-17% of HCV patients [154].

4.4. Effect of genotype on disease severity

In recent years, substantial evidence has emerged indicating the role of HCV genotypes in disease severity and sensitivity to the antiviral therapy [140,146]. Importantly, patients infected with any of the 6 genotype can develop advanced liver disease, including cirrhosis and HCC. However, some genotypes appear to be more severe compared to the other. For example, Chronic HCV patients infected with genotype 1b is reported to be associated with a more severe liver disease and a more aggressive course compared to genotype 2 (146,155-157]. HCV genotype 1b has also been reported to be associated with the development of advanced liver disease including cirrhosis and hepatocellular carcinoma (HCC) compared to other genotypes (157,158]. However, other investigators failed to arrive at the same conclusions for genotype 1b and therefore further studies are required to elucidate any role of HCV genotype in disease progression or severity [159-162]. A study from India, indicate that most of the chronic hepatitis patients related to genotype 3 is associated with significant steatosis (accumulation of fat within hepatocytes) and fibrosis [163]. Another study report steatosis regardless of genotypes, but resolution of viral infection from genotype 3a patients is associated with disappearance of steatosis and hence genotype 3a and steatosis are interlinked [164]. According to latest statistics 15% of Egyptians are infected with HCV and most of these patients have genotype 4 [165,166]. Genotype 4 has also been associated with a severe disease and increased development of HCC [165]. HCV Genotype 5 is associated with higher mean age compared to other HCV genotype infection [167]. Report from Hong Kong suggested prominence of genotype 6 in patients with thalassemia major and intravenous drug abusers [168].

4.5. HCV genotype and antiviral treatment response

The main objective of therapy administered to patients with chronic hepatitis C is to achieve a Sustained Virological Response (SVR). SVR is characterized by the clearance of serum HCV RNA at the end of therapy and maintained throughout the 6-month follow up period after completion of treatment [169]. The current treatment for HCV infection is two daily doses of

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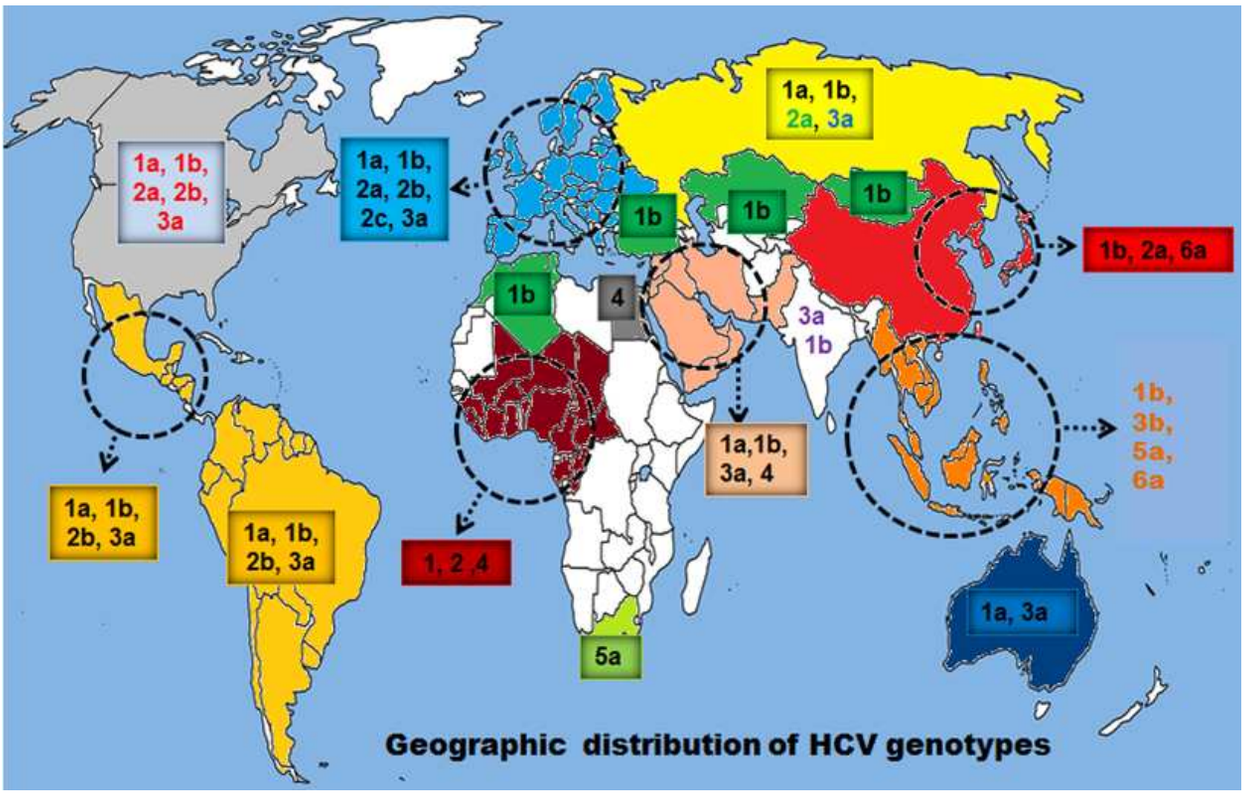


