Review

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Is response to antiviral treatment influenced by hepatitis B virus genotype?

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Recently released clinical practice guidelines and consensus conference statements point to the importance of hepatitis B virus (HBV) genotyping in therapeutic algorithms for the treatment of chronic hepatitis B. This information usually comes from post hoc analyses of clinical trials which were not designed to study associations with the HBV genotype. We have performed a literature search through to April 2009 and have selected randomized clinical trials of currently approved anti-HBV drugs providing information on HBV genotypes and (i) baseline characteristics of study subjects, (ii) any response to antiviral therapy, (iii) interaction between HBV genotypes and the type of therapy. There were several intrinsic features and weaknesses in the majority of clinical trials conducted so far which make it difficult to reach firm conclusions about the role of HBV genotypes in response to antiviral therapy. Indeed, most trials were necessarily multicenter in order to reach a sufficient statistical power, but pooling together patients of different ethnicities may have revealed false-positive associations between response to antiviral therapy and HBV genotype. Moreover, endpoint definitions, especially for the composite ones, varied substantially among studies, leading to lack of homogeneity. Finally, possible interactions between the type of therapy and the HBV genotype were only seldom analysed. The present review highlights several caveats regarding current indications proposed by the major clinical practice guidelines and consensus conference statements published thus far and emphasise the need for further long term studies in the field.

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Abbreviations: HBV, hepatitis B virus; HCV, hepatitis C virus; NUC's, nucleos(t)ide analogues; HIV, human immunodeficiency virus; HCC, hepatocellular carcinoma; HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; PEG, polyethylene glycole; IFN, interferon; LAM, lamivudine; LdT, telbivudine; ETV, entecavir; ADV, adefovir dipivoxil; TDF, tenofovir disoproxil fumarate; ALT, alanine aminotransferase; PCR, polymerase chain reaction.

Introduction

Hepatitis B virus (HBV) is responsible for a large number of chronic infections worldwide and its control and eradication are considered one of the major public health challenges of the 21st century [1]. Similarly to hepatitis C virus (HCV), HBV persistence and progression to chronic liver disease are thought to result from a combination of viral and host factors [2]. The evolutionary history of major hepatitis viruses in different human populations includes the origin of phylogenetic variants named genotypes. Several clinical and epidemiological observations suggest that genetic differences in viral genotypes may underlie differences in biological and clinical behaviours. More importantly, although for HCV infections genotyping has become an essential part of therapeutic algorithms [3], the evidence that HBV genotypes play any role in response to antiviral treatment is much less clear. The problem is further compounded by the fact that drugs which have exclusive antiviral activity against HBV, such as nucleos(t)ide analogues (NUC's), seem not to be influenced by genotypes, whereas interferon-α does [4]. The HBV-specific antiviral pharmacopoeia is limited to NUC's which display a broader genotypic coverage than the protease and non-nucleosidic inhibitors of human immunodeficiency virus (HIV) and HCV; however, the information provided in pivotal clinical trials is often incomplete. Here we shall review data on the therapeutic implications of HBV genotypes with the aim of discussing established tenets as well as controversial issues. Moreover, we shall briefly discuss whether the main characteristics of patients before starting treatment differed according to HBV genotypes.

HBV genotype geographical distribution and relevant clinical correlates

A genotype is a viral variant which sufficiently differs from other variants of the same virus to constitute a distinct phylogenetic group. This simple definition also implies that several virus isolates worldwide would fall in a particular genetic group to support single anecdotal reports and that there is evidence for spread of a specific genotype in particular transmission networks. In order to achieve this distinction, evidence of infection with a specific viral genotype should be provided in several independently infected individuals. The identification of new genotypes will henceforth require demonstration of a consistent independent genetic grouping. Until now,

