Hepatitis B virus (HBV) infection is a global health issue. Effective and individualized treatment of chronic hepatitis B to prevent progression to end-stage liver diseases and hepatocellular carcinoma is needed. HBV can be classified into eight genotypes (A–H) on the basis of genome sequence divergence. In addition, several naturally occurring HBV mutants have also been identified. The epidemiology of HBV genotypes and their implications for response to antiviral therapy have become increasingly recognized. Recent studies suggested that responses to standard interferon treatment in patients with genotype A or B are better than those with genotype C or D; however, conflicting results exist regarding the response to pegylated interferon. The influence of dose and duration on interferon-based therapy remains to be clarified. In addition to genotype, naturally occurring mutations such as precore and core promoter mutations have also attracted much attention, because they have been shown to affect the disease progression of HBV-related chronic liver disease and possibly the response to antiviral therapy. Here, we review the differences in antiviral therapeutic response among HBV genotypes and discuss the role of precore or core promoter mutations in response to antiviral therapy.

Hepatitis B virus (HBV) infection causes a wide spectrum of liver diseases [1,2]. Worldwide, the number of individuals infected with this virus has been estimated to be as high as 400 million [3,4]. Although safe and effective vaccines are available, HBV infection remains a global health threat. Thus, understanding the natural history of HBV infection and the individualization of chronic hepatitis B (CHB) therapy to prevent disease progression to end-stage liver disease and hepatocellular carcinoma (HCC) is of paramount importance. The natural and therapeutic outcomes of patients with CHB infection are determined by the interplay among viral, host and environmental factors. Recently, several HBV factors predictive of clinical outcomes have been identified, such as persistently high viral load, HBV genotype C, the core promoter (CP) A1762T/G1764A mutation and the deletion of the pre-S region of the HBV surface antigen gene [5–56]. HBV genotypes have attracted much attention as they could affect the disease progression of HBV-related chronic liver disease as well as the response to antiviral therapies [5–43,48,54,57–68]. Thus, an understanding of HBV genotype and its association with the response to antiviral therapy is clinically useful. Eight genotypes of HBV have been identified based on a sequence divergence >8% in the entire HBV genome and designated from A to H in the order of documentation [69–72]. An explorative analysis of published data has already drawn attention to the role of HBV genotype in determining the responses to treatment [62]. However, existing information is still insufficient. This review presents current lines of evidence regarding the various facets of HBV genotypes based on an analysis of significant articles published up to December 2007. Beyond HBV genotype, data regarding the association of naturally occurring HBV mutants such as precore stop codon and CP mutations with clinical outcomes are accumulating. Nevertheless, their correlation with antiviral responses deserves further examination [63–68,73–90]. In this article, we therefore...
update our previous systematic examination on HBV genotypes and discuss the role of precore or CP mutations in response to antiviral treatments [43,44].

Responses to antiviral therapy

Six drugs have been approved for the treatment of CHB: standard interferon (IFN)-α, lamivudine, adefovir dipivoxil, pegylated IFN-α2a (PEG-IFN-α2a), entecavir and telbivudine [91–102]. The influence of HBV genotype on current antiviral treatments is partly clarified on the basis of several parameters: normalization of serum alanine aminotransferase (ALT) level, reduction of serum HBV DNA, loss of hepatitis B antigen (HBsAg), HBeAg seroconversion, loss of hepatitis B surface antigen (HBsAg) and HBsAg seroconversion. Owing to the unique distribution of HBV genotypes in Asian and western countries, the therapeutic implications of the HBV genotype could only be compared between genotype B and C or genotype A and D. Fortunately, in recent large clinical trials, the influence of major HBV genotypes (A–D) in response to antiviral therapy has been examined.

