than 50%.[179,180] Longer duration of therapy is associated with improved durability of response: 10–15% with 4–6 months of therapy, 22–30% with 6–12 months of therapy and 30% with 24 months of therapy.[181–184]

Consensus statement 11

11-1 Durability of response is less than 50% in HBeAg negative patients. (Level 1b.)

11-2 Longer duration of therapy (>48 weeks) is associated with improved durability of response. (Level 2b.)

Pegylated IFN (PEG IFN)

Twenty four weeks of PEG IFN-α-2a monotherapy had higher rate of combined response (loss of HBeAg, suppression of HBV DNA <500 000 copies/mL and ALT normalization) compared to standard IFN-α-2a.[185] Another study with 24 weeks of PEG IFN-α-2b monotherapy also showed a higher rate of HBeAg loss and HBV DNA suppression compared to standard IFN-α-2b.[169]

Controlled studies comparing the 48 weeks of PEG IFN-α-2a and LVD in HBeAg positive and negative patients revealed that PEG IFN had a higher rate of sustained response.[170,171] Seroconversion of HBeAg (32% vs 19%), ALT normalization (41% vs 28% in HBeAg positives and 59% vs 44% in HBeAg negatives), HBV DNA suppression (HBV DNA <10 000 copies/mL, 32% vs 22% in HBeAg positives; HBV DNA <20 000 copies/mL, 43% vs 29% in HBeAg negatives) and negative HBV DNA (14% vs 5% in HBeAg positives and 19% vs 7% in HBeAg negatives) were more frequent in PEG IFN treated patients.

Differences were reported in outcome of the antiviral treatment of patients infected with different genotypes; genotype B is associated with a higher rate of antiviral response to IFN treatment than HBV genotype C among Asian patients with HBeAg positive chronic hepatitis B.[169,186,187] In multicenter trials comparing combination therapy of PEG IFN-α-2b and LVD versus PEG IFN-α-2b alone, it was shown that treatment with PEG IFN-α-2b is the best therapy to achieve HBsAg clearance in patients with genotype A compared with D.[188,189]

Combination or sequential therapy

Combination of two antiviral agents with different mechanisms of action seems a logical approach to improve efficacy. In fact, simultaneous combination of LVD and PEG IFN has a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone. However, there is no difference in treatment response between the simultaneous combination of LVD and IFN or PEG IFN compared to IFN or PEG IFN alone (Table 5).[132,133,170]

There are several clinical trials of sequential therapy with LVD followed by IFN.[190–194] Common to all studies is that the sequential therapy had no advantage over IFN alone. Some studies have shown the suggestive evidence that sequential therapy had a higher rate of HBV suppression, ALT normalization and less frequent emergence of LVD-resistant mutant virus compared to LVD alone (Table 5).[190–194] However, because the study protocols and their results are variable, a conclusive result could not be drawn.

Table 5 Sequential therapy of lamivudine and interferon

		BR	SC	VR	LVD-R
Manesis *et al.* 2006	Sequential	39%	NA	28%	
($n=36$)[190]	IFN	22%	NA	19%	
Shi *et al.* 2006	Sequential	53%	NA	14%	0%
($n=162$)[191]	LVD	36%	NA	18%	23%
Yurdaydin *et al.* 2005	Sequential	51%	NA	54%	24%
($n=78$)[193]	LVD	41%	NA	59%	53%
Sarin *et al.* 2005	Sequential	40%	40%	40%	15%
($n=75$)[194]	LVD	14%	11%	16%	8%
Schalm *et al.* 2000	Sequential	50%	36%	55%	0%
($n=226$)[192]	IFN	50%	22%	49%	0%
	LVD	63%	19%	63%	31%

BR, biochemical response; IFN, interferon; LVD, lamivudine; LVD-R, lamivudine resistant mutation; NA, not applicable because hepatitis B e-antigen patients are studied; SC, seroconversion; VR, virological response.

Long-term outcome

The end-point of antiviral therapy is to prevent liver cirrhosis and HCC. Meta-analysis of five studies including 935 patients revealed that IFN treatment significantly decreased the incidence of cirrhosis with the combined risk ratio of 0.65 (95% confidence interval [CI] = 0.47–0.91).[195] Meta-analysis of 11 studies including 2082 patients revealed that IFN treatment significantly decreased the incidence of HCC with the combined risk ratio of 0.59 (95% CI = 0.43–0.81).[195] These results suggest that IFN prevents progression of liver disease to liver cirrhosis or delays the development of HCC, as long as it is within 4–7 years of follow up which is the length of follow up in these studies. Sustained response to IFN therapy was associated with increased survival.[175,181,196,197] To further elucidate the impact of IFN on the natural course of chronic hepatitis B, studies with larger populations followed for longer periods may be needed.

Consensus statement 12

12-1 IFN therapy prevents progression to cirrhosis or the development of HCC. (Level 1a.)

12-2 IFN therapy is associated with improved survival. (Level 1b.)

Adverse effects

The most frequent adverse effects are flu-like symptoms, fatigue, myelosuppression and dermal reaction at the injection site. Others include alopecia, depression and thyroid dysfunction. Less frequent but severe adverse events include interstitial pneumonitis, exacerbation of underlying autoimmune disorders, cerebral vascular events and flare of hepatitis.

REFERENCES

1 Lavanchy D. Hepatitis B virus epidemiology, disease burden, treatment, and current and emerging prevention and control measures. *J Viral Hepat* 2004; 11: 97–107.

2 McMahon BJ, Alward WL, Hall DB *et al.* Acute hepatitis B virus infection: relation of age to the clinical expression of disease and subsequent development of the carrier state. *J Infect Dis* 1985; 151: 599–603.

3 Shiffman RN, Shenkelle P, Overhage JM, Drimshaw J, Deshpande AM. Standard reporting of clinical practice guidelines: a proposal from the Conference on Guideline Standization. *Ann Intern Med* 2003; 139: 493–8.

4 McMahon BJ. The natural history of chronic hepatitis B virus infection. *Hepatology* 2009; 49: S45–55.

5 Fattovich G. Natural history and prognosis of hepatitis B. *Semin Liver Dis* 2003; 23: 47–58.

6 Chen DS. From hepatitis to hepatoma: lessons from type B viral hepatitis. *Science* 1993; 262: 369–70.

7 Hoofnagle JH, Doo E, Liang TJ, Fleischer R, Lok AS. Management of hepatitis B: summary of a clinical research workshop. *Hepatology* 2007; 45: 1056–75.

8 Lok AS. Chronic hepatitis B. *N Engl J Med* 2002; 346: 1682–3.

9 Lok AS, Heathcote EJ, Hoofnagle JH. Management of hepatitis B: 2000 – summary of a workshop. *Gastroenterology* 2001; 120: 1828–53.

10 Chang MH, Hsu HY, Hsu HC, Ni YH, Chen JS, Chen DS. The significance of spontaneous hepatitis B e antigen seroconversion in childhood: with special emphasis on the clearance of hepatitis B e antigen before 3 years of age. *Hepatology* 1995; 22: 1387–92.

11 Hui CK, Leung N, Yuen ST *et al.* Natural history and disease progression in Chinese chronic hepatitis B patients in immune-tolerant phase. *Hepatology* 2007; 46: 395–401.

12 Lok AS, Lai CL. A longitudinal follow-up of asymptomatic hepatitis B surface antigen-positive Chinese children. *Hepatology* 1988; 8: 1130–3.

13 Bortolotti F, Guido M, Bartolacci S *et al.* Chronic hepatitis B in children after e antigen seroclearance: final report of a 29-year longitudinal study. *Hepatology* 2006; 43: 556–62.

14 Chu CM, Hung SJ, Lin J, Tai DI, Liaw YF Natural history of hepatitis B e antigen to antibody seroconversion in patients with normal serum aminotransferase levels. *Am J Med* 2004; 116: 829–34.

15 Hoofnagle JH, Dusheiko GM, Seeff LB, Jones EA, Waggoner JG, Bales ZB. Seroconversion from hepatitis B e antigen to antibody in chronic type B hepatitis. *Ann Intern Med* 1981; 94: 744–8.

16 McMahon BJ. Epidemiology and natural history of hepatitis B. *Semin Liver Dis* 2005; 25 (Suppl 1): 3–8.

17 Fattovich G, Rugge M, Brollo L *et al.* Clinical, virologic and histologic outcome following seroconversion from HBeAg to anti-HBe in chronic hepatitis type B. *Hepatology* 1986; 6: 167–72.

18 Liaw YF, Chu CM, Huang MJ, Sheen IS, Yang CY, Lin DY. Determinants for hepatitis B e antigen clearance in chronic type B hepatitis. *Liver* 1984; 4: 301–6.

19 Lok AS, Lai CL, Wu PC, Leung EK, Lam TS. Spontaneous hepatitis B e antigen to antibody seroconversion and reversion in Chinese patients with chronic hepatitis B virus infection. *Gastroenterology* 1987; 92: 1839–43.

20 McMahon BJ, Holck P, Bulkow L, Snowball M. Serologic and clinical outcomes of 1536 Alaska Natives chronically infected with hepatitis B virus. *Ann Intern Med* 2001; 135: 759–68.

21 Yuen MF, Yuan HJ, Hui CK *et al.* A large population study of spontaneous HBeAg seroconversion and acute exacer-

bation of chronic hepatitis B infection: implications for antiviral therapy. *Gut* 2003; 52: 416–19.
22 Kao JH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes correlate with clinical outcomes in patients with chronic hepatitis B. *Gastroenterology* 2000; 118: 554–9.
23 Chu CJ, Hussain M, Lok AS. Hepatitis B virus genotype B is associated with earlier HBeAg seroconversion compared with hepatitis B virus genotype C. *Gastroenterology* 2002; 122: 1756–62.
24 Hsu YS, Chien RN, Yeh CT *et al.* Long-term outcome after spontaneous HBeAg seroconversion in patients with chronic hepatitis B. *Hepatology* 2002; 35: 1522–7.
25 Carman WF, Jacyna MR, Hadziyannis S *et al.* Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 1989; 2: 588–91.
26 Chan HL, Hussain M, Lok AS. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. *Hepatology* 1999; 29: 976–84.
27 Chen CH, Hung CH, Lee CM *et al.* Pre-S deletion and complex mutations of hepatitis B virus related to advanced liver disease in HBeAg-negative patients. *Gastroenterology* 2007; 133: 1466–74.
28 Marschenz S, Endres AS, Brinckmann A *et al.* Functional analysis of complex hepatitis B virus variants associated with development of liver cirrhosis. *Gastroenterology* 2006; 131: 765–80.
29 Chen CH, Changchien CS, Lee CM *et al.* Combined mutations in pre-s/surface and core promoter/precore regions of hepatitis B virus increase the risk of hepatocellular carcinoma: a case–control study. *J Infect Dis* 2008; 198: 1634–42.
30 Yuen MF, Tanaka Y, Shinkai N *et al.* Risk for hepatocellular carcinoma with respect to hepatitis B virus genotypes B/C, specific mutations of enhancer II/core promoter/precore regions and HBV DNA levels. *Gut* 2008; 57: 98–102.
31 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 patients with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
32 Yu MW, Chang HC, Liaw YF *et al.* Familial risk of hepatocellular carcinoma among chronic hepatitis B carriers and their relatives. *J Natl Cancer Inst* 2000; 92: 1159–64.
33 Chen CJ, Yang HI, Su J *et al.* Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. *JAMA* 2006; 295: 65–73.
34 Ishiguro S, Inoue M, Tanaka Y, Mizokami M, Iwasaki M, Tsugane S. Serum aminotransferase level and the risk of hepatocellular carcinoma: a population-based cohort study in Japan. *Eur J Cancer Prev* 2009; 18: 26–32.
35 Kao JH, Chen PJ, Lai MY, Chen DS. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. *Gastroenterology* 2003; 124: 327–34.
36 Yu MW, Yeh SH, Chen PJ *et al.* Hepatitis B virus genotype and DNA level and hepatocellular carcinoma: a prospective study in men. *J Natl Cancer Inst* 2005; 97: 265–72.
37 Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut* 2004; 53: 744–9.
38 Ohnishi K, Iida S, Iwama S *et al.* The effect of chronic habitual alcohol intake on the development of liver cirrhosis and hepatocellular carcinoma: relation to hepatitis B surface antigen carriage. *Cancer* 1982; 49: 672–7.
39 Yu MC, Yuan JM. Environmental factors and risk for hepatocellular carcinoma. *Gastroenterology* 2004; 127: S72–78.
40 Manno M, Camma C, Schepis F *et al.* Natural history of chronic HBV carriers in northern Italy: morbidity and mortality after 30 years. *Gastroenterology* 2004; 127: 756–63.
41 Chen YC, Sheen IS, Chu CM, Liaw YF. Prognosis following spontaneous HBsAg seroclearance in chronic hepatitis B patients with or without concurrent infection. *Gastroenterology* 2002; 123: 1084–9.
42 Huo TI, Wu JC, Lee PC *et al.* Sero-clearance of hepatitis B surface antigen in chronic carriers does not necessarily imply a good prognosis. *Hepatology* 1998; 28: 231–6.
43 Hui CK, Cheung WW, Zhang HY *et al.* Kinetics and risk of de novo hepatitis B infection in HBsAg-negative patients undergoing cytotoxic chemotherapy. *Gastroenterology* 2006; 131: 59–68.
44 Tanaka E, Umemura T. History and prevention of de novo hepatitis B virus-related hepatitis in Japan and the World. *Clin J Gastroenterol* 2008; 1: 83–6.
45 Umemura T, Kiyosawa K. Fatal HBV reactivation in a subject with anti-HBs and anti-HBc. *Intern Med* 2006; 45: 747–8.
46 Umemura T, Tanaka E, Kiyosawa K, Kumada H. Mortality secondary to fulminant hepatic failure in patients with prior resolution of hepatitis B virus infection in Japan. *Clin Infect Dis* 2008; 47: e52–56.
47 Taylor BC, Yuan JM, Shamliyan TA, Shaukat A, Kane RL, Wilt TJ. Clinical outcomes in adults with chronic hepatitis B in association with patient and viral characteristics: a systematic review of evidence. *Hepatology* 2009; 49: S85–95.
48 Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; 40: 14–30.
49 Chen DS. Hepatitis B vaccination: the key towards elimination and eradication of hepatitis B. *J Hepatol* 2009; 50: 805–16.
50 Lok AS. Natural history and control of perinatally acquired hepatitis B virus infection. *Dig Dis* 1992; 10: 46–52.
51 Koibuchi T, Hitani A, Nakamura T *et al.* Predominance of genotype A HBV in an HBV-HIV-1 dually positive