Figure 9. Worldwide geographic distribution of HCV genotypes. (Adapted from: Ref. 138,146,147 & 152)

ribavirin (non-specific anti-viral agent) combined with a weekly injection of interferon-α (standard or pegylated) [170]. The recommended treatment length depends on the virus genotype. Patients with genotypes 2 and 3 are almost three times more likely than patients with genotype 1 to respond to the combination of alpha interferon and ribavirin therapy [129, 164, 171, 172]. Notably, patients with genotype 2 and 3, a 24 week course of combination treatment is adequate, whereas a 48 week course is recommended for genotype 1 [172]. Although, the response of combination therapy against genotype 2 and 3 is better, still there are moderately high percentage of non-responders. The re-treatment response involving combination therapy in non-responders are still better in genotype 2 and 3 (50-60% SVR) compare to genotype 1 (14%) [173]. Genotype 4 presents advance liver disease in patients, and response to Interferon-Ribavirin combination therapy is moderate with ~60% SVR after 48 weeks treatment [174]. Similarly, among genotype 5 patients, the response rate achieved is more than 60% SVR, when treated with Interferon-Ribavirin combination therapy for 48 weeks [175]. HCV genotype 6 responds better to Interferon-Ribavirin combination therapy compare to genotype 1 with treatment duration of 48 weeks [176].

5. Hepatitis D virus

HDV was first discovered in 1977 among a group of patients infected with hepatitis B virus [177]. HDV depends on the HBV to provide hepatitis B surface antigen (HBsAg) for virion

assembly and propagation [178]. There are approximately 350 million chronic HBV carriers of the virus, according to the available data 5% (15 million) of these HBV carriers are infected with HDV [179,180]. HDV is highly endemic in Mediterranean countries, Middle East, Central Africa, and northern parts of South America [181]. In contrast, in industrialized countries, its prevalence is low and its transmission is often associated with intravenous drug use [182]. HDV is transmitted through blood and body fluids, quite similar to that of HBV [183]. Hepatitis D symptoms are identical to other viral hepatitis diseases and include jaundice, fever, malaise, dark urine and nausea [183].

5.1. Disease severity

HDV propagates in human hepatocytes as its natural host but only in presence of hepatitis B virus, causing severe acute, fulminant or chronic hepatitis leading to liver cirrhosis [179, 184, 185]. HDV infection can occur either as a co-infection with HBV or as a superinfection in patients with chronic HBV infection [179]. The individuals with HBV-HDV co-infection resulted in more severe acute disease and a higher risk of fulminant hepatitis than HBV alone (1983). Only 2% of co-infected patients resulted in chronic infection [186]. Super infection with HDV in HBV chronic carriers leads to higher incidence of cirrhosis and hepatocellular carcinoma [187]. A factor that may influence the course of disease is the genetic heterogeneity of HDV prevalent in different geographical areas [188].