Influence on therapy with interferon

Standard interferon

We first demonstrated that the response rate (normalization of ALT level, loss of HBeAg and undetectable serum HBV DNA level by using the hybridization assay 48 weeks post-treatment) to a 24-week IFN-α2b therapy (subcutaneously 5 million units [MU] three times weekly) was related to HBV genotypes, being 41% and 15% in Taiwanese genotype B and C patients, respectively (P=0.045) [9]. Young age and genotype B were predictive of a better response to IFN-α2a. A recent larger clinical data set revealed consistent findings [48]. A total of 119 patients received 5 MU of IFN-α2b daily for 4 weeks followed by 5 MU three times weekly for 28 weeks. The patients were followed up for 24 weeks post-treatment. By intention-to-treat analysis, the rate of HBeAg seroconversion was 30% versus 18% between genotype B and C at the end of treatment (P=0.14) and 44% versus 22% at the end of follow-up (P=0.02). Wai et al. [10] also compared the rate of HBeAg loss after IFN therapy (IFN-α2b for 16 weeks at a dose of 10 MU three times weekly) between genotype B and C in Chinese patients. They similarly found the response was better in patients with genotype B than genotype C (12/31 [39%] versus 7/42 [17%]; P=0.034).

Recently, human leukocyte antigen-DRB1 (HLA-DRB1) alleles and HBV genotype were determined in 126 patients with CHB who received IFN-α2a treatment subcutaneously at a dose of 3 MU three times weekly for 24 weeks [63]. They found that HLA-DRB1*07 allele and HBV genotype C were significantly associated with a lower rate of HBeAg seroconversion, normalization of serum ALT levels and reduction of serum HBV DNA (P<0.05). These data suggested that HBV genotype C, compared with genotype B, is associated with a lower response rate to IFN-α2a therapy.

A similar phenomenon was noted between HBV genotype A and D. Hou et al. [22] studied the role of HBV genotypes in 103 HBeAg-positive patients with CHB who received IFN treatment in Europe (46 infected with genotype A and 35 with genotype D). Response to IFN-α2a was more likely to occur in genotype A than genotype D (33% versus 11%; P=0.03). Recently, Erhardt et al. [58] reported that in 144 patients with HBV genotype A or D infection, the genotype was an important and independent predictor of IFN responsiveness. Sustained response (6 months after treatment) to standard IFN therapy (4.5–6 MU IFN-α2a three times weekly for at least 4 months) was higher in HBV genotype A compared with genotype D (49% versus 26%; P<0.005). Sustained response to IFN was 46% versus 24% (P=0.03) in HBeAg-positive hepatitis (n=99) and 59% versus 29% (P=0.05) in HBeAg-negative hepatitis (n=45) for HBV genotype A compared with genotype D. Multivariate logistic regression analysis identified HBV genotype A and high pretreatment ALT levels (>2× upper limit of normal) as independent positive predictive parameters of IFN response. Notably, an earlier European study on HBeAg-negative patients consistently showed that HBsAg clearance was found in two of 10 cases infected with genotype A, whereas all individuals who suffered a relapse after IFN therapy were infected with non-A genotypes [65]. Therefore, it is suggested that HBV genotype-dependent treatment regimen may further improve therapeutic efficacy in patients with CHB.

Pegylated interferon-α

Cooksley et al. [102] first showed that the response to 24 weeks PEG-IFN-α2a (90, 180 or 270 µg weekly) or standard IFN-α2a (4.5 MU three times weekly) therapy was higher in genotype B versus C (33% versus 21% and 25% versus 6%, respectively) in a Phase II clinical trial. In a subsequent Phase III multicentre study, the overall response rate for PEG-IFN-α2b (100 µg/week during weeks 1–31 and 50 µg/week during weeks 32–52) also differed according to HBV genotype (genotype A [47%], B [44%], C [28%] and D [25%]) [101]. Furthermore, the overall rate of HBsAg loss was 7%. Subgroup analysis revealed that loss of HBsAg also differed according to HBV genotype (genotype A [14%], B [9%], C [3%] and D [2%]) [54]. Supporting data came from another large Phase III clinical trial. Cooksley et al. [57] demonstrated that there was no statistically significant difference in the treatment response (HBeAg seroconversion at 24 weeks post-treatment) to a 48-week PEG-IFN-α2a (180 µg once weekly) therapy among four HBV genotypes (genotype A [52%], B [30%], C [31%] and D [22%]). However, a higher rate of treatment response in genotype A compared with the
other three genotypes was documented in a recent study with respect to HBsAg seroconversion in both HBeAg-positive (genotype A [22%], B [0%], C [2%] and D [0%]) and HBeAg-negative chronic hepatitis B patients (genotype A [18%], B [2%], C [3%] and D [0%]) [59]. In logistic regression analyses of the HBeAg-negative treatment cohort, baseline ALT and HBV DNA levels, age, gender and HBV genotype significantly influenced combined ALT normalization and HBV DNA level of <20,000 copies/ml at 24 weeks post-treatment with PEG-IFN-α2a and/or lamivudine therapy [103]. In a randomized, open-label control study, HBeAg-positive patients were assigned to receive PEG-IFN-α2b (1.0 µg/kg; n=115) or IFN-α2b (3 MU three times per week; n=115) for a 24-week period [64]. Multivariate analysis revealed that HBV genotype B and young age (<25 years) were two independent factors associated with sustained combined serum HBV DNA level <10^5 copies/ml, HBeAg loss and normal ALT levels at the end of follow-up in both treatment arms.