population compared with an HIV-1-negative counterpart in Japan. *J Med Virol* 2001; 64: 435–40.
52 Shibayama T, Masuda G, Ajisawa A *et al.* Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. *J Med Virol* 2005; 76: 24–32.
53 Kobayashi M, Arase Y, Ikeda K *et al.* Viral genotypes and response to interferon in patients with acute prolonged hepatitis B virus infection of adulthood in Japan. *J Med Virol* 2002; 68: 522–8.
54 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; 44: 326–34.
55 Sugauchi F, Orito E, Ohno T *et al.* Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. *Hepatol Res* 2006; 36: 107–14.
56 Suzuki Y, Kobayashi M, Ikeda K *et al.* Persistence of acute infection with hepatitis B virus genotype A and treatment in Japan. *J Med Virol* 2005; 76: 33–9.
57 Matsuura K, Tanaka Y, Hige S *et al.* Distribution of hepatitis B virus genotypes among patients with chronic infection in Japan shifting toward an increase of genotype A. *J Clin Microbiol* 2009; 47: 1476–83.
58 Sherlock S. The natural history of hepatitis B. *Postgrad Med J* 1987; 63 (Suppl 2): 7–11.
59 Sugiyama M, Tanaka Y, Kato T *et al.* Influence of hepatitis B virus genotypes on the intra- and extracellular expression of viral DNA and antigens. *Hepatology* 2006; 44: 915–24.
60 Sugiyama M, Tanaka Y, Kurbanov F *et al.* Direct cytopathic effects of particular hepatitis B virus genotypes in severe combined immunodeficiency transgenic with urokinase-type plasminogen activator mouse with human hepatocytes. *Gastroenterology* 2009; 136: 652–62. e3.
61 Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G. Fulminant hepatitis and late onset hepatic failure in Japan. *Hepatol Res* 2008; 38: 646–57.
62 Sato S, Suzuki K, Akahane Y *et al.* Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 1995; 122: 241–8.
63 Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. *N Engl J Med* 1991; 324: 1699–704.
64 Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; 324: 1705–9.
65 Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. Nucleotide sequence analysis of the precore region in patients with fulminant hepatitis B in the United States. *Gastroenterology* 1993; 105: 1173–8.
66 Feray C, Gigou M, Samuel D, Bernuau J, Bismuth H, Brechot C. Low prevalence of precore mutations in hepatitis B virus DNA in fulminant hepatitis type B in France. *J Hepatol* 1993; 18: 119–22.
67 Liang TJ, Hasegawa K, Munoz SJ *et al.* Hepatitis B virus precore mutation and fulminant hepatitis in the United States. A polymerase chain reaction-based assay for the detection of specific mutation. *J Clin Invest* 1994; 93: 550–5.
68 Chan HL, Sung JJ. Hepatocellular carcinoma and hepatitis B virus. *Semin Liver Dis* 2006; 26: 153–61.
69 Lok AS. Hepatitis B: liver fibrosis and hepatocellular carcinoma. *Gastroenterol Clin Biol* 2009; 33: 911–15.
70 Lok AS, McMahon BJ. Chronic hepatitis B. *Hepatology* 2007; 45: 507–39.
71 Chan HL, Tse CH, Mo F *et al.* High viral load and hepatitis B virus subgenotype ce are associated with increased risk of hepatocellular carcinoma. *J Clin Oncol* 2008; 26: 177–82.
72 Chen G, Lin W, Shen F, Iloeje UH, London WT, Evans AA. Past HBV viral load as predictor of mortality and morbidity from HCC and chronic liver disease in a prospective study. *Am J Gastroenterol* 2006; 101: 1797–803.
73 Chan HL, Hui AY, Wong ML *et al.* Genotype C hepatitis B virus infection is associated with an increased risk of hepatocellular carcinoma. *Gut* 2004; 53: 1494–8.
74 Sumi H, Yokosuka O, Seki N *et al.* Influence of hepatitis B virus genotypes on the progression of chronic type B liver disease. *Hepatology* 2003; 37: 19–26.
75 Tanaka Y, Mukaide M, Orito E *et al.* Specific mutations in enhancer II/core promoter of hepatitis B virus subgenotypes C1/C2 increase the risk of hepatocellular carcinoma. *J Hepatol* 2006; 45: 646–53.
76 Huang Y, Wang Z, An S *et al.* Role of hepatitis B virus genotypes and quantitative HBV DNA in metastasis and recurrence of hepatocellular carcinoma. *J Med Virol* 2008; 80: 591–7.
77 Livingston SE, Simonetti JP, McMahon BJ *et al.* Hepatitis B virus genotypes in Alaska Native people with hepatocellular carcinoma: preponderance of genotype F. *J Infect Dis* 2007; 195: 5–11.
78 Tseng TC, Kao JH. HBV genotype and clinical outcome of chronic hepatitis B: facts and puzzles. *Gastroenterology* 2008; 134: 1272–3. author reply 3.
79 Kew MC, Kramvis A, Yu MC, Arakawa K, Hodkinson J. Increased hepatocarcinogenic potential of hepatitis B virus genotype A in Bantu-speaking sub-saharan Africans. *J Med Virol* 2005; 75: 513–21.
80 Kramvis A, Kew MC, Bukofzer S. Hepatitis B virus precore mutants in serum and liver of Southern African Blacks with hepatocellular carcinoma. *J Hepatol* 1998; 28: 132–41.
81 Sanchez-Tapias JM, Costa J, Mas A, Bruguera M, Rodes J. Influence of hepatitis B virus genotype on the long-term outcome of chronic hepatitis B in western patients. *Gastroenterology* 2002; 123: 1848–56.

82 Ou JH, Laub O, Rutter WJ. Hepatitis B virus gene function: the precore region targets the core antigen to cellular membranes and causes the secretion of the e antigen. *Proc Natl Acad Sci U S A* 1986; 83: 1578–82.
83 Uy A, Bruss V, Gerlich WH, Köchel HG, Thomssen R. Precore sequence of hepatitis B virus inducing e antigen and membrane association of the viral core protein. *Virology* 1986; 155: 89–96.
84 Miyanohara A, Imamura T, Araki M, Sugawara K, Ohtomo N, Matsubara K. Expression of hepatitis B virus core antigen gene in Saccharomyces cerevisiae: synthesis of two polypeptides translated from different initiation codons. *J Virol* 1986; 59: 176–80.
85 Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the human hepatitis B virus. *Hepatol Res* 2010; 40: 14–30.
86 Lindh M, Andersson AS, Gusdal A. Genotypes, nt 1858 variants, and geographic origin of hepatitis B virus – large-scale analysis using a new genotyping method. *J Infect Dis* 1997; 175: 1285–93.
87 Okamoto H, Tsuda F, Akahane Y *et al.* Hepatitis B virus with mutations in the core promoter for an e antigen-negative phenotype in carriers with antibody to e antigen. *J Virol* 1994; 68: 8102–10.
88 Buckwold VE, Xu Z, Chen M, Yen TS, Ou JH. Effects of a naturally occurring mutation in the hepatitis B virus basal core promoter on precore gene expression and viral replication. *J Virol* 1996; 70: 5845–51.
89 Parekh S, Zoulim F, Ahn SH *et al.* Genome replication, virion secretion, and e antigen expression of naturally occurring hepatitis B virus core promoter mutants. *J Virol* 2003; 77: 6601–12.
90 Kosaka Y, Takase K, Kojima M *et al.* Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology* 1991; 100: 1087–94.
91 Karayiannis P, Alexopoulou A, Hadziyannis S *et al.* Fulminant hepatitis associated with hepatitis B virus e antigen-negative infection: importance of host factors. *Hepatology* 1995; 22: 1628–34.
92 Laskus T, Rakela J, Nowicki MJ, Persing DH. Hepatitis B virus core promoter sequence analysis in fulminant and chronic hepatitis B. *Gastroenterology* 1995; 109: 1618–23.
93 Laskus T, Rakela J, Persing DH. The stem-loop structure of the cis-encapsidation signal is highly conserved in naturally occurring hepatitis B virus variants. *Virology* 1994; 200: 809–12.
94 Liu CJ, Kao JH, Lai MY, Chen PJ, Chen DS. Precore/core promoter mutations and genotypes of hepatitis B virus in chronic hepatitis B patients with fulminant or subfulminant hepatitis. *J Med Virol* 2004; 72: 545–50.
95 Pollicino T, Zanetti AR, Cacciola I *et al.* Pre-S2 defective hepatitis B virus infection in patients with fulminant hepatitis. *Hepatology* 1997; 26: 495–9.
96 Kalinina T, Riu A, Fischer L, Will H, Sterneck M. A dominant hepatitis B virus population defective in virus secretion because of several S-gene mutations from a patient with fulminant hepatitis. *Hepatology* 2001; 34: 385–94.
97 Bock CT, Tillmann HL, Maschek HJ, Manns MP, Trautwein C. A preS mutation isolated from a patient with chronic hepatitis B infection leads to virus retention and misassembly. *Gastroenterology* 1997; 113: 1976–82.
98 Zhang K, Imazeki F, Fukai K *et al.* Analysis of the complete hepatitis B virus genome in patients with genotype C chronic hepatitis in relation to HBeAg and anti-HBe. *J Med Virol* 2007; 79: 683–93.
99 Baptista M, Kramvis A, Kew MC. High prevalence of 1762(T) 1764(A) mutations in the basic core promoter of hepatitis B virus isolated from black Africans with hepatocellular carcinoma compared with asymptomatic carriers. *Hepatology* 1999; 29: 946–53.
100 Liu CJ, Chen BF, Chen PJ *et al.* Role of hepatitis B virus precore/core promoter mutations and serum viral load on noncirrhotic hepatocellular carcinoma: a case–control study. *J Infect Dis* 2006; 194: 594–9.
101 Guo X, Jin Y, Qian G, Tu H. Sequential accumulation of the mutations in core promoter of hepatitis B virus is associated with the development of hepatocellular carcinoma in Qidong, China. *J Hepatol* 2008; 49: 718–25.
102 Tong MJ, Blatt LM, Kao JH, Cheng JT, Corey WG. Basal core promoter T1762/A1764 and precore A1896 gene mutations in hepatitis B surface antigen-positive hepatocellular carcinoma: a comparison with chronic carriers. *Liver Int* 2007; 27: 1356–63.
103 Tong MJ, Blatt LM, Kao JH, Cheng JT, Corey WG. Precore/basal core promoter mutants and hepatitis B viral DNA levels as predictors for liver deaths and hepatocellular carcinoma. *World J Gastroenterol* 2006; 12: 6620–6.
104 Yang HI, Yeh SH, Chen PJ *et al.* REVEAL-HBV Study Group. Associations between hepatitis B virus genotype and mutants and the risk of hepatocellular carcinoma. *J Natl Cancer Inst* 2008; 100: 1134–43.
105 Fang ZL, Sabin CA, Dong BQ *et al.* HBV A1762T, G1764A mutations are a valuable biomarker for identifying a subset of male HBsAg carriers at extremely high risk of hepatocellular carcinoma: a prospective study. *Am J Gastroenterol* 2008; 103: 2254–62.
106 Bläckberg J, Kidd-Ljunggren K. Mutations within the hepatitis B virus genome among chronic hepatitis B patients with hepatocellular carcinoma. *J Med Virol* 2003; 71: 18–23.
107 Zhang KY, Imazeki F, Fukai K *et al.* Analysis of the complete hepatitis B virus genome in patients with genotype C chronic hepatitis and hepatocellular carcinoma. *Cancer Sci* 2007; 98: 1921–9.
108 Fang ZL, Sabin CA, Dong BQ *et al.* Hepatitis B virus pre-S deletion mutations are a risk factor for hepatocellular carcinoma: a matched nested case–control study. *J Gen Virol* 2008; 89: 2882–90.

