Table 5 Gain in hepatocellular carcinoma (HCC) free survival by sustained virological response as a function of age and fibrosis stage

Age (y)	Life expectancy	F0/1	F2	F3	F4
Males					
30	48.7	2.48	7.66	12.40	15.98
40	39.1	2.52	7.68	12.41	15.96
50	29.9	1.68	5.75	9.45	12.14
60	21.4	0.84	3.38	5.95	8.14
70	14.0	0.40	1.70	3.26	4.98
80	8.0	0.15	0.67	1.40	2.38
Females					
30	55.3	1.45	5.60	10.52	15.73
40	45.5	1.46	5.61	10.51	15.69
50	36.0	0.93	4.24	8.17	12.44
60	26.9	0.44	2.52	5.17	8.39
70	18.2	0.22	1.30	2.81	4.95
80	10.6	0.08	0.52	1.18	2.24

Expressed in years, life expectancy was that in the Japanese general population in 2000. The gain in HCC free survival was the difference in expected cumulative HCC free survival with and without attaining a sustained virological response.

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GI SNAPSHOT

Answer

From question on page 386

Figure 1 shows a water soluble contrast enema, which revealed obstruction at the rectosigmoid, apparently caused by an extrinsic pelvic mass.

An emergency laparotomy for large bowel obstruction was carried out. At operation she was found to have a dilated small and large bowel with a cut off at the rectosigmoid. The cause of the obstruction was a hugely enlarged fibroid uterus which was incarcerated in the pelvis. A subtotal hysterectomy was performed to relieve the obstruction. The compressed large bowel was found to be healthy and did not necessitate resection. She eventually made a complete recovery, enjoying normal bowel function.

Histology confirmed a hugely enlarged uterus distorted by a single 11×9×7.5 cm leiomyoma with no evidence of dysplasia or malignancy.

Gynaecological disease and its treatment is a relatively common cause of bowel obstruction. The commonest cause is gynaecological malignancy, particularly that of the ovaries and complications of treatment such as adhesions and radiotherapy.

Leiomyomas or fibroids are benign smooth muscle tumours of the uterine myometrium and are a common condition in women especially over the age of 40 years. Small leiomyomas are present in more than 20% of women over the age of 40 years and usually remain asymptomatic. However, bowel obstruction secondary to benign uterine leiomyomas may occur, albeit rarely.

This should be considered early in female patients and if rapid sustained resolution of the clinical features of obstruction does not occur, the condition should be treated aggressively with surgical intervention.

Prevention and Treatment of Hepatocellular Carcinoma

Masao Omata and Haruhiko Yoshida

Viral hepatitis, by either hepatitis C virus (HCV) or hepatitis B virus (HBV), is the dominant cause of hepatocellular carcinoma (HCC). This is to say that HCC may be prevented by controlling viral infection. Horizontal transmission of HCV has become obsolete owing to the discovery of the virus. Vertical transmission of HBV during delivery has been effectively prevented by vaccination and immunization of neonates. The efficacy of interferon therapy against HCV was recently much improved. We now possess several powerful antiviral drugs against HBV. There has been progress also in the treatment of HCC, and together with advances in diagnostics facilitating HCC detection at an early stage, tumor nodules can often be completely removed either by medical ablation or surgical resection. Nevertheless, recurrence of HCC after apparently curative treatment is extraordinarily frequent, since the remaining liver is still at a particularly high risk of HCC. An effective treatment of HCC should include measures to control *de novo* carcinogenesis. (*Liver Transpl 2004;10:S111–S114.*)

Hepatocellular carcinoma (HCC) is unique among cancers in that the acquired factors are directly responsible for carcinogenesis in the majority of cases. In particular, HCV infection forms the predominant basis of HCC development in various countries, whereas HBV is the main etiology in regions where its infection is prevalent. In Japan, HCV is causative in 80% and HBV in 10% cases of HCC.[1] This implies two important things. First, it is possible to distinguish the patients at high risk for HCC, facilitating efficient screening for HCC development. Second, HCC can be prevented, at least theoretically, by controlling the acquired factor, viral infection.

Prevention of Viral Infection

Strategies for HCC prevention can be made at two levels: the prevention of virus infection and the treatment of viral hepatitis. In Japan and other countries, neonates from HBV-positive mothers are treated with the combination of hepatitis B vaccination and hepatitis B immune globulin (HBIG).[2] This procedure has been practiced in Japan since the 1980s. It effectively prevents the infection during delivery, the main route of HBV transmission, although intrauterine infection remains a possibility. Thus, the rate of mother–neonate vertical transmission of HBV has been reduced down to one-twentieth of the previous rate. The prevalence of an HBV carrier in Japan is significantly lower among the younger generation than among the older ones. HBV prevention is said to be one of the most conspicuous achievements of public health policy.

In Japan, HCV transmission also seems to have been decreasing for a couple of decades, possibly owing to the improved hygienics in general medical practice. However, we were not able to effectively prevent the blood transfusion-mediated HCV infection, or non-A, non-B hepatitis, as it was called then, until the discovery of HCV in the late 1980s.[3] With subsequent improvements in HCV detection, the occurrence of HCV infection has become virtually obsolete, although transmission through intravenous drug abuse remains a threat.

Taken together, transmission of HBV and of HCV has been effectively controlled for years in Japan, and the incidence of HCC will doubtless be decreased in the future. Nevertheless, the incidence of HCC has been rising since the 1970s and has not yet started to decline. This is because the risk of HCC in infected patients is actually increasing as the patients grow older. Consequently, treatment for viral hepatitis remains a matter of great clinical importance.

Treatment of Chronic Hepatitis C

HCC in Japan is characterized by the 8:1 predominance of type C over type B, while the prevalence of HBV and HCV infection in the Japanese general population is estimated at about 1.5% each. Consequently, the odds ratio for HCC as against the uninfected population is 500 with HCV infection and 65 with HBV infection. The risk ratio for lung cancer posed by smoking is about 10. *Helicobacter pylori* infection may be associated with stomach cancer with a similar risk ratio. Thus, patients with HCV infection can be considered to constitute a super-high-risk group. The risk of HCC does differ among patients with HCV infection: it is

Abbreviations: HCC, hepatocellular carcinoma; HBIG, hepatitis B immune globulin; ALT, alanine aminotransferase; PEG, polyethyleneglycolated; HBV, hepatitis B virus; HCV, hepatitis C virus.

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negligible in asymptomatic healthy carriers and as high as 6% per year in cirrhotic patients.[4-6] The latter value is more than 2,000 times the incidence of HCC in the population without hepatitis virus infection. Thus, hepatitis virus infection, especially infection by HCV, is by far the strongest acquired risk factor known for any carcinogenesis.

Shortly after the discovery of HCV, the effectiveness of interferon therapy against hepatitis C virus infection was confirmed.[7,8] Interferon monotherapy was licensed by the Japanese national health insurance program in 1992, and more than 200,000 patients with chronic hepatitis C were treated. By the licensed 6-month protocol of interferon monotherapy, about 30% of the treated patients showed sustained virologic response. Pretreatment factors predictive for the response were extensively studied, showing that serum low virus load and non-1b HCV genotypes are the strongest factors facilitating the virologic response.

In 1994, we set up a national surveillance program for HCC development among chronic hepatitis C patients and enrolled about 2,900 biopsy-proven cases; 2,400 of them received interferon treatment, showing a sustained virologic response rate of 33% on average.[6,9,10] The reduction in the risk of HCC by interferon therapy was confirmed by multivariate Cox proportional hazard regression controlling for age, gender, and the stage of liver fibrosis.[6] Compared to untreated patients, the risk of HCC was reduced by half among the interferon-treated patients as a whole, and down to one-fifth among sustained virologic responders. We also confirmed histologically the resolution of cirrhosis following sustained virologic response.[9] About 10% of the patients showed sustained normalization of serum alanine aminotransferase (ALT) levels after interferon therapy in spite of continued HCV viremia. HCC incidence was reduced also among these patients as compared to the untreated ones. However, longer-term observation revealed that active hepatitis may recrudesce in the biochemical responders with viremia.

