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Review

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.

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Abbreviation index: HCC: Hepatocellular carcinoma, HCV: Hepatitis C virus, HLA: Human leucocyte antigen, HVR: Hypervariable region, NCR: non-coding region, IFN-α: Interferon-α, IRES: Internal ribosome entry site, IVDU: Intravenous drug users, PCR: Polymerase chain reaction, RFLP: Restriction fragment length polymorphism

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.[1] In Greece the prevalence of anti-HCV ranges from 0.6 to 7.5% in different geographic regions (mean rate 1.9%).[2] 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%.[3]

At least 80% of infected persons will develop chronic disease.[4] Cirrhosis occurs in 20-50% of chronic HCV patients after a period of 20 years[5] and the annual incidence of hepatocellular carcinoma in cirrhotics is approximately 3%.[6] 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 aminoacids 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.[9] The second proteinase encoded by NS2 is responsible for the cleavage of the polyprotein in the NS2/NS3.[9] 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 genome[10] 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[10] but only 6% in the 5'NCR. As 5'NCR is the most conserved region it has became 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] Simmonds[10]) (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

Simmonds	Chiron	Okamoto	Enomoto
1a	I	I	K-PT
1b	II	II	K-1
1c	nc*	nc*	nc*
2a	III	III	K-2a
2b	III	IV	K-2b
2c	III	nc*	nc*
3a	IV	V	nc*
3b	IV	VI	nc*
4	nc*	nc*	nc*
5a	V	nc*	nc*
6a	nc*	nc*	nc*

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[19] were recognized and subtype numbers raised to about 80.[20] For a new subtype to be assigned it should be different in the phylogenetic analysis in at least two genomic regions.[21] 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[22] (Figure 1). Genotype 1 accounts for 50% of the cases and genotypes 2 and 3 for 20% each.[23] 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.[24] In Greece, genotype 1b is responsible for 47% of cases, followed by 3a (22%) and 1a (11%).[25] In cental 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.[26] In South-East Asian countries 1b is the most prevalent with the exception of the Philippines where 1a predominates.[27] 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[28] and 10 and 11 in Indonesia.

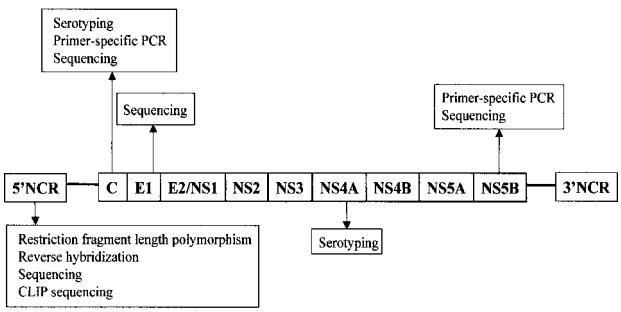

Figure 1. Worldwide geographic distribution of HCV genotypes.

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.[29,30] 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.[25,30] 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.[25,29]

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).[31]

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 HCV typing in special populations such as immunocompromised and immunodialysed people. In addition, no

Figure 2. HCV genotyping assays and the genomic regions used for every assay.

serotyping method can determine the subtypes included in the genotypes.

In clinical practice, the only indispensable distinction is between genotype 1 and genotypes 2 and 3 in order to choose the optimal therapeutic regimen. All the above mentioned systems have concordant results with respect to this distinction.

Mixed genotype infections from a major and a minor HCV population accounts for 4-17% of HCV patients.[32] Methods used for the detection of mixed genotype infections are type-specific PCR, RFLP, LiPA, serological methods and sequence analysis of high number of clones. The prevalence of mixed genotype infections is dependent on the sensitivity of the method and the group of HCV infected persons. Patients on haemodialysis, thalassaemia patients with multiple transfusions, IVDUs and haemophiliacs have the highest rate of mixed genotype infections.

4. CLINICAL SIGNIFICANCE OF GENETIC HETEROGENEITY OF HCV

4.1. Diagnostic assays

4.1.1. Anti-HCV antibody detection

The serological assays employ antigens from structural and non-structural regions of HCV. However, all the antigens employed are derived from genotype 1 and possibly these assays are less efficient in detecting non–1 genotypes. Antibody reactivity to 5-1-1 and c100-3 is reduced in patients infected with types 2b and 3a.[33] Many investigators claim that the sensitivity of serological methods is low in geographical areas with a high prevalence of non-1 HCV genotypes. It is also possible that the current screening assays may not detect unidentified genotypes.

