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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.

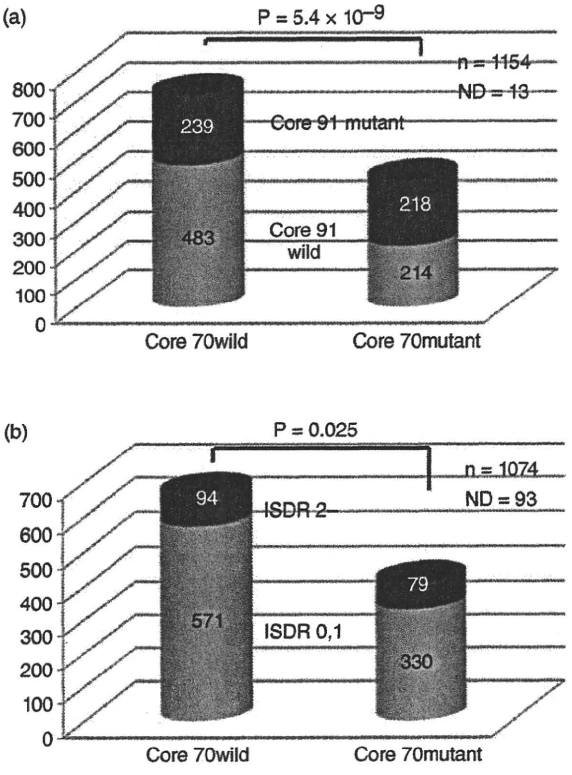


Figure 1 Association of core amino acid 70, amino acid 91 and interferon sensitivity determining region (ISDR). The relationship between hepatitis C virus core 70 and core 91 wild type and mutant amino acids (a) and the ISDR (b) were examined. Statistical significance was assessed using the χ^2-test. 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 (x10^4/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.

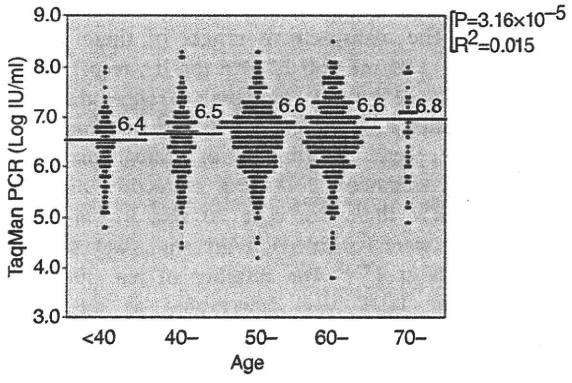


Figure 2 Relationship between age and virus titer. Virus titers were plotted according to age. The median titer within each 10-year age group is shown as horizontal bars.

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-

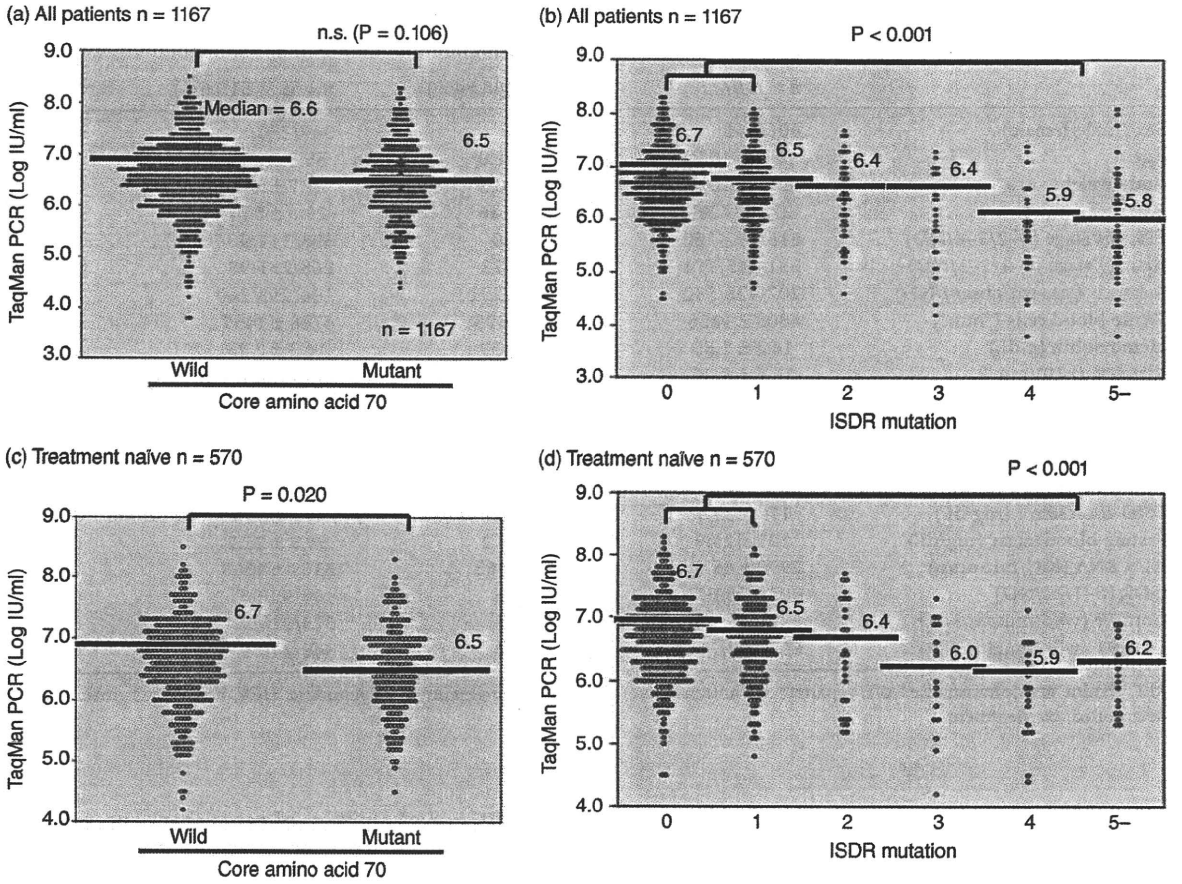


Figure 3 Analysis of virus load by core amino acid 70 substitution and number of amino acid substitutions in the interferon sensitivity determining region (ISDR). Virus titers of all 1167 patients were classified according to core 70 wild type and mutant amino acids (a) or by the number of substitutions 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.