About 70% of HCV found in Japan belongs to the 1b genotype. Infection with this genotype is usually accompanied by high serum virus load and resistance to interferon therapy. Sustained virologic response rate in 1b genotype high-virus-load infection is less than 10% with the licensed 6-month interferon monotherapy, in marked contrast to the rate of 60% in non-1b genotype infection.[11] Combination with ribavirin is known to improve the efficacy of interferon.[12] In Japan, the duration of the combination therapy is currently limited to 6 months, resulting in a response rate of only about 15% in 1b genotype high-virus-load infection. In the near future, however, polyethyleneglycolated (PEG) interferon will be introduced in Japan. PEG interferon can be given on a once-a-week basis and improvement is anticipated in compliance.[13] A sustained virologic response rate of over 40% is expected with the combination of PEG interferon and ribavirin for 48 weeks in 1b genotype high-virus-load infection.[14]

Activity of hepatic inflammation and the progression rate of fibrosis varies widely among individual patients with HCV infection. Some patients never have active hepatitis in their lifetime. An asymptomatic healthy carrier at 70 years of age has virtually no possibility of ever developing HCC or liver failure. On the other hand, patients at 40 years with moderate liver fibrosis stand a substantial risk of HCC development sometime in their lifetimes. The indication of interferon therapy must be considered on the basis of expected lifetime risk of HCC development, which is dependent on the present fibrosis stage and life expectancy, together with the possibility of achieving sustained virologic response deduced from viral genotype and load. If liver biopsy is not feasible, the stage of liver fibrosis can be estimated from laboratory data, the platelet count, or prothrombin activity, in particular.

Treatment of Chronic Hepatitis B

Although HBV infection is a definite risk factor for HCC development, the risk ratio can be crudely estimated at one-eighth that of HCV infection. The stage of liver fibrosis and the risk of HCC are not as strongly associated in chronic hepatitis B as in hepatitis C.[15] Thus, it is less easy to distinguish a high-risk group of HCC among chronic hepatitis B patients. Interferon had been used to treat chronic hepatitis B before it was applied to chronic hepatitis C. Interferon does facilitate the seroconversion of HBe antigen to HBe antibody, but the efficacy is rather limited. It is controversial whether interferon therapy reduces HCC development in chronic hepatitis B patients.

Lamivudine, an inhibitor of RNA-dependent DNA polymerase, effectively suppresses hepatic inflammation caused by HBV.[16] The exacerbation following the appearance of resistant virus has been a difficult problem in lamivudine therapy but it can now be circumvented by novel antiviral agents, such as adefovir and entecavir. HBV-mediated hepatocarcinogenesis is believed to be associated with the integration of viral DNA into the host genome. Suppression of inflammation and regeneration may diminish the chance of DNA integration and thus the risk of HCC, although the effect remains to be confirmed in clinical trials.

Treatment of HCC

Diagnosis and treatment of HCC is detailed in other articles and will not be reiterated here. Complete removal of HCC nodules can be achieved by surgical resection or by medical ablation. Microscopic intrahepatic metastasis, which will result in early-phase recurrence, is not infrequent but the risk can be reduced by detecting HCC at an early stage. This can be facilitated by the recognition of high-risk patients. Nevertheless, recurrence is distressingly frequent after apparently curative surgery or ablation. A distinctive characteristic of HCC is the fact that the recurrence rate does not decline with time after initial treatments.[17] Most cases of late-phase recurrence are thought to be due to metachronous multicentric, or *de novo,* carcinogenesis. This is quite understandable, because the remaining liver, often cirrhotic, is still at high risk of the cancer. The rate of metachronous recurrence after complete resection or ablation is estimated at as high as 20% per year, presenting an extremely high risk group.

An obvious solution to the difficulty will be liver transplantation. At least theoretically, liver transplantation provides each patient with a completely cancer-free liver with normal function. Currently, the presence of HCC per se, when extrahepatic metastasis can be ruled out, is not considered a contraindication, but an indication for liver transplantation. However, the feasibility of liver transplantation is limited worldwide by the scarcity of tissue donors. Living-related liver transplantation is an alternative choice but not always possible. Moreover, in both cadaver and living-related liver transplantation, the control of hepatitis viruses, especially HCV, is particularly difficult with postoperative administration of an immunosuppressant.

Another approach is the combined treatment of HCC and viral hepatitis. After the complete ablation of HCV-related HCC with percutaneous ethanol injection,[18] we treated 49 patients with interferon monotherapy.[19] A low HCV-RNA titer was an inclusion criterion, and a sustained virologic response was achieved in 14 (29%) patients. The rate of first recurrence of HCC did not differ between the interferon-treated patients and untreated controls. The recurrence was vigorously treated with ethanol ablation in each patient. The rates of second or third recurrence were different between the two groups. The interferon-treated patients as a whole had a survival rate of 68% at 5 years and 53% at 7 years, whereas untreated patients had a survival rate of 48% at 5 years and 23% at 7 years. Survival rates in sustained virologic responders were 78% at 5 years and 68% at 7 years. These results suggest that metachronous carcinogenesis was suppressed by the elimination of HCV, leading to prolonged life expectancy. Improvement in liver function was another factor contributing to the good prognosis among the responders. Today, with interferon therapies with improved efficacy, we can anticipate more favorable outcomes.

Conclusions

Prevention and treatment of HCC should consist of three levels of strategies. At the first level (prevention of hepatitis virus infection), we already have effective measures, promising a future decrease in HCC incidence. At the second level (treatment of viral hepatitis), we have recently achieved considerable improvements in interferon therapy against HCV and antiviral agents against HBV. The strategy at the third level (treatment of HCC) should include the prevention of recurrence.

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Neither hepatitis C virus genotype nor virus load affects survival of patients with hepatocellular carcinoma

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Objective **Hepatitis C virus (HCV) genotype and virus load, the strongest determinants of the efficacy of interferon therapy, have been presumed to be associated with risk for hepatocellular carcinoma (HCC). This study was conducted to elucidate whether these two factors are capable of predicting the prognosis of patients with HCC.**

Methods **A total of 371 patients with HCV infection (258 men and 113 women; median age, 66 years; range, 37–88 years) who developed HCC between January 1993 and December 1999 were enrolled. Overall survival and recurrence-free survival were analysed with the Cox proportional hazard regression according to HCV genotype (type 1 versus type 2) and virus load (above versus below 100 kIU/ml).**

Results **Of the 371 patients, 346 received locoregional treatments (ethanol injection, microwave, radiofrequency, or surgery), and 307 achieved complete response as determined by subsequent imaging studies. The remaining 25 patients underwent arterial embolization or chemotherapy. Cox proportional hazard regression showed that neither genotype ($P = 0.814$) nor virus load ($P = 0.958$) were significant predictors for survival ($P = 0.814$ and 0.958, respectively) and recurrence ($P = 0.505$ and 0.736, respectively).**

Conclusions **Neither genotype nor virus load of HCV affected prognosis of HCC patients.** ***Eur J Gastroenterol Hepatol*** **16:459–466 © 2004 Lippincott Williams & Wilkins**

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Keywords: hepatitis C virus genotype, hepatitis C virus virus load, hepatocellular carcinoma, survival, recurrence

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Introduction

In 1989, hepatitis C virus (HCV) was shown to be the predominant agent of non-A, non-B hepatitis, causing cirrhosis and hepatocellular carcinoma (HCC) worldwide [1–3]. With elucidation of the nucleotide sequence of this RNA virus, several genotypes of varied geographical prevalence were found, and the quantitative determination of serum HCV-RNA was established [4].