In the third-generation assay ELISA3, 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.[34]

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.[35] 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.[36]

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 1994[77]	Italy	111	CHC	Retrospective
Mita 1994[78]	Italy	148	CHC+ Cirrhosis+HCC	Retrospective
Feray 1995[79]	France	60	LT	Retrospective
Cathomas 1996[80]	Switzerland	69	CHC	Retrospective
Caccamo 1996[81]	Italy	35	LT	Retrospective
Silini 1996[82]	Italy	593+166+219	CHC+HCC+Cirrhosis	Cross-sctional Case-control
Gane 1996[83]	UK	149	LTR	Prospective
Simmonds 1996[84]	UK	610	CHC	Retrospective
Zein 1996[85]	USA	48	HCC	Retrospective
Zein 1996[86]	USA	179	CHC	Retrospective
Hatzakis 1996[87]	Greece	17+87+23	HCC+CHC Cirrhosis	Retrospective Case-control
Kobayashi 1996[88]	Japan	140	CHC	Prospective
Booth 1997[89]	UK	29	CHC	Retrospective
Gordon 1997[90]	USA	42	LTR	Retrospective
Donato 1997[91]	Italy	172	HCC	Retrospective
Bruno 1997[92]	Italy	163	Cirrhosis	Prospective
Pageaux 1997[93]	France	22	LTR	Prospective
Tassopoulos 1998[94]	Greece	152	CHC	Retrospective
Berg 1998[95]	Germany	79	LTR	Prospective
Bellentani 1999[96]	Italy	6917	General Population	Prospective
Prieto 1999[97]	Spain	81	LTR	Prospective
Belli 2000[98]	Italy	89	LTR	Prospective

CHC: Chronic hepatitis C, LTR: Liver transplant recipients

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

Source	Location	No of pts	Type of pts	Type of study
Yamada 1994[99]	Japan	251	CHC	Retrospective
Yotsunanagi 1995[100]	Japan	72+131	HCC+CHC	Retrospective
Takano 1995[101]	Japan	124	CHC	Prospective
Zhou 1996[102]	USA	124	LTR	Retrospective
Guido 1996[103]	Italy	59	CHC	Retrospective
Lee 1996[104]	Korea	138	CHC+Cirrhosis+HCC	Retrospective Case-control
Zeuzeum 1996[105]	Germany	97	CHC	Retrospective
Romeo 1996[106]	Italy	197	CHC	Retrospective
Lau 1996[107]	USA	438	CHC	Cross-sectional
Boker et al 1997[108]	Germany	71	LTR	Prospective
Mangia 1997[109]	Italy	213	CHC	Retrospective
Benvegnu 1997[110]	Italy	429	CHC+Cirrhosis	Prospective
Lopez-Labrador 1997[111]	Spain	414	CHC	Cross-sectional
Naoumov 1997[112]	UK	1438	Cirrhosis	Prospective
Gayowski 1997[113]	USA	47	LTR	Prospective
Poynard 1997[114]	France	2235	CHC	Retrospective
Serfaty 1998[115]	France	668	Cirrhosis	Prospective
Vargas 1998[116]	USA	202	LTR	Prospective
De Moliner 1998[117]	Italy	96	CHC	Retrospective
Kleter 1998[118]	Netherlands	293	CHC	Retrospective
Roffi 1998[119]	Italy	1368	CHC	Retrospective
Costes 1999[120]	France	25	LTR	Prospective
Dutta 1999[121]	Australia	17	HCC	Case-control
Reid 1999[122]	USA	28+38	HCC+Cirrhosis	Retrospective
Adinolfi 2000[123]	Italy	324	CHC	Retrospective