eight HBV genotypes have been identified and are numbered alphabetically from A to H [5]. HBV genotypes differ by at least 8% from each other [6] and several subtypes (at least 24) have been described, except for genotypes E and G [7]. Not unexpectedly, HBV genotypes and subtypes show a distinct geographical distribution, with genotype A being typically isolated in Northern Europe and countries with a strong prevalence of populations of Northern European origin, including the USA, but is occasionally seen also in the Indian subcontinent. Genotype D is highly prevalent in Eastern Europe, the Mediterranean countries and the Middle East, whereas genotypes B and C are typical of China and Japan. Infections with genotypes E, F, G and H are rarer and usually observed in West Africa, Central-South America, Central Europe and Southern USA, and Central America, respectively [7]. Infection with more than one genotype is possible [8] and there is evidence that super-infection may be accompanied by acute exacerbation of the underlying chronic disease, suggesting that adaptive immunity may not always be protective across genotypes [9]. Recombination among genotypes is possible and it has been shown that recombination between genotypes B and C has led to the generation of two different strains with distinct geographic distribution [10]. A relationship between HBV genotypes and clinical outcome of hepatitis B has been reported but most studies have compared genotypes B and C or genotypes D and A, because of their geographical distribution, indicating that genotypes A and B are generally associated with a more benign course of infection [1]. Indeed, there is evidence that cirrhosis and HCC are more frequent in carriers of genotype C than B [11–13] and a recent long-term prospective study on a large number of HBV carriers from Taiwan showed that HBV genotype C was associated with an increased risk of hepatocellular carcinoma (HCC) [14]. While strong epidemiological and clinical data support clear clinical differences between genotypes B and C, the evidence in favour of genotype A being more benign than D is softer, and to determine whether clinically significant differences in the natural history are true will require large longitudinal studies in European and North American countries. Indeed, a study performed among Alaska Natives suggested the opposite, with the odds of HCC being 4.7 times greater in adults with genotype A (95% confidence interval (CI) = 1.4–16.0) and 11.7 times greater in adults with genotype F (95% CI = 5.4–25.4) compared to those with genotype D [15]. Moreover, the molecular virological mechanisms that contribute to these clinical differences among HBV genotypes are far from being determined.

Is antiviral treatment outcome influenced by viral genotype?

Treatment objectives in chronic HBV infection are to obtain hepatitis B e antigen (HBeAg) loss or anti-HBe seroconversion in HBeAg-positive patients which is often associated with liver disease remission [1]. Although HBeAg-positive patients are commonly observed in Asia and Northern Europe, the typical European or Mediterranean patient would be anti-HBe positive and therefore other therapeutic endpoints should be considered. Complete and sustained suppression of HBV replication, and improvement in necroinflammatory activity and fibrosis of the liver, which are associated with delayed disease progression, are valuable short-term end points [4,16]. Ideally, HBsAg and anti-HBs seroconversion is now considered the objective closest to a cure and can be observed in a respectable proportion of patients after complete viral suppression is achieved and stable after treatment discontinuation.

Genotype and interferon treatment

Large multicenter trials of peginterferon (PEG-IFN) α showed that in patients treated with PEG-IFN-α-2b there was a statistically significant association between viral genotype and sustained HBeAg loss. Thus, when all interferon-treated patients were examined independently of concomitant lamivudine treatment, the highest rate of HBeAg clearance at the end of follow-up occurred in patients infected with genotype A (47%), followed by genotype B (44%), C (28%) and D (25%). Further analyses of the same study population demonstrated that HBsAg clearance was also closely linked to viral genotype, being highest in genotype A (14%) compared to B (9%), C (3%) and D (2%) [17]. A re-evaluation of the data carried out approximately 3 years later indicated that among patients who cleared HBeAg in the initial study, 96% of those with genotype A were still HBeAg-negative and 58% were HBsAg-negative, whereas the same endpoints were achieved in 86% and 14% of patients with genotype B, 67% and 0% of patients with genotype C and 76% and 6% of those with genotype D, respectively [18]. These data indicate that durable HBeAg loss after interferon therapy occurs most frequently in patients with genotypes A and B, and this is associated with a greater chance for HBsAg clearance upon prolonged follow-up [17]. A recent meta-analysis [19] and a pooled analysis of over 1200 patients [20] provide compelling support for the fact that genotype A is the most treatment-responsive genotype in HBeAg-positive hepatitis B. Genotype A is relatively uncommon in HBeAg-negative cases, but non-D genotypes, particularly C, appear to have higher rates of sustained virological response in this form of chronic hepatitis B. The reasons for the different rates of virological response according to genotype remain unclear but may relate to changes in viral sequences during interferon therapy that affect host immune responses [21]. An alternative but rather speculative explanation could be that different routes of HBV transmission, mostly horizontal for genotype A vs vertical for other genotypes, could influence T-cell reactivity to HBV proteins which may explain the clinical observations of higher HBsAg loss during treatment of patients infected with HBV genotype A with PEG-IFN. T-cell exhaustion following exposure to high viral antigen concentration in the peripheral blood, as it occurs for vertically transmitted genotypes, may be responsible for inefficient responses [22].