109 Chen BF, Liu CJ, Jow GM, Chen PJ, Kao JH, Chen DS. High prevalence and mapping of pre-S deletion in hepatitis B virus carriers with progressive liver diseases. *Gastroenterology* 2006; 130: 1153–68.
110 Mun HS, Lee SA, Jee Y *et al.* The prevalence of hepatitis B virus preS deletions occurring naturally in Korean patients infected chronically with genotype C. *J Med Virol* 2008; 80: 1189–94.
111 Takahashi K, Akahane Y, Hino K, Ohta Y, Mishiro S. Hepatitis B virus genomic sequence in the circulation of hepatocellular carcinoma patients: comparative analysis of 40 full-length isolates. *Arch Virol* 1998; 143: 2313–26.
112 Zanetti AR, Tanzi E, Manzillo G *et al.* Hepatitis B variant in Europe. *Lancet* 1988; 2: 1132–3.
113 Carman WF, Zanetti AR, Karayiannis P *et al.* Vaccine-induced escape mutant of hepatitis B virus. *Lancet* 1990; 336: 325–9.
114 Yamamoto K, Horikita M, Tsuda F *et al.* Naturally occurring escape mutants of hepatitis B virus with various mutations in the S gene in carriers seropositive for antibody to hepatitis B surface antigen. *J Virol* 1994; 68: 2671–6.
115 Hsu HY, Chang MH, Liaw SH, Ni YH, Chen HL. Changes of hepatitis B surface antigen variants in carrier children before and after universal vaccination in Taiwan. *Hepatology* 1999; 30: 1312–17.
116 McMahon G, Ehrlich PH, Moustafa ZA *et al.* Genetic alterations in the gene encoding the major HBsAg: DNA and immunological analysis of recurrent HBsAg derived from monoclonal antibody-treated liver transplant patients. *Hepatology* 1992; 15: 757–66.
117 Carman WF, Trautwein C, van Deursen FJ *et al.* Hepatitis B virus envelope variation after transplantation with and without hepatitis B immune globulin prophylaxis. *Hepatology* 1996; 24: 489–93.
118 Ghany MG, Ayola B, Villamil FG *et al.* Hepatitis B virus S mutants in liver transplant recipients who were reinfected despite hepatitis B immune globulin prophylaxis. *Hepatology* 1998; 27: 213–22.
119 Jongerius JM, Wester M, Cuypers HT *et al.* New hepatitis B virus mutant form in a blood donor that is undetectable in several hepatitis B surface antigen screening assays. *Transfusion* 1998; 38: 56–9.
120 Chemin I, Trépo C. Clinical impact of occult HBV infections. *J Clin Virol* 2005; 34 (Suppl 1): S15–21.
121 Torresi J, Earnest-Silveira L, Civitico G *et al.* Restoration of replication phenotype of lamivudine-resistant hepatitis B virus mutants by compensatory changes in the "fingers" subdomain of the viral polymerase selected as a consequence of mutations in the overlapping S gene. *Virology* 2002; 299: 88–99.
122 Hsu CW, Yeh CT, Chang ML, Liaw YF. Identification of a hepatitis B virus S gene mutant in lamivudine-treated patients experiencing HBsAg seroclearance. *Gastroenterology* 2007; 132: 543–50.
123 Chu CM, Yeh CT, Tsai SL *et al.* HBsAg seroclearance in asymptomatic carriers of high endemic areas: appreciably high rates during a long-term follow-up. *Hepatology* 2007; 45: 1187–92.
124 Iloeje UH, Yang HI, Su J *et al.* Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; 130: 678–86.
125 Yuen MF, Ng IO, Fan ST *et al.* Significance of HBV DNA levels in liver histology of HBeAg and Anti-HBe positive patients with chronic hepatitis B. *Am J Gastroenterol* 2004; 99: 2032–7.
126 Lai CL, Chien RN, Leung NW *et al.* A one-year trial of lamivudine for chronic hepatitis B. Asia Hepatitis Lamivudine Study Group. *N Engl J Med* 1998; 339: 61–8.
127 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. *N Engl J Med* 2003; 348: 800–7.
128 Marcellin P, Chang TT, Lim SG *et al.* Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. *N Engl J Med* 2003; 348: 808–16.
129 Marcellin P, Lau GKK, Bonino F *et al.* Peginterferon alfa-2a alone, lamivudine alone and the two in combination in patients with HBeAg negative chronic hepatitis B. *N Engl J Med* 2004; 351: 1206–17.
130 Lau GK, Piratvisuth T, Luo KX *et al.* Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2005; 352: 2682–95.
131 Sherman M, Yurdaydin C, Sollano J *et al.* Entecavir for treatment of lamivudine-refractory, HBeAg-positive chronic hepatitis B. *Gastroenterology* 2006; 130: 2039–49.
132 Lai CL, Gane E, Liaw YF *et al.* Telbivudine versus lamivudine in patients with chronic hepatitis B. *N Engl J Med* 2007; 357: 2576–88.
133 Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. *J Hepatol* 2003; 38: 322–7.
134 Liaw YF, Sung JJ, Chow WC *et al.* Lamivudine for patients with chronic hepatitis B and advanced liver disease. *N Engl J Med* 2004; 351: 1521–31.
135 Leung NW, Lai CL, Chang TT *et al.* Extended lamivudine treatment in patients with chronic hepatitis B enhances hepatitis B e antigen seroconversion rates: results after 3 years of therapy. *Hepatology* 2001; 33: 1527–32.
136 Liaw YF, Chang TT, Wu SS *et al.* Long-term entecavir therapy results in reversal of fibrosis/cirrhosis and continued histologic improvement in patients with HBeAg(+) and (–) chronic hepatitis B: results from studies ETV-022, -027 and -901. *Hepatology* 2008; 48: 706A. abst 894.
137 Lok AS, Zoulim F, Locarnini S *et al.* Hepatitis B Virus Drug Resistance Working Group. *Hepatology* 2007; 46: 254–65.
138 Yuan HJ, Lee WM. Molecular mechanisms of resistance to antiviral therapy in patients with chronic hepatitis B. *Curr Mol Med* 2007; 7: 185–97.

139 Chang TT, Lai CL, Chien RN *et al.* Four years of lamivudine treatment in Chinese patients with chronic hepatitis B. *J Gastroenterol Hepatol* 2004; 19: 1276–82.
140 Yatsuji H, Noguchi C, Hiraga N *et al.* Emergence of a novel lamivudine-resistant hepatitis B virus variant with a substitution outside the YMDD motif. *Antimicrob Agents Chemother* 2006; 50: 3867–74.
141 Gaia S, Marzano A, Smedile A *et al.* Four years of treatment with lamivudine: clinical and virological evaluations in HBe antigen-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2004; 20: 281–7.
142 Tipples GA, Ma MM, Fischer KP, Bain VG, Kneteman NM, Tyrrell DL. Mutation in HBV RNA-dependent DNA polymerase confers resistance to lamivudine *in vivo*. *Hepatology* 1996; 24: 714–17.
143 Chayama K, Suzuki Y, Kobayashi M *et al.* Emergence and takeover of YMDD motif mutant hepatitis B virus during long-term lamivudine therapy and re-takeover by wild type after cessation of therapy. *Hepatology* 1998; 27: 1711–16.
144 Matsumoto A, Tanaka E, Rokuhara A *et al.* Efficacy of lamivudine for preventing hepatocellular carcinoma in chronic hepatitis B: a multicenter retrospective study of 2795 patients. *Hepatol Res* 2005; 32: 173–84.
145 Ono-Nita SK, Kato N, Shiratori Y *et al.* YMDD motif in hepatitis B virus DNA polymerase influences on replication and lamivudine resistance: a study by *in vitro* full-length viral DNA transfection. *Hepatology* 1999; 29: 939–45.
146 Lok AS, Hussain M, Cursano C *et al.* Evolution of hepatitis B virus polymerase gene mutations in hepatitis B e antigen-negative patients receiving lamivudine therapy. *Hepatology* 2000; 32: 1145–53.
147 Ono-Nita SK, Kato N, Shiratori Y *et al.* Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. *J Clin Invest* 1999; 103: 1635–40.
148 Delaney WE 4th, Yang H, Westland CE *et al.* The hepatitis B virus polymerase mutation rtV173L is selected during lamivudine therapy and enhances viral replication *in vitro*. *J Virol* 2003; 77: 11833–41.
149 Zollner B, Petersen J, Schafer P *et al.* Subtype-dependent response of hepatitis B virus during the early phase of lamivudine treatment. *Clin Infect Dis* 2002; 34: 1273–7.
150 Kobayashi M, Akuta N, Suzuki F *et al.* Virological outcomes in patients infected chronically with hepatitis B virus genotype A in comparison with genotypes B and C. *J Med Virol* 2006; 78: 60–7.
151 Yeh CT, Chien RN, Chu CM, Liaw YF. Clearance of the original hepatitis B virus YMDD-motif mutants with emergence of distinct lamivudine-resistant mutants during prolonged lamivudine therapy. *Hepatology* 2000; 31: 1318–26.
152 Fung SK, Chae HB, Fontana RJ *et al.* Virologic response and resistance to adefovir in patients with chronic hepatitis B. *J Hepatol* 2006; 44: 283–90.
153 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir Dipivoxil 438 Study Group.Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. *N Engl J Med* 2005; 352: 2673–81.
154 Hadziyannis SJ, Tassopoulos NC, Heathcote EJ *et al.* Adefovir Dipivoxil 438 Study Group. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B for up to 5 years. *Gastroenterology* 2006; 131: 1743–51.
155 Angus P, Vaughan R, Xiong S *et al.* Resistance to adefovir dipivoxil therapy associated with the selection of a novel mutation in the HBV polymerase. *Gastroenterology* 2003; 125: 292–7.
156 Lee YS, Suh DJ, Lim YS *et al.* Increased risk of adefovir resistance in patients with lamivudine-resistant chronic hepatitis B after 48 weeks of adefovir dipivoxil monotherapy. *Hepatology* 2006; 43: 1385–91.
157 Chen CH, Wang JH, Lee CM *et al.* Virological response and incidence of adefovir resistance in lamivudine-resistant patients treated with adefovir dipivoxil. *Antivir Ther* 2006; 11: 771–8.
158 Yeon JE, Yoo W, Hong SP *et al.* Resistance to adefovir dipivoxil in lamivudine resistant chronic hepatitis B patients treated with adefovir dipivoxil. *Gut* 2006; 55: 1488–95.
159 Chang TT, Gish RG, de Man R *et al.* BEHoLD AI463022 Study Group A comparison of entecavir and lamivudine for HBeAg-positive chronic hepatitis B. *N Engl J Med* 2006; 354: 1001–10.
160 Tenney DJ, Rose RE, Baldick CJ *et al.* Long-term monitoring shows hepatitis B virus resistance to entecavir in nucleoside-naïve patients is rare through 5 years of therapy. *Hepatology* 2009; 49: 1503–14.
161 Lai CL, Shouval D, Lok AS *et al.* BEHoLD AI463027 Study Group. Entecavir versus lamivudine for patients with HBeAg-negative chronic hepatitis B. *N Engl J Med* 2006; 9 (354): 1011–20.
162 Innaimo SF, Seifer M, Bisacchi GS, Standring DN, Zahler R, Colonno RJ. Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob Agents Chemother* 1997; 41: 1444–8.
163 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naïve patients with hepatitis B. *Hepatology* 2006; 44: 1656–65.
164 Tenney DJ, Rose RE, Baldick CJ *et al.* Two-year assessment of entecavir resistance in Lamivudine-refractory hepatitis B virus patients reveals different clinical outcomes depending on the resistance substitutions present. *Antimicrob Agents Chemother* 2007; 51: 902–11.
165 Suzuki F, Suzuki Y, Akuta N *et al.* Changes in viral loads of lamivudine-resistant mutants during entecavir therapy. *Hepatol Res* 2008; 9 (38): 132–40.
166 Hoofnagle JH, Peters M, Mullen KD *et al.* Randomized, controlled trial of recombinant human alpha-interferon in patients with chronic hepatitis B. *Gastroenterology* 1988; 95: 1318–25.