CHC: Chronic hepatitis C, LTR: Liver transplant recipients

ciated 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.[41] Viral factors predictive of response to IFN-α are; pre-treatment viral load,[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.[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.[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%.[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.[46] IFN-α and ribavirin combination treatment for six months achieved similar rates of sustained response of 16% for genotype 1 in naove 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).[47] In another study, combination therapy in naove patients achieved a 13% rate of sustained response in genotype 1b while for 2 and 3 the rates were 43% and 53% respectively.[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-

tients 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 naove 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[63] 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.[66]
- Pre-transplantation quasispecies may be a predictor of more rapid progression of HCV-induced hepatitis and graft fibrosis after liver transplantation.[67]
- 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.[68]

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 (intrafamiliar, sexual, vertical from mother to child, nosocomial etc).[69] 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.[70] 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.[71]

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.[72] It has also been observed that the immune protection is not sufficient against challenge from the homologous strain.[73] 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.[74] 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.[75] Several promising approaches based on molecular technology have been explored, such as naked DNA, recombinant viruses, recombinant proteins, viruslike particles and peptides.[76]

REFERENCES

1. Van Der Poel CL, Reesinck HW, Lelie PN, et al. Anti-hepatitis C antibodies and non-A non-B post-transfusion hepatitis in The Netherlands. Lancet 1989; 2:297-298.
2. Papadimitropoulos V, Gioustozi A, Papaioannou C et al. Prevalence of HCV infection in the general population of various regions of Greece. In: Hepatitis C 1998. Hadziyannis SJ (ed), Athens 1998:163-170
3. Saeed AA, Admawi A, Al Rasheed A, et al. Hepatitis C virus infection in Egyptian volunteer blood donors in Riyandh. Lancet 1991; 338:459-460.
4. Alter AJ, Margolis HS, Krawczynski K, et al. The natural history of community-acquired hepatitis C in the United States. N Eng J Med 1992; 327:1899-1905.
5. Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alfa: A multicenter randomized, controlled trial. N Engl J Med 1989; 321:1506-1510.
6. Fattovich G, Giustina G, Degos F et al. Effectiveness of interferon alfa on incidence of hepatocellular carcinoma and decompensation in cirrhosis type C. J Hepatol 1997; 27:201-205.
7. Tsukiyama-Kohara K, Likuza N, Kohara M, Nomoto A. Internal ribosomal entry site within hepatitis C virus RNA. J Virol 1992; 66:1476-1483.
8. Weiner AJ, Brauer MJ, Rosenblatt J, et al. Variable and hypervariable domains are found in the regions of HCV corresponding to the flavivirus envelope and NS1 proteins and the pestivirus envelope glycoproteins. Virology 1991; 180:842-848.
9. Houghton M, Weiner A, Han J, et al. Molecular biology of the hepatitis C virus: implications for diagnosis development and control of viral disease. Hepatology 1991; 14:381-388.
10. Simmonds P. Variability of hepatitis C virus. Hepatology 1995; 21:570-583.
11. Brechot C. Hepatitis C virus. Molecular biology and genetic variability. Dig Dis Sci 1996; 41:6S-21S.
12. Simmonds P, Mcomish F, Yap PL, et al . Sequence variability in the 5' non coding region of hepatitis C virus: identification of a new virus type and restrictions on sequence diversity. J Gen Virol 1993; 74:661-668.
13. Simmonds P, Holmes EC, CHA TA, et al. Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS5 region. J Gen Virol 1993; 74:2391-2399.
14. Simmonds P, Smith DB, Mcomish F, et al. Identification of genotypes of hepatitis C virus by sequence comparison in the core, E1 and NS5 regions. J Gen Virol 1994; 75:1053-1061.
15. Ohno T, Lau JYN. The gold standard accuracy and the current concepts. Hepatitis C virus genotype and viraemia. Editorial. Hepatology 1996; 24:1312-1315.
16. Smith Db, Pontisso P. Heterogeneity of hepatitis C virus. Baillere's Clin Gastroenterol 1996; 10:243-255.
17. Enomoto N, Takada A, Nakao T, Date T. There are two major types of hepatitis C virus in Japan. Biochem Bio-