5.2. Genome organization

The HDV virion is a spherical particle of about 36-nm in diameter, which contains an envelope (HBsAg) and a nucleocapsid containing an RNA genome in complex with HDAg [189-191]. HDV particles consist of a negative sense, circular, single-strand RNA genome, approximately 1.7 kb in length. HDV anti-genome contains a unique open reading frame that encodes the small (sHD) and large hepatitis delta (LHD) proteins. The sHD and LHD correspond respectively to the small-p24 and the large-p27 hepatitis delta proteins [192]. The LHD amino acid sequence is identical to sHD except with the extension of 19 to 20 amino acids at carboxy-terminal end [192, 193]. sHD is required for viral replication and might promote RNA polymerase II elongation of nascent HDV RNA, while LHD inhibits HDV RNA replication and is required for HDV RNA packaging with the HBV envelope protein [194].

5.3. HDV genotypes and geographic distribution

To date researchers have identified 8 major clades or genotypes of hepatitis delta virus based on phylogenetic analysis and are labeled as HDV-1 to HDV-8 [195]. These genotypes are mostly defined based on the analysis of 357 nucleotide semi-conserved region of HDV genome [187,196,197] (Fig. 10).The sequence differences among these genotypes are significant; there is a divergence of 40% in nucleotide sequence and 35% in amino acid sequence of HDAg. Distribution of genotype 1 is ubiquitous which includes European, North American, African, and some Asian isolates [187,196-199]. Genotype 2 has been found in Japan, Taiwan and Yakoutia (Russia) [200]. Genotype 3 has been found exclusively in Central and South

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America [187]. Genotype 4 was mostly prevalent in Japan and Taiwan [200,201]. Genotype 5-8 has been exclusively found in Africa [195] (Fig. 11).

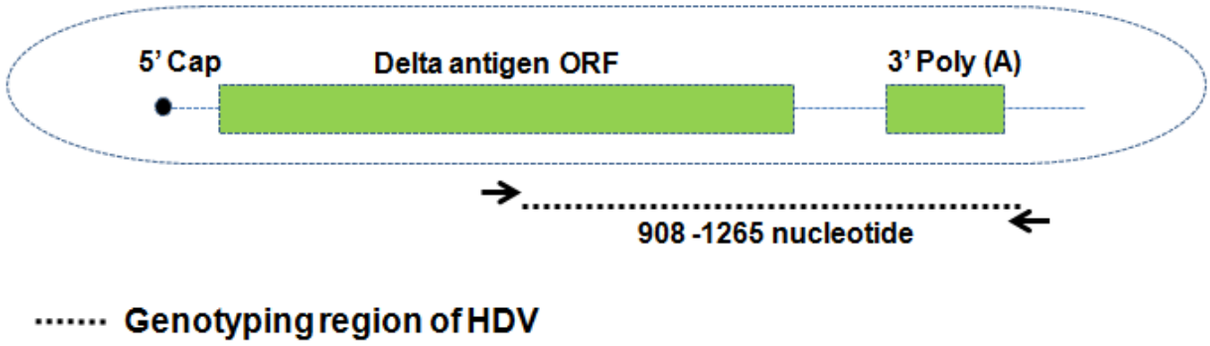


Figure 10. Genotyping region of HDV: The most widely used region for genotyping is spanning 908-1265 nucleotide, which encodes the second half of HDAg protein indicated by dotted line (…). (Adapted from: Ref. 190)