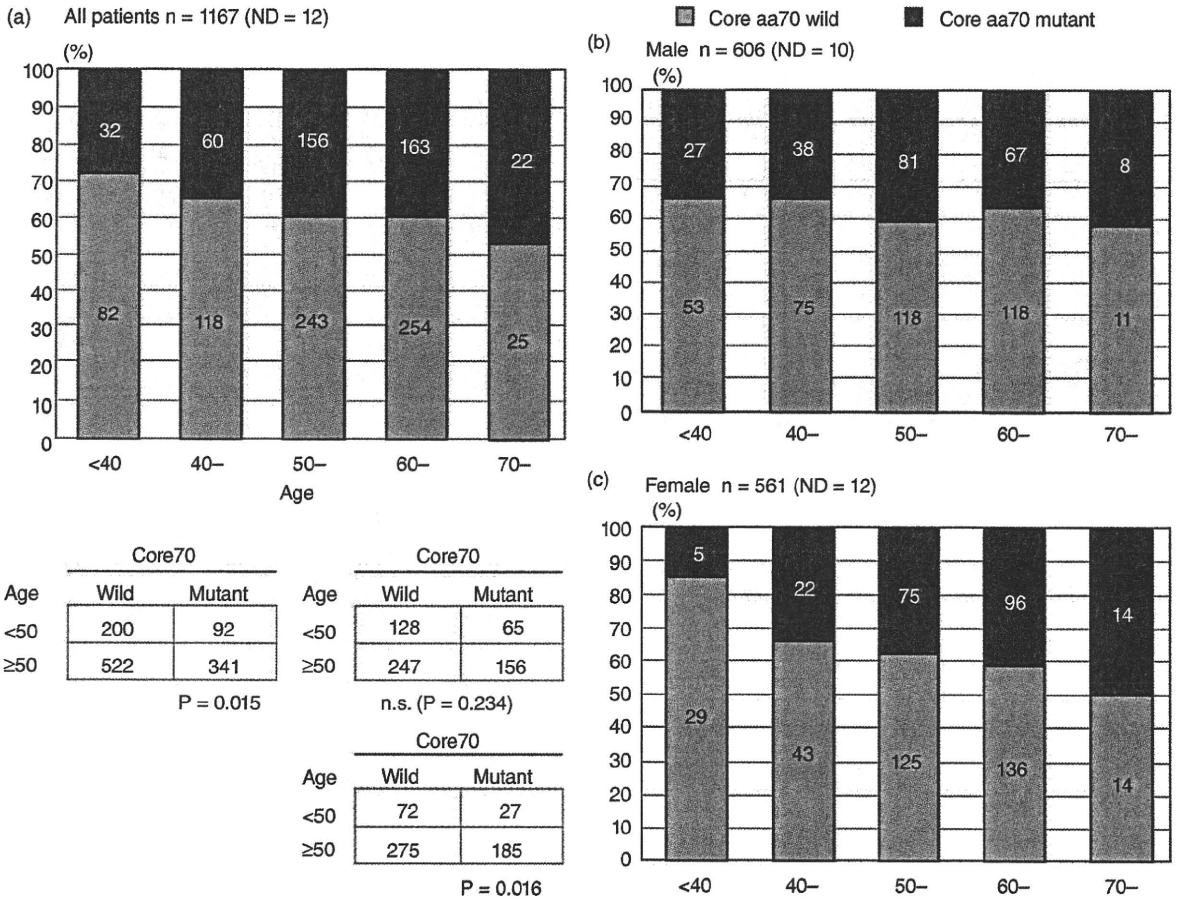
Analysis of viral titer and a.a. sequences in the core and ISDR region

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



Age	Core70 Wild	Core70 Mutant
<50	200	92
≥50	522	341

P = 0.015

Age	Core70 Wild	Core70 Mutant
<50	128	65
≥50	247	156

n.s. (P = 0.234)

Age	Core70 Wild	Core70 Mutant
<50	72	27
≥50	275	185

P = 0.016

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 ($P = 3.16 \times 10^{-5}$, $R^2 = 0.015$,