Genotype and treatment with nucleos(t)ide analogues

Besides standard or pegylated interferon, current treatment options include three nucleoside analogues [lamivudine (LAM), telbivudine (LdT) and entecavir (ETV)], and two nucleotide analogues [adefovir dipivoxil (ADV) and tenofovir disoproxil fumarate (TDF)]. A previous meta-analysis on both observational studies and clinical trials published up to 2007 failed to detect a genotype effect on treatment responses to analogues [19]. However, that study did not take into account that different studies evaluated different endpoints and it did not include in the analysis continuous responses such as ALT normalization and serum DNA level reductions. We have therefore reviewed the results from clinical trials published up to 2009 with data on the association between HBV genotypes and response to therapy, giving a more complete picture of all the study endpoints, in order to identify, if possible, a single pattern of response to therapy according to HBV genotype. Moreover, we have briefly described the baseline characteristics of HBV infected patients according to the HBV genotypes.

Literature search

We performed a literature search through to April 2009 on PubMed using combinations of the keywords "HBV", "genotype", "treatment", "pegylated interferon", "lamivudine", "adefovir", "entecavir", "telbivudine", "tenofovir", with no search restrictions. We selected only randomised clinical trials with any information provided on HBV genotypes and (i) baseline characteristics of study subjects, (ii) any response to antiviral therapy and (iii) interaction with the type of therapy. In addition, we reviewed the references from the retrieved articles and relevant reviews to identify additional studies. Overall, we went through the full text of 32 articles from 20 different clinical trials. We excluded 17 papers that did not report any useful information for analyses on HBV genotypes. Fifteen papers from 13 different clinical trials were eventually included in this review.

Data extraction and methods

For each study we recorded information on the publication year, study location, period of accrual, weeks of treatment, patient age range, type of therapy and doses, HBeAg-status, studied endpoint(s) and treatment endpoint(s) by HBV genotype.

We extracted any available information (including *p*-values) from tables and text on the differences in baseline characteristics and treatment endpoints by HBV genotype. For studies investigating the interaction between HBV genotypes and type of therapy, *p*-value for interaction was extracted and reported in our database as well. Among the 15 reviewed papers, 11 reported data for the analyses on HBV genotypes, while the remaining four papers just stated in the text that at the multivariate analysis no association with HBV genotype was found for the studied endpoints.

For binary outcomes, when crude data were reported without any *p*-value for the statistical analysis, we constructed the frequency table and calculated the *p*-value for Chi-Square test. *P*-values <0.05 were considered statistically significant, unless specified. Chi-Square test was performed using SAS software, version 8.2 (SAS Institute, Inc., Cary, NC, USA).

Baseline patient characteristics and HBV genotypes

Several trials reported the main patient characteristics before starting antiviral therapy, according to HBV genotype (Table 1), including HBeAg-status, serum HBV-DNA levels, presence of advanced fibrosis or cirrhosis, resistance to lamivudine and ALT levels. Although one study did not provide *p*-values for the significance of differences in serum HBV-DNA and ALT levels among HBV genotypes [17], evidence from other reports suggested that all the investigated characteristics differed by HBV genotype. Patients with genotype D were more frequently HBeAg-negative compared to patients with other genotypes, while patients with genotype C seemed to be less frequently HBeAg-negative. By pooling frequencies from the four studies with available data [23–26], 134 out of 447 subjects with genotype A (30%) were HBeAg-negative, compared with 273/809 (34%) with genotype B, 355/1326 (27%) with genotype C and 648/917 (71%) with genotype D. Serum HBV-DNA levels seemed higher among patients with genotypes D and A, and lower in patients with genotypes C and B [17,27]. Prevalence of advanced fibrosis or cirrhosis was higher among carriers of genotypes A and C [26,28].

Response to therapies with NUC's and HBV genotypes

Results from studies which investigated the association between HBV genotypes and response to therapy with NUC's are presented in Table 2.

Among HBeAg-positive patients, the investigated endpoints were: HBeAg seroconversion, drug resistance, ALT normalization and PCR negativity. None of these endpoints differed significantly among HBV genotypes either with univariate or multivariate analysis (*p*-values >0.10). Just one study [29] presented the percentage of HBeAg seroconversion according to HBV genotype: it was slightly higher in patients with genotypes A and B compared to patients with genotypes C and D, although the association was not statistically significant.