167 Perrillo RP, Schiff ER, Davis GL *et al.* A randomized, controlled trial of interferon alfa-2b alone and after prednisone withdrawal for the treatment of chronic hepatitis B. The Hepatitis Interventional Therapy Group. *N Engl J Med* 1990; 323: 295–301.
168 Suzuki F, Arase Y, Akuta N *et al.* Efficacy of 6-month interferon therapy in chronic hepatitis B virus infection in Japan. *J Gastroenterol* 2004; 39: 969–74.
169 Zhao H, Kurbanov F, Wan MB *et al.* Genotype B and younger patient age associated with better response to low-dose therapy: a trial with pegylated/nonpegylated interferon-alpha-2b for hepatitis B e antigen-positive patients with chronic hepatitis B in China. *Clin Infect Dis* 2007; 44: 541–8.
170 Janssen HL, van Zonneveld M, Senturk H *et al.* Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; 365: 123–9.
171 Perrillo R, Tamburro C, Regenstein F *et al.* Low-dose, titratable interferon alfa in decompensated liver disease caused by chronic infection with hepatitis B virus. *Gastroenterology* 1995; 109: 908–16.
172 Hoofnagle JH, Di Bisceglie AM, Waggoner JG, Park Y. Interferon alfa for patients with clinically apparent cirrhosis due to chronic hepatitis B. *Gastroenterology* 1993; 104: 1116–21.
173 Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment in patients with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. *Ann Intern Med* 1993; 119: 312–23.
174 Janssen HL, Gerken G, Carreno V *et al.* Interferon alfa for chronic hepatitis B infection: increased efficacy of prolonged treatment. The European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 1999; 30: 238–43.
175 Niederau C, Heintges T, Lange S *et al.* Long-term follow-up of HBeAg-positive patients treated with interferon alfa for chronic hepatitis B. *N Engl J Med* 1996; 334: 1422–7.
176 Lok AS, Chung HT, Liu VW, Ma OC. Long-term follow-up of chronic hepatitis B patients treated with interferon alfa. *Gastroenterology* 1993; 105: 1833–8.
177 Lin SM, Tai DI, Chien RN, Sheen IS, Chu CM, Liaw YF. Comparison of long-term effects of lymphoblastoid interferon alpha and recombinant interferon alpha-2a therapy in patients with chronic hepatitis B. *J Viral Hepat* 2004; 11: 349–57.
178 Fattovich G, Giustina G, Realdi G, Corrocher R, Schalm SW. Long-term outcome of hepatitis B e antigen-positive patients with compensated cirrhosis treated with interferon alfa. European Concerted Action on Viral Hepatitis (EUROHEP). *Hepatology* 1997; 26: 1338–42.
179 Fattovich G, Farci P, Rugge M *et al.* A randomized controlled trial of lymphoblastoid interferon-alpha in patients with chronic hepatitis B lacking HBeAg. *Hepatology* 1992; 15: 584–9.
180 Hadziyannis S, Bramou T, Makris A, Moussoulis G, Zignego L, Papaioannou C. Interferon alfa-2b treatment of HBeAg negative/serum HBV DNA positive chronic active hepatitis type B. *J Hepatol* 1990; 11 (Suppl 1): S133–136.
181 Papatheodoridis GV, Manesis E, Hadziyannis SJ. The long-term outcome of interferon-alpha treated and untreated patients with HBeAg-negative chronic hepatitis B. *J Hepatol* 2001; 34: 306–13.
182 Brunetto MR, Oliveri F, Coco B *et al.* Outcome of anti-HBe positive chronic hepatitis B in alpha-interferon treated and untreated patients: a long term cohort study. *J Hepatol* 2002; 36: 263–70.
183 Manesis EK, Hadziyannis SJ. Interferon alpha treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. *Gastroenterology* 2001; 121: 101–9.
184 Lampertico P, Del Ninno E, Vigano M *et al.* Long-term suppression of hepatitis B e antigen-negative chronic hepatitis B by 24-month interferon therapy. *Hepatology* 2003; 37: 756–63.
185 Cooksley WG, Piratvisuth T, Lee SD *et al.* Peginterferon alpha-2a (40 kDa): an advance in the treatment of hepatitis B e antigen-positive chronic hepatitis B. *J Viral Hepat* 2003; 10: 298–305.
186 Kao JH, Wu NH, Chen PJ, Lai MY, Chen DS. Hepatitis B genotypes and the response to interferon therapy. *J Hepatol* 2000; 33: 998–1002.
187 Wai CT, Chu CJ, Hussain M, Lok AS. HBV genotype B is associated with better response to interferon therapy in HBeAg(+) chronic hepatitis than genotype C. *Hepatology* 2002; 36: 1425–30.
188 Flink HJ, van Zonneveld M, Hansen BE, de Man RA, Schalm SW, Janssen HL. Treatment with Peg-interferon alpha-2b for HBeAg-positive chronic hepatitis B: HBsAg loss is associated with HBV genotype. *Am J Gastroenterol* 2006; 101: 297–303.
189 Buster EH, Flink HJ, Cakaloglu Y *et al.* Sustained HBeAg and HBsAg loss after long-term follow-up of HBeAg-positive patients treated with peginterferon alpha-2b. *Gastroenterology* 2008; 135: 459–67.
190 Manesis EK, Papatheodoridis GV, Hadziyannis SJ. A partially overlapping treatment course with lamivudine and interferon in hepatitis B e antigen-negative chronic hepatitis B. *Aliment Pharmacol Ther* 2006; 23: 99–106.
191 Shi M, Wang RS, Zhang H *et al.* Sequential treatment with lamivudine and interferon-alpha monotherapies in hepatitis B e antigen-negative Chinese patients and its suppression of lamivudine-resistant mutations. *J Antimicrob Chemother* 2006; 58: 1031–5.
192 Schalm SW, Heathcote J, Cianciara J *et al.* Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. *Gut* 2000; 46: 562–8.

193 Yurdaydin C, Bozkaya H, Cetinkaya H *et al.* Lamivudine vs lamivudine and interferon combination treatment of HBeAg(-) chronic hepatitis B. *J Viral Hepat* 2005; **12**: 262–8.
194 Sarin SK, Kumar M, Kumar R *et al.* Higher efficacy of sequential therapy with interferon-alpha and lamivudine combination compared to lamivudine monotherapy in HBeAg positive chronic hepatitis B patients. *Am J Gastroenterol* 2005; **100**: 2463–71.
195 Yang YF, Zhao W, Zhong YD, Xia HM, Shen L, Zhang N. Interferon therapy in chronic hepatitis B reduces progression to cirrhosis and hepatocellular carcinoma: a meta-analysis. *J Viral Hepat* 2009; **16**: 265–2671.
196 Lin SM, Sheen IS, Chien RN *et al.* Long-term beneficial effect of interferon therapy in patients with chronic hepatitis B virus infection. *Hepatology* 1999; **29**: 971–5.
197 van Zonneveld M, Honkoop P, Hansen BE *et al.* Long-term follow-up of alpha-interferon treatment of patients with chronic hepatitis B. *Hepatology* 2004; **39**: 804–10.

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Original Article

Accumulation of refractory factors for pegylated interferon plus ribavirin therapy in older female patients with chronic hepatitis C

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Aim: Several host and viral factors have been reported to influence the effectiveness of pegylated interferon plus ribavirin combination therapy for chronic hepatitis C. In Japan, where the age of treated patients is comparatively high, recent studies have reported poor response to treatment in older female patients, but little is known about the relationship between advanced age in women and previously reported factors.

Methods: Using a database of 1167 patients chronically infected with hepatitis C virus (HCV) genotype 1b, we analyzed the amino acid sequences of the HCV core protein and interferon sensitivity determining region (ISDR) and examined the relationships among predictive factors.

Results: The proportion of patients with substitutions at core 70, which is associated with poor response to pegylated interferon plus ribavirin therapy, increased with age only in female patients. A similar trend was observed for ISDR wild type (wt). We also found that core 70 wt is associated with core 91 wt ($P = 5.4 \times 10^{-9}$) as well as ISDR wt ($P = 0.025$). HCV RNA levels were higher in patients with core and ISDR wt ($P < 0.001$). Furthermore, core amino acid mutations were associated with advanced fibrosis and higher inflammatory activity ($P = 0.028$ and 0.048, respectively) as well as higher gamma-glutamyltranspeptidase, alanine aminotransferase and low-density lipoprotein cholesterol levels ($P < 0.001$, 0.006 and 0.001, respectively).

Conclusion: A combination of factors account for poor response rate in older female patients in Japan. Elucidating the relationship between amino acid substitutions and metabolic alteration is an important step in understanding the mechanism of HCV interferon resistance.