While HCV infection rarely disappears spontaneously once it has become chronic [5,6], interferon therapy is capable of terminating chronic HCV infection [7]. The efficacy of interferon therapy depends on the HCV genotype and the virus load. For example, genotype 1 patients [8] or patients with a high titre [9] respond poorly to this type of therapy. Statistically, genotype 1 infection is strongly associated with high virus load, possibly reflecting a high viral replication rate.

HCV genotype 1 infection has been implicated in the rapid progression of liver damage [10] that consequently leads to a higher risk for HCC [11]. However, this issue remains controversial [12]. For example, several studies suggested that HCV genotype 1b infection was associated with more advanced histological changes in the liver and a higher risk for HCC development compared with other genotype infections [10,13–16]. While follow-up studies of liver graft reinfection by HCV demonstrated an association between genotype 1b and the rapid progression of chronic active hepatitis [17,18], other studies reported conflicting results that indicated no effect of HCV genotype on histological severity [10,19–21], the risk of developing HCC [22,23], or the progression of post-transplantation hepatitis [24].

Owing to recent advancement in the medical and surgical treatment for HCC, the malignancy is now often treated curatively. The remaining liver tissue, however, is still at very high risk for HCC unless the virus is completely eradicated or a normal liver is

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transplanted [25]. If the genotype 1 HCV infection is really considered a high risk factor for HCC development, the recurrence rate of HCC after apparently curative treatment of primary HCC should be higher for this genotype. Similarly, the presumed association between genotype 1 and the rapid progression of liver damage would result in a higher incidence rate of liver failure. Thus, prognosis of HCV-related HCC may differ according to HCV genotypes. This study examines the HCC recurrence rate and survival of patients treated for HCC with respect to HCV genotype and virus load.

Patients and methods

Patients

Patients with HCV infection who developed HCC for the first time and were treated in our institution between January 1993 and December 1999 were consecutively enrolled. HCV infection was diagnosed by anti-HCV seropositivity and confirmed by HCV-RNA determination. Patients with a history of heavy alcohol consumption (> 80 g/day) or those with other liver diseases such as hepatitis B virus infection, autoimmune hepatitis, or primary biliary cirrhosis were excluded. Patients treated for HCC at other hospitals were excluded. Those who had previously received interferon with sustained virological response showed significantly better prognosis (unpublished observation) and were also excluded from this study. During the same period, 18 patients received interferon therapy after the treatment for HCC, and they were also excluded. Thus, a total of 371 consecutive patients, 258 men and 113 women ranging in age from 37 to 88 years (median age, 66 years) were enrolled in this study (Table 1).

One hundred and fifty-three patients (41.2%) were referred from other hospitals for treatment and the remaining 218 developed HCC during surveillance with ultrasound in our out-patient clinic. Diagnosis of HCC was confirmed by artery-dominant hypervascularity on dual-phased computed tomography. Histological evaluation was performed in 304 patients, and HCC differentiation was classified as Edmondson grade I, grade II, grade III, or grade IV based on tumour biopsy specimens [26]. HCC-specific tumour markers α-fetoprotein (AFP), lectin fraction 3 of AFP (AFP-L3), and des-γ-carboxy prothrombin (DCP) were assessed using the cut-off values of 100 ng/ml, 10%, and 40 IU/l, respectively.

HCV genotyping

The HCV genotype was determined by dot-blot assay or serological grouping. In the dot-blot assay, HCV-RNA in serum was amplified by polymerase chain reaction, immobilized on a nylon filter, and hybridized with the oligonucleotide probes specific for HCV genotype 1a, genotype 1b, genotype 2a, and genotype 2b [25,27,28]. Serological grouping was performed using an enzyme-linked immunosorbent assay (ELISA) (Immucheck F-HCV Gr kit; Kokusai Shiyaku, Kobe, Japan) [29], which determined the reactivity between anti-HCV antibody in sample sera and the purified recombinant peptides C14-1 and C14-2. HCV type 1 was defined by positive reactivity with C14-1, which corresponded to HCV genotype 1 infection, and HCV type 2, reactive with C14-2, corresponded to HCV genotype 2 infection. In Japan, the majority of HCV cases are of genotype 1b, genotype 2a, or genotype 2b, in this order of frequency. The other genotypes are extremely rare. Thus, we assumed that type 1 indicated genotype 1b infection. Serological grouping did not distinguish between genotypes 2a and 2b.

Table 1 Demographic characteristics of the total 371 patients

Characteristic	Value
Age (years)	65.3 ± 7.67
Gender (male:female)	258:113
Alcohol consumption (< 50 g/day:more)	254:100[a]
Maximum size of tumour (mm)	31 ± 16
Number of tumours (solitary:multiple)	176:188
Differentiation (Edmondson I:II:III:IV)	90:169:40:5[b]
TNM stage (I:II:III:IV-A:IV-B)	74:130:57:104:5
Fibrosis staging (1:2:3:4)	7:24:60:221
Liver function (Child A:B:C)	168:145:57
Albumin (g/dl)	3.5 ± 0.5
Total bilirubin (mg/dl)	0.9 ± 0.5
Asparate aminotransferase (IU/l)	88 ± 44
Alaninetransferase (IU/l)	87 ± 51
Platelet count (× 10^4/μl)	10.2 ± 4.3
Prothrombin time (%)	70.7 ± 13.1
ICG R15 (%)	26.4 ± 14.8
AFP-positive (≥ 100 ng/ml)	94/368 (25.5%)
AFP-L3-positive (≥ 10%)	29/131 (22.1%)
DCP-positive (≥ 40 IU/l)	278/352 (79.0%)
Cancer therapy (PTA:TAE:Che)	346:23:2

ICG R15, indocyanine green retention at 15 min; AFP, α-fetoprotein; AFP-L3, lectin fraction 3 of AFP; DCP, des-γ-carboxy prothrombin; PTA, percutaneous tumour ablation; TAE, transcatheter arterial embolization, Che, systemic chemotherapy.
[a]Unknown for 17 patients. [b]Not evaluated in 67 patients.

Serum HCV-RNA quantification

Serum HCV-RNA was quantified by competitive reverse transcription-polymerase chain reaction (CRT-PCR) assay or a commercially available assay (Amplicore HCV assay; Roche Diagnostic System, Branchburg, New Jersey, USA). The HCV viral load was divided into high and low groups. A low virus load was defined as less than 100 000 copies/50 μl by CRT-PCR assay or less than 100 kIU/ml by Amplicore, and a high virus load was defined as otherwise. Correspondence between the two cut-off values was confirmed by paired measurement of more than 30 samples.

Treatment

Possibly curative treatment was indicated for 346 of the 371 patients: 265 received percutaneous ethanol injec-

tion therapy (PEIT), 36 received percutaneous microwave coagulation therapy (PMCT), 42 received radiofrequency ablation (RFA), and three received surgical resection. PEIT was performed under ultrasound (US) guidance using a 21-gauge needle (15 cm long; Silux, Tokyo, Japan) as described previously. PMCT was carried out under US guidance using a 15-cm long guide needle (14 gauge) according to the procedure described previously. RFA was executed under US guidance using a 15-cm long guide needle (16 gauge). These treatments were repeated once or twice a week until complete necrosis of the lesion(s) was confirmed with a safety margin of more than 5 mm by dynamic computed tomography [30]. Three hundred and seven patients achieved complete ablation or resection of all detected tumours (complete response), while 39 patients were left with viable lesions.

The remaining 25 patients of the total 371 patients did not receive curative treatment because of numerous lesions, distant metastases, or poor liver function. Twenty-three patients received transcatheter arterial embolization and two patients received systemic chemotherapy.