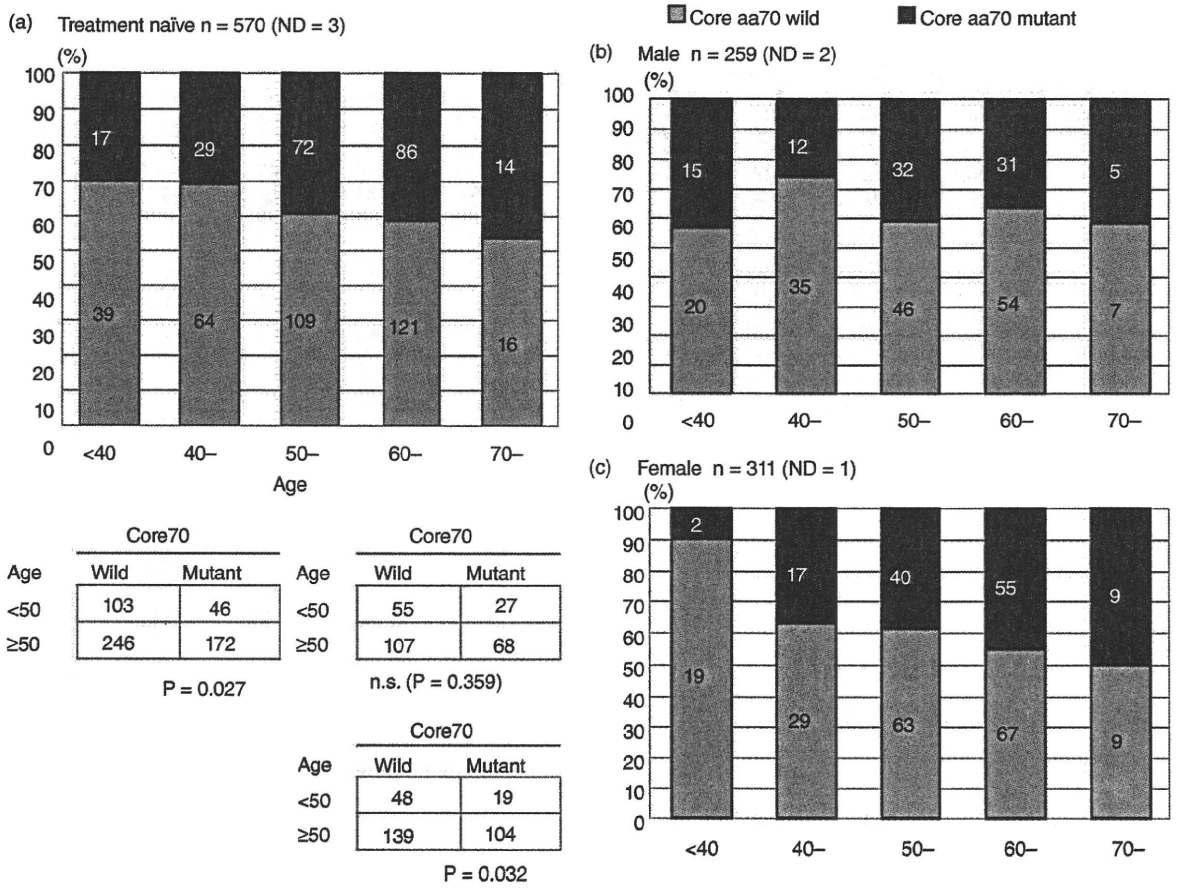


Figure 5 Age-dependent increase in core amino acid 70 mutants in treatment-naive female patients. Percentage of core wild type (arginine) and mutant amino acid were analyzed as in Figure 5 using only interferon treatment-naive patients. Results for all 561 patients (a), as well as for male (b) and female (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-

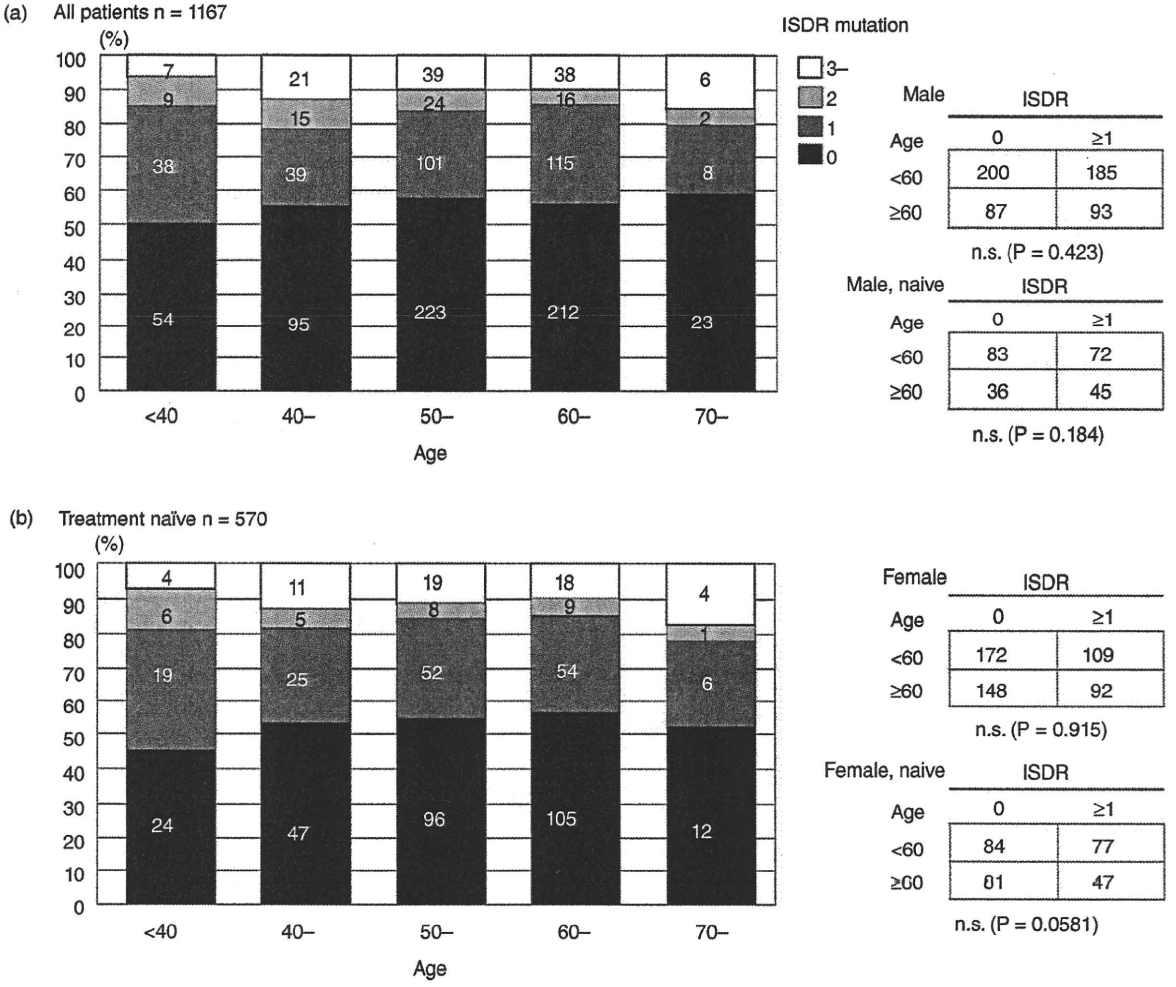


Figure 6 Age-dependent increase in number of amino acid substitutions in the interferon sensitivity determining region (ISDR). The relationship between age and the number of amino acid substitutions in the ISDR was examined. All patients (a) and only naive patients (b) were analyzed. Statistical analysis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