Key words: combination therapy, core protein, genotype 1b, interferon sensitivity determining region, low-density lipoprotein cholesterol

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INTRODUCTION

HEPATITIS C VIRUS (HCV) is a causative agent of acute and chronic hepatitis as well as liver cirrhosis and hepatocellular carcinoma.[1–3] The single stranded RNA genome encodes one large open reading frame that is processed into at least 10 proteins by host and viral enzymes.[4,5] Some viral proteins are known to affect the outcome of pegylated interferon (PEG IFN) plus ribavirin combination therapy, the current standard of care for chronic hepatitis.[6–8] The number of amino acid substitutions in the IFN sensitivity determining region (ISDR) of the NS5A protein, which was initially reported to affect IFN monotherapy,[9,10] has recently been reported to affect PEG IFN plus ribavirin combination therapy as well.[11–14]

NS5A PKR binding domain (PKRBD),[15–19] variable region 3 (V3),[20–23] IFN/ribavirin resistance determining region (IRRDR),[24,25] and E2 PKR-eIF2α phosphorylation homology domain (PePHD)[26] have also been reported to affect therapy outcome, although these results need to be confirmed. More recently, amino acid (a.a.) substitutions in the core protein have been reported to negatively affect IFN plus ribavirin therapy.[27,28] Substitution at a.a. 70 of the core protein (core 70) has been reported to be associated with non-virological response (NVR), and this finding was confirmed by several groups.[29–31]

Several cytokines and adipokines have also been reported to be associated with the effectiveness of therapy. For instance, tumor necrosis factor (TNF)-α expression has been reported to be elevated in patients with HCV infection, and high expression levels are associated with poor response to IFN therapy.[32] IP-10 has also been reported to associate with response to therapy in patients with HCV and HIV co-infection.[33] Leptin and adiponectin levels are also reportedly associated with the effect of combination therapy.[34,35] In addition to these factors, there are many studies reporting relationships between common polymorphisms in the human genome and outcome of IFN therapy.[36–44] Among them, single nucleotide polymorphisms (SNP) in the interleukin (IL)-28B locus discovered through genome-wide association studies appear to have a large effect on outcome of PEG IFN plus ribavirin combination therapy[42–44] as well as spontaneous eradication of HCV.[45]

In addition to the above viral and host genetic factors, several metabolic factors such as obesity,[34] insulin resistance[46] and low-density lipoprotein (LDL) cholesterol levels[28,47] have been reported to be correlated with the effect of combination therapy. Furthermore, higher gamma-glutamyltranspeptidase (γ-GTP) levels, often associated with fatty liver, have also been reported to be associated with treatment outcome.[48,49] Although these factors may be mutually interdependent, their relationships with viral factors have not yet been analyzed.

Recent papers have reported poor response to therapy in older female patients,[50–52] but little is known about the relationship between age, sex and other predictive factors. To analyze these associations, we constructed a database consisting of 1425 patients with chronic hepatitis C. Using this database, we analyzed the relationship between viral and metabolic data and found that a.a. substitutions in the core and ISDR are associated with metabolic change, which may be related to disease progression and response to therapy.

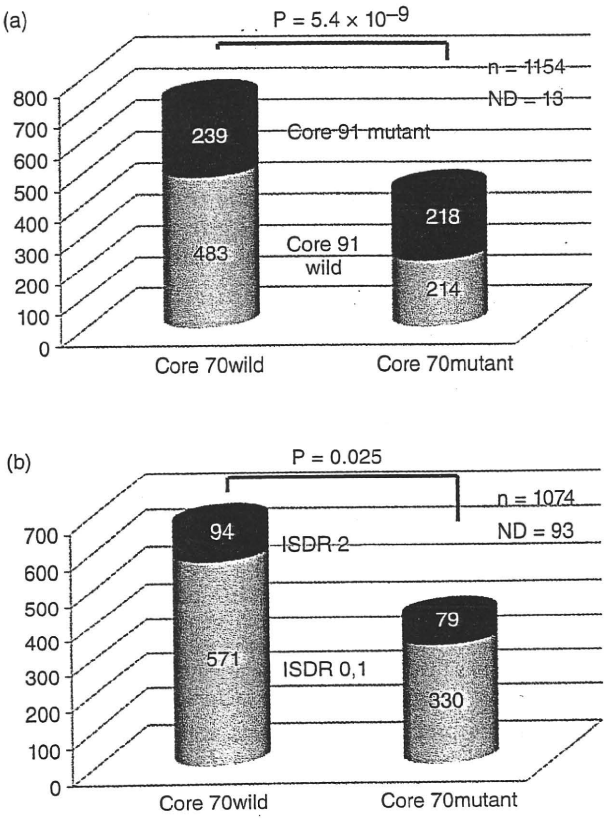


Figure 1 Association of core amino acid 70, amino acid 91 and interferon sensitivity determining region (ISDR). The relationship between hepatitis C virus core 70 and core 91 wild type and mutant amino acids (a) and the ISDR (b) were examined. Statistical significance was assessed using the χ^2-test. ND, not determined due to polymerase chain reaction or sequence calling failure.

Table 1 Clinical profile of 1167 patients

	All patients $n = 1167$	Tx naive $n = 570$ (48.84%)	Prev. tx $n = 597$ (51.16%)	*P*-value
Sex (male/female)	606/561	259/311	347/250	1.45E-05
Age	55.1 ± 10.7	55.2 ± 11.0	55.0 ± 10.5	0.604
Body weight	60.6 ± 10.8	59.5 ± 10.5	61.7 ± 11.0	0.001
BMI	27.0 ± 7.38	24.3 ± 5.46	29.6 ± 8.02	0
Fibrosis stage (0–2/3–4/ND)	815/192/160	422/78/70	393/114/90	0.005
Activity stage (0–1/2–3/ND)	531/465/171	263/234/73	268/231/98	0.803
Steatosis (present/absent/ND)	207/428/532	103/175/292	104/253/240	0.034
White blood cells (/mm^3)	4808 ± 1428	4871 ± 1395	4748 ± 1457	0.127
Hemoglobin (g/dL)	14.1 ± 1.88	14.0 ± 1.39	14.3 ± 2.23	0.001
Platelets (×10^4/mm^3)	16.6 ± 5.06	16.5 ± 5.31	16.7 ± 4.82	0.288
ALT (IU/L)	66 ± 52	67 ± 48	65 ± 55	0.265
AST (IU/L)	65 ± 54	58 ± 37	71 ± 66	0.001
γ-GTP (IU/L)	56 ± 58	57 ± 62	55 ± 54	0.942
Albumin (g/dL)	4.00 ± 0.375	4.04 ± 0.402	3.97 ± 0.347	0.001
Total cholesterol (mg/dL)	173 ± 32.1	175 ± 32.7	172 ± 31.6	0.206
Fasting blood sugar (mg/dL)	101 ± 24.9	102 ± 27.2	99.8 ± 22.2	0.715
HCV RNA (KIU/mL: amp)	2999 ± 4523	2822 ± 4365	3169 ± 4668	0.048
ISDR (0–1/≥2/ND)	908/178/81	440/85/45	468/93/36	0.863
Core 70 (wild/mutant/ND)	722/433/12	349/218/3	373/215/9	0.509
Core 91 (wild/mutant/ND)	697/457/13	349/217/4	348/240/9	0.39

ALT, alanine aminotransferase; AST, aspartate aminotransferase; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; ND, not determined; tx., treatment.

METHODS

Study subjects

WE COLLECTED DATA from 1425 participating patients with chronic hepatitis C from 16 centers in Japan. Inclusion criteria included testing positive for HCV RNA over a period of more than 6 months and testing negative for both hepatitis B virus surface antigen and anti-HIV antibody. Patients with confounding liver conditions were excluded, as well as patients who were lost to follow up or who did not have high viral load (≥5 log IU/mL) for HCV genotype 1b (Fig. 1). Patient data was not used when we failed to determine core 70, core 90 and ISDR sequences. In total, data from 1167 patients were included in the analysis. All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

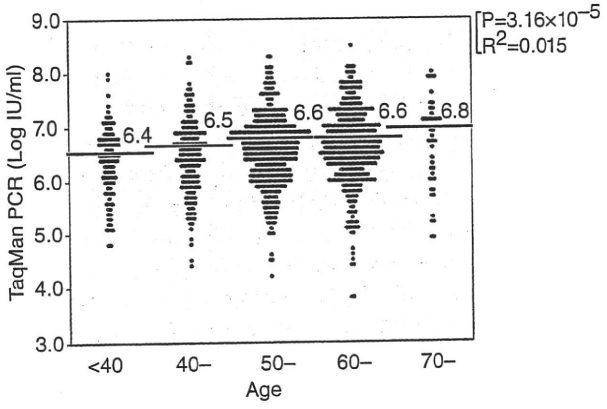


Figure 2 Relationship between age and virus titer. Virus titers were plotted according to age. The median titer within each 10-year age group is shown as horizontal bars.

Patients received weekly injections of PEG IFN-α-2b for either 48 or 72 weeks using the following doses: 60 μg for 35–45 kg bodyweight; 80 μg for 46–60 kg; 100 μg for 61–75 kg; 120 μg for 76–90 kg; and 150 μg for 91–120 kg. Ribavirin was administrated p.o., and the dose was determined based on the patient's bodyweight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Ribavirin dosage was reduced when hemoglobin levels reduced to 10.0 g/dL and stopped if hemoglobin levels reached 8.5 g/dL. Bio-

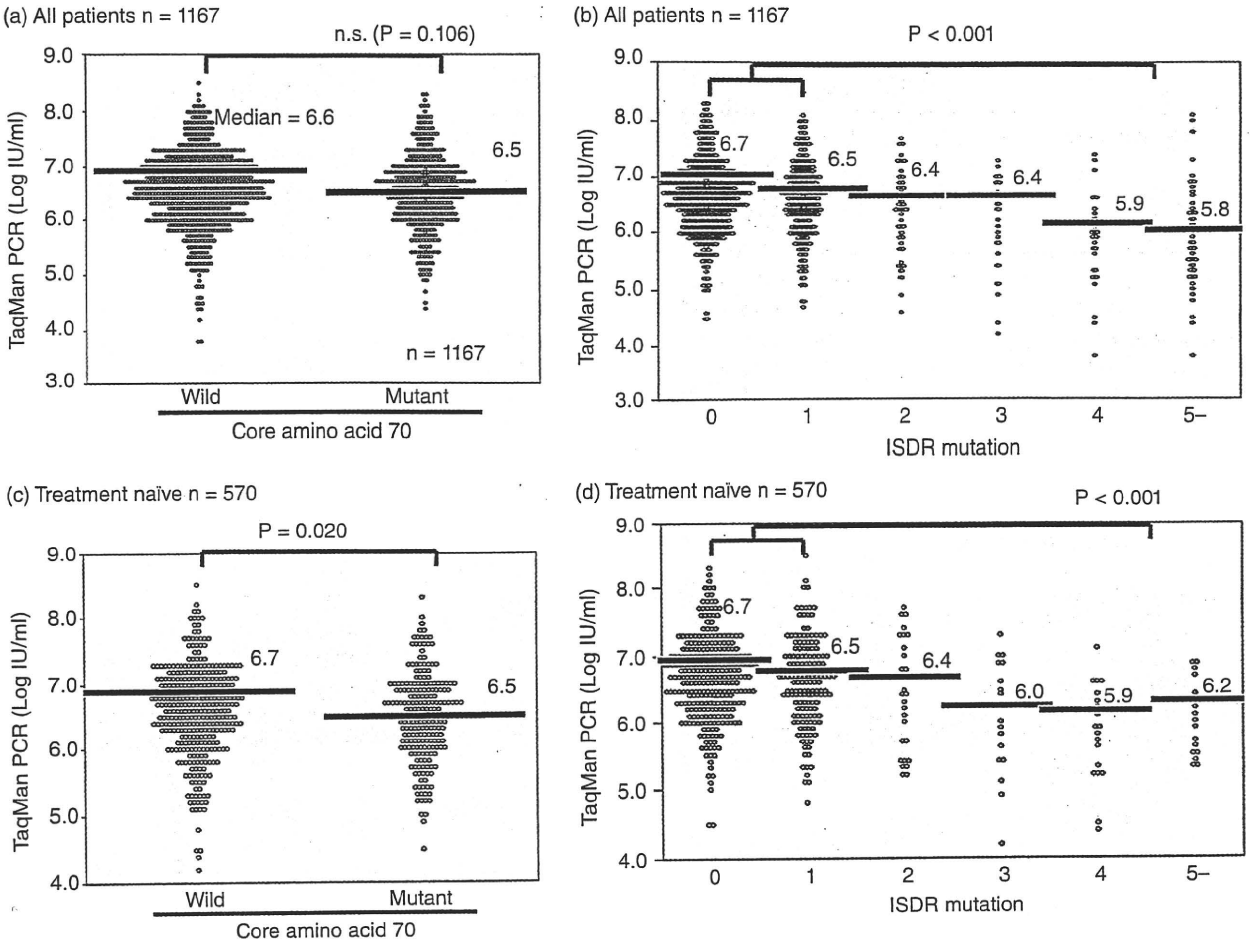


Figure 3 Analysis of virus load by core amino acid 70 substitution and number of amino acid substitutions in the interferon sensitivity determining region (ISDR). Virus titers of all 1167 patients were classified according to core 70 wild type and mutant amino acids (a) or by the number of substitutions in the ISDR (b). The 570 interferon therapy naive patients were also examined separately (c,d).

chemical tests were performed by center, and pathological diagnosis was made according to the criteria of Desmet *et al.*[53] Successful treatment was ascertained based on sustained virological response (SVR), defined as HCV RNA negative 6 months after cessation of therapy.