Taking these lines of evidence together, although the data are straightforward for genotypes A and D, they are more heterogeneous in patients with genotypes B and C. The association between HBV genotypes with the response to PEG-IFN-based therapy awaits further clinical trials. The relevant data and level of evidence in patients with HBeAg-positive and -negative CHB are summarized in Tables 1 and 2, respectively. The level of evidence is based on the quality as well as the content of the cited paper (Table 3).

Influence on therapy with nucleos(t)ide analogues

Lamivudine

Ample data are now available on whether HBV genotype affects the outcome of lamivudine therapy, the development of the lamivudine-resistant tyrosine-methionine-aspartate-aspartate (YMDD) mutation, and the occurrence of breakthrough hepatitis accompanying the emergence of drug-resistant YMDD mutants [11–15,18,28,34,35,40,41,60]. Overall, in HBeAg-positive patients, the available data do not show a higher frequency of drug resistance in genotype A compared with genotype D nor toward genotype B compared with genotype C. In HBeAg-negative patients, treatment response also does not differ between genotype A versus D or genotype B versus C.

### Treatment outcomes

Previous data suggested that genotype B had a slightly better virological response to lamivudine than genotype

| Table 1. Responses to interferon-based therapy for HBeAg-positive chronic hepatitis B: focus on treatment duration and regimen |
|---------------------------------|-------|-----------------|-------|-------|-----------------|-----------------|
|                                | B     | C Level of evidence* | A     | D     | Level of evidence* | Reference       |
| Standard interferon            |       |                  |       |       |                  |                 |
| Suppression of HBV DNA         | Better | Worse           | II-1  | Better | Worse           | [9,10,48,58,63,68] |
| HBeAg loss or seroconversion   | Better | Worse           | II-1  | Better | Worse           |                 |
| HBsAg loss or seroconversion   | Better | Worse           | II-1  | Better | Worse           |                 |
| Pegylated interferon           |       |                  |       |       |                  | [54,57,59,64]   |
| Suppression of HBV DNA         | Controversial | II-1 | Better | Worse | II-1             |                 |
| HBeAg loss or seroconversion   | Better | Worse           | II-1  | Better | Worse           |                 |
| HBsAg loss or seroconversion   | Better | Worse           | II-1  | Better | Worse           |                 |

*The level of evidence is based on the quality and content of the cited paper (Table 3 [78]). It should be noted that the definition of each treatment parameter to standard interferon could be different from that to pegylated interferon (for details, please refer to reference [62]). HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus.

| Table 2. Responses to interferon-based therapy for HBeAg-negative chronic hepatitis B |
|---------------------------------|-------|-----------------|-------|-------|-----------------|-----------------|
|                                | B or C | Level of evidence* | A     | D     | Level of evidence* | Reference       |
| Standard interferon            |       |                  |       |       |                  |                 |
| Suppression of HBV DNA         | NA     | II-2             | Better | Worse | II-2             | [58,65]         |
| HBsAg loss or seroconversion   | Comparable | II-2 | Better | Worse | II-2             |                 |
| Pegylated interferon           |       |                  |       |       |                  | [103]           |
| Suppression of HBV DNA         | Controversial | II-2 | Better | Worse | II-2             |                 |
| HBsAg loss or seroconversion   | Comparable | II-2 | Better | Worse | II-2             |                 |

*The level of evidence is based on the quality and content of the cited paper (Table 3 [78]). It should be noted that the definition of each treatment parameter to standard interferon may be different from that to pegylated interferon (for details, please refer to reference [62]). HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NA, not applicable.
long-term lamivudine therapy [18]. Breakthroughs were similar in patients with genotypes B and C in 154 HBeAg-positive patients receiving lamivudine treatment. In a Japanese study, severe breakthrough hepatitis with lamivudine resistance was reported. These findings were in line with our data and data reported by others [11,13,18,41,60].