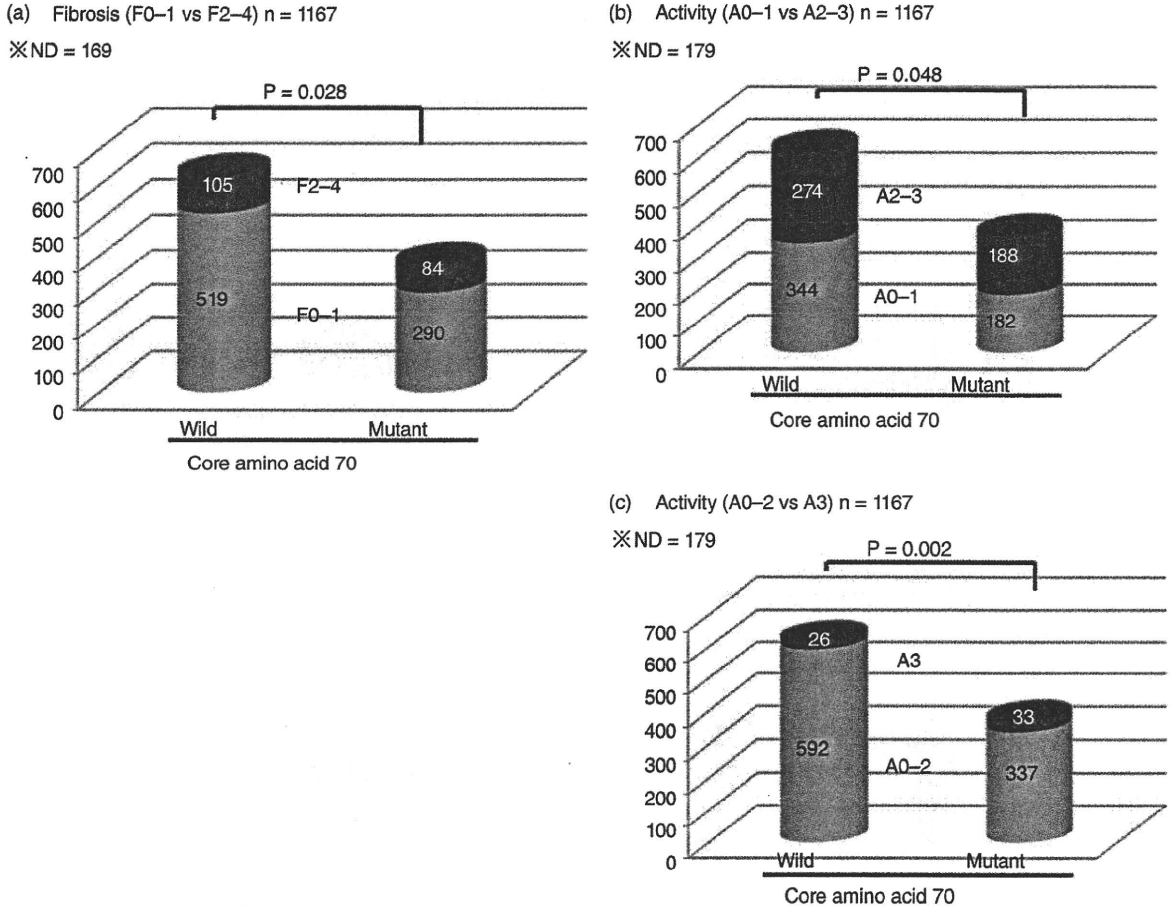


Figure 7 Histological findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and degree of fibrosis (F0–1 and F2–4) (a) and activity (b,c) were examined. Activity was divided into A0–1 and A2–3 (b) or A0–2 and A3 (c) and compared with amino acid 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

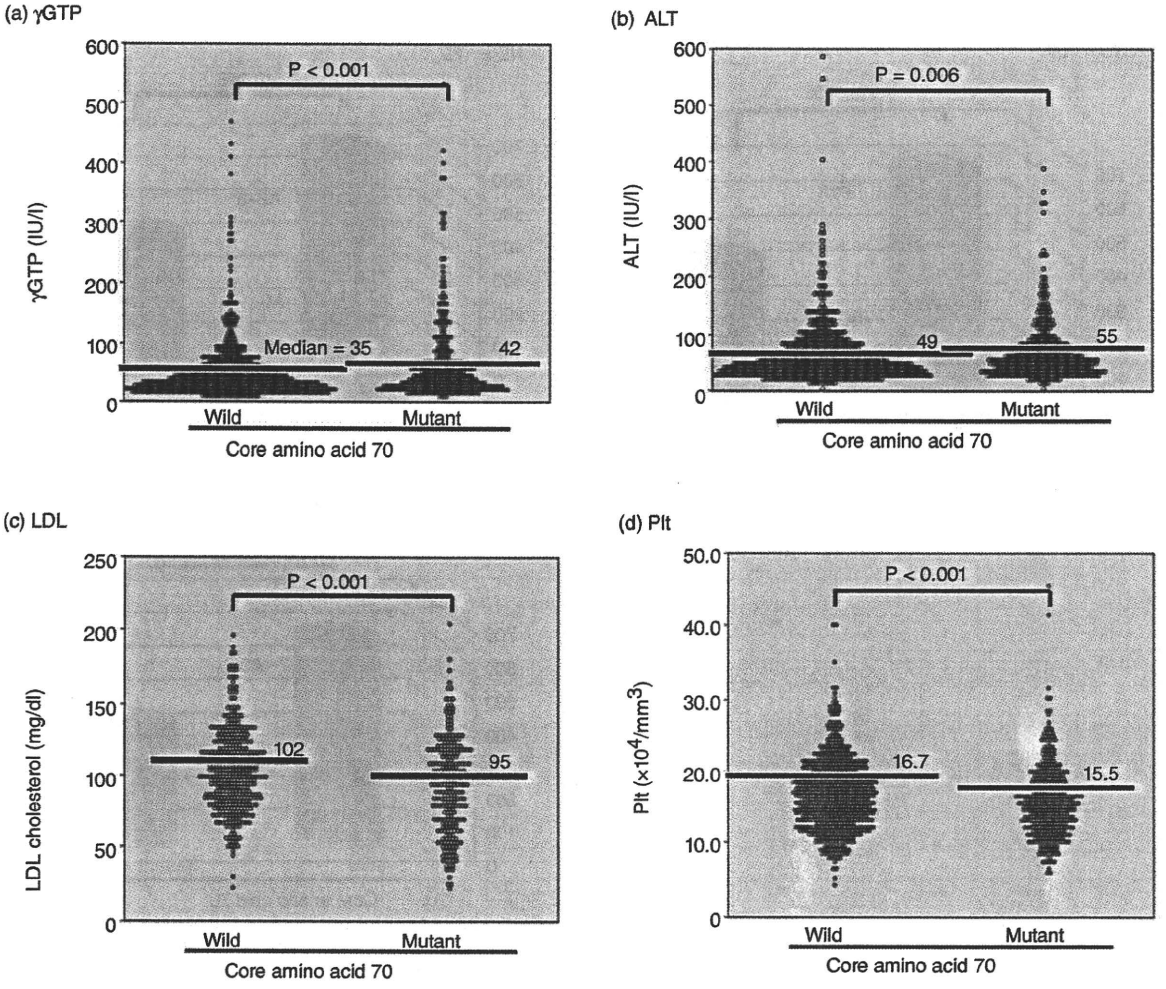


Figure 8 Relationship between blood test findings and core amino acid 70 substitutions. Relationships between core amino acid 70 (wild type or mutant) and gamma-glutamyltranspeptidase (γ-GTP) (a), alanine aminotransferase (ALT) (b), low-density lipoprotein (LDL) cholesterol (c) and platelet count (Plt)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