Analysis of viral titer and a.a. sequences in the core and ISDR region

The HCV RNA level was analyzed using reverse transcription polymerase chain reaction (RT-PCR)-based methods (Amplicor Hepatitis C Virus test: Roche Diagnostics, Basel, Switzerland; high range test: Cobas Amplicor, Roche Diagnostics, Basel, Switzerland; or TaqMan RT-PCR test: Applied Biosystems, Foster city, CA, USA). The measurement ranges of these assays were 5–5000 KIU/mL and 1.2–7.8 log IU, respectively. For values exceeding the measurable range, the titer was determined after dilution of the serum samples.

Sequences were determined by direct sequencing of PCR fragments following extraction and RT of serum HCV RNA. For core 70 and 91, arginine and leucine were considered wild type (wt) according to Akuta *et al.*[27,28] The number of a.a. substitutions in the ISDR was determined as described previously.[9,10,53]

Statistical analysis

The χ^2-test and Mann–Whitney *U*-test were applied to detect significant associations using PASW ver. 18

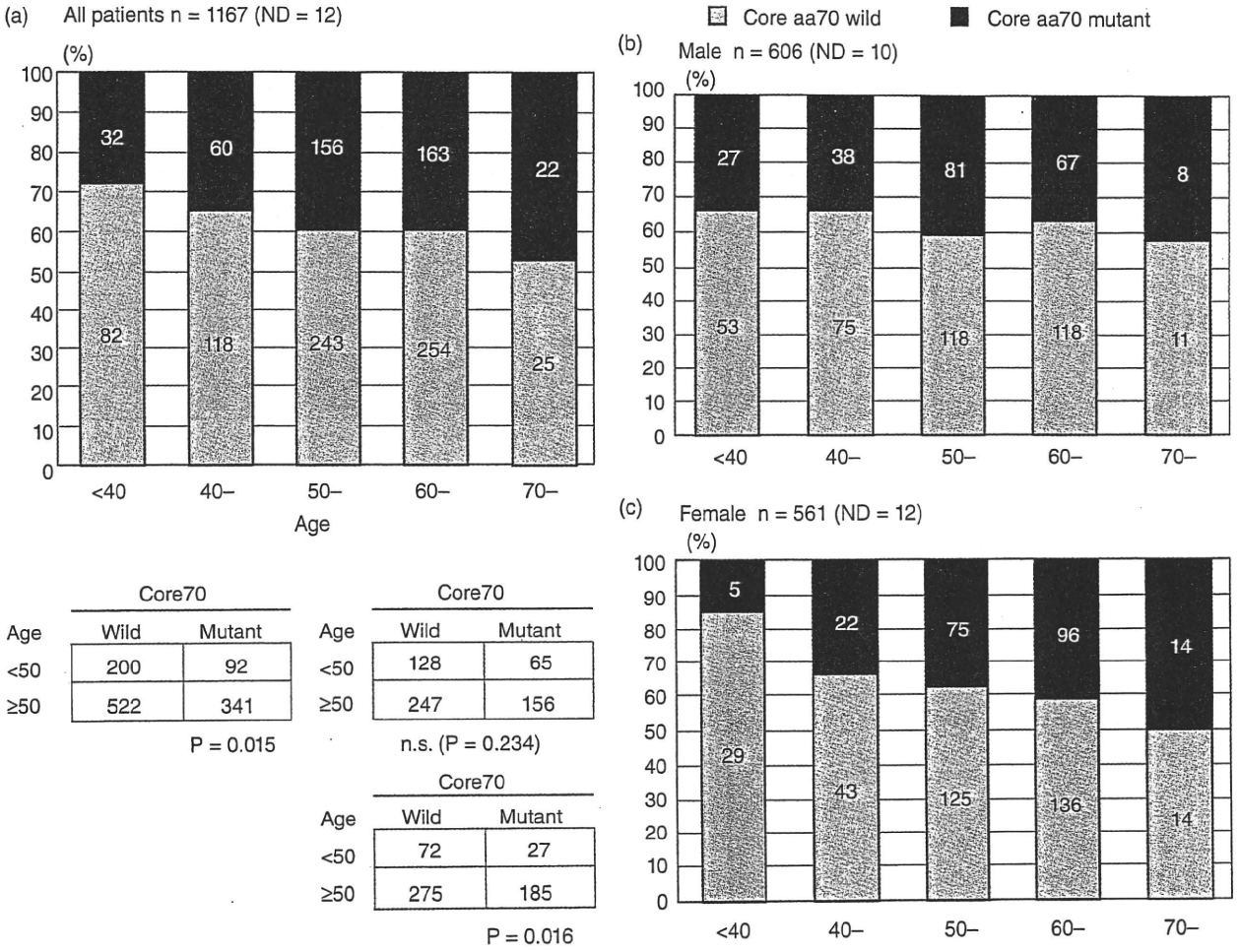


Figure 4 Age-dependent increase in core amino acid 70 mutants in female patients. Percentages of core wild type (arginine) and mutant amino acids for all patients (a), as well as for male (b) and female (c) patients are shown. Note that the age-dependent increase in mutant frequency was observed only in female patients. Statistical analysis was performed by χ^2-test. ND, not determined.

(SPSS, Chicago, IL, USA). All statistical analyses were two sided, and $P < 0.05$ was considered significant. Simple and multiple regression analyses were used to examine the association between viral substitutions and clinical factors using $P < 0.05$ as the criterion for inclusion in the multivariate model. Continuous variables were split into indicator variables based on the median, except for age which was divided into 10-year intervals. Multivariate logistic regression analysis was performed using the Design package in R (www.r-project.org) with fast backward elimination and validation based on AIC score.

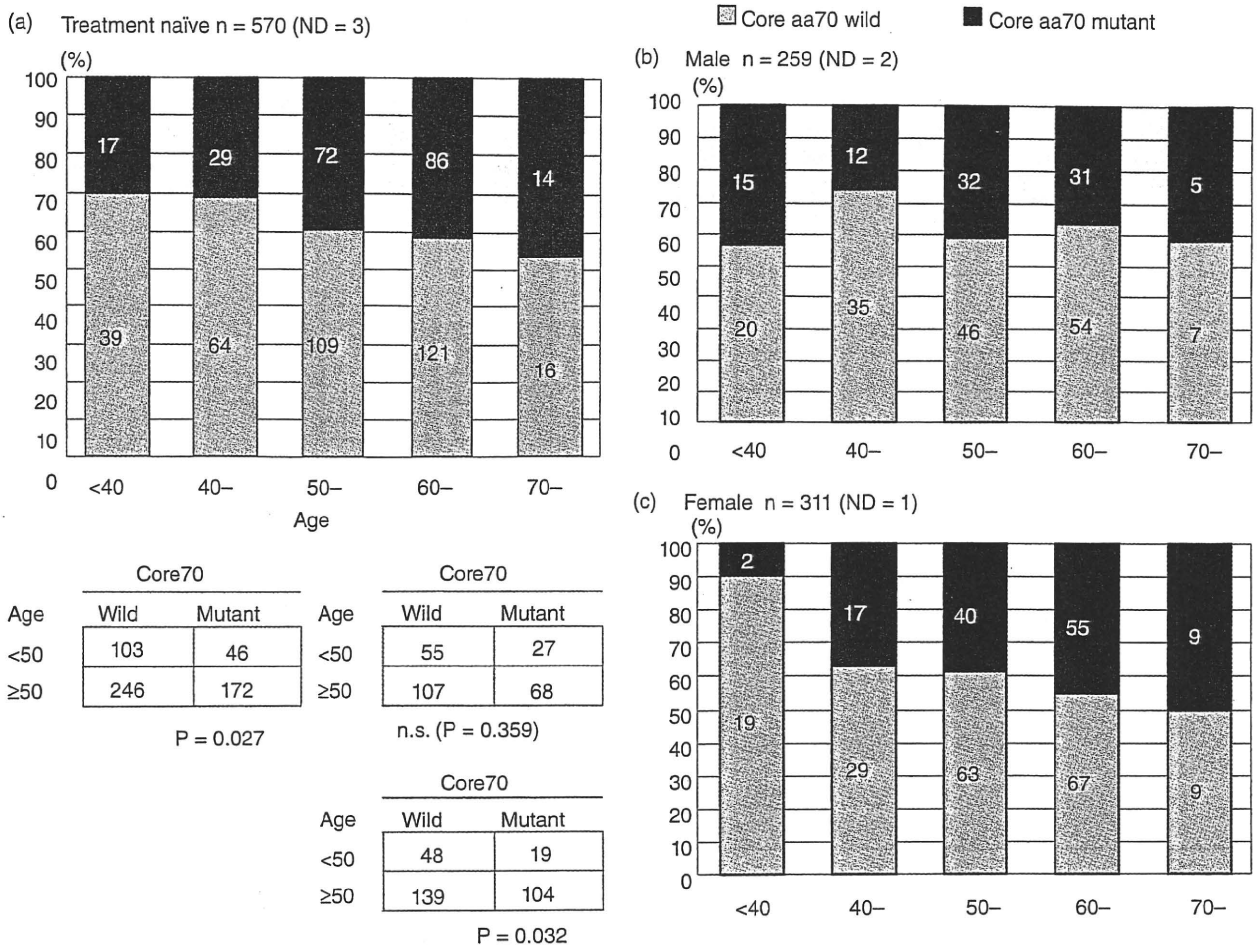
RESULTS

Patient characteristics

PATIENT PROFILES ARE shown in Table 1. Results are presented separately for patients who were naive to IFN therapy and those who had had previous IFN therapy but failed to eradicate the virus.

Virus titer and a.a. substitutions in the core and the ISDR

We found a significant positive correlation between patient age and virus titer ($P = 3.16 \times 10^{-5}$, $R^2 = 0.015$,



Age	Core70 Wild	Core70 Mutant
<50	103	46
≥50	246	172

P = 0.027

Age	Core70 Wild	Core70 Mutant
<50	55	27
≥50	107	68

n.s. (P = 0.359)

Age	Core70 Wild	Core70 Mutant
<50	48	19
≥50	139	104

P = 0.032

Figure 5 Age-dependent increase in core amino acid 70 mutants in treatment-naive female patients. Percentage of core wild type (arginine) and mutant amino acid were analyzed as in Figure 5 using only interferon treatment-naive patients. Results for all 561 patients (a), as well as for male (b) and female (c) patients are shown. ND, not determined.

Fig. 2). Wt core 70 was associated with wt core 91, with 40% of patients wt for both core 70 and core 91 and 20% of patients non-wt for both (Fig. 1, $P = 5.4 \times 10^{-9}$). Virus titer did not differ in patients with wt core 70 compared to non-wt when all patients were included (Fig. 3a), but when treatment-naive patients were analyzed separately, virus titer was significantly higher in patients with core 70 wt ($P = 0.02$, Fig. 3c). We found a significant negative linear relationship between virus titer and the number of substitutions in the ISDR ($P < 0.001$, Fig. 3b), regardless of treatment history ($P < 0.001$, Fig. 3d).

Amino acid substitution and age

The proportion of patients with core 70 substitutions increased with age among female patients (Figs 4,5), and the proportion of patients without substitutions in the ISDR tended to increase with age among treatment-naive females ($P = 0.0581$, Fig. 6).