**Table 3. Quality (level) of evidence**

<table>
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<tr>
<th>Grade</th>
<th>Definition</th>
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<tbody>
<tr>
<td>I</td>
<td>Evidence from multiple well-designed randomized controlled trials, each involving a number of participants to be of sufficient statistical power [78]</td>
</tr>
<tr>
<td>II</td>
<td>Evidence from at least one large well-designed clinical trial with or without randomization (II-1), from cohort or case-control analytic studies (II-2) or well-designed meta-analysis (II-3) [78]</td>
</tr>
<tr>
<td>III</td>
<td>Evidence based on clinical experience, descriptive studies or reports of expert committees [78]</td>
</tr>
<tr>
<td>IV</td>
<td>Not rated [78]</td>
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</table>

C (23% versus 11%, P not significant) [11]. Two studies from Hong Kong indicated that HBV genotype had no effect on the response to lamivudine therapy [18,34]. In Spain, Buti et al. [40] suggested that the outcome after lamivudine treatment was comparable between genotype A and D. These data imply that HBV genotype might have no substantial effect on the response to lamivudine treatment. However, Chien et al. [12] reported that the sustained response rate to lamivudine was much higher in patients with genotype B than those with genotype C (38/62 [61%] versus 5/20 [20%], P=0.009). Age ≤ 36 years and an additional lamivudine treatment over 8 months correlated with a higher sustained response rate.

**Drug resistance**

Zöllner et al. [14,15,35] initially reported the HBV serotype adw (exclusively genotype A in Europe) to be associated with a 20-fold greater risk of lamivudine resistance than ayw (mainly genotype D in Europe). However, the authors later found that the risk of emergence of the YMDD mutation was only slightly higher in genotype A patients than in genotype D and the difference was noted only during the first year of lamivudine treatment [40]. If therapy was extended to ≥ 2 years, the proportion of YMDD mutation was not different between genotype A and D [40], indicating that lamivudine resistance takes longer to emerge in genotype D patients [35,40]. Accordingly, HBV serotype does not correlate with the risk of lamivudine resistance. These findings were in line with our data and data reported by others [11,13,18,41,60].

**Breakthrough hepatitis with lamivudine resistance**

In a Japanese study, severe breakthrough hepatitis accompanying the emergence of YMDD mutants only occurred in four (2%) of the 185 patients with genotype C [13]. In a report from Hong Kong, the chances of YMDD mutations with virological and biochemical breakthroughs were similar in patients with genotypes B and C in 154 HBeAg-positive patients receiving long-term lamivudine therapy [18].

**Adefovir dipivoxil**

One study has addressed the influence of genotype on the response to adefovir dipivoxil therapy [16]. Westland et al. [16] analysed the frequency and distribution of genotypes in patients from two multinational Phase III studies of adefovir dipivoxil. They found that the reductions in serum HBV DNA level did not correlate with HBV genotype; similarly, there was no statistical difference in HBeAg seroconversion rates among patients with different genotypes.

**Entecavir**

One recent study evaluated the association between HBV genotype and the response to entecavir therapy [61]. Again, the reduction of serum HBV DNA level and histological improvement did not differ among HBV genotypes in both HBeAg-positive and -negative patients.

**Telbivudine**

Although the results of a study of the efficacy of telbivudine versus lamivudine have just been published [104,105], data regarding its correlation with HBV genotypes were not available. Nevertheless, there was no apparent association of HBV genotype with resistance mutation pattern [105].

**Influence on therapy with thymosin α1**

Chien et al. [66] recently demonstrated that in 98 patients with CHB randomly allocated to three groups (26-week or 52-week course of thymosin α1 1.6 mg two times a week and untreated controls), genotype B, precore mutation and thymosin α1 treatment were independent factors associated with combined ALT normalization plus seroclearance of HBeAg and HBV DNA by stepwise logistic regression analysis.