ACKNOWLEDGMENTS

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ORIGINAL ARTICLE

Evaluation of long-term entecavir treatment in stable chronic hepatitis B patients switched from lamivudine therapy

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Abstract
Purpose Current Japanese guidelines recommend that patients should be switched from lamivudine to entecavir when they meet certain criteria. This analysis examines the efficacy and safety of long-term entecavir therapy in patients who were switched to entecavir after 24 weeks' lamivudine therapy in Japanese studies ETV-047 and ETV-060.
Methods The Phase II Japanese study ETV-047 assessed the efficacy of different entecavir doses when compared with lamivudine. A total of 33 Japanese patients who received lamivudine 100 mg daily in ETV-047 entered the open-label rollover study ETV-060 and subsequently received treatment with entecavir 0.5 mg daily. Hepatitis B virus (HBV) DNA suppression, alanine aminotransferase (ALT) normalization, hepatitis B e antigen (HBeAg) seroconversion, and resistance were evaluated among patients with available samples for up to 96 weeks. Safety was assessed throughout the treatment period.
Results After 96 weeks of entecavir therapy in ETV-060, 90% of patients achieved HBV DNA <400 copies/mL as compared to 21% of patients who completed 24 weeks of lamivudine therapy in ETV-047. Increasing proportions of patients achieved ALT normalization and HBeAg seroconversion following long-term entecavir treatment. No patients experienced virologic breakthrough, and substitutions associated with entecavir resistance were not

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observed in patients with detectable HBV DNA. Entecavir was well tolerated during long-term treatment.

Conclusions Switching lamivudine-treated patients with chronic hepatitis B to entecavir results in increased virologic suppression with no evidence of resistance through 2 years of entecavir therapy. These findings support recommendations in the current Japanese treatment guidelines that stable lamivudine patients should be switched to entecavir.

Keywords Japanese · Chronic hepatitis B · Entecavir · Lamivudine · Switch

Introduction

Chronic hepatitis B virus (HBV) infection affects more than 350 million people worldwide, and is a leading cause of liver-related mortality [1]. Although Japan has one of the lowest prevalence rates for chronic hepatitis B (CHB) (0.8%) among Asian countries, it is still estimated that over 1 million people are chronically infected with HBV [2]. These individuals are at an increased risk of developing cirrhosis, liver failure or hepatocellular carcinoma (HCC) [3].

Lamivudine was the first nucleoside analog introduced for the treatment of CHB. In clinical trials, it demonstrated superior efficacy to placebo for HBV DNA suppression, alanine aminotransferase (ALT) normalization and hepatitis B e antigen (HBeAg) seroconversion [4, 5]. However, a major limitation of lamivudine therapy is the development of resistance, which occurs in up to 70% of patients through 4 years of therapy [6]. Entecavir is a potent inhibitor of HBV replication [7]. In global Phase III studies, entecavir demonstrated superior histologic, virologic and biochemical responses when compared with lamivudine in nucleoside-naïve patients and lamivudine-refractory patients at 48 weeks [8–10]. In the Japanese Phase II study ETV-047, treatment with entecavir resulted in a superior reduction in HBV DNA as compared to lamivudine [11]. In contrast to lamivudine, entecavir has been shown to have a high genetic barrier to resistance; the cumulative probability of resistance through 5 years of treatment has been reported to be 1.2% [12]. The genetic barrier is lower in patients who are infected with lamivudine-resistant HBV and consequently higher resistance rates are observed in this population with long-term treatment [12].

Current Japanese treatment guidelines recommend that all treatment-naïve CHB patients with ALT levels $\geq$31 IU/L should be treated, dependent on their viral load. The thresholds for treatment are HBV DNA ≥ 5 $\log_{10}$ copies/mL in HBeAg-positive patients, ≥ 4 $\log_{10}$ copies/mL in HBeAg-negative patients, and ≥ 3 $\log_{10}$ copies/mL in cirrhotic patients [13]. Lamivudine, adefovir, and entecavir are currently approved for the treatment of CHB in Japan. Entecavir 0.5 mg once daily is the first choice therapy for treatment-naïve HBeAg-positive and negative patients aged 35 years or older. In treatment-naïve patients <35 years, the guidelines recommend treating first with interferon for HBeAg-positive patients, and treating HBeAg-negative patients with HBV DNA ≥ 7 $\log_{10}$ copies/mL with entecavir until undetectable HBV DNA is achieved, followed by a combination of entecavir and interferon for 4 weeks, and finally interferon monotherapy for 20 weeks. HBeAg-negative patients with HBV DNA <7 $\log_{10}$ copies/mL should be monitored or can receive interferon therapy. For patients who are lamivudine experienced, but not necessarily resistant, the guidelines also recommend that patients can be switched to entecavir 0.5 mg daily if they have received lamivudine therapy, and have HBV DNA <2.1 $\log_{10}$ copies/mL. Patients with HBV DNA ≥ 2.1 $\log_{10}$ copies/mL can also be switched to entecavir 0.5 mg once daily if they do not have viral breakthrough. Limited data on the efficacy of entecavir in this patient population are available; however, the design of the Japanese study ETV-047 and the rollover study ETV-060 presents an opportunity to assess the efficacy of this treatment option. This report examines the long-term efficacy, safety and resistance of entecavir 0.5 mg daily among patients who were directly switched from lamivudine following 24 weeks' treatment in ETV-047.

Materials and methods

Study population

Study ETV-047 was a Phase II, randomized, double-blind study conducted to evaluate the dose–response relationship of entecavir and compare the antiviral activity and safety of entecavir to lamivudine in Japanese patients with CHB. In ETV-047, 137 patients were randomized to receive one of three entecavir doses [0.01 mg ($n = 35$), 0.1 mg ($n = 34$) or 0.5 mg ($n = 34$), once daily] or lamivudine [100 mg ($n = 34$), once daily] for 24 weeks. The study design and complete inclusion criteria have been described previously [11]. Briefly, eligible patients had HBeAg-positive or -negative CHB with compensated liver disease, HBV DNA ≥ 7.6 $\log_{10}$ copies/mL by PCR assay, <12 weeks' prior therapy with anti-HBV nucleoside analogs and ALT levels 1.25–10 × upper limit of normal (ULN). After completion of treatment in ETV-047, all patients were eligible to enroll immediately in the rollover study ETV-060, with no gap in dosing.