phys Res Commun 1990; 170:1021-1025.
18. Okamoto H, Okada S, Sugiyama Y, et al. Nucleotide sequence of the genomic RNA of hepatitis C virus isolated from a human carrier: comparison with reported isolates for conserved and divergent regions. J Gen Virol 1991; 72:2697-2704.
19. Okamoto H, Miyakawa Y, Mayumi M. Molecular virology of hepatitis C virus. Viral Hepat Rev 1997; 3:51-62.
20. Smith DB, Davidson F, Simmonds P. Hepatitis C virus variants and the role of genotyping. J Hepatol 1995; 23(suppl):26-31.
21. Simmonds P, Alberti A, Bonino F, et al. A proposed system for the nomenclature of hepatitis C virus genotypes. Hepatology 1994; 19:1321-1324.
22. Mcomish F, Yap PL, Dow BC, et al. Geographical distribution of hepatitis C virus genotypes in blood donors: an international collaborative survey. J Clin Microbiol 1994; 32:884-892.
23. Kleter GEM, Van Doom U, Brouwer JT, et al. Sequence analysis of the 5' untranslated region in isolates of at least four genotypes of hepatitis C virus in the Netherlands. J Clin Microbiol 1994; 32:306-310.
24. Nousbaum JB, Pol S, Nalbas B, et al. Hepatitis C virus 1b infection in France and Italy. Ann Intern Med 1995; 122:161-168.
25. Sinani C, Savvas S, Spanou E, et al. Epidemiology of HCV genotypes in Greece. In: Hepatitis C 1997. Hadziyannis SJ (ed). Athens 1997:189-197.
26. PM Kao JH, Tsai SL, Chen PJ, et al. A clinicopathological study of chronic non-A, non-B (type C) hepatitis in Taiwan: comparison between posttransfusion and sporadic patients. J Hepatol 1994; 21:244-249.
27. Greene WK, Cheong MK, NG V, Yap KW. Prevalence of hepatitis C virus sequence variants in South-East Asia. J Gen Virol 1995; 76:211-215.
28. Tokita H, Okamoto H, Tsuda F, et al. Hepatitis C virus variants from Vietnam are classifiable into the seventh, eighth and nine major genetic groups. Proc Natl Acad Sci USA 1994; 91:11022-11026
29. Haushofer AC, Kopty C, Hauer R, et al. HCV genotypes and age distribution in patients of Vienna and surrounding areas. J Clin Virol 2001; 20:41-47.
30. Elghouzzi MH, Bouchardeau F, Pillonel J, et al. Hepatitis C virus: routes of infection and genotypes in a cohort of anti-HCV-positive french blood donors. Vox Sang 2000; 79:138-144.
31. Forns X, Bukh J. Methods for determining the hepatitis C virus genotype. Viral Hepat Rev 1998; 4:1-19.
32. Blatt LM, Mutchnick MG, Tong MJ et al. Assessment of hepatitis C virus RNA and genotype from 6807 patients with chronic hepatitis C in United States. J Viral Hepat 2000; 7:196-202.
33. Zein NN, Rakela J & Persing DH. Genotype dependent serologic reactivities in patients infected with hepatitis C virus in the United States. Mayo Clin Proc 1995; 70:449-452.
34. Makris K, Kouvelis I, Drakopoulos E, et al. Frequency and characteristics of post-transfusionhepatitis in Greece with emphasis in hepatitis C: comparing second-and third generation assays. Transfus Med 1995; 5:213-224.
35. Pawlotsky JM. Measuring hepatitis C viraemia in clinical samples: Can we trust the assays? Hepatology 1997; 26:1-4.
36. Mellor J, Hawkins A, Simmonds P. Genotype dependence of hepatitis C virus load measurement in commercially available quantitative assays. J Clin Microbiol 1999, 37:2525-2532.
37. Zekri AR, Bahnassy AA, Shaarawy SM, et al. Hepatitis C virus genotyping in relation to neu-oncoprotein overexpression and the development of hepatocellular carcinoma. J Med Microbiol 2000; 49:89-95.
38. Michitaka K, Durazzo M, Tillmann HL, et al. Analysis of hepatitis C virus genome in patients with autoimmune hepatitis type 2. Gastroenterology 1994, 106:1603-1610.
39. Zehender G, De Maddalena C, Monti G, et al. HCV genotypes in bone marrow and peripheral blood mononuclear cells of patients with mixed cryoglobulinemia. Clin Exp Rheumatol 1995; 13(Suppl 13):S87-S90.
40. Poynard T, Leroy V, Cohard M. Meta-analysis of interferon randomized trials in the treatment of viral hepatitis C. effects of dose and duration. Hepatology 1996; 24:778-789.
41. Pawlotsky JM, Thorval FR, Bastie A. Factors affecting treatment responses to interferon-a in chronic hepatitis C. J ID 1996; 174:1-7.
42. Kobayashi Y, Watanabe S, Konishi M. Quantitation and typing of serum hepatitis C virus RNA in patients with chronic hepatitis C treated with interferon-b. Hepatology 1993; 18:1319-1325.
43. Conjeevaram HS, Everhart JE, Hoofnagle JH. Predictors of a sustained beneficial response to interferon alfa therapyvin chronic hepatitis C. Hepatology 1995; 22:1326-1329.
44. Pontisso P, Gerotto M, Chemello L. Hepatitis C virus genotypes HCV-1a and HCV-1b: the clinical point of view. J Infect Dis 1995; 171:760.
45. Chemello L, Bonetti P, Cavalletto L, et al. Randomized trial comparing three different regimens of alpha-2a-interferon in chronic hepatitis C. Hepatology 1995; 22:700-706.
46. Davis GL, Lau JYN. Factors predictive of a beneficial response to therapy of hepatitis C. Hepatology 1997; 26(suppl 1):122S-127S.
47. Davis GL, Esteban-Mur R, Rustgi V, et al. Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. N Engl J Med 1998; 339:1493-1439.
48. Reichard O, Norkrans G, Fryden A, et al. Randomized, double-blind, placebo controlled trial of interferon a-2b with and without ribavirin for chronic hepatitis C. Lancet 1998; 351:83-87.
49. EASL international consensus conference on hepatitis C. Paris 26-28 February 1999. Consensus statement. J Hepatol 1999; 30:956-961.
50. Zylbeberg H, Chaix ML, Brechot C. Infection with hepatitis C virus genotype 4 is associated with a poor response

