Genetic heterogeneity of hepatitis C virus and its clinical significance

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SUMMARY
The hepatitis C virus (HCV) displays high genetic heterogeneity. Many classification systems have been used but the most widely accepted is that of Simmonds with 11 genotypes and 80 subtypes. Techniques used to determine the HCV genotype are molecular biology based (genotyping techniques) and serological (serotyping techniques). This viral diversity has epidemiological and clinical implications and has been associated with the severity of liver disease, prognosis, diagnostic tests, response to treatment and failure to generate an effective protective vaccine. Commercially available HCV RNA reverse transcription/PCR qualitative and quantitative first-generation assays underestimated the HCV RNA level of genotypes 2 to 6. The second generation methods have corrected this problem. HCV genotype 1b is the predominant genotype in Western Europe and has been associated with a more severe clinical course of liver disease, cirrhosis and hepatocellular carcinoma. Genotypes 1 and 4 have been associated with a low response rate to IFN-α or to the combination of ribavirin and IFN-α. Consequently, the duration of treatment has been tailored according to genotype and viral load. Explaining the possible pathogenetic mechanisms involved in the different clinical profiles of HCV genotypes has been the subject of many studies. HCV circulates as a mixed population of HCV molecules termed quasispecies derived from multiple point mutations. Multiple quasispecies have been implicated in both chronic and acute HCV infection leading to severe prognosis and to the evolution to chronicity respectively.

Key words: Hepatitis C virus, genotypes, subtypes, geographical distribution, quasispecies


1. INTRODUCTION
The hepatitis C virus (HCV) was discovered in 1989. It is transmitted by exposure to blood or blood products, and persons who are using illicit drugs and share needles may acquire HCV infection.

The antibody prevalence against HCV (anti-HCV) is encountered in 0.4-1.5% of healthy blood donors in Western Europe but the rate is estimated to be higher in southern Europe. In Greece the prevalence of anti-HCV ranges from 0.6 to 7.5% in different geographic regions (mean rate 1.9%). In some endemic areas, such as the Middle East, north-east Asia and South Africa, the prevalence of HCV infection is as high as 30%.

At least 80% of infected persons will develop chronic disease. Cirrhosis occurs in 20-50% of chronic HCV patients after a period of 20 years and the annual incidence of hepatocellular carcinoma in cirrhotics is approximately 3%. Moreover, HCV infection seems to be associated with autoimmune diseases including essential mixed cryoglobulinaemia. Thus morbidity and mortality are caused by HCV infection worldwide.

In their efforts to identify the host and viral factors
associated with the outcome of the disease, investigators found that the genetic heterogeneity of HCV is related to disease prognosis and pathogenicity, as well as to response to treatment. They found that they could tailor the duration of treatment according to the genetic form of the virus in order to achieve optimal cost-effective results. Also, characterization of the genetic heterogeneity of HCV could play a major role in the development of a broadly effective HCV vaccine.

2. GENOMIC ORGANISATION OF HCV

Hepatitis C virus (HCV) is a positive sense, single-stranded RNA virus, consisting of 9400 nucleotides, which is classified as belonging to the Flaviviridae family. The HCV genome has a single open reading frame coding for a polyprotein of 3010 amino acids which is cleaved after translation into structural and non-structural proteins necessary for viral replication and virion formation. The 5' non coding region (5'NCR) is a well-conserved region and is important for the initiation of translation and ribosomal binding.7 The structural domain contains the core and envelope regions (E1 and E2/NS1). The envelope region E2 has two hypervariable regions (HVR1 and HVR2) for genotype 1 and one hypervariable region for the remaining genotypes with a sequence variability of more than 50%.8 The non-structural domain contains the non-structural regions NS2, NS3, NS4 and NS5. The NS3 region encodes the serine proteinase which is responsible for the cleavage of the polyprotein into NS4a, NS4b, NS5a, and NS5b.7 The second proteinase encoded by NS2 is responsible for the cleavage of the polyprotein in the NS2/NS3.7 The NS5b region encodes the viral polymerase.