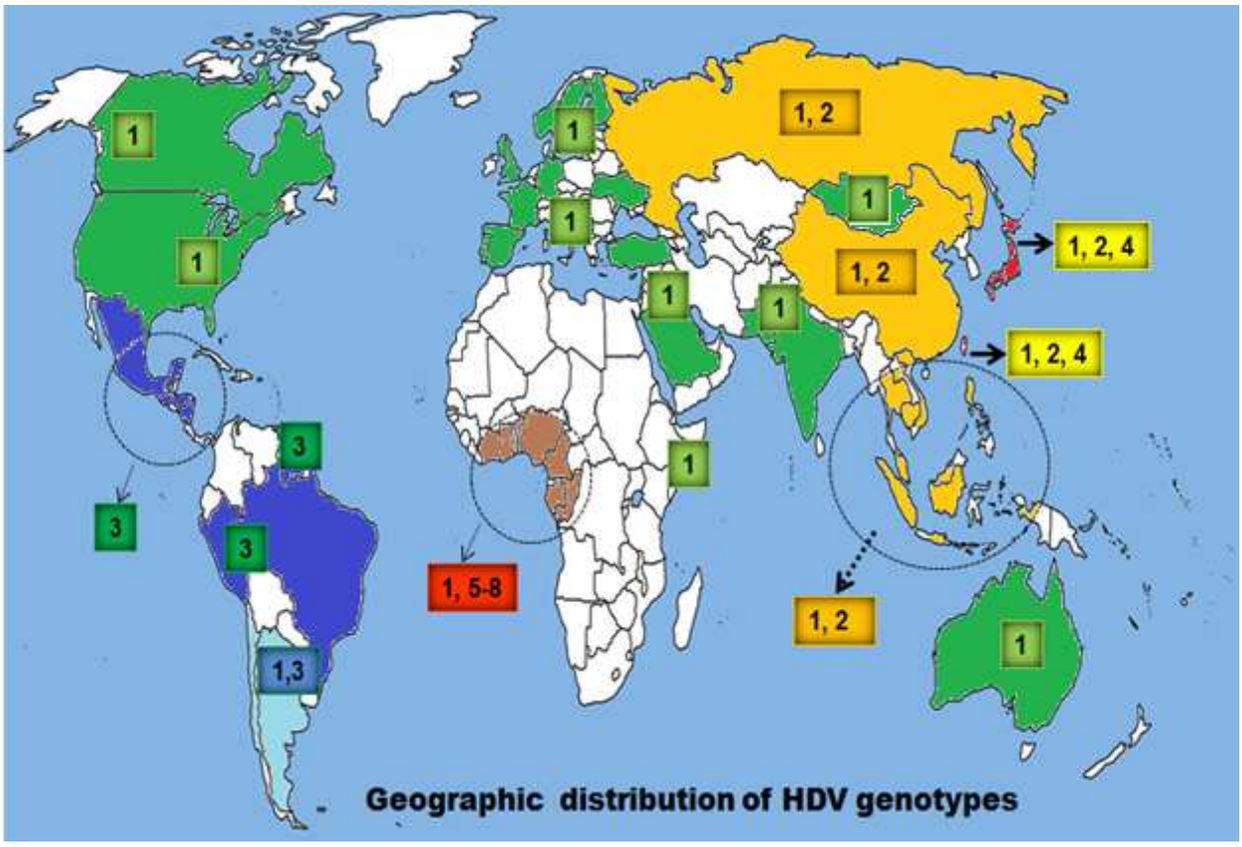


Figure 11. Worldwide geographic distribution of HDV. (Adapted from: Ref. 181 & 195)

5.4. Effect of genotype on disease severity

The genetic variant of hepatitis D virus along with geographical location and transmission route have been identified as important determinants of disease severity [202,203]. At one geographic location genotype 1 has been associated with fulminant hepatitis and hepatocellular carcinoma [196], while in other locality the same genotype have a mild clinical course [197,199]. The diversity observed among genotype HDV-2 has been associated with a less aggressive course, lower ALT values and HDV RNA levels than genotype HDV-1[196,204]. Genotype HDV-3 is endemic in northern South America and is associated with a severe clinical course [184]. Genotype HDV-4 (previously labeled HDV-IIb) also showed variable severity depending upon the geographic location [200,201,205]. Genotype HDV (5-8), mostly observed in African patients, most patients suffered from active chronic hepatitis or cirrhosis [181,195].