to interferon-a. Ann Intern Med 2000; 132:845-846.
51. Enomoto N, Sakuma I, Asahina Y. Mutations in the nonstructural protein 5A gene and response to Interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996; 334:77-81.
52. Tanji Y, Kaneko T, Shimotohno K. Phosphorylation of hepatitis C virus-encoded non-structural protein NS5. J Virol 1995; 69:3980-3986.
53. Liaw YF, Lee CS, Tsai SL. T-cell mediated autologous hepatocytotoxicity in patients with chronic hepatitis C virus infection. Hepatology 1995; 22:1368-1373.
54. Ballardini G, Groff P, Pontisso P, et al. Hepatitis C virus (HCV genotype, tissue HCV antigens hepatocellular expression of HLA-A, B, C, and intercellular adhesion-1 molecules. Clues to pathogenesis of hepatocellular damage and response to interferon treatment in patients with chronic hepatitis C. J Clin Invest 1995; 95:2067-2075.
55. Missale G, Cariani E, Lamonaca V, et al. Effects of interferon treatment on the antiviral T-cell response in hepatitis C virus genotype 1b and genotpe 2c-infected patients. Hepatology 1997; 26:792-797.
56. Buratti E, Gerotto M, Pontisso P, et al. In vivo translational efficiency of different hepatitis C virus 5'-UTRs. FEBS Lett 1997; 411:275-180.
57. Matsumoto M, Hsieh TY, Zhu N, et al. Hepatitis C virus core protein interacts with the cytoplasmic tail of lymphotoxin betaR. J Virol 1997; 71:1301-1309.
58. Mondelli MU, Cerino A, Lisa A, et al. Antibody responses to hepatitis C virus hypervariable region 1: evidence for cross-reactivity and immune mediated sequence variation. Hepatology 1999; 30:537-545.
59. Neumann AU, Lam NP, Dahari H et al. Differences in viral dynamics between genotypes 1 and 2 of hepatitis C virus. J Infect Dis 2000; 182:28-35.
60. Di Martino V, Brenot C, Samuel D et al. Influence of liver hepatitis C virus RNA and hepatitis C virus genotype on FAS-mediated apoptosis after liver transplantation for hepatitis C. Transplantation 2000; 70:1390-1396.
61. Rodriguez-Lopez M, Riezu Boj JI, Ruiz M, et al. Immunogenicity of variable regions of hepatitis C virus proteins: selection of peptide epitopes to assess hepatitis C virus genotypes by Elisa. J Gen Virol 1999; 80:727-738.
62. Kurosaki M, Enomoto N, Marumo F, Sato C. Evolution and selection of hepatitis C virus variants in patients with chronic hepatitis C. Virology 1994; 205:161-169.
63. Kato N, Sekiya H, Ootsuyama Y, et al. Humoral immune response to hypervariable region 1 of the putative envelope glycoprotein (gp70) of hepatitis C virus. J virol 1993; 67:3923-3930.
64. Weiner A, Erickson Al, Kansopon J, et al. Persistent hepatitis C virus infection in a chimpanzee is associated with emergence of a cytotoxic T lymphocyte escape T lymphocyte escape mutant. PNAS USA 1995; 92:2755-2759.
65. Honda M, Kaneko S, Sakai A, et al. Degree of diversity of hepatitis C virus quasispecies and progression of liver disease. Hepatology 1994; 20:1144-1151.