3. GENOTYPES, SUBTYPES AND QUASISPECIES

One possible reason for the high rate of chronic liver disease is viral persistence that may lie in the high mutation rate and resulting altered antigenicity of the virus which allows it to evade the hosts immune system. The rate of mutation's is about 10⁻³ substitutions/site/year in the entire genome10 while the mutation rate is 10 times higher in the hypervariable region. 5'NCR, core and NS5 are relatively conserved regions. The variation observed in the nucleotide sequence of various isolates of HCV has led to their classification into distinct genotypes which differ from each other by as much as 34% in nucleotide positions over the entire viral genome. Each of the major genotypes can be divided into several subtypes which differ by about 23% in nucleotide sequence. The viral isolates within one subtype (termed quasispecies - they are not identical but display up to 9% diversity from each other)11 are derived from an original strain by point mutations.

Determination of the genotype and subtype can be performed by sequence analysis of the whole genome. However, this work is laborious and not practicable for routine use. It now appears that any of the following regions could be used for identification of genotypes and would produce equivalent results. The regions are the 5’NCR,12 NS5,13 E1,14 core and NS3.15 The diversity between genotypes, subtypes and isolates varies according to the genomic variability of the region. Sequence divergence is about 35% in the regions E1, NS4 and NS5 but only 6% in the 5’NCR. As 5’NCR is the most conserved region it has become clear that it allows for segregation of genotypes but not of subtypes.16

3.1. Classification systems

Different systems of HCV genotyping have been proposed (by Chiron,9 Enomoto,17 Okamoto,18 Simmonds10) (Table 1). The most widely accepted is that of Simmonds which is based on the sequence variability of the 222 base pairs of the NS5 region, performed by direct sequencing method on PCR (polymerase chain reaction) product and which enabled the construction of a phylogenetic tree with six main branches or genotypes. In this system the major genotypes are numbered by arabic numerals 1 to 6 and the subtypes are identified by small alphabetic letters a, b and c, in both cases in order of their discovery. The first described was genotype 1a (prototype HCV). The comparison of different classification systems for

Table 1. Classification systems of the HCV genotypes

<table>
<thead>
<tr>
<th>Simmonds</th>
<th>Chiron</th>
<th>Okamoto</th>
<th>Enomoto</th>
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</thead>
<tbody>
<tr>
<td>1a</td>
<td>I</td>
<td>I</td>
<td>K-PT</td>
</tr>
<tr>
<td>1b</td>
<td>II</td>
<td>II</td>
<td>K-1</td>
</tr>
<tr>
<td>1c</td>
<td>nc*</td>
<td>nc*</td>
<td>nc*</td>
</tr>
<tr>
<td>2a</td>
<td>III</td>
<td>III</td>
<td>K-2a</td>
</tr>
<tr>
<td>2b</td>
<td>III</td>
<td>IV</td>
<td>K-2b</td>
</tr>
<tr>
<td>2c</td>
<td>III</td>
<td>nc*</td>
<td>nc*</td>
</tr>
<tr>
<td>3a</td>
<td>IV</td>
<td>V</td>
<td>nc*</td>
</tr>
<tr>
<td>3b</td>
<td>IV</td>
<td>VI</td>
<td>nc*</td>
</tr>
<tr>
<td>4</td>
<td>nc*</td>
<td>nc*</td>
<td>nc*</td>
</tr>
<tr>
<td>5a</td>
<td>V</td>
<td>nc*</td>
<td>nc*</td>
</tr>
<tr>
<td>6a</td>
<td>nc*</td>
<td>nc*</td>
<td>nc*</td>
</tr>
</tbody>
</table>

nc* = no classified
HCV genotypes is shown in Table 1.