Patient follow-up

After the initial treatment was completed, patients were regularly screened for HCC recurrence through the evaluation of HCC-specific tumour markers AFP, AFP-L3, and DCP every 1–2 months [31,32], abdominal ultrasonography every 3 months, and dynamic computed tomography every 4–6 months. If HCC recurrence was suspected, magnetic resonance imaging and/or angiographic studies were performed. US-guided tumour biopsy was also performed when necessary. When recurrence was detected, patients received further treatment for HCC: PEIT, PMCT, or RFA when the criteria were met. Otherwise, transcatheter arterial embolization, systemic chemotherapy, or radiation therapy was performed when indicated. Whenever possible, patients received additional treatment for recurrent HCC.

Statistical analysis

The difference in frequency distribution was analysed using the two-tailed chi-square test or Fisher's exact test. Differences in means were evaluated by an unpaired Student's t test. Survival time was measured from the start of the initial treatment of HCC to the time of event or 31 December 2000, whichever came first. Cumulative overall and recurrence-free survival curves were made according to the Kaplan–Meier method, and the difference was analysed with the log-rank test. Predictive factors for survival and recurrence were evaluated by Cox proportional hazard regression. $P < 0.05$ was considered significant.

Results

HCV genotypes

HCV-RNA genotyping was performed in 328 patients. The number of patients for results of genotype were: genotype 1a, two patients (0.61%); genotype 1b, 266 patients (81.1%); genotype 2a, 48 patients (14.6%); and genotype 2b, 12 patients (3.66%). The serotype was determined in 351 patients. Type 1 comprised 280 patients (79.8%), and 71 patients (20.2%) were type 2. Both genotyping and serotyping were performed in 316 patients. The genotype could not be determined in eight patients, although serum HCV-RNA was positive in the genotype-non-specific reverse transcription-polymerase chain reaction assay. Overall, 291 patients (78%) had HCV type 1 infection, 72 patients (19%) had HCV type 2 infection, and eight patients (2%) were undetermined.

The clinical backgrounds of patients are presented in Table 2, including information grouped according to HCV genotype. There was no significant difference in age, gender, tumour size, tumour number, TNM stage, alcohol consumption, fibrous staging, histological findings, liver function, tumour marker levels, and received treatment between the type 1 and type 2 groups.

Virus load

The serum virus load was determined in 307 patients by CRT-PCR and in 64 patients by Amplicore. Results demonstrated that 201 patients (54%) had a low virus load and 170 patients (46%) had a high virus load.

Table 2 also presents the background of patients grouped according to virus load. The prothrombin time was better ($P = 0.001$) and DCP positivity was higher ($P = 0.001$) in patients with a high virus load. There was no significant difference in other variables between the two groups.

Cumulative overall survival

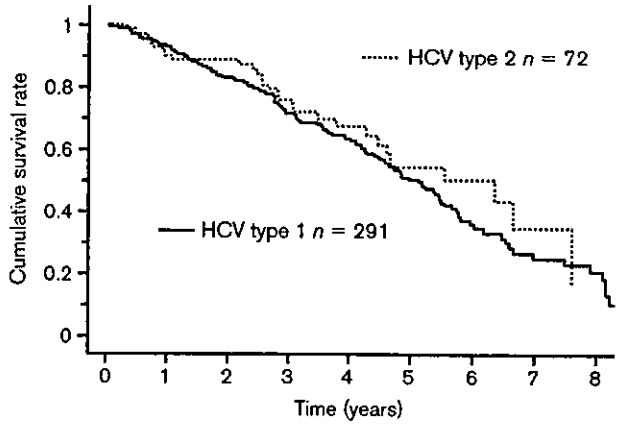
The patients were followed up for 5.3 ± 2.7 years on average. The cumulative survival rates of all 371 HCC patients at 1, 3, and 5 years were 92.4%, 71.7%, and 48.9%, respectively. There was no difference in cumulative survival between patients with HCV type 1 and type 2 infection ($P = 0.391$, Fig. 1). Figure 2 compares the cumulative survival of patients with high and low virus loads. Again, there was no significant difference ($P = 0.913$). The causes of death are summarized in Table 3. HCC was the predominant cause of death, followed by liver failure while HCC was controlled. The frequency distribution of causes of death did not differ between HCV genotypes or virus load. The effects of HCV genotype and virus load on cumulative overall survival were further evaluated by multivariate analysis (see later).

Table 2 Demographic characteristics: comparison between hepatitis C virus type 1 and type 2, between high virus load and low virus load

	Type 1 (n = 291)	Type 2 (n = 72)	P	Low virus load (n = 201)	High virus load (n = 170)	P
Age (average)	65.4 ± 7.77	65.5 ± 7.32	0.990	65.1 ± 7.93	65.7 ± 7.41	0.501
Gender (male:female)	198:93	53:19	0.360	140:61	118:52	0.960
Alcohol consumption (< 50 g/day:more)	204:77	45:22	0.171	134:61	122:42	0.237
Size of tumours (mm)	30.1 ± 16.2	30.5 ± 14.9	0.738	30.4 ± 15.9	30.1 ± 16.1	0.969
Number of tumours (solitary:multiple)	125:98	35:27	0.959	98:99	80:87	0.726
Differentiation (Edmondson I:II:III:IV)	71:131:32:3	22:30:8:2	0.819	41:87:27:4	48:76:15:1	0.076
TNM stage (I:II:III:IV-A:IV-B)	61:101:42:83:3	13:26:14:17:2	0.833	43:67:30:57:3	33:62:27:46:2	0.941
Fibrosis staging (1:2:3:4)	6:17:50:174	1:7:13:47	0.777	3:14:33:123	4:10:30:101	0.779
Liver function (Child A:B:C)	130:113:47	33:32:7	0.425	89:77:34	78:71:21	0.418
Albumin (g/dl)	3.5 ± 0.5	3.5 ± 0.4	0.482	3.5 ± 0.5	3.5 ± 0.5	0.244
Total bilirubin (mg/dl)	0.9 ± 0.5	0.9 ± 0.4	0.602	1.0 ± 0.5	0.9 ± 0.4	0.127
Asparate aminotransferase (IU/l)	88 ± 44	93 ± 42	0.593	92 ± 44	86 ± 44	0.063*
Alaninetransferase (IU/l)	87 ± 51	89 ± 54	0.947	92 ± 56	82 ± 46	0.060*
Platelet count ($\times 10^4/\mu l$)	10.1 ± 4.3	10.3 ± 4.5	0.683	10.2 ± 4.2	10.2 ± 4.5	0.450
Prothrombin time (%)	70.7 ± 13.6	69.0 ± 13.0	0.222	68.1 ± 13.6	73.4 ± 12.8	0.001*
ICG R15 (%)	26.8 ± 14.8	26.7 ± 14.8	0.659	28.1 ± 15.9	25.3 ± 13.6	0.192
AFP-positive (≥ 100 ng/ml)	78/287 (27.2%)	13/72 (18.1%)	0.112	47/198 (23.7%)	47/169 (27.8%)	0.372
AFP-L3-positive (≥ 10%)	19/96 (19.8%)	8/28 (28.6%)	0.322	10/54 (18.5%)	19/77 (24.7%)	0.403
DCP-positive (≥ 40 IU/l)	221/278 (79.5%)	57/71 (80.3%)	0.883	164/191 (85.9%)	118/165 (71.5%)	0.001*
Cancer therapy (PTA:TAE:Che)	271:18:2	71:1:0	0.200	188:12:1	158:11:1	0.973

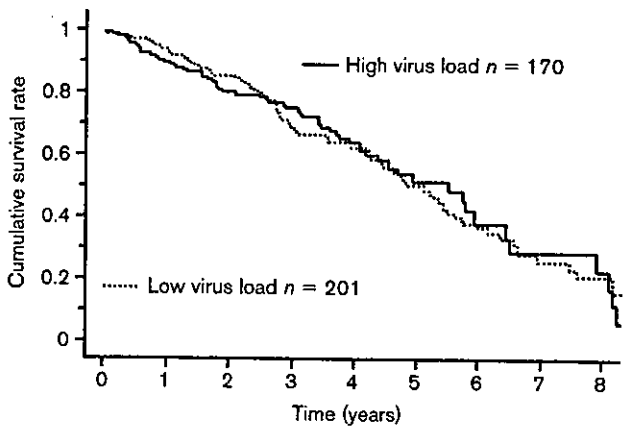
ICG R15, indocyanine green retention at 15 min; AFP, α-fetoprotein; AFP-L3, lectin fraction 3 of AFP; DCP, des-γ-carboxy prothrombin; PTA, percutaneous tumour ablation; TAE, transcatheter arterial embolization; Che, systemic chemotherapy.