**Influence on combination therapy with interferon-α and lamivudine**

For patients with HBeAg-positive CHB, Erhardt et al. [58] demonstrated genotype A to be an independent parameter for sustained response after IFN monotherapy. Nevertheless, after combination therapy with lamivudine, the favourable results became less clear as the sustained rate of HBeAg seroconversion was 22% and 18% for genotype A and D, respectively [58]. In HBeAg-negative patients, three studies revealed higher antiviral response rates for genotype A versus D [58,65,103]. However, the combination therapy of PEG-IFN-α2a plus lamivudine showed contradictory results [103]. Therefore, the influence of hepatitis B genotypes on IFN-based combination therapies is not yet clear [62]. The combination of lamivudine with IFN could even be disadvantageous for genotype A patients.

In brief summary, HBV genotype correlates well with the response to standard IFN but not to
HBV genetic variability and antiviral therapy

nucleoside/nucleotide-based therapy (Tables 1 and 2). The association between HBV genotype and response to PEG-IFN remains controversial. One crucial issue may be the dose and duration of IFN-based therapy. To address this issue, future genotype-based stratification trials should be considered with emphasis on the influence of dose and duration. Finally, further studies should also focus on the contribution of viral factors other than genotype to the treatment outcome of antiviral agents [23,41,60].

Clinical correlates of precore and core promoter mutations

In addition to genotype, naturally occurring mutations such as precore and CP mutations also attract much attention as each of them has been shown to affect the disease progression of HBV-related chronic liver disease and possibly the response to antiviral therapy [65–68,77–90]. For example, several mutations in the X gene of the HBV genome are frequently found in patients with advanced liver disease, suggesting that these mutants may play a certain role in the pathogenesis of HBV infection. Among these mutations, the double nucleotide mutation A1762T/G1764A in the CP region affecting codons 130 and 131 of the hepatitis B X gene (xK130M and xV131I) is of particular interest [56]. Many studies have demonstrated that this mutation is associated with decreased HBeAg expression, enhanced viral replication and more severe liver damage [45]. Apart from this CP A1762T/G1764A mutation, mutations in other parts of the CP region including C1653T and T1753A/C/G have become increasingly associated with the outcome of chronic HBV infection (Figure 1) [56]. The role of the G1896A mutation in the progression of liver disease is still not clarified. Most of the previous studies examined the association between precore or CP mutations and the development of advanced liver disease, however, the influence of these mutations on the response to antiviral therapy remains ill-defined.

The implications of pretreatment precore and CP mutations for the response to IFN as well as lamivudine therapy are summarized in Tables 4 and 5, respectively. Overall, approximately half of the studies showed that precore or CP mutations correlated with the response to IFN or lamivudine therapy. Representative studies are shown as discussed below.

Influence on therapy with interferon

Lok et al. [80] examined whether the presence of precore mutants could affect IFN-induced HBeAg clearance in 106 HBeAg-positive Chinese patients. Twenty patients (10%) had the precore G1896A mutant. During a follow-up period of 1–7 years, 75% of those with precore mutant and 26% of those with wild-type HBV, respectively, cleared HBeAg (P<0.0001). Sustained antiviral response was achieved in 55% and 17% of patients who had precore mutant and wild-type HBV, respectively (P=0.04). Their findings suggested that HBeAg-positive Chinese patients with the precore G1896A mutant were more likely to clear HBeAg.

The CP region was analysed from a large series of 96 patients with CHB (64 HBeAg-positive patients and 32 HBeAg-negative patients) treated with IFN-α [76]. IFN response (suppression of HBV DNA and ALT normalization) correlated well with serum HBV DNA levels, HBsAg levels and the number of mutations in the entire basal CP especially the nucleotide (nt) region between 1753–1766 and mutations at nt 1762 and 1764. The prediction of IFN response was possible on the basis of nt 1764 in 77% of the HBeAg-positive patients and 78% of the HBeAg-negative patients. Their data suggested that HBV mutations located within the CP are determinants of a response to IFN therapy.