The rollover study ETV-060 was designed to provide open-label entecavir for patients who had completed therapy in the Japanese Phase II program. Patients who completed 24 weeks of treatment in ETV-047 enrolled in ETV-060 and received 0.5 mg entecavir once daily. After 96 weeks of treatment in study ETV-060, patients could complete the study and were eligible to receive commercially available entecavir, which was approved by Japanese health authorities while study ETV-060 was ongoing.

The current analysis describes results for a subset of 33 patients who received lamivudine for 24 weeks in ETV-047 and entecavir 0.5 mg once daily for up to 96 weeks in ETV-060.

Efficacy analyses

Efficacy assessments evaluated the proportions of patients who had available samples (non-completer = missing) every 24 weeks through 120 weeks' treatment. Efficacy end points assessed included HBV DNA <400 copies/mL by PCR assay, ALT normalization (≤1.0 × ULN), HBeAg seroconversion among patients who were HBeAg-positive at baseline, and hepatitis B surface antigen (HBsAg) loss. Serum HBV DNA was determined by Roche Amplicor® PCR assay (Roche Diagnostics K.K., Tokyo, Japan; limit of quantification = 400 copies/mL) in a central laboratory. Clinical laboratory tests, PCR assays for HBV DNA, and serologic tests for HBV were performed at SRL, Inc. (Tokyo, Japan), the central clinical laboratory designated by the trial sponsor. On-treatment testing for resistance was carried out using a direct-sequencing PCR method.

Safety analyses

Safety analyses include the incidence of adverse events, serious adverse events, laboratory abnormalities, and discontinuations due to adverse events on-treatment throughout treatment in study ETV-060. On-treatment ALT flares were defined as ALT >2 × baseline and >10 × ULN.

Resistance analysis

Resistance testing was performed using a direct-sequencing PCR method. Paired samples from all patients with HBV DNA ≥400 copies/mL were analyzed for substitutions associated with entecavir or lamivudine resistance at week 96 (72 weeks of entecavir therapy) or week 120 (96 weeks of entecavir therapy). Patients who discontinued therapy prior to week 120 had their last on-treatment sample analyzed. All patients with virologic breakthrough (≥1 $\log_{10}$ increase from nadir on two consecutive measurements) were also tested for resistance.

Results

Study population

Of the 34 patients in ETV-047 who received treatment with lamivudine 100 mg once daily for 24 weeks, 33 entered ETV-060 and received treatment with entecavir 0.5 mg once daily. Two patients discontinued treatment during ETV-060: one due to an adverse event (depression) and the other due to insufficient effect. In addition, one patient completed treatment at week 76 (52 weeks of entecavir therapy) after meeting the criteria for protocol-defined complete response (undetectable HBV DNA by PCR assay, undetectable HBeAg and normal serum ALT).

Baseline demographic and disease characteristics for the switch cohort are presented in Table 1. The majority of patients (82%) in the cohort were male with a mean age of 43 years. The mean duration of entecavir therapy was 105.9 weeks (range 25–141 weeks). Baseline mean HBV DNA and ALT levels were 7.9 $\log_{10}$ copies/mL and 184 IU/L, respectively. Ninety-one percent of patients were HBeAg-positive and 88% had HBV genotype C infection.

Virologic end points

After completion of 24 weeks of lamivudine treatment in ETV-047, 21% (7/33) of patients in the switch cohort had achieved HBV DNA <400 copies/mL (Fig. 1). Following the switch to entecavir, the proportion of patients achieving HBV DNA <400 copies/mL increased to 82% (27/33) by week 48 (24 weeks of entecavir therapy). Viral suppression

Table 1 Baseline (pretreatment) demographics and disease characteristics: switch cohort

Characteristic	ETV-047/-60 lamivudine to entecavir switch cohort (n = 33)
Age, mean (years)	42.7
Male, n (%)	27 (82)
Ethnicity Japanese, n (%)	33 (100)
Entecavir treatment periods, mean (range) (weeks)	105.9 (25–141)
HbeAg-positive, n (%)	30 (91)
HBV DNA by PCR, mean $\log_{10}$ copies/mL (SD)	7.9 (0.80)
ALT (IU/L), mean (SD)	184.8 (132.9)
HBV genotype, n (%)	
A	2 (6)
B	2 (6)
C	29 (88)
Others	0

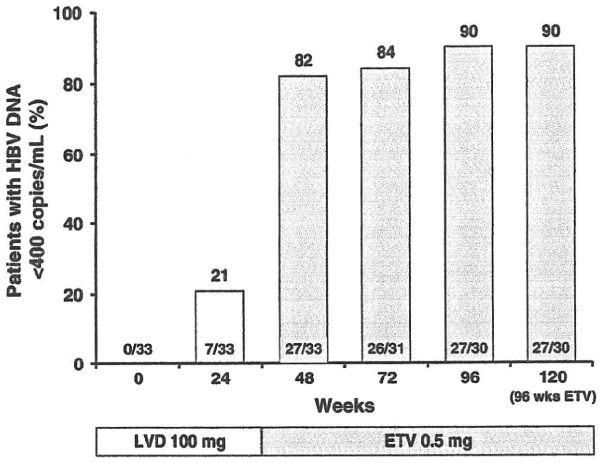

Fig. 1 Proportion of patients with HBV DNA <400 copies/mL through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples. *ETV* entecavir, *HBV* hepatitis B virus, *LVD* lamivudine