Fig. 1



Cumulative survival was compared between hepatitis C virus (HCV) type 1 (solid line, n = 291) and HCV type 2 (dotted line, n = 72) infection. Log-rank test results indicated there was no significant difference (P = 0.3914).

Fig. 2



Cumulative survival was compared between patients with high virus load (solid line, n = 170) and with low virus load (dotted line, n = 201). Log-rank test results indicated there was no significant difference (P = 0.9129).

Cumulative recurrence-free survival

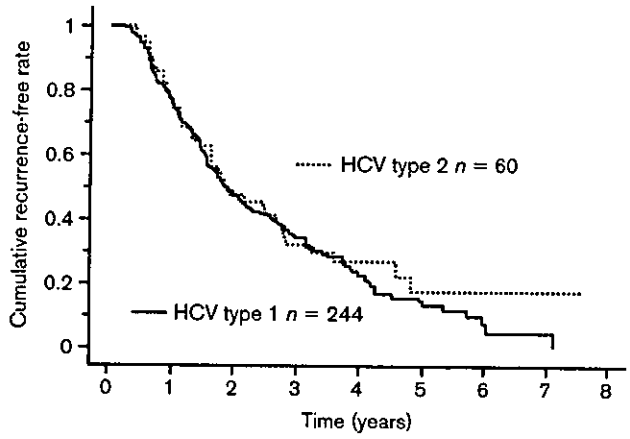
Some patients did not achieve complete response to the initial treatment (i.e. viable tumour remained). Therefore, cumulative recurrence-free survival was examined only in the 307 patients who achieved complete response to PEIT, PMCT, RFA, or surgical resection. Of these 307 patients, there was no difference in cumulative survival between patients with HCV type 1 and type 2 infection (P = 0.212), and between patients with high and low virus loads (P = 0.817).

The cumulative recurrence-free survival rates of the 307 patients 1, 3, and 5 years after treatment were 77.3%, 34.2%, and 15.5%, respectively. The proportion of patients who achieved complete response to the initial treatment was similar for patients with HCV type 1 and type 2 infection (245/289 and 61/72, P = 0.991). Recurrence-free survival was compared between the two groups (Fig. 3), and the log-rank test indicated no significant difference (P = 0.472). The proportion of patients who achieved complete response was also similar for patients with high and low virus loads (144/170 and 166/201, P = 0.736). Recurrence-free survival did not differ between the two groups (Fig. 4, P = 0.911).

Table 3 Cause of death compared between type 1 and type 2, and between high and low virus load

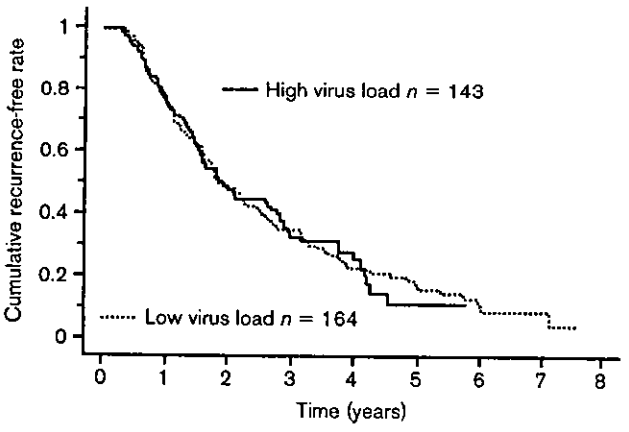
	Tumour progression	Liver failure	Varices rupture	Liver-unrelated death	Total	*P*
Genotype 1	77 (55.0%)	32 (22.9%)	5 (3.57%)	26 (18.6%)	140 (100%)	0.351
Genotype 2	18 (66.7%)	7 (25.9%)	0 (0%)	2 (7.4%)	27 (100%)	
Virus load low	57 (54.3%)	27 (25.7%)	4 (3.81%)	17 (16.2%)	105 (100%)	0.746
Virus load high	39 (60.0%)	12 (18.5%)	3 (4.62%)	11 (16.9%)	65 (100%)	

Fig. 3



Cumulative recurrence-free survival was compared between hepatitis C virus (HCV) type 1 (solid line, $n = 244$) and HCV type 2 (dotted line, $n = 60$) groups. The log-rank test results indicated no significant difference ($P = 0.4717$).

Fig. 4



Cumulative recurrence-free survival rates were compared between patients with high virus load (solid line, $n = 143$) and low virus load (dotted line, $n = 164$). The log-rank test results indicated there was no significant difference ($P = 0.9112$).

Multivariate analysis

The effect of genotype and virus load on overall survival and recurrence-free survival was investigated using the multivariate analysis and Cox proportional hazard regression model. As presented in Table 4, older age, advanced tumour grade, poor liver function, and AFP positivity before the initial treatment for HCC were associated with poor survival. Neither HCV genotype (type 1 versus type 2, risk ratio = 1.062, $P = 0.814$) nor virus load (low versus high, risk ratio = 1.011, $P = 0.958$) was significant. Furthermore, neither factor was significant when assessed separately. The same factors were also assessed for HCC recurrence. As presented in Table 5, male gender and AFP positivity were associated with the risk of recurrence. Neither HCV genotype (type 1 versus type 2, risk ratio = 1.145, $P = 0.505$) nor virus load (low versus high, risk ratio = 0.945, $P = 0.736$) was significant.

Discussion

We analysed whether HCV genotype (1b versus 2a/2b) and virus load affected the prognosis of HCC patients with HCV infection. The present results indicate that these factors were not significantly related to overall survival or HCC recurrence.

Bruno *et al.* studied the frequency distribution of HCV genotype in a cohort of 163 patients with cirrhosis and prospectively observed HCC development [33]. They reported, based on multivariate analysis, that infection with HCV genotype 1b was the strongest risk factor for HCC development, followed by older age, male gender, and interferon treatment. However, it should be noted that 82 (51%) of the 163 patients received interferon therapy. Genotype 1b infection is known to be associated with resistance to interferon. Since interferon therapy reduces the risk for HCC, especially when a sustained virological response is achieved, this form of therapy may have been a confounding factor in their study [34,35]. In contrast, Yoshida *et al.* surveyed the HCC development of 2890 patients with chronic hepatitis C and found that interferon therapy was associated with a significant reduction in HCC risk. Genotype 1b infection was significantly associated with lower virological response rate to interferon therapy. However, when stratified according to the history of interferon therapy and its outcome, neither HCV genotype nor virus load was found to be a significant predictor of HCC development [36].