We have previously compared the full-length HBV genomes in 18 genotype B patients (10 responders and eight non-responders) who had received 24-week IFN 5 MU three times weekly and were followed monthly for 12 months post-treatment [79]. Among the CP region, we found that CP A1762T/G1764A mutations were not common in the HBeAg-positive population (two of the 10 responders and one of the eight non-responders) and did not correlate with treatment outcome (reduction of combined serum HBV DNA, ALT normalization and HBeAg seroconversion). The CP T1753C mutation was

![Figure 1. Commonly encountered mutations of hepatitis B virus basal core promoter region](image)
uncommon and was noted in only two responders and one non-responder.

The relationship between HBV genetic characteristics and the outcome of short (16 weeks) or prolonged (32 weeks) treatment with standard IFN-α was examined in a prospective cohort of 103 European patients with HBeAg-positive CHB [68]. Logistic regression analysis identified genotype A as a positive predictor of short (16 weeks) treatment response ($P=0.001$), having a greater effect than baseline HBV DNA or ALT levels.

In contrast, the response to prolonged IFN-α treatment was similar between HBV genotypes. Baseline CP A1762T/G1764A mutation was found more commonly in responders than non-responders (31% versus 15%, $P=0.049$).

Wai et al. [10] examined the predictive role of the CP mutation as well as genotype on the response to IFN therapy. However, the presence of pretreatment CP mutations did not correlate with the clearance of HBeAg. The pretreatment CP region of HBV from 26

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<tr>
<td>Brunetto</td>
<td>Case control; IFN</td>
<td>56 CHB (15 precore wild-type and 21 mutant); DNA suppression</td>
<td>IFN response noted in 0% and 19% of the patients infected with wild-type and mutant HBV, respectively</td>
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<td>Lok</td>
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<td>106 HBeAg-positive; CHB HBeAg loss</td>
<td>IFN response noted in 17% and 55% of the patients infected with precore wild-type and mutant HBV, respectively</td>
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<tr>
<td>Aikawa</td>
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<td>31 CHB; DNA suppression</td>
<td>IFN response comparable between precore mutant and mixed precore/wild-type HBV</td>
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<tr>
<td>Kanai</td>
<td>Case series; IFN</td>
<td>46 HBeAg-positive; CHB HBeAg loss</td>
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<td>[77]</td>
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<td>Kako</td>
<td>Case series; IFN</td>
<td>44 HBeAg-positive and 24 HBeAg-negative; CHB DNA suppression</td>
<td>IFN response better in HBeAg-negative patients compared with HBeAg-positive patients; precore G1896A mutant sensitive to IFN</td>
<td>III</td>
<td>[84]</td>
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<tr>
<td>Erhardt</td>
<td>Case series; IFN</td>
<td>96 CHB (64 HBeAg-positive and 32 HBeAg-negative); DNA suppression and ALT normalization</td>
<td>IFN response similar between precore wild-type and mutant HBV</td>
<td>III</td>
<td>[76]</td>
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<tr>
<td>Shindo</td>
<td>Case control; IFN</td>
<td>23 CHB; HBeAg seroconversion, DNA suppression and ALT normalization</td>
<td>Precore wild-type and mutant HBVs had similar sensitivity to IFN</td>
<td>II-2</td>
<td>[85]</td>
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<td>Kuwahara</td>
<td>Case series; lamivudine</td>
<td>45 CHB; HBeAg loss</td>
<td>Lamivudine response more effective in precore mutant compared with wild-type HBV</td>
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<td>Shin</td>
<td>Case series; lamivudine</td>
<td>260 consecutive CHB; viral breakthrough</td>
<td>Pretreatment CP mutation and low HBV DNA level associated with low viral breakthrough</td>
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<tr>
<td>Chen</td>
<td>Case series; lamivudine</td>
<td>74 HBeAg-positive; CHB; HBeAg loss</td>
<td>Pretreatment high ALT, G1896A mutant and HBV DNA level were major determinants of response</td>
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<td>[88]</td>
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<td>Yuen</td>
<td>Case series; lamivudine</td>
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<td>[89]</td>
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<td>Fukai</td>
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<td>49 CHB; DNA suppression</td>
<td>Precore and enhancer I mutation correlated with good response</td>
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<td>[90]</td>
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<td>Thompson</td>
<td>Case series; lamivudine</td>
<td>85 CHB, 45% HBeAg-negative; DNA suppression and development of YMDD mutation</td>
<td>Patients infected with precore mutation developed YMDD mutation earlier</td>
<td>III</td>
<td>[67]</td>
</tr>
</tbody>
</table>