Among HBeAg-negative patients, the investigated endpoints were: drug resistance, ALT normalization and PCR negativity. The GLOBE study [30] found a significantly lower risk of LdT-resistance in patients with genotype C compared to patients with genotypes non-C at the univariate analysis. This association, however, disappeared after adjustment for potential confounders in multivariate analysis. No other study reported any significant association in this group of patients.

Drug resistance, serum HBV-DNA level reductions, and PCR negativity have been further cumulatively investigated on HBeAg-positive and -negative patients: again, no significant association with HBV genotypes was highlighted.

Response to IFN-based therapies and HBV genotypes

Results from studies which investigated the association between HBV genotypes and response to IFN-based therapies are presented in Table 3.

Among HBeAg-positive patients, the investigated endpoints were: HBeAg seroconversion, HBeAg loss, serum HBV-DNA level reduction, PCR negativity, ALT normalization and composite endpoints. Differences in percentages of HBeAg seroconversion among HBV genotypes have been found in one Chinese multicenter study [31]: patients with genotype B had more often HBeAg seroconversion than subjects with genotype C. In another multicenter study [29], however, the percentage of subjects with HBeAg seroconversion was similar between genotypes B and C, and slightly higher for genotypes A than D, although the difference was not statistically significant. Percentage of subjects with HBeAg loss significantly differed among genotypes in the two studies with available data [17,32], with genotype A patients having higher probability of HBeAg loss than genotypes C and D patients, and genotype B patients having higher probability of HBeAg loss than genotype C patients in one Chinese study [31]. This last result was not confirmed in another multicenter study [32]. In the same Chinese study, patients with genotype B reported significantly higher serum HBV-DNA level reduction, higher rate of PCR negativity and ALT normalization than patients with genotype C. When considering the composite endpoints (HBeAg seroconversion + PCR negativity and HBeAg loss + PCR negativity + ALT normalization), patients with genotypes A and B have been found to respond better to therapy compared to genotypes C and D.

Table 1. Baseline characteristics of patients and HBV genotypes.

Study name (reference)		No. of patients	HBeAg pos./neg.	Baseline characteristic	Baseline characteristic by HBV genotype	Significance (p-value[o])
GLOBE (Lai, 2007)	[23]	1370	Both	Prevalence of HBeAg-negative patients	A: 26 (32%) B: 118 (33%) C: 175 (25%) D: 121 (56%) Other/missing: 6 (35%)	YES ($p < 0.0001$*)
– (Hou, 2008)[a]	[24]	332	Both	Prevalence of HBeAg-negative patients	B: 10 (8%) C: 31 (15%)	YES ($p < 0.004$*)
102 and 103 (Marcellin, 2008)	[25]	641	Both	Prevalence of HBeAg-negative patients	A: 42 (42%) B: 39 (53%) C: 41 (37%) D: 235 (73%) Other/missing: 18 (51%)	YES ($p < 0.0001$*)
AI463022 BEHoLD, AI463027 BEHoLD, AI463026 BEHoLD (Schiff, 2008)	[26]	1633	Both	Prevalence of HBeAg-negative patients	A: 66 (25%) B: 106 (42%) C: 108 (35%) D: 292 (77%) F: 3 (9%) Other/missing: 63 (55%)	YES ($p < 0.0001$*)
GS-98-437 (Westland, 2003)	[27]	511	Positive	Serum HBV-DNA levels ($\log_{10}$ copies/ml)	A: 8.44 B: 8.25 C: 7.83 D: 8.47 E: 7.11 F: 7.66 G: 9.49	YES ($p < 0.0001$)
HBV 99-01 (Flink, 2006)	[17]	266	Positive	Serum HBV-DNA levels ($\log_{10}$ copies/ml)	A: 9.1 B: 8.3 C: 8.3 D: 9.5	UNKNOWN
GS-98-438 (Westland, 2003)	[27]	184	Negative	Serum HBV-DNA levels ($\log_{10}$ copies/ml)	A: 6.44 B: 6.51 C: 6.52 D: 7.16 E: 7.22 F: 6.83	YES ($p = 0.0001$)
HBV 99-01 (Buster, 2007)	[28]	239	Positive	Prevalence of patients with advanced fibrosis	A: 38 (49%) B: 4 (19%) C: 10 (28%) D: 15 (16%)	YES ($p < 0.001$)
AI463022 BEHoLD, AI463027 BEHoLD, AI463026 BEHoLD (Schiff, 2008)	[26]	1633	Both	Prevalence of patients with advanced fibrosis or cirrhosis	A: 42 (16%) B: 24 (10%) C: 51 (17%) D: 56 (15%) F: 10 (29%) Other/missing: 18 (16%)	YES ($p = 0.02$*)
AI463022 BEHoLD, AI463027 BEHoLD, AI463026 BEHoLD (Schiff, 2008)	[26]	1633	Both	Prevalence of Lamivudine refractory patients	A: 69 (27%) B: 40 (16%) C: 55 (18%) D: 101 (27%) F: 7 (20%) Other/missing: 14 (12%)	YES ($p = 0.002$*)
HBV 99-01 (Flink, 2006)	[17]	266	Positive	Baseline ALT ($\times$ ULN)	A: 4.2 B: 4.2 C: 3.9 D: 4.6	UNKNOWN