In the current study, we did not directly assess the relationship between HCC risk and HCV genotype. Nevertheless, our finding that genotype was not asso-

Table 4 Multivariate analysis of predictors for overall survival

Variable	Risk ratio	95% confidence interval	*P*
Gender (male vs female)	1.479	0.967–2.260	0.071
Age (≥ 66 years vs < 66 years)	1.547	1.056–2.267	0.025*
Differentiation (III/IV vs I/II)	2.086	1.310–3.323	0.002*
TNM stage (3/4 vs 1/2)	1.176	0.803–1.722	0.404
Liver function (Child B/C vs Child A)	2.107	1.388–3.200	0.0005*
Fibrosis staging (4 vs 1/2/3)	1.099	0.685–1.763	0.696
AFP (positive vs negative)	2.328	1.543–3.511	< 0.001*
DCP (positive vs negative)	1.165	0.488–2.781	0.730
Genotype (1 vs 2)	1.062	0.644–1.750	0.814
Virus load (low vs high)	1.011	0.679–1.505	0.958

Independent variables for overall survival of 371 patients were analysed with Cox proportional hazard regression. AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin.

Table 5 Multivariate analysis of predictors for hepatocellular carcinoma recurrence

Variable	Risk ratio	95% confidence interval	*P*
Gender (male vs female)	1.477	1.010–2.159	0.044*
Age (≥ 66 years vs < 66 years)	1.057	0.758–1.474	0.745
Differentiation (III/IV vs I/II)	1.392	0.891–2.174	0.146
TNM stage (3/4 vs 1/2)	1.170	0.835–1.637	0.361
Liver function (Child B/C vs Child A)	0.950	0.676–1.335	0.768
Fibrosis staging (4 vs 1/2/3)	1.214	0.842–1.751	0.300
AFP (positive vs negative)	1.572	1.027–2.405	0.037*
DCP (positive vs negative)	1.484	0.908–2.425	0.115
Genotype (1 vs 2)	1.145	0.769–1.706	0.505
Virus load (low vs high)	0.945	0.679–1.315	0.736

Independent variables for hepatocellular carcinoma recurrence of 307 patients demonstrating complete response to the initial treatment were analysed with Cox proportional hazard regression. AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin.

ciated with survival or recurrence may have implications concerning this issue. First, if genotype 1b was strongly associated with the rapid exacerbation of liver function, there should have been an effect on survival. As presented in Table 3, liver function is a strong predictor of survival. Poor liver function will affect survival by causing death from liver failure in patients with controlled HCC or by making additional treatment for recurrent HCC unfeasible. We did not find such difference. Second, we can assume that not a few cases of HCC recurrence were actually multicentric carcinogenesis. Thus, if the relationship between genotype 1 and HCC risk was strong, there should have been an effect on recurrence. Since such an effect was not observed in the current study, the association between HCV genotype and the rapid progression of liver damage or risk for HCC does not appear to be strong, if any.

Toyoda *et al.* compared the characteristics of small HCC (≤ 2 cm in diameter) between patients with HCV type 1 ($n = 53$) and type 2 ($n = 23$) infections, and reported that HCC in type 2 infection was of a more progressive nature (i.e. poorer differentiation, hypervascularity, and shorter doubling time). This study also indicated that intrahepatic metastases were significantly frequent in type 2 infection. However, the investigators failed to find a difference in survival or overall recurrence, suggesting that the difference in HCC characteristics, if any, may not be of major clinical importance [37]. As presented in Table 2, we did not find a genotypical difference in HCC characteristics.

Nevertheless, our data do not exclude the possibility that genotype is associated with risk for HCC. Of the subjects in this study, the prevalence of genotype 1 was 291/363 (i.e. 80.2%) with a 95% confidence interval of 75.7–84.1%. This value is somewhat greater than the values found in chronic hepatitis C patients without HCC in Japan [36]. For example, Yoshida *et al.* reported the prevalence of genotype 1 among Japanese chronic hepatitis patients as 1270/1814 (70.0%, 95% confidence interval = 67.8–72.1%). Silini *et al.* examined the HCV genotype of 978 Italian patients, including 166 with HCC, and found that genotype 1b was more prevalent among patients with HCC than it was in cirrhotic or non-cirrhotic patients without HCC ($P < 0.001$ and $P < 0.01$, respectively) [11]. Thus, with all the discrepancies in findings, this issue warrants further investigation. It should be mentioned that interferon therapy is more effective for non-genotype 1b than for genotype 1b and sustained virological response is associated with reduced risk of HCC. Thus, interferon therapy may result in an increase in the

proportion of genotype 1b among HCC patients compared with the non-HCC population even if the risk of HCC is similar.

Recent reports suggest interferon therapy inhibits HCC recurrence after curative treatment [38,39]. With sustained virological response, interferon therapy will suppress multicentric carcinogenesis in treated HCC patients as well as de-novo carcinogenesis in non-HCC patients. While HCC patients with HCV type 2 have a risk of HCC recurrence similar to those with type 1, interferon therapy is much more effective in the former. Although HCC patients usually have background cirrhosis and the efficacy of interferon therapy is relatively low, combination therapy with ribavirin and/or the use of PEG interferon are more efficient than conventional monotherapy. Thus, an antiviral treatment may be well indicated for curatively treated HCC patients with HCV type 2 infection.

In conclusion, HCV genotype (type 1 versus type 2) and virus load (high versus low) did not affect the cumulative survival or cumulative recurrence-free survival of HCC patients who achieved complete response to initial treatment of the tumour.

Conflict of interest
None declared.

Authors' contributions
Study design by M. Akamatsu, Y. Shiratori, M. Omata. Data collection by M. Akamatsu, S. Shiina, T. Teratani, R. Tateishi, S. Obi, S. Sato, Y. Koike, T. Fujishima, T. Ishikawa. Data analysis by M. Akamatsu, H. Yoshida, R. Tateishi. Manuscript by M. Akamatsu, H. Yoshida.

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UDP-Glucuronosyltransferase 1A7 Genetic Polymorphisms Are Associated with Hepatocellular Carcinoma in Japanese Patients with Hepatitis C Virus Infection

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ABSTRACT

Purpose: **Genetic polymorphisms of UDP-glucuronosyltransferase 1A7 (UGT1A7), which detoxifies endogenous and environmental carcinogens, have been reported to be associated with hepatocellular carcinoma (HCC) in German populations. On the other hand, we reported that interleukin-1β (*IL-1β*) gene polymorphisms were associated with hepatitis C virus (HCV)-related HCC. In this study, we evaluated the association of both genes with the risk of HCC in Japanese HCV-infected patients.**

Experimental Design: **Genetic polymorphisms of *UGT1A7* and *IL-1β* were investigated in 280 Japanese patients (122 with HCC and 158 without HCC) with chronic HCV infections, by use of standard PCR-based genotyping techniques.**

Results: **We designated the *UGT1A7*1* allele (a haplotype conferring higher activity) as *H* and the **2*, **3*, and **4* alleles (haplotypes conferring lower activity) as *L*. The proportions of *UGT1A7 L/L* and *H/L* alleles (genotypes) in patients with HCC (25% and 45%, respectively) were higher than those in patients without HCC (15% and 39%, respectively) with odds ratios of 2.73 (95% confidence interval, 1.40–5.35) and 1.80 (95% confidence interval, 1.05–3.09), respectively, compared with the *UGT1A7 H/H* alleles. Multivariate analyses revealed that *UGT1A7 L/L* and *IL-1β/−31T/T−511C/C* genotypes, the presence of cirrhosis, age >60 years, male sex, and α-fetoprotein >20 μg/ml were associated with the presence of HCC (odds ratios, 2.33, 2.67, 4.20, 3.12, 3.09, and 2.90, respectively).**

Conclusion: **The *UGT1A7* polymorphisms together with *IL-1β* were associated with the presence of HCC in Japanese HCV-infected patients.**

INTRODUCTION

It is estimated that more than 170 million people worldwide are chronically infected with the hepatitis C virus (HCV). The most important consequence of chronic HCV infection is progressive liver fibrosis leading to cirrhosis and finally to hepatocellular carcinoma (HCC), which has significant morbidity and mortality (1–4). Many factors, such as alcohol intake, older age at time of infection, male gender, and co-infection with the human immunodeficiency virus or hepatitis B virus accelerate disease progression in HCV infection (5–8). In addition, host genetic factors have recently been reported to increase the risk of HCC (9–15).