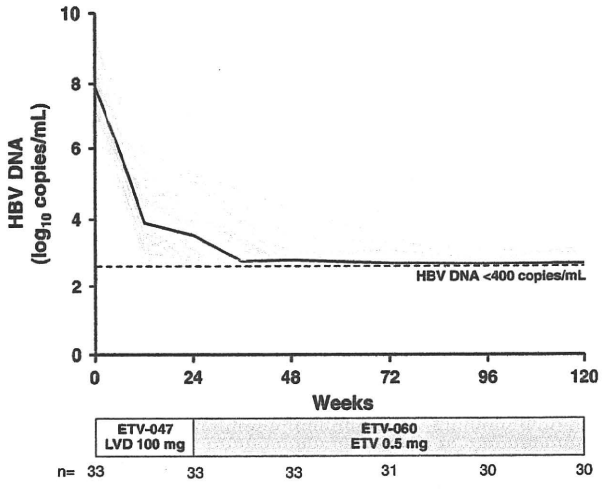

Fig. 2 HBV DNA suppression through week 120 (96 weeks of entecavir therapy). Individual patient HBV DNA profiles are plotted in *gray*. Mean HBV DNA levels are represented by the *solid black line*. *ETV* entecavir, *HBV* hepatitis B virus, *LVD* lamivudine

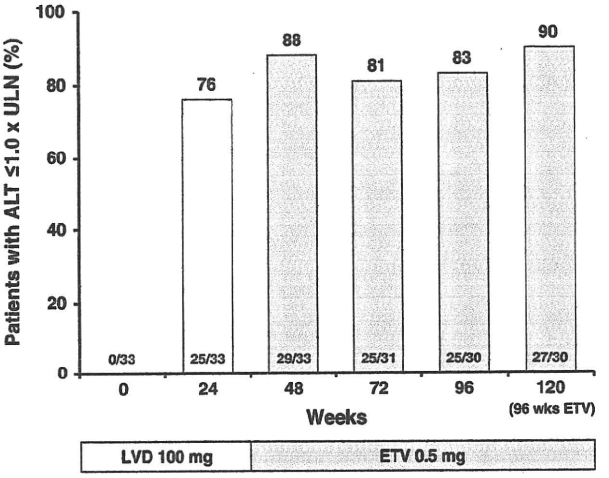

Fig. 3 Proportion of patients with ALT normalization (≤1.0 × ULN) through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples. *ALT* alanine aminotransferase, *ETV* entecavir; *LVD* lamivudine, *ULN* upper limit of normal

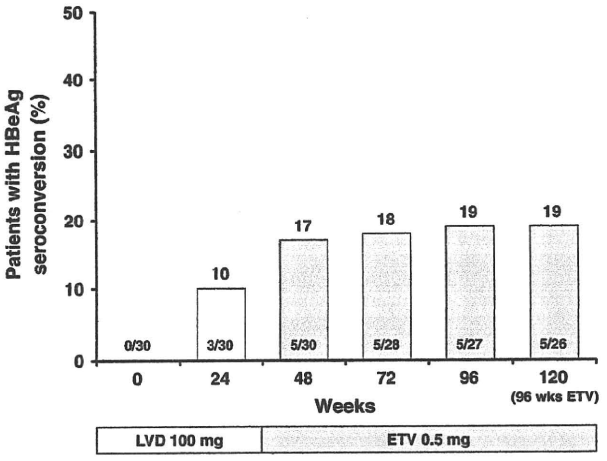

Fig. 4 Proportion of patients with HBeAg seroconversion through 120 weeks of therapy (ETV-047 to ETV-060). *Denominators* represent patients with available samples among the 30 patients HBeAg-positive at baseline. *ETV* entecavir, *HBeAg* hepatitis B e antigen, *LVD* lamivudine

was maintained with longer entecavir treatment, with 84% (26/31) and 90% (27/30) achieving HBV DNA <400 copies/mL at weeks 72 and 120, respectively (48 and 96 weeks of entecavir therapy). Mean HBV DNA levels decreased from a baseline of 7.90 to 3.52 $\log_{10}$ copies/mL after 24 weeks of lamivudine therapy in ETV-047, and reached 2.69 $\log_{10}$ copies/mL after 96 weeks of entecavir therapy in ETV-060 (week 120; Fig. 2). No viral breakthrough was observed during entecavir therapy.

Biochemical end points

ALT normalization (≤1.0 × ULN) was demonstrated in 76% (25/33) of patients after 24 weeks of lamivudine therapy in ETV-047 (Fig. 3). Following treatment with entecavir in ETV-060, ALT normalization was maintained in 90% (27/30) of patients achieving this end point by week 120. Minor fluctuations in the proportion of patients achieving ALT normalization were attributed to patients discontinuing entecavir therapy during the course of study ETV-060.

Serologic end points

HBeAg seroconversion was assessed among the 30 patients in the switch cohort who were HBeAg-positive at baseline in ETV-047 (Table 1; Fig. 4). Three patients (10%)

achieved HBeAg seroconversion during the initial 24-week lamivudine treatment period in ETV-047 (Fig. 4). Following switch to entecavir in ETV-060, two additional patients developed HBeAg seroconversion by week 120 (96 weeks of entecavir therapy). None of the patients in the switch cohort experienced HBsAg loss during treatment in ETV-047 or ETV-060.

Resistance

Four of the 33 patients who received entecavir therapy in ETV-060 had HBV DNA ≥400 copies/mL either at treatment discontinuation or at week 120. One patient discontinued therapy at week 68 (44 weeks of entecavir therapy) due to insufficient effect. HBV DNA prior to treatment discontinuation was 3.1 $\log_{10}$ copies/mL, however, resistance testing revealed no substitutions associated with entecavir resistance. The remaining three patients had HBV DNA ≥400 copies/mL at weeks 96 and 120; however, only two patients had samples available for testing. Neither patient's samples had substitutions associated with entecavir or lamivudine resistance either at weeks 96 or 120.

Safety

Entecavir was well tolerated during long-term treatment and the safety profile of patients in the switch cohort was consistent with that previously reported for patients who received continuous entecavir therapy in studies ETV-047 and ETV-060 (Table 2). Serious adverse events (Meniere's disease, subcutaneous abscess and ALT flare) were reported in three patients (9.1%). The most frequently reported adverse events during treatment in ETV-060, occurring in ≥10% of patients, were nasopharyngitis (76%), diarrhea (21%), back pain (18%), influenza (18%), and allergic rhinitis (15%). One patient discontinued entecavir therapy due to depression, which the investigator considered was possibly related to entecavir therapy. An ALT flare (ALT >2 × baseline and >10 × ULN) occurred in one patient at week 18, and was judged a serious adverse event by the investigator, but was not associated with a change in HBV DNA. No deaths were reported during the study.