[a] Study conducted in China.
[o] p-value for difference among genotypes.
* p-value was not presented in the original paper. We calculated it by reported data using the Chi-Square test.

Among HBeAg-negative patients, one study [33] investigated virological response and the composite endpoint virological response + ALT normalization. The authors found a significant difference among HBV genotypes for both the investigated endpoints: patients with genotypes B and C had a better response to therapy compared to patients with genotype D; patients with genotypes A and D had a similar response to therapy. All in all, preliminary conclusions can be drawn that while genotype B appears to perform better than C in Chinese populations, the data is insufficient in non-Chinese populations. More individual data linking genotype to ethnic background will need to become available.

Interaction between HBV genotype and antiviral therapy

Four studies tried to assess whether there was an interaction between HBV genotypes and type of treatment in determining

Table 2. Response to therapies with nucleos(t)ide analogues and HBV genotypes.

Study name (reference)		Nucleos(t)ide/ control group (no. of patients)	Weeks of treatment	Treatment endpoint	Endpoint definition	Treatment endpoint by HBV genotype	Significance (p-value[o])
HBeAg-positive patients							
GS-98-437 (Westland, 2003)	[27]	Adefovir/none (511)	48	HBeAg seroconversion	Loss of serum HBeAg and appearance of anti-HBe	From 7% to 20% among genotypes A–D	NO ($p = 0.25$)
Peginterferon Alfa-2a HBeAg-Positive Chronic Hepatitis B Study Group (Lau, 2005)	[29]	Lamivudine/ none (272)	48	HBeAg seroconversion	Loss of serum HBeAg and appearance of anti-HBe antibody	A: 3 (20%) B: 17 (23%) C: 29 (18%) D: 3 (18%)	NO ($p = 0.81$*)
GLOBE (Liaw, 2009)	[34]	Telbivudine/ Lamivudine (1367)	104	HBeAg seroconversion		Not reported	NO (multivariate $p > 0.10$)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (458)	104	HBeAg seroconversion		Not reported	NO (multivariate $p > 0.10$)
AI463026 BEHoLD (Sherman, 2008)	[35]	Entecavir/ Lamivudine (286)	96	Drug resistance	Virologic breakthrough defined as increased in HBV DNA of $\geqslant 1$ $\log_{10}$ in copies/ml from the on-treatment nadir, as determined by at least two sequential measurements or the last on-treatment measurements	Not reported	NO (multivariate p not significant)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (458)	104	Drug resistance	Emergence of treatment-associated resistance mutations		NO (multivariate $p > 0.10$)
GLOBE (Liaw, 2009)	[34]	Telbivudine/ Lamivudine (1367)	104	ALT normalization		Not reported	NO (multivariate $p > 0.10$)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (458)	104	ALT normalization		Not reported	NO (multivariate $p > 0.10$)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (458)	104	PCR negativity	Proportion of patients with undetectable HBV DNA by PCR assay (<300 copies/ml)	Not reported	NO (multivariate $p > 0.10$)
HBeAg-negative patients							
GS-98-438 (Hadziyannis, 2006)	[36]	Adefovir/ placebo (184)	240	Drug resistance	Presence of adefovir-resistance mutations (N236T or A181V)	Not reported	NO (multivariate $p > 0.10$)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (222)	104	Drug resistance	Emergence of treatment-associated resistance mutations	Not reported	YES OR = 0.17, $p = 0.0099$ for C vs non-C (univariate analysis) NO at multivariate analysis
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (222)	104	ALT normalization		Not reported	NO (multivariate $p > 0.10$)
GLOBE (Zeuzem, 2009)	[30]	Telbivudine/ none (222)	104	PCR negativity	Proportion of patients with undetectable HBV DNA by PCR assay (<300 copies/ml)	Not reported	NO (multivariate $p > 0.10$)
Both HBeAg-positive and -negative patients							
(Hou, 2008)[a]	[24]	Telbivudine/ Lamivudine (332)	104	Drug resistance	Viral breakthrough with identified treatment-emergent resistance mutations	B: 15 (12%) C: 17 (8%)	NO (multivariate p not significant)
GLOBE (Liaw, 2009)	[34]	Telbivudine/ Lamivudine (1367)	104	Drug resistance	Viral breakthrough with the emergence of treatment-associated resistance mutations	Not reported	NO (multivariate $p > 0.10$)
GS-98-437 GS-98-438 (Westland, 2003)	[27]	Adefovir/ none (695)	48	Serum HBV-DNA levels reduction ($\log_{10}$ copies/ml)	Change between baseline levels of serum HBV-DNA and HBV-DNA levels after 48 weeks of therapy	A: −3.58 B: −3.42 C: −3.65 D: −3.68 E: −3.60 F: −4.23 G: −3.67	NO ($p = 0.90$)
GLOBE (Liaw, 2009)	[34]	Telbivudine/ Lamivudine (1367)	104	PCR negativity	Proportion of patients with undetectable HBV DNA by PCR assay (<300 copies/ml)	Not reported	NO (multivariate $p > 0.10$)