66. Moribe T, Hayashi N, Kanazawa Y, et al. Hepatitis C viral complexity detected by single-strand conformation polymorphism response to interferon therapy. Gastroenterology 1995; 108:789-795.
67. Pelletier SJ, Raymond DP, Crabtree TD et al. Pretransplantation hepatitis C virus quasispecies may be predictive of outcome after liver transplantation. Hepatology 2000; 32:375-381.
68. Farzi P, Shimoda A, Colana A et al. The outcome of acue hepatitis C predicted by the evolution of the viral quasispecies. Science 2000; 288:339-344.
69. Allander T, Gruber A, Naghavi M, et al. Frequent patient to patient transmission of hepatitis C virus in a haematology ward. Lancet 1995; 345:603-607.
70. Bronowicki JP, Venard V, Botte C, et al. Patient-to-patient transmission of hepatitis C during colonoscopy. N Engl J Med 1997; 337:237-240
71. Katsoulidou A, Paraskevis D, Kalapothaki V, et al. Molecularepidemiology of a hepatitis C outbreak in a haemodialysis Cohort Study on Viral Hepatitis. Nephrol Dial Transplant 1999; 14:1188-1194.
72. Farci P, Alter HJ, Gonvidarajan S, et al. Lack of protective immunity against reinfection with hepatitis C virus. Science 1992; 258:135-140.
73. Farci P, Alter Hj, Wong DC, et al. Pevention of hepatitis C virus infection in chimpanzees after antibody-mediated in vitro neutralization. PNAS USA 1994; 91:779-786.
74. Weiner A, Geysen HM, Christopherson C, et al. Evidence for immune selection of hepatitis C virus (HCV) putative envelope glycoprotein variants: potential role in chronic HCV infections. PNAS 1992; 89:3468-3472
75. Choo Ql, Kuo G, Ralston R, et al. Vaccination of chimpanzees against infection by the hepatitis C virus. Proc Natl Acad Sci USA 1994; 91:1294-1298.
76. Lechmann M, Liang J. Vaccine development for hepatitis C. Sem Liver Dis 2000; 20:211-226.
77. Pozzato G, Kaneko S, Moretti M, et al. Different genotypes of hepatitis C virus are associated with different severity of chronic liver disease. J Med Virol 1994; 43:291-296.
78. Mita E, Hayashi N, Kanazawa Y, et al. Hepatitis C virus genotype and RNA titer in the progression of type C chronic liver disease. J Hepatol 1994; 21:468-473.
79. Feray C, Gigou M, Samuel D, et al. Influence of the genotypes of hepatitis C virus on the severity of recurrent liver disease after liver transplantation. Gastroenterology 1995; 108:1088-1096.
80. Cathomas G, McGandy CE, Terraciano LM, et al. Detection and typing of hepatitis C RNA in liver biopsies and its relation to histopathology. Virchows Arch 1996; 429:353-358.
81. Caccamo L, Gridelli B, Sampietro M, et al. Hepatitis C virus genotypes and reinfection of the graft during long-term follow-up in 35 liver transplant recipients. Transpl Int 1996; 9:S204-S209.
82. Silini E, Bottelli R, Asti M. Hepatitis C virus genotypes and risk for hepatocellular carcinoma in cirrhosis: a case-control study. Gastroenterology 1996; 111:199-205.
83. Gane EJ, Naoumov NV, Qian KP. A longitudinal analysis of hepatitis C virus replication following liver trans-