Table 2 Summary of safety in ETV-060: switch cohort

On-treatment in ETV-060	Patients, *n* (%)
Any adverse events	33 (100)
Clinical adverse events	33 (100)
Laboratory adverse events	33 (100)
Grade 3/4 clinical adverse event	1 (3)
Grade 3/4 laboratory adverse event	5 (15)
Clinical serious adverse event[a]	3 (9)
Discontinuations due to adverse events	1 (3)
Deaths	0
ALT flares[b]	1 (3)

[a] Including ALT flares

[b] ALT >2 × baseline and >10 × ULN

Discussion

Profound long-term suppression of HBV DNA is required for patients to meet the goals of CHB therapy, which are to prevent cirrhosis, hepatic failure, HCC and liver-related death [14–16]. A major concern with long-term therapy is the increasing risk of selecting resistance mutations, especially for therapies with a low-genetic barrier to resistance, such as lamivudine. The current analysis presents results for a cohort of Japanese patients who were switched directly from lamivudine to long-term entecavir therapy. The results show that this switch cohort achieved additional HBV DNA suppression after the switch to entecavir. The proportion of patients with HBV DNA <400 copies/mL increased from 21% after 24 weeks of lamivudine treatment to 82% following an additional 24 weeks of entecavir treatment. Mean HBV DNA decreased from 3.52 $\log_{10}$ copies/mL at week 24 to 2.80 $\log_{10}$ copies/mL at week 48. Rates of HBV DNA suppression were maintained in this cohort, with 90% of patients achieving HBV DNA <400 copies/mL through 96 weeks of entecavir therapy (week 120). These results are comparable to those achieved by the cohort of patients who received entecavir 0.5 mg once daily in the Japanese Phase II studies and the rollover study ETV-060 [17]. At baseline in ETV-060, 56% of this cohort had achieved HBV DNA <400 copies/mL, increasing to 83% through 96 weeks of entecavir therapy. Among patients with abnormal ALT levels at ETV-060 baseline, 88% of patients in the entecavir 0.5 mg cohort achieved normalized ALT levels at week 96 as compared to 90% of patients in the switch cohort. Rates of HBeAg seroconversion at week 96 in ETV-060 were also similar (20 vs. 19%, respectively). These rates of viral suppression also show comparison favorably to those reported for the global nucleoside-naïve cohorts treated for a similar period of time [18, 19]. The potent antiviral activity of entecavir and its high genetic barrier to resistance is expected to minimize the potential for resistance in the switch cohort, allowing long-term therapy for patients. Liver biopsies were not obtained from patients in the switch cohort; however, the histologic benefits of long-term entecavir therapy have been recently reported for a cohort of naïve Japanese patients in the ETV-060 rollover study [20]. Following treatment with entecavir 0.5 mg daily for 3 years, all patients experienced histologic improvement and 57% experienced improvement in fibrosis score. In

addition, the results from a separate global study have confirmed the histologic benefits of long-term entecavir treatment [21].

Previous Japanese (ETV-052/-060) and global (ETV-026) studies have examined the efficacy of entecavir in lamivudine-refractory patients. In these studies, entecavir demonstrated efficacy, with 54% of Japanese patients achieving HBV DNA <400 copies/mL through 3 years' treatment [10, 22]. However, as a result of the lower genetic barrier in these patients, a major drawback of entecavir therapy in this population is the development of resistance. The cumulative probabilities of genotypic entecavir resistance among lamivudine-refractory patients were 33% through 3 years' treatment in Japanese patients and 51% through 5 years' treatment among patients in the global cohort [12, 22]. In the current study, no entecavir- or lamivudine-associated resistance substitutions were detected after 96 weeks of entecavir treatment. However, in contrast to the previous studies where the majority of patients had high baseline HBV DNA and documented lamivudine resistance [10, 23], patients in the switch cohort received entecavir after achieving variable degrees of HBV DNA suppression with 24 weeks of lamivudine therapy. Therefore, the fact that no resistance has been observed in this cohort to date is not unexpected. This observation is consistent with an analysis of lamivudine-refractory patients enrolled in the worldwide lamivudine-refractory study ETV-026. Patients with baseline HBV DNA <7 $\log_{10}$ copies/mL had a higher probability of achieving HBV DNA <300 copies/mL as compared to those who had baseline HBV DNA ≥7 $\log_{10}$ copies/mL (73 vs. 16%) [24]. Furthermore, among the 42 entecavir-treated patients in ETV-026 who achieved HBV DNA <300 copies/mL through 96 weeks of therapy, only one patient subsequently developed entecavir resistance.

Current recommendations on the treatment of patients with documented lamivudine resistance suggest that patients should receive a second drug without cross resistance. The combination of lamivudine and adefovir has been shown to be superior to adefovir monotherapy for the treatment of lamivudine resistance, especially in preventing the selection of adefovir resistance [25–27]. Although only short-term clinical data are available for tenofovir, rates of viral suppression among lamivudine-experienced or -resistant patients who received tenofovir monotherapy do not differ significantly from those of treatment-naïve patients [28, 29]. Small studies have also shown pegylated interferon alpha-2a to be a safe and beneficial treatment option for lamivudine-experienced patients [30]. However, the treatment options for Japanese lamivudine-resistant patients are more limited, since neither tenofovir nor pegylated interferon alpha-2a are currently approved in Japan.

The Japanese guidelines recommend that patients with detectable YMDD mutations should receive treatment with a combination of lamivudine and adefovir [13]. However, the guidelines also allow patients who have received <3 years of lamivudine therapy, have HBV DNA <400 copies/mL, and no breakthrough hepatitis or YMDD mutations to switch directly to entecavir. The results presented in this analysis suggest that the strategy of switching to entecavir is an effective one that may avoid the additional cost and potential toxicity of combination treatment with lamivudine and adefovir. Among patients in the switch cohort, 96 weeks of entecavir treatment was well tolerated and the safety profile was comparable with previous experience in Japanese patients. One patient experienced an ALT flare (ALT >2 × baseline and >10 × ULN) 18 weeks after initiating entecavir, which was not associated with a change in HBV DNA. This low rate of ALT flares is consistent with previous findings and demonstrates that lamivudine-treated patients can be switched safely to entecavir with a minimal risk of such flares [10, 22].

In summary, the data from the switch cohort presented in this analysis demonstrate that CHB patients can be switched from lamivudine to long-term entecavir. The treatment with entecavir resulted in increased rates of virologic suppression with no evidence of resistance through 2 years of therapy. These findings support recommendations in the current Japanese treatment guidelines that patients on stable lamivudine therapy with no YMDD mutations should be switched to entecavir.

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