Initially six main genotypes (1-6) and 12 subtypes were described. Then on the basis of sequence analysis of the regions NS5 and E1, genotypes 7-11 were recognized and subtype numbers raised to about 80. For a new subtype to be assigned it should be different in the phylogenetic analysis in at least two genomic regions. However, following the description of the new genotypes 7-11, many investigators considered that it was more suitable for genotypes 7, 8, 9 and 11 to be assigned as subtypes of genotype 6 and for genotype 10 to be a subtype of genotype 3.

3.2. Geographic distribution of genotypes

There are differences in the relative prevalence of genotypes and subtypes in distinct geographic areas. 1a, 1b, 2a, 2b and 3a are the most prevalent types in Western Europe and North America (Figure 1). Genotype 1 accounts for 50% of the cases and genotypes 2 and 3 for 20% each. In the United States type 1a is the predominant one. Italy, France, and the Netherlands are predominantly infected by 1b. In southern and eastern Europe type 1b is the most prevalent. In Greece, genotype 1b is responsible for 47% of cases, followed by 3a (22%) and 1a (11%). In central Europe genotypes 2 and 3 are absent and almost all infected individuals have genotype 1. In the Middle East and Central Africa, genotype 4 has the highest prevalence rate and in Southern Africa genotype 5 is the most prevalent. In the Far East 1b, 2a and 2b are the most widely distributed. In South-East Asian countries 1b is the most prevalent with the exception of the Philippines where 1a predominates. Genotype 3 with 9 subtypes (a-i) has been observed in Singapore, Thailand, Bangladesh, and eastern India. The most recently discovered genotypes are limited to the Asian continent. Genotypes 7, 8 and 9 have been found in Vietnam and Thailand and 10 and 11 in Indonesia.

Genotype 6 has been isolated in a relatively small geographic area of Hong Kong, Singapore and Macao.

Determination of the distribution of HCV genotypes has been helpful in understanding the epidemiology of the virus. For example, the age-related distribution of HCV genotypes in Western Europe is suggestive of changes over the last 40 years. The relative prevalence of genotypes 1a and 3a has increased in the population. The increasing drug addiction rate over this period may explain this observation, since there is evidence that both types are most prominent among intravenous drug users (IVDUs). Genotype 1a accounts for 51-53% and genotype 3a for 40-45% among IVDUs. Genotype 1b seemed to be spread by transfusion of blood and blood products or nosocomial infection, since it accounts for the majority of HCV infected persons with a history of hospitalization. Also, patients with genotype 1 are older than patients with genotype 2 or 3 and patients with subtype 1b are older than patients with subtype 1a or 3a.

3.3. Genotype determination techniques

Two categories of techniques can be used to determine HCV genotype in the serum: molecular biology based techniques (genotyping techniques) and serological techniques (serotyping techniques).

3.3.1. Genotyping techniques

Genotyping techniques are the following (Figure 2):

- Primer-specific PCR
- Restriction fragment length polymorphism (RFLP)
- Reverse hybridisation technique
- Sequencing method

The main advantages of the above mentioned molecular biology-based genotyping techniques are direct access to viral genome sequence information, high sensitivity and the possibility of subtyping. The shortcomings are that they are laborious and expensive and require technical skills and adequate laboratory equipment.

3.3.2. Serotyping techniques

The major advantages of the serotyping methods are: (a) they are feasible in low levels of viraemia or in the resolved HCV infections; and (b) they are less expensive and easier to perform than methods based on PCR. Their disadvantages are low sensitivity and inability to perform HCVtyping in special populations such as immunocompromised and immunodialysed people. In addition, no
In the third-generation assay ELISA, a recombinant NS5 antigen was added to the four antigens of the second-generation assay. The third generation assay is less influenced by the genotype.

4.1.2. HCV RNA detection assays

The commercially available reverse transcription PCR qualitative and quantitative first-generation methods underestimated the viral level of individuals infected with non-1 genotypes. These discrepancies may be explained by differences in the efficiency of virus amplification methods for different genotypes. In fact, probes and primers have been designed to match genotype 1 sequences. The new version of the branched signal DNA amplification method (Quantiplex HCV RNA 2.0, Chiron Diagnostics, Emeryville, California) and the second-generation reverse transcription quantitative PCR-based assay (Amplicor HCV Monitor 2.0, Roche Molecular Systems) have corrected this problem and they are virtually unaffected by the genotype.