The human UDP-glucuronosyltransferases (UGTs) represent an enzyme superfamily that is capable of catalyzing the glucuronidation of diverse compounds, including therapeutic drugs, endogenous metabolites (*e.g.*, bilirubin and steroid hormones), and known human carcinogens, such as heterocyclic and polycyclic hydrocarbons and heterocyclic amines (16–21). UGTs can catalyze the conjugation of hydrophobic compounds of divergent chemical classes to form water-soluble β-D-glucopyranosiduronic acids. These metabolites then undergo renal or biliary elimination from the body. Because of this function, UGTs have been regarded as major biochemical factors in cellular defense and detoxification.

The N129K (codon 129: AAT→AAG), R131K (codon 131: CGA→AAA), and W208R (codon 208: TGG→CGG) polymorphisms of the *UGT1A7* gene encode enzymes with lower carcinogen detoxification activities and are associated with certain cancers. In Caucasians and African-Americans, the *UGT1A7*3* (129AAG-131AAA-208CGG) and *UGT1A7*4* (129AAT-131CGA-208CGG) alleles (haplotypes) are related to orolaryngeal cancer (22). In Germans, the *UGT1A7*3* allele has been found to be associated with colorectal (23) and pancreatic cancer (24). UGTs are also directly involved in liver diseases. Genetic variations in the UGT promoters are associated with mild and more severe forms of Gilbert's syndrome (25). The *UGT1A7*3* allele has been identified in association with HCC in Germans (13). On the other hand, we have reported that a single-nucleotide polymorphism (SNP) in the interleukin-1β (*IL-1β*) gene is associated with HCV-related HCC in a Japanese population (11).

In this study, we first aimed to assess the association between the reported polymorphisms of the *UGT1A7* gene and

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the presence of HCC in HCV-infected Japanese patients. We then combined *UGT1A7* and *IL-1β* polymorphism information with clinical information in an attempt to improve the diagnosis of the risk for HCC in HCV-infected patients.

PATIENTS AND METHODS

Patients. To assess genetic polymorphisms related to HCC in HCV-infected patients, we studied 280 consecutive Japanese patients (194 men and 86 women; age range, 24–86 years; median age, 60 years) with chronic HCV infections (122 with HCC and 158 without HCC) who visited the outpatient clinic of the University of Tokyo Hospital between November 2000 and November 2001. To obtain an estimate of the genetic distribution of reference alleles in the general Japanese population, we also obtained DNA samples from 60 healthy individuals who visited our hospital (48 men and 12 women; age range, 24–53 years; median age, 31 years) with no history of liver diseases. We obtained written informed consent for DNA genotyping from all subjects. We also obtained approval for this study from the institutional ethics committee.

All of the patients were positive for HCV antibody, as determined by a second-generation enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan) and were negative for hepatitis B surface antigen (Abbott Laboratories, North Chicago, IL). HCV RNA was measured with the Amplicor HCV assay, version 1 (Roche, Tokyo, Japan), and HCV genotypes were determined by a genotyping assay (SRL Laboratory Co., Tokyo, Japan). Any patient with a daily ethanol intake of ≥80 g for a period longer than 10 years was considered to have a positive history of alcohol abuse. The following clinical parameters were obtained for each patient at the time of whole-blood collection: age, gender, alcohol intake, serum albumin level, serum total bilirubin level, serum alanine aminotransferase (ALT) level, serum α-fetoprotein (AFP) level, prothrombin time, platelet count, and HCV serotype and serum viral load, as measured with the Amplicor-HCV monitor assay. Liver biopsies were performed on 194 patients within 6 months, and the diagnosis of liver cirrhosis was made based on liver histology according to the criteria of Desmet (7) and Scheuer *et al.* (8). In patients without biopsy specimens, the diagnosis of cirrhosis was based on the presence of clinical manifestations: portal hypertension (*e.g.*, varices, encephalopathy, or ascites), biochemical abnormalities (elevated serum bilirubin, decreased serum albumin, or prolonged prothrombin time), and obvious morphological changes in the liver, as detected by hepatic imaging (*e.g.*, ultrasonography, computed tomography, arteriography, or magnetic resonance imaging). HCC was diagnosed based on several imaging modalities and was confirmed histologically in sonography-guided fine-needle biopsy specimens from all 122 patients. Initial screening examinations confirmed that no other cancers were present in any of the patients.

Polymorphism Genotyping. Genomic DNA was extracted from 100 μl of whole blood by use of the SepaGene kit (Sanko Junyaku, Tokyo, Japan) according to the manufacturer's instructions. Extracted DNA was dissolved in 20 μl of 10 mM Tris-HCl buffer (pH 8.0) containing 1 mM EDTA, and the DNA samples were stored at −30°C until use. The genetic polymorphisms in *UGT1A7* were determined by direct sequencing of amplified gene fragments.

Polymorphisms have been reported in 12 codons of the *UGT1A7* gene: P11P, R68K, G115S, N129K, R131K, E139D, A144A, L147L, A169T, G173C, W208R, and T255T (13, 26, 27). Eight of the 12 codon produce amino acid changes. We used the primers F1 (Ref. 13; 5′-gcggctcgagccacttactatattataggagct-3′) and R1 (Ref. 13; 5′-gcggatatccataggcactggcttttcctgatgaca-3′) to amplify the specific *UGT1A7* fragment that covers all 12 codons. A 917-bp fragment of *UGT1A7* was amplified by PCR with extracted genomic DNA as the template. PCR was performed with Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Uppsala, Sweden). The thermocycling conditions were as follows: 94°C for 10 min, followed by 30 cycles of 94°C for 30 s, 57°C for 60 s, and 72°C for 60 s. To verify the size of the PCR product, the amplicon was visualized on a 12.5% polyacrylamide gel with the appropriate size markers. For polymorphism determinations, direct sequencing was performed bidirectionally with 10 ng of QIAquick Spin-purified (Qiagen, Hilden, Germany) PCR product and the BigDye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA), together with the primers S129F (5′-accattgcgaagtgc-3′), S129R (5′-acaattaagccacagg-3′), S208F (5′-ccctgctcctctttcctatg-3′), and S208R (5′-tgaaaaaatagggge-3′), followed by detection on an ABI 310 automated sequencer (PE Applied Biosystems; Ref. 11). Polymorphisms in the promoter region of the *IL-1β* gene were determined as described previously (11).

Statistical Analysis. We used a multivariate logistic regression model to calculate the statistical power required to detect the contribution of a SNP to the risk of harboring HCC, while including other known risk factors for HCC. SNP status was assigned continuous values of $X = 0$, 1, or 2, which represent homozygous for an allele, heterozygous, or homozygous for a different allele, respectively. The required sample size, n, for the multivariate logistic regression analysis was calculated using the following formula (28):

$$n = \frac{(Z_{1-\alpha/2} + Z_{1-\beta})^2}{[P(1-P)\beta^{*2}(1-R^2)]} \quad \text{(A)}$$

where Z_u is the upper uth percentile of the standard normal distribution; P is the proportion of patients with HCC when $X = 1$; β^* is the size of the effect of an SNP; and R^2 is the multiple correlation coefficient (we used 0.009 from our data) relating X with the following four covariates: the presence of cirrhosis, male gender, high serum AFP level, and advanced age (29–31). When the effect size of a SNP was assumed to be 0.69, which corresponded to an odds ratio (OR) of 2, the required sample size was calculated to be 75 or 100 for a statistical power of 80 or 90%, respectively. When the effect size of an SNP was assumed to be 1.39, which corresponded to an OR of 4, the required calculated sample size was 18 or 25 for a statistical power of 80 or 90%, respectively. On the basis of these calculations, our sample size was sufficient for conditions under which the OR of an SNP exceeded 2.