Core 70 a.a. substitution and histological findings

Fibrosis stage and activity were higher in patients with core 70 mutants ($P = 0.028$ and $P = 0.048$, respectively; Fig. 7). There was no apparent correlation between his-

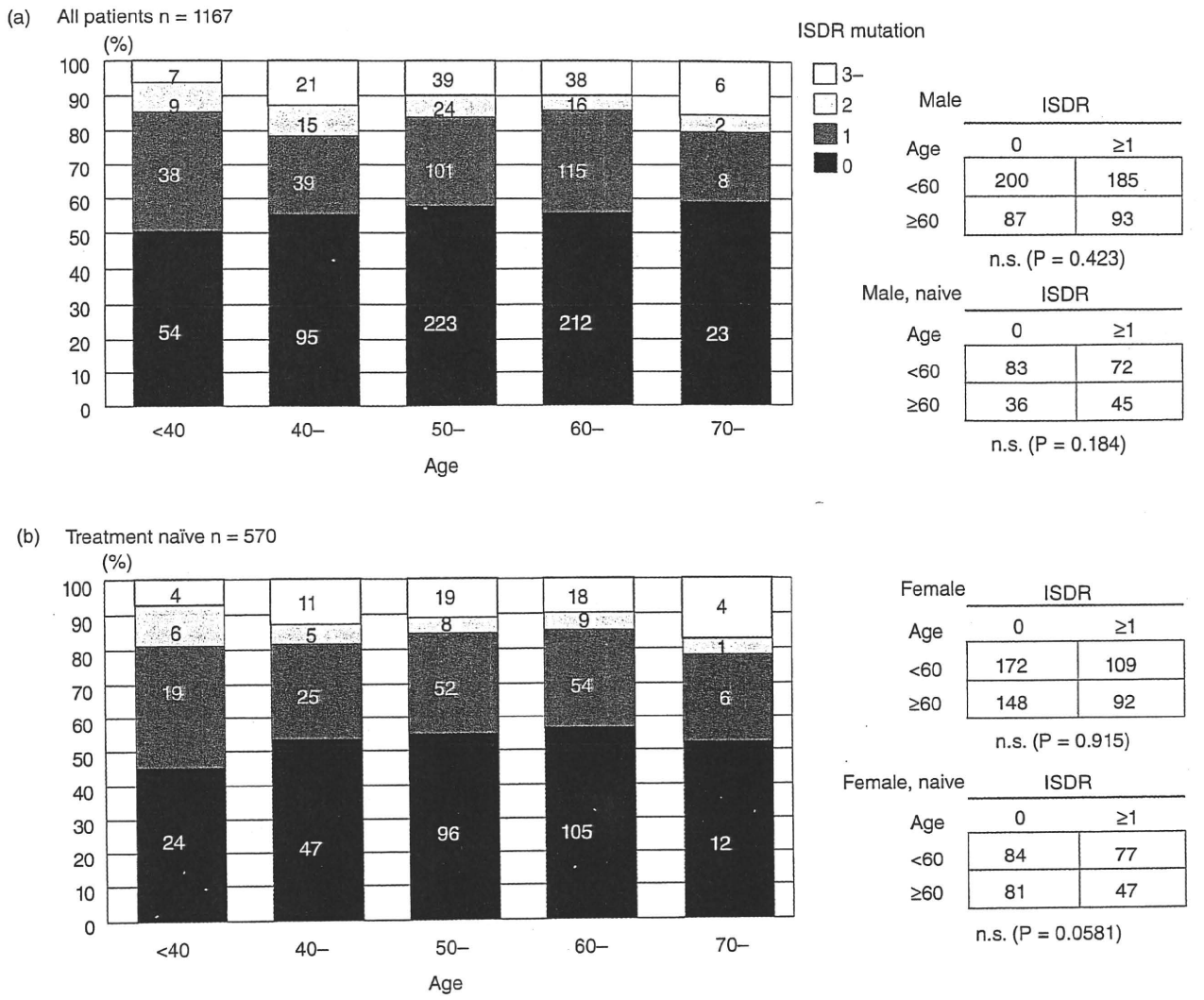


Figure 6 Age-dependent increase in number of amino acid substitutions in the interferon sensitivity determining region (ISDR). The relationship between age and the number of amino acid substitutions in the ISDR was examined. All patients (a) and only naive patients (b) were analyzed. Statistical analysis was performed using the χ^2-test.

tological findings and the number of a.a. substitutions in the ISDR (data not shown).

Correlation between viral a.a. substitutions and clinical conditions

We compared γ-GTP, ALT, LDL cholesterol levels and other clinical conditions between patients with core 70 wild and mutant types (Fig. 8). ALT and γ-GTP levels were significantly higher in patients with core 70 substitutions (Fig. 8a,b). In contrast, LDL cholesterol levels and platelet counts were significantly higher in patients with core 70 wt (Fig. 8c,d). However, only sex, fibrosis, γ-GTP and core 91 substitution were independently associated with core 70 substitution (Table 2). Only viral load and core 70 substitutions are independent predictive factors for the presence of two or more ISDR substitutions (Table 3).

DISCUSSION

WE FOUND THAT factors previously reported to be associated with poor response to IFN-based treatment for chronic hepatitis C tended to be most strongly associated with older female patients. Studies on difficult-to-treat older female patients have so far only been reported in Japan, probably due to the rela-

Table 2 Factors associated with HCV core protein amino acid 70 substitutions

Variable	Simple			Multiple			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age (in 10-year increments)	331	1.1	0.3536				
Sex (male vs female)	365	1.58	0.04178	214	2.09	(1.11–3.95)	0.0234
BMI (kg/m^2)	363	0.763	0.2229				
Diabetes	312	1.77	0.08053				
Fibrosis (F0–1 vs F2–4)	252	2.12	0.007444	214	2.18	(1.15–4.13)	0.017
Activity (A0–1 vs A2–4)	246	1.73	0.04849				
ALT (IU/L)	329	0.866	0.5461				
Platelets (×104/mm^3)	329	0.937	0.7836				
γ-GTP (IU/L)	305	1.69	0.03427	214	1.59	(0.841–3.02)	0.153
Albumin (g/dL)	190	0.765	0.3981				
Fasting blood sugar (mg/dL)	250	0.898	0.6878				
TaqMan PCR (log IU/mL)	327	0.748	0.2232				
HDL cholesterol (mg/dL)	202	1.64	0.1025				
LDL cholesterol (mg/dL)	165	1.25	0.5085				
Total cholesterol (mg/dL)	321	0.907	0.6847				
Core 91 (wild vs others)	365	2.22	0.000393	214	2.68	(1.43–5.02)	0.002
ISDR (0,1 vs >1)	343	1.82	0.03102	214	1.85	(0.853–4)	0.1197

Simple and multiple logistic regression were used to examine the association between substitution at core amino acid 70 and patient and viral factors.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; HDL, high-density lipoprotein; ISDR, interferon sensitivity determining region; LDL, low-density lipoprotein; ND, not determined; OR, odds ratio.

Table 3 Factors associated with viral ISDR substitutions (0–1 vs >1 mutations)

Variable	Simple			Multiple			
	n	OR	*P*	*n*	OR	(95% CI)	*P*
Age (in 10-year increments)	311	1	0.9735				
Sex (male vs female)	345	0.644	0.1247				
BMI (kg/m^2)	343	1.14	0.6254				
Diabetes	293	0.818	0.6509				
Fibrosis (F0–1 vs F2–4)	235	1.28	0.4545				
Activity (A0–1 vs A2–4)	229	1.3	0.4281				
ALT (IU/L)	309	1.15	0.646				
Platelets (×104/mm^3)	309	0.668	0.1707				
γ-GTP (IU/L)	287	1.47	0.2115				
Albumin (g/dL)	172	0.979	0.9622				
Fasting blood sugar (mg/dL)	233	1.36	0.3641				
TaqMan PCR (log IU/mL)	307	0.517	0.02527	305	0.529	(0.30–0.95)	0.03223
HDL cholesterol (mg/dL)	189	1.23	0.617				
LDL cholesterol (mg/dL)	152	0.463	0.1199				
Total cholesterol (mg/dL)	303	0.656	0.1537				
Core 70 (wild vs others)	343	1.82	0.03102	305	1.82	(1.01–3.3)	0.04763
Core 91 (wild vs others)	344	0.699	0.2038				

Simple and multiple logistic regression was used to examine the association between the number of substitutions in the ISDR region and patient and viral factors.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; γ-GTP, gamma-glutamyltranspeptidase; HCV, hepatitis C virus; HDL, high-density lipoprotein; ISDR, interferon sensitivity determining region; LDL, low-density lipoprotein; ND, not determined; OR, odds ratio.

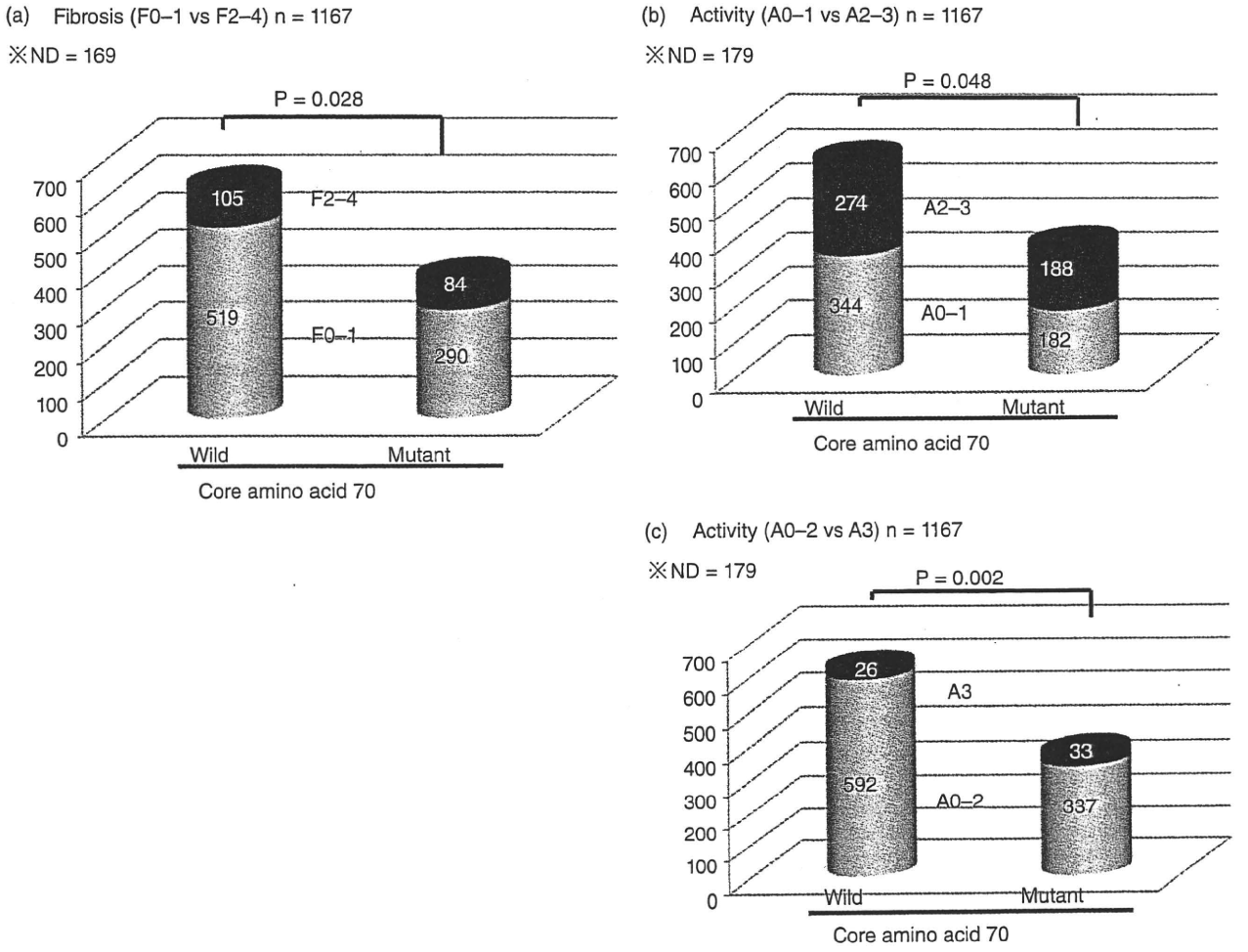


Figure 7 Histological findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and degree of fibrosis (F0–1 and F2–4) (a) and activity (b,c) were examined. Activity was divided into A0–1 and A2–3 (b) or A0–2 and A3 (c) and compared with amino acid 70. ND, not determined.

tively higher age at treatment. The mechanism underlying this association is unknown. Recently, SNP in the IL-28B locus were found to be associated with response to combination therapy as well as to spontaneous eradication of the virus,[42–44] although differences in the eradication rate between men and women have not been reported so far. We have previously reported that incidence of wt core 70 is significantly higher in patients with the IL-28 protective allele.[54] Therefore, it seems reasonable that the wt core 70 confers a selective advantage for the virus in patients with the IL-28 protective allele. During the time when IFN monotherapy was still the standard treatment, female sex, or perhaps the lower iron concentration associated with female sex, had been reported as one of the predictive factors for a favorable response to monotherapy.[55–57] It is possible that spontaneous eradication of the virus occurs during the natural course of chronic hepatitis through IFN produced naturally as a result of liver inflammation in young female patients with wt core 70, resulting in accumulation of core mutant viruses as the patient ages. Further prospective observations are necessary to address this issue.