5.5. HDV Genotype and antiviral treatment response

There is no specific treatment for HDV infection. Treatment of chronic delta hepatitis is not very effective using long-term administration of high doses of interferon-alpha [206]. It is not known whether some HDV genotypes might be more susceptible to therapy than others. Although, there is no specific vaccine for HDV but vaccination of persons at high risk of acquiring HBV will prevent the acquisition of HDV infection.

6. Hepatitis E virus

HEV infection is a significant public health problem in many parts of the world, especially in developing countries [207-209]. It is estimated that about 2 billion people live in areas endemic for HEV [207,208]. HEV is transmitted via the fecal-oral route and target population includes young to middle aged adults [210]. Patients infected with HEV present with nausea, vomiting, anorexia, jaundice, abdominal pain, fever, and hepatomegaly [211]. It causes large outbreaks of acute hepatitis but usually resolves without any therapy [212-214]. The infected individual therefore develops antibodies that protect against future infection.

6.1. Disease severity

The clinical presentation of this disease ranges from subclinical to fulminant hepatic failure [215,216]. HEV is a major cause of fulminant hepatitis in endemic areas such as India and Bangladesh [216]. Hepatitis E has a mortality rate of 1-4% in the general population [211]. Increased morbidity and mortality is observed in chronic liver disease patients superinfected with HEV [215]. The endemic area is frequently associated with increased incidence and severity in pregnant women with around 15-20% mortality rate [217]. More than one-fourth of affected women have obstetric complications, such as premature rupture of membranes and intrauterine growth restriction [218,219]. It is unknown why HEV causes severe disease in pregnant women.

6.2. Genome organization

HEV is a spherical, non-enveloped virus of about 27–34 nm, classified currently as the sole member of the genus *Hepevirus* in the family *Hepeviridae* [210,220,221]. Its genome is a single stranded, positive-sense 5′capped RNA of approximately 7.2 kb in length [222,223]. It contains three overlapping open reading frames (ORF1, ORF2 and ORF3) flanked by short 5′ and 3′ untranslated regions (UTRs) [224,225] (Fig. 12). The ORF1 encodes a large non-structural protein with several putative functional motifs and domains such as methyltransferase, papain-like cysteine protease (PCP), RNA helicase and RNA dependent RNA polymerase (RdRp) [226]. The ORF2 encodes HEV capsid protein of 660 amino acids and encapsidate the viral RNA genome [227]. The ORF3 of HEV encodes a small protein of 123 amino acids that is essential for viral infectivity *in vivo*. Both the ORF2 and ORF3 proteins are translated from a single bicistronic subgenomic RNA [228].

6.3. HEV genotypes and geographic distribution

Four major genotypes (genotypes 1-4) of mammalian HEV have been identified on the basis of complete genome sequences [229,230]. Recently, partial sequence of HEV conserved region (i.e. ORF1 and ORF2) reflects nucleotide sequence heterogeneity and hence genotyping of HEV is possible without determining the entire genomic sequence [231,232] (Fig. 13). Regions within nucleotides 171–221, 280–310 and 6461–6495 were most conserved and represented the best targets for primer or probe design for genotyping and quantitation [232].