4.2. Severity of liver disease

There are multiple factors that may influence the progression of the disease including age at infection, sex, duration of infection, mode of acquisition, host immunity, co-existing infections and alcohol intake.

However, the impact of genotype on the severity and the progression of liver disease has already been thoroughly investigated. Genotype 1b has been considered by many investigators as more pathogenic since it has been associated with advanced liver disease, including liver cirrhosis and hepatocellular carcinoma (HCC) compared to genotype 2 (Table 2). Yet, the longer duration of the infection observed in genotype 1 infected persons may be responsible rather than the viral type per se. Moreover, most studies are retrospective (Table 2) and the cases included are sporadic so that it is difficult to define the onset of infection. Another confounding factor is that most studies are performed in tertiary referral centres where patients with advanced liver disease are selected for specialized treatment. The information about the potential pathogenic role of some genotypes offered by case-control prospective studies is difficult to obtain because of the slow progression of the disease. In three prospective studies where cirrhotics were followed up to evaluate their progression to HCC, it was shown that patients infected with 1b were more prone to develop HCC (Table 2). Longitudinal studies also showed that patients undergoing liver transplantation have a more rapid recurrence and progression leading to cirrhosis when they are infected with 1b (Table 2).
Other investigators failed to demonstrate any relationship between genotype 1b and the more severe outcome of liver disease (Table 3). Poynard et al did not detect any relationship between the viral type and the evolutionary rate to fibrosis (Table 3). Eight further studies including 2 prospective ones did not show any findings confirming the association of 1b with HCC (Table 3). Other reports did not confirm the role of genotype 1 in causing rapid recurrence and progression of liver disease after liver transplantation (Table 3).

Genotype 4, mostly distributed among patients coming from the Middle East, has also been associated with a severe disease outcome and an increased development of HCC.37

The clinical significance of mixed genotype HCV infection has not yet been established but it seems that, like genotype 1, there is significantly higher serum HCV RNA concentration and unfavorable outcome of liver disease when compared with genotype 2 and 3.32

The implication of viral genotypes in the development of autoimmune disorders associated with chronic hepatitis C has also been investigated. Genotype 2 has been associated with the development of autoimmune hepatitis with positive liver-kidney microsomal antibodies.38 Genotypes 1b and 2a have also been associated with the development of essential mixed cryoglobulinemia.39 However both observations need further confirmation.

In conclusion, the influence of viral genotype in the pathogenesis of liver disease is still controversial. A meta-analysis of the most reliable of the above reports would be necessary for the evaluation of the role of viral genotypes in the course and the outcome of chronic hepatitis C. Certain strains of HCV may indeed have enhanced virulence and experimental models to assess this hypothesis in the laboratory are essential.

### 4.3. Response to antiviral treatment

Sustained response to interferon-alpha (IFN-α) treatment ranges from 10% to 20%.40 Many factors are asso-