*The level of evidence is based on the quality as well as content of the cited paper (Table 3 [78]). ALT, alanine aminotransferase; CHB, chronic hepatitis B; CP, core protein; HBeAg, hepatitis B e antigen HBV, hepatitis B virus; IFN, interferon.
HBeAg-positive patients (18 sustained and eight non-sustained responders) treated with IFN for 12 weeks was analysed [74]. Before treatment, 16 patients had a CP region identical to a consensus sequence of the corresponding genotype A. There was no association between specific CP mutations and the response to IFN.

Fourteen HBeAg-positive patients received IFN-α2a (six responders and eight non-responders) [75]. Six responders had lower HBeAg levels than non-responders. Five of six responders (83%) and none of the non-responders had A1762T/G1764A CP mutations (0/8, \( P<0.003 \)), indicating that the CP mutation might correlate with the response to IFN therapy.

Earlier studies showed that the precore G1896A stop codon mutant could be associated with a poor response to IFN therapy in patients with HBeAg-negative CHB; however, the results seem controversial. Recently, Erhardt et al. [76] performed sequence analysis of several HBV subgenomic regions in 96 HBeAg-positive and HBeAg-negative patients treated with standard IFN. The overall sustained response rate to IFN was 30% with no significant difference between HBeAg-positive and HBeAg-negative patients. IFN responsiveness correlated with the number of mutations in the CP, especially the nt region 1753–1766 and mutations at nt 1762 and 1764. Sustained response to IFN was associated with a high number of mutations in the CP and the nt region 1753–1766 as well as mutations at nucleotide 1764 in HBeAg-positive patients. In contrast, sustained response to IFN correlated with a low number of mutations in the CP and nt region 1753–1766 and a wild-type sequence at nt 1764 in HBeAg-negative patients. In addition, IFN response did not correlate with the occurrence of the precore G1896A mutation. These data suggested that mutations located within the CP of

<table>
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<th>Table 5. Reported associations between pretherapy core promoter mutations and treatment outcomes</th>
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<td><strong>Author (year)</strong></td>
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<tr>
<td>Kanai (1996)</td>
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<td>Liu (2005)</td>
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<td>Thompson (2007)</td>
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<td>Hou (2007)</td>
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</table>

*The level of evidence is based on the quality as well as content of the cited paper (Table 3 [78]). ALT, alanine aminotransferase; CHB, chronic hepatitis B; CP, core protein; HBeAg, hepatitis B e antigen; HBV, hepatitis B virus; IFN, interferon; nt, nucleotide.
the HBV genome could determine a response to IFN therapy; however, whether a similar phenomenon holds true in the era of PEG-IFN awaits further studies.

Influence on therapy with lamivudine

We retrospectively reviewed data from 103 HBeAg-positive CHB patients (C-JL & J-HK unpublished data). Pretreatment virological profiles were mean serum HBV DNA level 7.32 ±1.91 log10 copies/ml, HBV genotype B/C 70%/30%, precore G1896A mutation 23% and CP A1762T/G1764A mutation 21%. The precore mutation was found to correlate with the loss of HBeAg post-treatment, in comparison with wild type (75% versus 52%, P=0.045), but not the CP mutation.

A cohort of 85 CHB patients (HBeAg-negative in 45% and CP A1762T/G1764A mutation in 63%) receiving at least 6 months lamivudine was studied in Australia [67]. The median follow-up period was 19 months. The study found that the precore G1896A mutation, high baseline viral load and persistent viraemia after 6 months of treatment were associated with the development of lamivudine resistance. However, the presence of a pretreatment CP mutation was not associated with the suppression of HBV DNA or development of the YMDD mutation.

Variables including CP and precore mutations were evaluated in 60 chronic HBeAg-positive patients with genotype C [73]. Out of the 60 patients, 30 were treated with lamivudine and the remaining 30 age- and sex-matched patients served as controls. The cumulative rates of HBeAg loss at 18 months were significantly higher in the lamivudine group (60.9%) compared with the control group (24.5%, P=0.03) and was especially pronounced in patients infected with CP mutant virus having the wild-type precore sequence (P=0.04). Multivariate analysis revealed that the CP mutation was an independent factor for HBeAg loss.