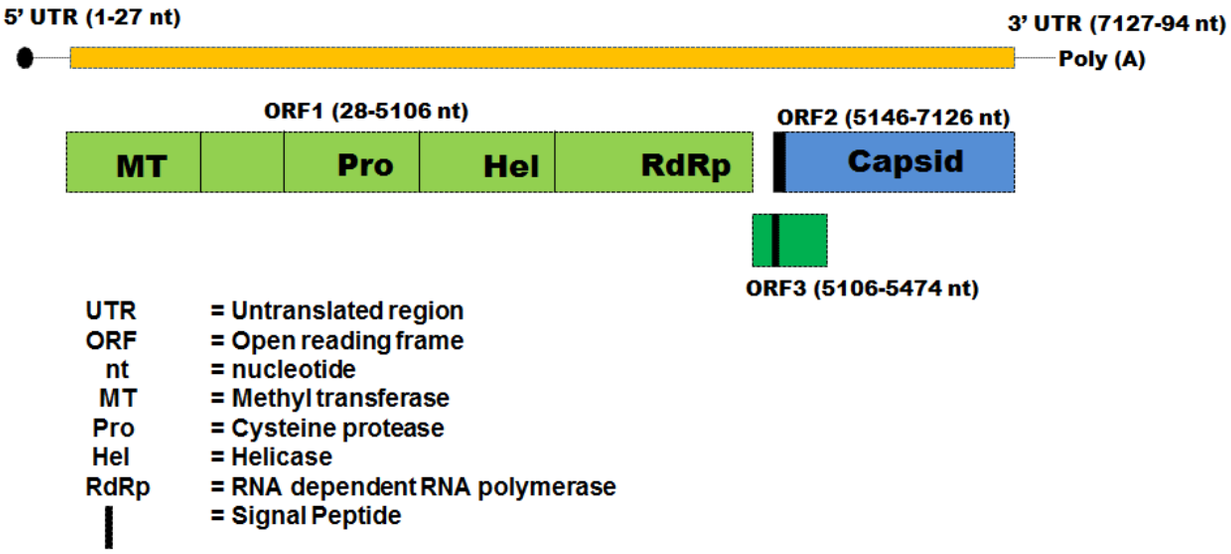


Figure 12. Genomic organization of HEV. HEV RNA is capped at the 5′ untranslated region (UTR) and polyadenylated at the 3′ UTR. ORF1 encodes the nonstructural polyprotein; methyltransferase (MeT), papain-like cysteine protease (PCP), RNA helicase (Hel) and RNA dependent RNA polymerase (RdRp). ORF2 encodes the viral capsid protein. ORF3 encodes a small regulatory phosphoprotein.(Adapted from: Ref. 222, 223 & 224)

Genotype 1 is mostly prevalent in Asia and Africa [230,233,234]. Genotype 2 is found in Central and South America as well in African countries (Fig. 14). Both genotype 1 and 2 outbreaks are

the result of efficient human-to-human feco-oral transmission mostly in developing countries [231,234]. In industrialized countries, HEV genotype 1 infection is rare, therefore its presence there is treated as imported infectious disease [231]. Genotype 3 includes human and swine HEV strains from industrialized countries [235,236]. Finally, genotype 4 includes human and swine HEV strains from Asia, particularly China, Taiwan and Japan [234,237]. HEV strains of genotype 3 and 4 are maintained among animal species and occasionally infect humans probably due to inefficient cross-species transmission [235].

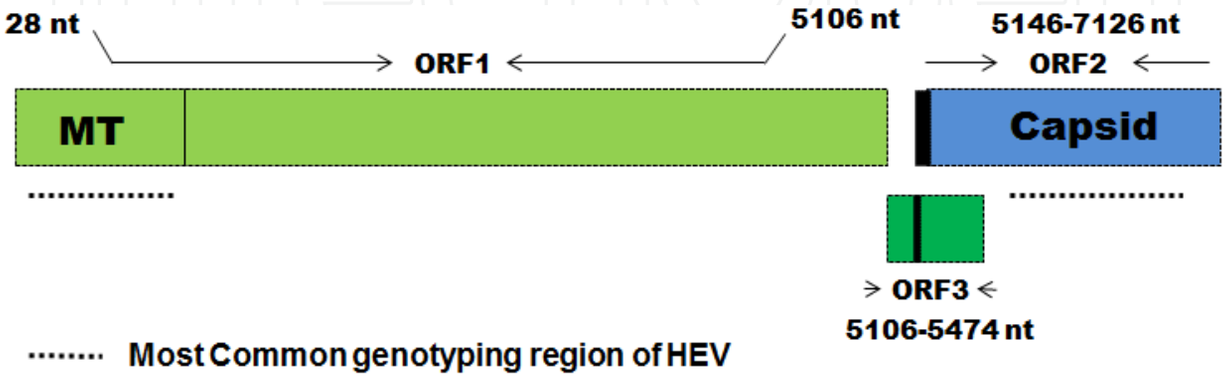


Figure 13. Genotyping region of HEV. The most widely used HEV genomic regions for genotyping are indicated by dotted line (…).