| Table 2. Studies that showed an association between genotype 1b and severity of liver disease |
|---------------------------------|-------|-----------|-----------|-----------|
| Source                          | Location | No of pts | Type of pts | Type of study |
| Pozzato 199477                  | Italy    | 111       | CHC        | Retrospective |
| Mita 199478                     | Italy    | 148       | CHC+ Cirrhosis+HCC | Retrospective |
| Feray 199579                    | France   | 60        | LT         | Retrospective |
| Cathomas 199680                 | Switzerland | 69     | CHC        | Retrospective |
| Caccamo 199681                  | Italy    | 35        | LT         | Retrospective |
| Silini 199682                   | Italy    | 593+166+219 | CHC+HCC+Cirrhosis | Cross-sectional |
| Gane 199683                     | UK       | 149       | LTR        | Prospective |
| Simmonds 199684                 | UK       | 610       | CHC        | Retrospective |
| Zein 199685                     | USA      | 48        | HCC        | Retrospective |
| Zein 199686                     | USA      | 179       | CHC        | Retrospective |
| Hatzakis 199687                 | Greece   | 17+87+23  | HCC+CHC Cirrhosis | Retrospective |
| Kobayashi 199688                | Japan    | 140       | CHC        | Prospective |
| Booth 199789                    | UK       | 29        | CHC        | Retrospective |
| Gordon 199790                   | USA      | 42        | LTR        | Retrospective |
| Donato 199791                   | Italy    | 172       | HCC        | Retrospective |
| Bruno 199792                    | Italy    | 163       | Cirrhosis  | Prospective |
| Pageaux 199793                  | France   | 22        | LTR        | Prospective |
| Tassopoulos 199884              | Greece   | 152       | CHC        | Retrospective |
| Berg 199885                     | Germany  | 79        | LTR        | Prospective |
| Bellentani 199986               | Italy    | 6917      | General Population | Prospective |
| Prieto 199987                   | Spain    | 81        | LTR        | Prospective |
| Belli 200088                    | Italy    | 89        | LTR        | Prospective |

CHC: Chronic hepatitis C, LTR: Liver transplant recipients
associated with response to therapy. Host factors predictive of a beneficial response to IFN-α are the following: young age, short duration of infection, absence of cirrhosis, mild histological score, IVDU, low iron content of the liver and low gamma-gt.\textsuperscript{41} Viral factors predictive of response to IFN-α are; pre-treatment viral load,\textsuperscript{42} HCV genome diversity (quasispecies) and specific genotypes. Patients infected with genotype 1 have a lower rate of response compared with genotypes 2 and 3.\textsuperscript{43} Despite the higher rate of complete response of the genotype 1a compared with 1b, the rate of sustained response is similar in both genotypes.\textsuperscript{44} In a therapeutic trial comparing three different regimens of IFN-α, sustained response for genotype 1b ranged from 9 to 28%, for genotype 2a-c from 42 to 46% and for genotype 3 from 67 to 100%.\textsuperscript{45} In a review of 15 trials where short therapeutic regimens have been used (usually of six months’ duration), sustained response to IFN-a was estimated to be 18% for genotype 1 and 55% for genotypes 2 and 3.\textsuperscript{46} IFN-α and ribavirin combination treatment for six months achieved similar rates of sustained response of 16% for genotype 1 in naïve patients. The results of combined treatment for six months were better in relapsed patients (30% for genotype 1 and 69% and 73% for genotypes 2 and 3 respectively).\textsuperscript{47} In another study, combination therapy in naïve patients achieved a 13% rate of sustained response in genotype 1b while for 2 and 3 the rates were 43% and 53% respectively.\textsuperscript{48}

As a consequence of the studies reported above, the Consensus Conference held in Paris in February 1999 has suggested that the duration of therapy should be dependent on genotype and the level of viraemia. In patients with genotype 2 or 3, six months of treatment is recommended regardless of the level of viraemia. In pa-