In this study, we found that each of the previously reported predictive factors that we examined also correlated with HCV a.a. substitutions. Interestingly, a.a. substitutions in the virus are associated with metabolic factors such as LDL and high-density lipoprotein cholesterol and fatty liver-related γ-GTP, and in particular, we found that substitution in the core protein (and possibly ISDR) is correlated with LDL cholesterol. The virus appears to influence expression of genes involved

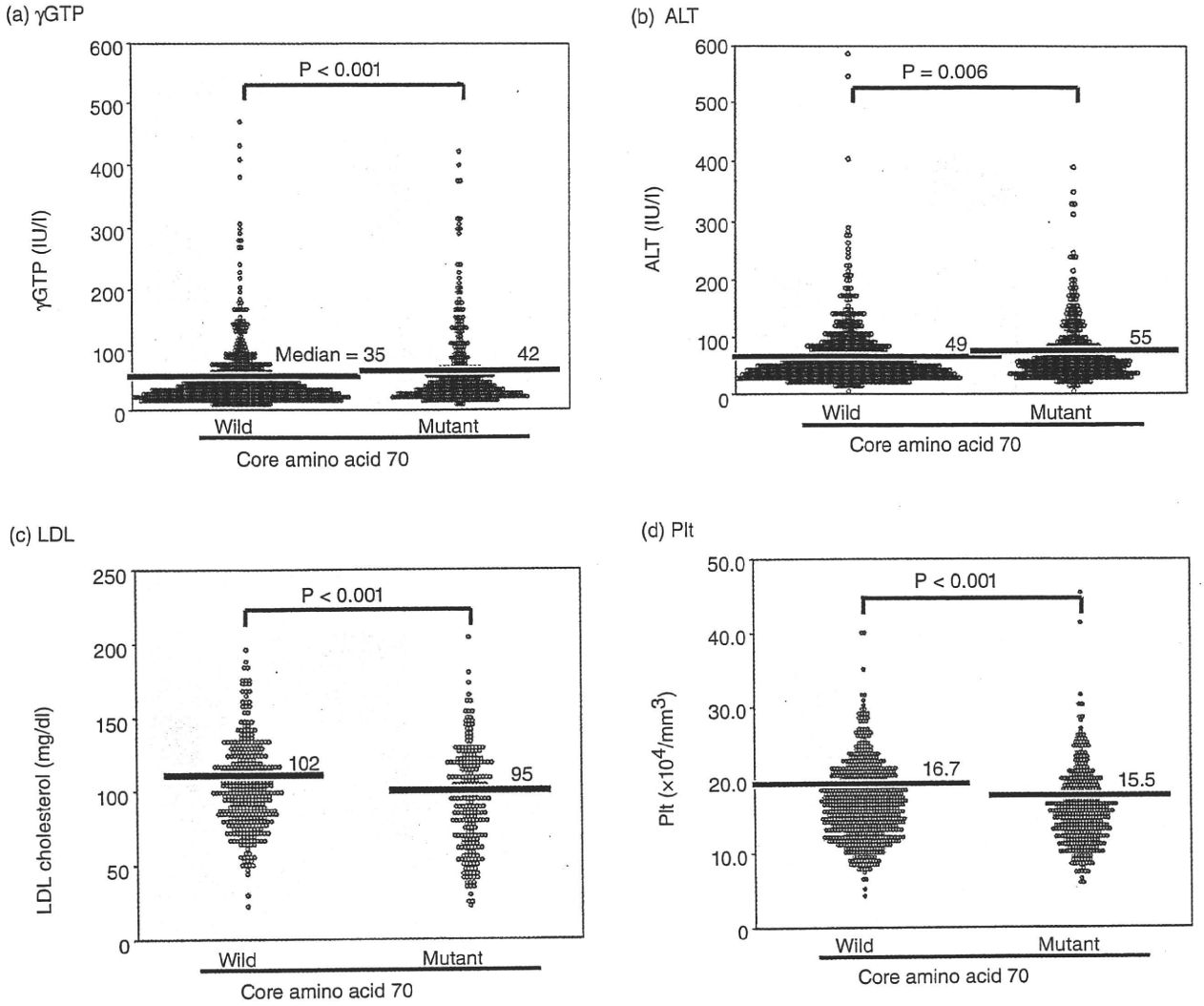


Figure 8 Relationship between blood test findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and gamma-glutamyltranspeptidase (γ-GTP) (a), alanine aminotransferase (ALT) (b), low-density lipoprotein (LDL) cholesterol (c) and platelet count (Plt) (d) were examined. Bars represent the median.

in host cell lipid metabolism to enhance its own replication and secretion.[58] Consequently, metabolic changes induced by infection by different strains of HCV should be investigated further to understand viral mechanisms of IFN resistance and to develop effective personalized therapies.

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REFERENCES

1 Barrera J, Bruguera M, Ercilla M *et al.* Persistent hepatitis C viremia after acute self-limiting posttransfusion hepatitis C. *Hepatology* 1995; 21: 639–44.
2 Niederau C, Lange S, Heintges T *et al.* Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology* 1998; 28: 1687–95.
3 Kiyosawa K, Sodeyama T, Tanaka E *et al.* Interrelationship of blood-transfusion, non-A, non-B hepatitis and

hepatocellular-carcinoma – analysis by detection of antibody to hepatitis-C virus. *Hepatology* 1990; **12**: 671–5.
4 Penin F, Dubuisson J, Rey FA, Moradpour D, Pawlotsky JM. Structural biology of hepatitis C virus. *Hepatology* 2004; **39**: 5–19.
5 Suzuki T, Aizaki H, Murakami K, Shoji I, Wakita T. Molecular biology of hepatitis C virus. *J Gastroenterol* 2007; **42**: 411–23.
6 Hadziyannis S, Sette HJ, Morgan T *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–55.
7 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958–65.
8 Jensen DM, Marcellin P, Freilich B *et al.* Re-treatment of patients with chronic Hepatitis C who do not respond to peginterferon-alpha 2b a randomized trial. *Ann Intern Med* 2009; **150**: 528–40.
9 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis-C virus 1b – sensitivity to interferon is conferred by amino-acid substitutions in the NS5A region. *J Clin Invest* 1995; **96**: 224–30.
10 Enomoto N, Sakuma I, Asahina Y *et al.* 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.
11 Yen YH, Hun CH, Hu TH *et al.* Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209–2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. *Aliment Pharmacol Ther* 2008; **27**: 72–9.
12 Akuta N, Suzuki F, Hirakawa M *et al.* A matched case-controlled study of 48 and 72 weeks of peginterferon plus ribavirin combination therapy in patients infected with HCV genotype 1b in Japan: Amino acid substitutions in HCV core region as predictor of sustained virological response. *J Med Virol* 2009; **81**: 452–8.
13 Hung CH, Lee CM, Lu SN *et al.* Mutations in the NS5A and E2-PePHD region of hepatitis C virus type 1b and correlation with the response to combination therapy with interferon and ribavirin. *J Viral Hepat* 2003; **10**: 87–94.
14 Hayashi K, Katano Y, Ishigami M *et al.* Mutations in the core and NS5A region of hepatitis C virus genotype 1b and correlation with response to pegylated-interferon-alpha 2b and ribavirin combination therapy. *J Viral Hepat* 2010.
15 de Rueda PM, Casado J, Paton R *et al.* Mutations in E2-PePHD, NS5A-PKRBD, NS5A-ISDR, and NS5A-V3 of hepatitis C virus genotype 1 and their relationships to pegylated interferon-ribavirin treatment responses. *J Virol* 2008; **82**: 6644–53.
16 Berg T, Marques AM, Hohne M, Wiedenmann B, Hopf U, Schreier E. Mutations in the E2-PePHD and NS5A region of hepatitis C virus type 1 and the dynamics of hepatitis C viremia decline during interferon alfa treatment. *Hepatology* 2000; **32**: 1386–95.
17 MacQuillan GC, Niu XW, Speers D *et al.* Does sequencing the PKRBD of hepatitis C virus NS5A predict therapeutic response to combination therapy in an Australian population? *J Gastroenterol Hepatol* 2004; **19**: 551–7.
18 Sarrazin C, Herrmann E, Bruch K, Zeuzem S. Hepatitis C virus nonstructural 5A protein and interferon resistance: a new model for testing the reliability of mutational analyses. *J Virol* 2002; **76**: 11079–90.
19 Jenke ACW, Moser S, Orth V, Zilbauer M, Gerner P, Wirth S. Mutation frequency of NS5A in patients vertically infected with HCV genotype 1 predicts sustained virological response to peginterferon alfa-2b and ribavirin combination therapy. *J Viral Hepat* 2009; **16**: 853–9.
20 Layden-Almer JE, Kuiken C, Ribeiro RM *et al.* Hepatitis C virus genotype 1a NS5A pretreatment sequence variation and viral kinetics in African American and white patients. *J Infect Dis* 2005; **192**: 1078–87.
21 Vuillermoz I, Khattab E, Sablon E *et al.* Genetic variability of hepatitis C virus in chronically infected patients with viral breakthrough during interferon-ribavirin therapy. *J Med Virol* 2004; **74**: 41–53.
22 Puig-Basagoiti F, Forns X, Furcic I *et al.* Dynamics of hepatitis C virus NS5A quasispecies during interferon and ribavirin therapy in responder and non-responder patients with genotype 1b chronic hepatitis C. *J Gen Virol* 2005; **86**: 1067–75.
23 Wohnsland A, Hofmann WP, Sarrazin C. Viral determinants of resistance to treatment in patients with hepatitis C. *Clin Microbiol Rev* 2007; **20**: 23–38.
24 El-Shamy A, Sasayama M, Nagano-Fujii M *et al.* Prediction of efficient virological response to pegylated interferon/ribavirin combination therapy by NS5A sequences of hepatitis C virus and anti-NS5A antibodies in pre-treatment sera. *Microbiol Immunol* 2007; **51**: 471–82.
25 El-Shamy A, Nagano-Fujii M, Sasase N, Imoto S, Kim SR, Hotta H. Sequence variation in hepatitis C virus nonstructural protein 5A predicts clinical outcome of pegylated interferon/ribavirin combination therapy. *Hepatology* 2008; **48**: 38–47.
26 Yang SS, Lai MY, Chen DS, Chen GH, Kao JH. Mutations in the NS5A and E2-PePHD regions of hepatitis C virus genotype 1b and response to combination therapy of interferon plus ribavirin. *Liver International* 2003; **23**: 426–33.
27 Akuta N, Suzuki F, Sezaki H *et al.* Predictive factors of virological non-response to interferon-ribavirin combination therapy for patients infected with hepatitis C virus of genotype 1b and high viral load. *J Med Virol* 2006; **78**: 83–90.