6.4. Genotype variations and disease severity

The severity of HEV associated acute hepatitis not only depends on the status of the host's immune system but also on viral factors such as genotypes. Hepatitis outbreaks in developing countries have been caused primarily by HEV genotype 1. Most of the patients suffering from hepatitis E caused by genotypes 1 are older children and young adults with mortality rate 1%[231,238]. A study from India reported that the viral load of genotype 1was significantly higher in pregnant patients than in non-pregnant patients, and high viral load might be a reason for severe liver disease in pregnant patients [239]. Genotype 2 is Mexican origin and mostly prevalent in central American and African countries, like genotype 1, it is mostly self-limiting. HEV genotype 3 is prevalent in industrialized nations and is rarely pathogenic. Recently, it has been reported that genotype 4 infected patients showed more severe form of the viral hepatitis than genotype 3 [240,241].

Most of the patients in this group belong to older age (~60 years) and the mortality rate is relatively high (6-10%) [231,242]. Japanese study suggested that the silent substitutions of U3148 and C5907 in the genotype 3 and 4 HEV strains are closely associated with the occurrence of fulminant and severe cases and found that C5907 is associated with high HEV load [243]. Thus, the genetic changes in hepatitis E virus genome may affect the effectiveness of virus transmission and hence the severity of HEV-associated hepatitis. Therefore, examination of HEV genotype is considered necessary in order to predict the clinical course and the outcome.

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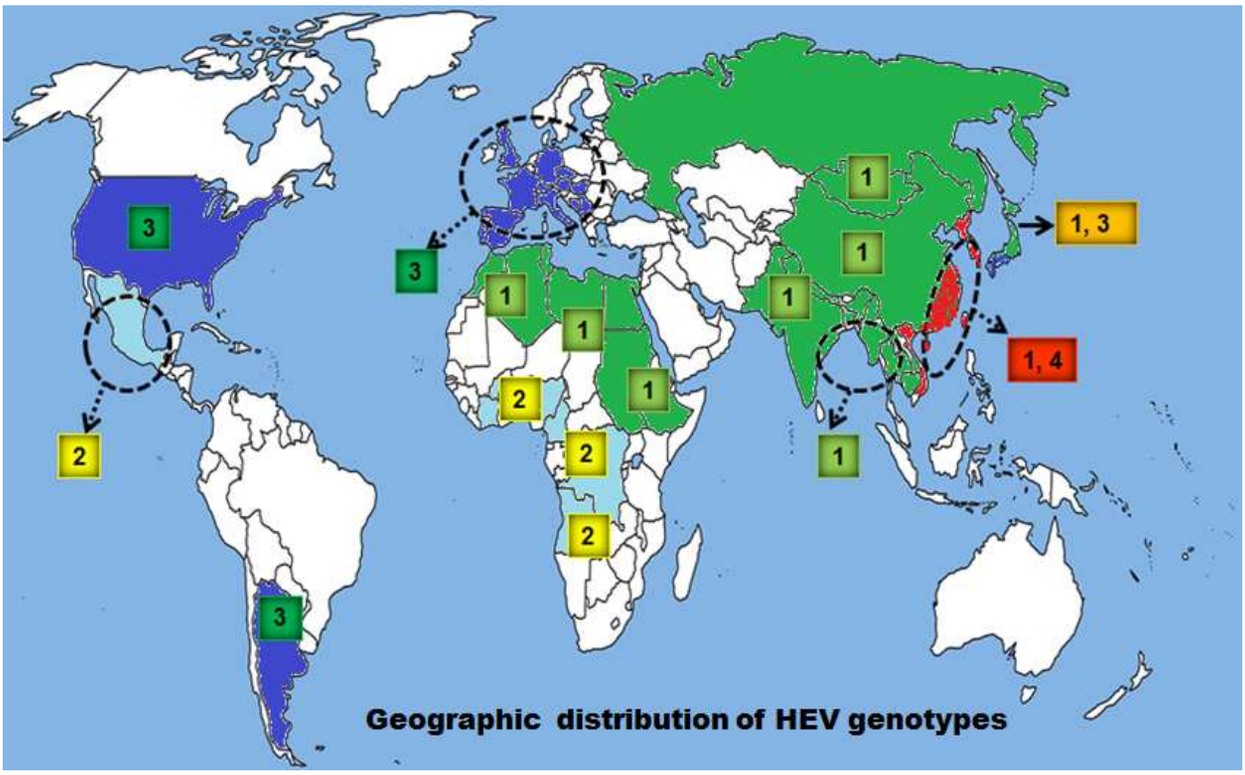