### Table 3. Studies that failed to show an association between genotype 1b and severity of liver disease

<table>
<thead>
<tr>
<th>Source</th>
<th>Location</th>
<th>No of pts</th>
<th>Type of pts</th>
<th>Type of study</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yamada 1994\textsuperscript{99}</td>
<td>Japan</td>
<td>251</td>
<td>CHC</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Yotsunanagi 1995\textsuperscript{100}</td>
<td>Japan</td>
<td>72+131</td>
<td>HCC+CHC</td>
<td>Retrospective</td>
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<tr>
<td>Takano 1995\textsuperscript{101}</td>
<td>Japan</td>
<td>124</td>
<td>CHC</td>
<td>Prospective</td>
</tr>
<tr>
<td>Zhou 1996\textsuperscript{102}</td>
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<td>124</td>
<td>LTR</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Guido 1996\textsuperscript{103}</td>
<td>Italy</td>
<td>59</td>
<td>CHC</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Lee 1996\textsuperscript{104}</td>
<td>Korea</td>
<td>138</td>
<td>CHC+Cirrhosis+HCC</td>
<td>Retrospective Case-control</td>
</tr>
<tr>
<td>Zeuzeum 1996\textsuperscript{105}</td>
<td>Germany</td>
<td>97</td>
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<td>Retrospective</td>
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<tr>
<td>Romeo 1996\textsuperscript{106}</td>
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<td>197</td>
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<td>Lau 1996\textsuperscript{107}</td>
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<td>Cross-sectional</td>
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<td>Boker et al 1997\textsuperscript{108}</td>
<td>Germany</td>
<td>71</td>
<td>LTR</td>
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<tr>
<td>Mangia 1997\textsuperscript{109}</td>
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<tr>
<td>Benvegnu 1997\textsuperscript{110}</td>
<td>Italy</td>
<td>429</td>
<td>CHC+Cirrhosis</td>
<td>Prospective</td>
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<tr>
<td>Lopez-Labrador 1997\textsuperscript{111}</td>
<td>Spain</td>
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<td>Naoumov 1997\textsuperscript{112}</td>
<td>UK</td>
<td>1438</td>
<td>Cirrhosis</td>
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<td>Gayowski 1997\textsuperscript{113}</td>
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<td>Poynard 1997\textsuperscript{114}</td>
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<td>Kleter 1998\textsuperscript{118}</td>
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<td>25</td>
<td>LTR</td>
<td>Prospective</td>
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<tr>
<td>Dutta 1999\textsuperscript{121}</td>
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<td>17</td>
<td>HCC</td>
<td>Case-control</td>
</tr>
<tr>
<td>Reid 1999\textsuperscript{122}</td>
<td>USA</td>
<td>28+38</td>
<td>HCC+Cirrhosis</td>
<td>Retrospective</td>
</tr>
<tr>
<td>Adinolfi 2000\textsuperscript{123}</td>
<td>Italy</td>
<td>324</td>
<td>CHC</td>
<td>Retrospective</td>
</tr>
</tbody>
</table>

CHC: Chronic hepatitis C, LTR: Liver transplant recipients
patients with genotype 1, 6 months of therapy is sufficient if the level of viraemia is low (<2 million copies/ml) while 12 months of treatment is recommended if the level of viraemia is high (>2 million copies/ml).49

In conclusion, the only clinical application of the viral genotyping routinely used is the distinction between genotype 1 and genotypes 2 and 3 in order to predict the outcome of therapy and to choose the optimal duration of therapeutic regimen.

Studies from the Middle East including patients with genotype 4 revealed that treatment with IFN-a and ribavirin achieved a sustained response rate of only 5% in naive patients.50 It seems, therefore, that genotype 4 has a poor response rate and the same therapy recommendations as for genotype 1 should be followed.

Evidence has been provided, mainly by Japanese investigators, that variability in the NS5 region (nt 2209-2248) affects response to treatment in patients with genotype 1b.51 The evidence that numerous mutations of this region are related to low viral load suggests that this region has been involved in viral replication. It has also been demonstrated that nt 2209-2248 region is important for the phosphorylation of the NS5.52

4.4. Potential pathogenetic mechanisms for the different clinical profiles of the HCV genotypes

- Immunological studies have shown that in HCV infected persons there is a vigorous immune response mediated by the cytotoxic CD8+ lymphocytes and the HLA I histocompatibility system against the viral proteins.53 It has been suggested that liver tissue from patients infected with genotype 1b shows higher expression of HLA-A, -B, -C and intercellular adhesion –1 molecules (ICAM-1).54 Genotype 1b might indeed be more cytopathic than other genotypes and this finding has also been associated with low response to IFN-a of this genotype.

- Cellular immune response to IFN-a in vitro is more vigorous in patients with genotype 2c compared with 1b.55

- The efficacy of HCV polyprotein translation regulated by the Internal ribosome entry site (IRES) is genotype-related. It is lower in genotype 3 compared to 1 and 2.56

- Core protein might interact with the tumor necrosis factor (TNF) related lymphocytotoxic receptor of the hepatocyte and this binding would differ among different genotypes.57 So the activation of TNF in the hepatocyte is genotype-related.