For the treatment of HBeAg-negative patients with lamivudine, Lok et al. [106] reported that both the duration of treatment and the presence of the CP mutation were associated with lamivudine resistance. They also found that some patients with lamivudine-resistant mutations had reversion of the precore G1896A stop codon mutation with reappearance of HBeAg. Our recent study on HBeAg-positive patients further showed that lamivudine therapy could result in the rapid development of the CP A1762T/G1764A mutation of the HBV genome, but this mutation could revert to wild type gradually after cessation of therapy [107]. In addition, there was no significant change of precore sequences before and during lamivudine therapy.

In summary, positive correlation between precore and CP mutations and the response to IFN therapy was noted in half of these studies. Nevertheless, the level of evidence was not high (Tables 4 and 5). Besides, only a few studies addressed the influence of CP mutations on the response to nucleos(t)ide analogue therapy. Because relevant data are limited, further studies should focus on the contribution of these viral factors to the treatment outcomes of existing antiviral agents.

Issues needing further research

Genotype-dependent treatment strategies should be tested in prospective clinical trials

Several lines of evidence consistently demonstrated that HBV genotype correlates with the response to IFN-based therapy. Thus, if no contraindications are present, IFN should be considered as the first-line therapy in all genotype A or B patients and particularly in individuals who are HBeAg-positive. However, this recommendation remains to be confirmed by future prospective genotype-dependent clinical trials.

Accumulation of evidence needed

Data regarding the correlation of HBV genotypes with treatment responses have rapidly accumulated in the past few years. Nevertheless, these results were mostly obtained from regional studies with limited genotypes and the number of patients was relatively small. Therefore, further studies specifically designed to elucidate the clinical relevance of HBV genotypes regarding the treatment outcomes and recruiting a large number of patients with suitable representation of geographically diverse HBV genotypes are warranted. As for naturally occurring mutations such as precore and CP mutations, relevant studies are just beginning and more data are needed.

Exploration of biological significance of HBV genotypes, precore and CP mutations

To explain the mechanisms underlying the pathogenesis and treatment outcomes of different HBV genotypes and naturally occurring mutations such as precore and CP mutations, we assume that the replication efficiency and the host immune selection pressure of different HBV genotypes, precore and CP mutations, relevant studies are just beginning and more data are needed.

The role of mixed genotype, precore or CP mutation infections

Apart from single HBV genotype, precore or CP mutation infection, a significant proportion of patients have more than two HBV genotypes or mixed wild-type/mutant infections. The relationship between precore/CP mutations and the response to IFN therapy was investigated by Kanai et al. [77]. Forty-six consecutive patients with HBeAg-positive CHB received...
IFN-α2a. Pretreatment mutations in the CP region were searched for in five HBV DNA clones propagated from each patient. HBeAg loss was noted at 6 months after IFN-α2a therapy in 11 (61%) of the 18 patients with the precore mutation and in 12 (43%) of the 28 patients without mutation. Of the 28 patients without any precore mutations, 19 with mutations in the CP region in all five HBV DNA clones lost HBeAg more frequently than the remaining nine who had at least one clone among the five that lacked such mutations (58 versus 11%, P<0.05). Thus, it was suggested that HBeAg-positive patients with pre-existing precore or CP mutants responded better to IFN. Whether mixed HBV genotype, precore or CP mutant infections correlate with treatment outcomes remains to be clarified.

Conclusions

Differences exist in the clinical and virological characteristics among HBV genotypes. Therefore, determining HBV genotype in patients with chronic HBV infection would help clinicians gain information for etiological, clinical and virological investigations. From the therapeutic point of view, if responses to a given antiviral agent can be predicted on the basis of HBV genotype, therapy can then be individualized to spare the cost and adverse effects of ineffective treatment. Regarding the role of naturally occurring mutations such as precore and CP mutations in response to antiviral therapy, more data are needed before drawing definite conclusions. Finally, the molecular and virological mechanisms contributing to the clinical phenotypes of HBV genotypes and naturally occurring mutations need further study.

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Disclosure statement

The authors declare no conflicts of interest.

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