OR, odds ratio.
*p-value was not presented in the original paper. We calculated it by reported data using the Chi-Square test.
[a] Study conducted in China.
[o] p-value for difference among genotypes.

Table 3. Response to IFN-based therapies and HBV genotypes.

Study name (reference)		IFN/control group (no. of patients)	Months of treatment	Treatment endpoint	Endpoint definition	Treatment endpoint by HBV genotype	Significance (p-value[b])
HBeAg-positive patients							
Peginterferon Alfa-2a HbeAg-Positive Chronic Hepatitis B Study Group (Lau, 2005)	[29]	Pegylated IFN-α-2a/ Lamivudine (814)	48 weeks	HBeAg seroconversion	Loss of HBeAg and appearance of anti-HBe antibody	A: 19 (34%) B: 64 (28%) C: 122 (25%) D: 7 (19%)	NO ($p = 0.37^{*}$)
Peginterferon Alfa-2a HbeAg-Positive Chronic Hepatitis B Study Group (Lau, 2005)	[29]	Pegylated IFN-α-2a/ none (271)	48 weeks	HBeAg seroconversion	Loss of HBeAg and appearance of anti-HBe antibody	A: 12 (52%) B: 23 (30%) C: 50 (31%) D: 2 (22%)	NO ($p = 0.18^{*}$)
Peginterferon Alfa-2a HbeAg-Positive Chronic Hepatitis B Study Group (Lau, 2005)	[29]	Pegylated IFN-α-2a/ +Lamivudine/none (271)	48 weeks	HBeAg seroconversion	Loss of HBeAg and appearance of anti-HBe antibody	A: 4 (22%) B: 24 (29%) C: 43 (28%) D: 2 (18%)	NO ($p = 0.84^{*}$)
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ IFN-a-2b (230)	24 weeks	HBeAg seroconversion		B: 33.3% C: 12.9%	YES ($p = 0.0012$)
HBV 99-01 (Janssen, 2005)	[32]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b + Lamivudine (266)	52 weeks	HBeAg loss	Loss of serum HBeAg, as tested by EIA	A: 42 (47%) B: 10 (44%) C: 11 (28%) D: 26 (25%)	YES OR (95% CI) = 2.4 (1.3–4.6) for A vs D 3.6 (1.4–8.9) for A vs C 2.2 (0.7–7.0) for B vs C
HBV 99-01 (Flink, 2006)	[17]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b + Lamivudine (266)	52 weeks	HBsAg loss	Loss of serum HBsAg at the end of follow-up	A: 13 (14%) B: 2 (9%) C: 1 (3%) D: 2 (2%)	YES (p for difference between genotypes A and D = 0.006)
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ IFN-α-2b (230)	24 weeks	HBeAg loss		B: 22 (36.7%) C: 22 (12.9%)	YES ($p = 0.0004$)
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ IFN-α-2b (230)	24 weeks	Serum HBV-DNA level reduction (log_{10} copies/ml)	Change between baseline levels of serum HBV-DNA and HBV-DNA levels after 48 weeks (24 therapy + 24 follow-up)	B: −2.23 C: −0.88	YES ($p < 0.0001$)
HBV 99-01 (Buster, 2007)	[28]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b + Lamivudine (239)	26 weeks	HBeAg seroconversion + serum HBV DNA < 10,000 copies/ml	Loss of HBeAg and appearance of anti-HBe + serum HBV DNA level < 10, 000 copies/ml	A: 23 (30%) B: 5 (26%) C: 1 (3%) D: 6 (6%)	YES RR (95% CI) = 11.3 (1.4–92.6) for A vs C 4.3 (1.4–13.2) for A vs D 12.1 (1.2–118.3) for B vs C 4.6 (1.1–18.4) for B vs D
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b (230)	24 weeks	HBeAg loss + serum HBV-DNA < 100,000 copies/ml + normal ALT levels		B: 16 (31.7%) C: 13 (7.7%)	YES OR (95% CI) = 0.19 (0.08–0.46) for C vs B
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b (230)	24 weeks	PCR negativity	Serum HBV DNA levels < 100,000 copies/ml	B: 28 (46.7%) C: 28 (16.5%)	YES ($p < 0.0001$)
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b (230)	24 weeks	PCR negativity	Serum HBV DNA levels < 1,000 copies/ml	B: 17 (28.3%) C: 11 (6.5%)	YES ($p < 0.0001$)
– (Zhao, 2007)[a]	[31]	Pegylated IFN-α-2b/ Pegylated IFN-α-2b (230)	24 weeks	ALT normalization		B: 34 (56.7%) C: 45 (26.5%)	YES ($p = 0.0002$)
HBeAg-negative patients							
Peginterferon Alfa-2a HbeAg-Negative Chronic Hepatitis B Study Group (Bonino, 2007)	[33]	Pegylated IFN-α-2a/ Pegylated IFN-α-2a + Lamivudine (294)	48 weeks	Virological response	HBV-DNA level <20,000 copies/ml at the end of treatment and at 24 weeks post-treatment	A: 6 (35%) B: 33 (45%) C: 70 (62%) D: 29 (33%)	YES (multivariate $p = 0.006$) OR (95% CI) = 3.3 (1.7–6.5) for C vs D
Peginterferon Alfa - 2a HbeAg-Negative Chronic Hepatitis B Study Group (Bonino, 2007)	[33]	Pegylated IFN-α-2a/ Pegylated IFN-α-2a + Lamivudine/ Lamivudine (518)	48 weeks	ALT normalization +HBV DNA level of <20,000 copies/ml		Not reported	YES (multivariate $p < 0.001$) OR (95% CI) = 0.42 (0.1–1.2) for A vs B 0.33 (0.1–0.9) for A vs C 0.97 (0.3–2.7) for A vs D 0.79 (0.5–1. 3) for B vs C 2.31 (1.3–4. 2) for B vs D 2.9 (1.7–5.0) for C vs D