- The two hypervariable regions existing in genotype 1 versus only one in the remaining genotypes might be consistent with the more severe clinical expression of this genotype. Genotype 1 might escape more efficiently from the immune system and/or the IFN-a therapy by the development of new mutations and the selection of immune escape mutants.

- There is evidence of a wider and more efficient antibody response against hypervariable region in patients with genotypes 2 and 3 compared with those with genotype 1.58

- Viral dynamics during IFN-a treatment are different in genotypes 1 and 2. The antiviral effectiveness of IFN-a in blocking virus production, the free virion clearance rate and HCV infected cell death rate were all significantly higher for genotype 2 than for genotype 1.59

- It has been shown that after liver transplantation, HCV type 1b may induce more severe FAS-mediated apoptosis than other genotypes.60

Only one study has not verified the various pathogenicities among genotypes, but showed that the immunogenic properties of structural and non-structural proteins may vary among genotypes but on average all the genotypes have the same immunogenicity.61

Further explanation of the clinical profile of genotypes on a molecular basis is the subject of many studies which are still in progress.

4.5. Quasispecies

The studies of the evolution of the quasispecies during the course of infection have provided data for the emergence and selection of HCV strains.62 The mutations in the hypervariable region that encompasses linear B cell epitopes may be associated with the selection of escape mutants that evade humoral immune response. In addition, escape mutants that evade cellular immune response may appear.64 Consequently, infection with a strain of the virus does not seem to prevent the infected individual from reinfection with another strain.

The following observations suggest the failure of the host immune system to eradicate a heterogeneous viral population:

- A high number of quasispecies have been associated with more severe chronic liver disease irrespective of the level of viraemia and the duration of the disease.65
• Heterogeneous viral population has been associated with the failure in IFN response.  
• Pre-transplantation quasispecies may be a predictor of more rapid progression of HCV-induced hepatitis and graft fibrosis after liver transplantation.  
• Acute resolving hepatitis has been associated with no evolution of heterogeneous HCV population, whereas progression to chronicity correlated with early genetic evolution of quasispecies during the acute phase of the infection.

Finally, the sequence analysis of the mutations in the HVR1 may be considered as the “signature” of the isolate and may be a useful tool to trace the source of HCV transmission (intrafamilial, sexual, vertical from mother to child, nosocomial etc).  

Genotyping and sequence analysis of HCV isolates provided evidence for a patient-to-patient transmission of HCV during colonoscopy. The index case and the two other infected patients had a sequence homology of 100%, strongly suggesting a common source of infection.  

HCV genotype determination and sequencing of a part of NS5 region revealed the route of transmission in a hepatitis C outbreak which occurred in a hemodialysis unit in Greece.

4.6. Role of genetic heterogeneity in HCV vaccine development

The genetic heterogeneity of HCV accounts for the lack of inducing an effective immune response against HCV. It has been shown that the neutralizing antibody elicited by a specific HCV isolate, is isolate-specific and fails to induce a protective immunity against reinfection by heterologous strains.  

It has also been observed that the immune protection is not sufficient against challenge from the homologous strain. This finding has shown that genetic variation of the virus leads to the development of escape mutants that circumvent the immune response. Neutralizing antibodies are directed against the hypervariable region and their efficacy is limited by the high rate of mutations in this region.  

An effective multivalent vaccine should activate broad humoral and multispecific cellular immunity that includes both T helper and cytotoxic T-cell responses. Given the high degree of HCV genetic heterogeneity, this vaccine should generate a cross-protective immunity against various HCV genotypes. This could be achieved either by using antigens from highly conserved regions or by including antigens from different genotypes.  

Several promising approaches based on molecular technology have been explored, such as naked DNA, recombinant viruses, recombinant proteins, viruslike particles and peptides.

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