plantation. Gastroenterology 1996; 110:167-177.
84. Simmonds P, Mellor J, Craxi A. Epidemiological clinical and therapeutic association of hepatitis C types in western European patients. J Hepatol 1996; 24:517-524.
85. Zein NN, Poteruch JJ, Gross JB, et al. Increased risk of hepatocellular carcinoma in patients infected with hepatitis C genotype 1b. Am J Gastroenterol 1996; 91:2560-2562.
86. Zein NN, Rakela J, Krawitt EL, et al. Hepatitis C virus genotypes in the United States: epidemiology, pathogenicity, and response to interferon therapy. Collaborative study Group.Ann Intern Med 1996; 125:634-639.
87. Hatzakis A, Katsoulidou A, Kaklamani E at al. Hepatitis C virus 1b is the dopminant genotype in HCV-related carcinogenesis: a case control study. Int J Cancer 1996; 68:51-53.
88. Kobayashi M, Tanaka E, Sodeyama T, et al The natural course of chronic hepatitis C: a comparison between patients with genotypes 1 and 2 hepatitis C viruses. Hepatology 1996; 23:695-699.
89. Booth JC, Foster GR, Levine T, et al. The relationship of histology to genotype inchronic HCV infection. Liver 1997; 17:144-151.
90. Gordon FD, Poterucha JJ, Germer J et al. Relationship between hepatitis C genotype and severity of recurrent hepatitis C after liver transplantatio. Transplantation 1997; 63:1419-1423.
91. Donato F, Tagger A, Chiesa R et al. Hepatitis B and C virus infection, alcohol drinking, and hepatocellular carcinoma: a case control study in Italy. Hepatol 1997; 26:579-584.
92. Bruno S, Silini E, Crosignani A, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. Hepatology 1997; 25:754-758.
93. Pageaux GP, Ducos J, Mondain AM, et al. Hepatitis C virus genotypes and quantitation of serum hepatitis C virus RNA in liver translant recipients. Liver Transpl Surg 1997; 3:501-505.
94. Tassopoulos NC, Papatheodoridis GV, Katsoulidou A, et al. Factors associated with severity and disease progression in chronic hepatitis C. Hepatogastroenterol 1998; 45:1678-83.
95. Berg T, Hopf U, Bechstein WO, et al. Pretransplant virviological markers hepatitis C virus genotype and viraemia level are not helpful in predicting individual outcome after orthotipic liver transplantation. Tranplantation1998; 66:225-228.
96. Bellentani S, Ponzato G, Saccoccio G, et al. Clinical course and risk factors of hepatitis C virus related liver disease in the general population: report from the Dionysos study. Gut 1999; 44:874-880.
97. Prieto M, Berenguer M, Ryon JM, et al. High incidence of allograft cirrhosis in hepatitis C virus genotype 1b infection following transplantation:relationship with rejection episodes. Hepatol 1999; 29:250-256.
98. Belli LS, Zavaglia C, Alberti AB, et al. Influence of immunogenetic backround on the outcome of recurrent hepatitis C after liver transplantation. Hepatol 2000; 31:1345-1350.
99. Yamada M, Kakumu S, Yoshioka K, et al. Hepatitis C virus genotypes are not responsible for development of serious liver disease. Dig Dis Sci 1994; 39:234-239.
100. Yotsuyanagi H, Koike k, Yasuda K et al. Hepatitis C virus genotypes and development of hepatocellular carcinoma. Cancer 1995; 76:1352-1355.
101. Takano S, Yokosuka O, Imazeki F, et al. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. Hepatology 1995; 21:650-655.
102. Zhou S, Terrault NA, Ferrell L, et al. Severity of liver diseasein liver transplantation recipientas with hepatitis C virus infection: relationshipto genotype and level of viraemia. Hepatol 1996; 24:1041-1046.
103. Guido M, Rugge M, Thung SN, et al. Hepatitis virus serotypes and liver pathology. Liver 1996; 16:353-357.
104. Lee DS, Sung YC, Whang YS. Distribution of HCV Genotypes among blood donors, patients with chronic liver disease , hepatocellular carcinoma, and patients on maintenance hemodialysis in Korea. J Med Virol 1996; 49:55-60.
105. Zeuzeum S, Franke A, Lee JH, et al. Phylogenetic analysis of hepatitis C virus isolates andtheir correlation to viraemia, liver function tests and histology. Hepatol 1996; 24:1003-1009.
106. Romeo R, Colombo M, Rumi M, et al. Lack of association between type of hepatitis C virus, serum load and severity of liver disease. J Viral Hepat 1996; 3:183-190.
107. Lau JYN, Davis GL, Prescott LE, et al. Distribution of hepatitis C virus genotypes determined by line probe assay in patients with chronic hepatitis C seen at tertiary referral centers in the United States. Ann Intern Med 1996; 124:868-876.
108. Boker KH, Dalley G, Bahr G et al. Long-term outcome of hepatitis C virus infection after liver transplantation, Hepatol 1997; 25:203-210.
109. Mangia A, Cascavilla I, Lezzi G et al. HCV genotypes in patients with liver disease of different stages and severity. J Hepatol 1997; 26:1173-1178.
110. Benvegnu L, Pontisso P, Cavalletto D, et al. Lack of correlation between hepatitis C virus genotypes and clinical course of hepatitis C virus related cirrhosis. Hepatology 1997; 25:211-215.
111. Lopez-Labrador FX, Ampurdanes S, Forns X. Hepatitis C virus (HCV) genotypes in Spanish patients with HCV infection: relationship between HCV genotype 1b, cirrhosis and hepatocellular carcinoma. J Hepatol 1997; 27:959-965.
112. Naoumov NV, Chokshi S, Metivier F, et al. Hepatitis C virus infection in the development of hepatocellular carcinoma in cirrhosis. J Hepatol 1997; 27:331-336.
113. Gayowski T, Singh N, Marino IR, et al. Hepatitis C virus genotypes in liver transplant recipients: impact on posttransplant recurrence, infections, response to interferon-alpha therapy and outcome. Transplantation 1997; 64:422-426.
114. Poynard T, Bedossa P, Opolon P. Natural history of liver