Table 3 (*continued*)

Study name (reference)	IFN/control group (no. of patients)	Months of treatment	Treatment endpoint	Endpoint definition	Treatment endpoint by HBV genotype	Significance (p-value[b])
Peginterferon Alfa-2a HbeAg-Negative Chronic Hepatitis B Study Group (Bonino, 2007) [33]	Pegylated IFN-α-2a/ Lamivudine (346)	48 weeks	ALT normalization +HBV DNA level of <20,000 copies/ml		B: 38 (41%) C: 46 (38%) D: 16 (14%)	YES (multivariate $p < 0.001$) OR (95% CI) = 5.9 (2.7–13.1) for B vs D 4.6 (2.2–9.5) for C vs D
Peginterferon Alfa-2a HbeAg-Negative Chronic Hepatitis B Study Group (Bonino, 2007) [33]	Pegylated IFN-α-2a/ Pegylated IFN-α-2a + Lamivudine (304)	48 weeks	ALT normalization + HBV DNA level of <20,000 copies/ml	One year after treatment	Not reported	YES OR (95% CI) = 2.58 (0.73–9.20) for A vs D 3.69 (1.54–8.79) for B vs D 5.46 (2.46–12.1) for C vs D

CI, confidence intervals; OR, odds ratio; RR, relative risk.
[a] Study conducted in China.
[b] p-value for difference among genotypes.
[*] p-value was not presented in the original paper. We calculated it by reported data using the Chi-Square test.

the response to therapy. Studied endpoints included histological improvement, PCR negativity, HBeAg seroconversion and composite endpoints.