Figure 14. Worldwide geographic distribution of HEV: (Adapted from: Ref. 230,231,233,234 & 236)

6.5. HEV genotype and antiviral treatment response

Since most of the cases are self-limiting, acute HEV does not require antiviral therapy in immunocompetent patients related to any genotype. The immunosuppressed patients should be recommended ribavirin, so that these patients should not progressed to cirrhosis. Liver transplantation is the only treatment option for patients with fulminant hepatic failure due to HEV. All the four mammalian HEV strains belong to one serotype. Thus, only one hepatitis E vaccine is needed for broad protection. The first phase of the immunoprophylaxis of HEV is already tested for safety and immunogenicity [244]. The next phase is to make it available in the market, especially in developing countries so that it should be effectively controlled.

7. Conclusions

The analysis of genomic sequence heterogeneity among different isolates of hepatitis viruses (A-E) may provide an opportunity to decipher the course and pathogenesis of the virus. Although, lot of information are available in this area but still there are many contradictions and discrepancies.

HAV: There are contradictory findings regarding the role of nucleotide substitutions and genotypic variations in disease severity among patients contracted with hepatitis A in different geographical location.

HBV: There are conflicting report about the role of HBV genotypes and severity of the disease based on geographical distribution. Based on multiple studies, compared to genotype A and B patients, C and D patients have a higher risk of disease progression (i.e. cirrhosis & HCC). Similarly, genotype A and B patients responded better to interferon therapy than C and D patients. It is important to note that few studies find no association between genotypes, disease severity and response to treatment regimen.

HCV: Patients infected with any of the 6 HCV genotype can develop advanced liver disease, including cirrhosis and HCC. However, some genotypes appear to be more severe compared to the other. For example, genotype 1b is reported to be associated with a more severe and a more aggressive course compared to genotype 2. Presently, it is recommended all the patients irrespective of genotype should be given treatment, however treatment length depends on the virus genotype.

HDV: There are contradictory outcomes about the role of HDV genotypes and disease severity. At one geographical location HDV genotype 1 is associated with fulminant hepatitis and HCC while at another location it is not severe. Other genotypes of HDV also have variable disease severity based on geographical location.

HEV: Although HEV is self-limiting illness still it causes severe illness in pregnant women with high mortality rate. Most of the pregnant women with aggressive course of the disease are associated with genotype 1. There are lack of information regarding severity in pregnant women and other genotypes. There are contradictory report regarding the severity of disease and genotype 3 and 4, although a report from Japan suggests, silent substitutions of U3148 and C5907 in the genotype 3 and 4 with response to fulminant hepatitis.

Therefore, the genomic heterogeneity of hepatitis viruses is not the only critical factor that leads to disease progression, severity and final outcome. Hence, in combination with viral genomic heterogeneity other factors such as age, sex, geographical distribution, genetic polymorphism and host immune elements may play a vital role in deciding the clinical implications and final outcome of the infection.

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