fibrosis progression in patients with chronic hepatitis C. Lancet 1997; 349:825-832.
115. Serfaty L. Aumaitre H, Chazouilleres O, et al. Determinants of outcome of compensated hepatitis C virus-related cirrhosis. Hepatology 1998:27:1435-1440.
116. Vargas HE, Laskus T, Wang LF, et al. The influence of hepatitis C virus genotypes on the outcome of liver transplantation. Liver transpl Surg 1998; 4:22-27.
117. De Moliner L, Pontisso P, De Salvo GL, et al. Serum and liver HCV RNA in patients with chronic hepatitis C: correlation with clinical and histological features. Gut 199; 42:856-860.
118. Kleter B, Brouwer JT, Nevens F, et al. Hepatitis C virus genotypes: epidemiological and clinical associations. Benelux Study Group on Treatment of Chronic Hepatitis C. Liver 1998; 18:32-38.
119. Roffi L, Ricci A, Ogliari C et al. HCV genotypes in Northern Italy: a survey of 1368 histologically proven chronic hepatitis C patients. J Hepatol 1998; 29:701-706.
120. Costes V, Durand L, Pageaux GP et al. Hepatitis C virus genotypes and quantification of hepatitis C RNA in liver transplant recipients. Relationship with histologic outcome of recurrent hepatitis C. Am J Clin Pathol 1999; 111:252-258.
121. Dutta Ubyth K, Kench J et al. Risk factors for development of hepatocellular carcinoma among Australians with hepatitis C: a case-control study. Aust N Z J Med 1999; 29:300-307.
122. Reid AE, Koziel MJ, Aiza I, et al. Hepatitis C virus genotypes and viraemia and hepatocellular carcinoma in the United States. Am J Gastroenterol 1999; 94:1619-1626.
123. Adinolfi LE, Utili R, Andreana A, et al. Eur J Gastroenterol Hepatol; 2000: 12:389-304.