Two [25,34] out of the four studies [25,31,33,34] focused on response to therapy with NUC's. In the first one [25], histological improvement was more common in patients treated with TDF than in patients treated with ADV for all genotypes but B; for patients with this latter genotype the difference in histological improvement between the two treatments was −4.4% (95% CI: −26.3%; 17.5%); among the other genotypes, the highest difference between the two treatments was observed for genotype C [8.1% (−9.1%; 25.3%)]. Similarly, in the same study the lower difference between the two treatments (in favour of TDF) for PCR negativity and for the combined endpoint PCR negativity + histological improvement was observed for patients with genotype B, while the highest difference between treatments was observed for patients with genotype A. However, the p-value for interaction between the HBV genotype and treatment was not statistically significant. The GLOBE study [34] compared LdT with LAM and found that the HBeAg seroconversion rate was significantly higher in patients treated with LdT compared to patients treated with LAM only for carriers of HBV genotype C ($p = 0.03$), while no difference between the two treatments was found for the other genotypes.

Two studies on IFN-based therapies [31,33] evaluated difference in treatments according to HBV genotypes. The first one [33], which included HBeAg-negative patients, found a significant genotype-therapy interaction ($p = 0.03$): ALT normalization + PCR negativity occurred more frequently after treatment with PEG-IFN-α-2a plus LAM than after treatment with LAM alone in patients with HBV genotype D (OR; 95% CI: 3.5; 1.3–9.1). The association was opposite in patients with genotype B (OR; 95% CI: 0.4; 0.1–1.2). In a further study comparing PEG vs standard IFN [31], patients treated with standard IFN had a significantly higher response rate (HBeAg seroconversion + PCR negativity + ALT normalization) than patients treated with PEG-IFN just among patients with HBV genotype C ($p = 0.02$).

Conclusive remarks

The present review discloses several intrinsic features and weaknesses of the clinical trials conducted so far which make it difficult to reach firm conclusions on the role of HBV genotypes in response to antiviral therapy. First, HBV genotype distribution varied significantly among different populations. Indeed, almost all trials were multicenter and pooled together patients of different ethnicities. If for any reason differences in response to therapy depend on ethnicity, then a false-positive association with the HBV genotype may be discovered since overlap between ethnicity and genotype is very high. Adjusting by ethnicity in multivariate analysis may lead to co-linearity. Stratified analysis by ethnic group may be a solution, but it would significantly lower the statistical power. Large studies within the same geographical populations are needed to clarify this point. Second, most studies did not provide information on response to therapy by HBV genotype, especially when no difference in treatment endpoints by genotype was present. This precludes obtaining a pooled estimate of the association between each endpoint and HBV genotype: it is indeed possible that each study found no significant association, but this might be the result of their low statistical power, and combining them in a meta-analysis of individual data may lead to a significant result. Third, endpoint definitions, especially for the composite ones, varied substantially among studies, leading to lack of homogeneity. A consensus in endpoint definition would be highly desirable, at least within large multicenter clinical trials. Fourth, possible interactions between the type of therapy and the HBV genotype have seldom been analysed. Among the four studies which gave a result, three reported a somewhat different response to different therapies according to the HBV genotype. It is therefore possible that specific treatments may be more effective on some HBV genotypes and not on others. This should be further investigated in future studies, possibly within ongoing large multicenter clinical trials. This last point is extremely important as most clinical trials are still running at the time of this review, even though the follow-up for some of them is limited to subgroups of patients and therefore conclusions may presently be premature.

Nevertheless, current evidence does suggest that potentially better responders to PEG-IFN α treatment can be identified by HBV genotyping; although patients with "unfavourable" genotypes should not be denied treatment with IFN, as they may have a benefit in the long term by achieving a durable anti-HBs seroconversion [37]. Besides this consideration, the present analysis highlights several caveats regarding current indications put forward by the major clinical practice guidelines and consensus development conference

statements published thus far, and emphasise the need for further long term investigations into this matter.

Key messages

- There are at least eight HBV genotypes which are geographically distributed.
- Genotype A and B may be associated with a more benign clinical course than D and C but these data may be biased by ethnicity.
- Genotype A and B may respond to IFN-based therapies better than C and D, while NUC therapy does not appear to be influenced by genotype.
- The above statement is limited by several caveats:
  - Ethnicity and genotype may overlap.
  - Clinical trials were not designed to analyse response by genotype.
  - Variability of endpoint definitions.
  - Interaction between type of therapy and genotype.

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