The associations between the clinical parameters (age, gender, alcohol intake, cirrhosis, serum albumin, serum total bilirubin, serum ALT, serum AFP, prothrombin time, platelet

Table 1 Patient demographics

Variables[a]	With HCC[b] (n = 122)	Without HCC (n = 158)	P
Sex (male)	93 (76)	101 (64)	0.026[c]
Cirrhosis	81 (66)	77 (49)	0.003[c]
Alcohol > 80 g/day	18 (15)	20 (13)	0.612[c]
HCV serotype 1	90 (74)	111 (70)	0.517[c]
Age (years)	62 (46–83)	56 (24–86)	0.023[d]
HCV load (IU)	377 (1–1448)	362 (45–1321)	0.543[d]
Albumin (g/dl)	3.6 (2.6–4.4)	4.1 (2.6–4.7)	0.034[d]
Total bilirubin (mg/dl)	0.8 (0.3–3.5)	0.6 (0.3–1.8)	0.004[d]
Alanine aminotransferase (units/l)	88 (16–280)	66 (10–429)	0.043[d]
α-Fetoprotein (μg/l)	25 (3–759)	7 (1–281)	0.031[d]
Prothrombin time (%)	70 (51–100)	85 (52–100)	0.028[d]
Platelet count (×10⁴/μl)	9.9 (4.3–24.4)	14.8 (5.5–34.3)	0.041[d]

[a] Proportion of male sex, presence of cirrhosis, alcohol >80 g/day, and HCV genotype 1 are shown as frequency (percentage). Age, HCV load, albumin, total bilirubin, alanine aminotransferase, α-fetoprotein, prothrombin, and platelet count are shown as median (range).

[b] HCC, hepatocellular carcinoma; HCV, hepatitis C virus

[c,d] Ps were calculated by [c]χ^2 test and [d]Mann–Whitney U test.

count, HCV serotype, serum viral load, *IL-1β* genotype, and *UGT1A7* genotype) and the presence of HCC were evaluated using Student's t test, the Mann–Whitney U test, one-way ANOVA, and the χ^2 test. The association between the genotype of each locus and the presence of HCC was evaluated using the χ^2 test. The Cochrane–Armitage test was used to test for trends. Possible confounding effects among these variables were adjusted using a multivariate logistic regression model, and the ORs and 95% confidence intervals (CIs) were calculated. $P < 0.05$ was considered significant in the two-tailed test. The Hardy–Weinberg equilibria of alleles at individual loci were evaluated using the HWE program.[3] The haplotype frequencies for pairs of alleles were estimated using the EH software for estimating haplotype frequencies.[3] Linkage disequilibrium coefficients ($D' = D/D_{min\ or\ max}$) were calculated using the 2BY2 program.[3] All other data analyses were performed using SPSS, version 10.0 (SPSS Inc., Chicago, IL) and the R statistical package.[4]

RESULTS

Patient Characteristics. There were no significant differences in alcohol intake, HCV serotype, or viral load between the HCV-infected patients with and without HCC. In patients with HCC, the factors age, male sex, the proportion of patients with cirrhosis, and serum total bilirubin, serum ALT, and serum AFP levels were higher than in patients without HCC; serum albumin levels, prothrombin time, and platelet count were lower than in patients without HCC (Table 1).

***UGT1A7* Genetic Polymorphisms in the Japanese Population.** We examined the 12 reported polymorphisms harboring codons in exon 1 of the *UGT1A7* gene in all 340 subjects (280 patients and 60 healthy individuals) by direct sequencing of the amplified 917-bp fragments. We found polymorphisms in only four codons: P11P, N129K, R131K, and W208R. The polymorphism in codon 11 was a silent change, and polymorphisms of the three other *UGT1A7* codons defined the alleles (haplotypes) *UGT1A7*2* (129K-131K-W208), *UGT1A7*3* (129K-131K-208R), and *UGT1A7*4* (N129-R131–208R). The alleles at the individual loci were all in Hardy–Weinberg equilibrium in both the patients and healthy individuals ($P > 0.05$).

Codons 129 and 131 were in complete linkage disequilibrium ($D' = 1$). The 129–131/AAG(K)-AAA(K) polymorphisms (38%) were observed more frequently than was the 208CGG(R) polymorphism (24%). The proportion of patients with *UGT1A7*4* was only ~1.8% in our study. The *UGT1A7*2/*4* and **4/*4* alleles were absent in our 340 individuals (Table 2). The allele frequencies in the patients without HCC were similar to those of healthy individuals ($\chi^2 = 4.925$; $P = 0.669$).

Association between *UGT1A7* Genetic Polymorphisms and HCC. The distribution of the *UGT1A7* alleles (genotype) and the *IL-1β* genotype with regard to the presence of HCC is shown in Table 2. The frequencies of the *UGT1A7*2/*3* and **1/*3* alleles (genotypes) in patients with HCC (15 and 30%, respectively) were higher than those in patients without HCC (4 and 20%, respectively), with ORs of 6.08 (95% CI, 2.22–16.64) and 2.35 (95% CI, 1.26–4.35), respectively, compared with the **1/*1* alleles (genotypes). Interestingly, the frequency of the **2/*3* allele had a higher OR (>6) than the homozygous **2* or **3* allele.

The estimated frequencies of the *UGT1A7*3* and **2* alleles (haplotypes) were higher in patients with HCC (28 and 19%, respectively) than in patients without HCC (17 and 14%, respectively), with ORs of 2.09 (95% CI, 1.38–3.18) and 1.71 (95% CI, 1.07–2.74), respectively, compared with the *UGT1A7*1* allele, which suggests a positive correlation between these alleles and the presence of HCC (Table 2).

The associations between the *UGT1A7* polymorphisms and enzymatic activities are known. The *UGT1A7*1* allele is related to higher activity, and the remaining alleles (**2*, **3*, and **4*) are related to lower activity. On the basis of these associations, for clarity we designated the former allele as "*H*," which represents higher UGT1A7 activity, and the latter alleles as "*L*," which represents lower activity. The proportions of *UGT1A7 L/L* (genotype, homozygous for the lower-activity alleles **2*, **3*, and **4*) and *UGT1A7 H/L* (genotype, heterozygous for higher- and low-

[3] ftp://linkage.rockefeller.edu/software.

[4] www.r-project.org.

Table 2 Association of *UGT 1A7*[a] and *IL-1*β polymorphisms with HCC in HCV-infected patients

Polymorphisms	Patients with HCV, *n* (%)		OR (95% CI)[b] with *vs.* without	Healthy (*n* = 60), *n* (%)
	With HCC (*n* = 122)	Without HCC (*n* = 158)		
UGT1A7 alleles (genotype)				
**1/*1*	36 (30%)	73 (46%)	1.00	30 (50%)
**1/*2*	18 (15%)	24 (15%)	1.52 (0.73–3.16)	12 (20%)
**1/*3*	37 (30%)	32 (20%)	2.35 (1.26–4.35)	11 (19%)
**1/*4*	0	6 (4%)		0
**2/*2*	5 (4%)	7 (5%)	1.45 (0.43–4.88)	2 (3%)
**2/*3*	18 (15%)	6 (4%)	6.08 (2.22–16.64)	2 (3%)
**2/*4*	0	0		0
**3/*3*	6 (5%)	6 (4%)	2.03 (0.61–6.73)	3 (5%)
**3/*4*	2 (1%)	4 (2%)	1.01 (0.18–5.80)	0
**4/*4*	0	0		0
UGT1A7 alleles (categorized genotype)				
H/H[c]	36 (30%)	73 (46%)	1.00	30 (50%)
H/L	55 (45%)	62 (39%)	1.80 (1.05–3.09)	23 (38%)
L/L	31 (25%)	23 (15%)	2.73 (1.40–5.35)	7 (12%)
UGT1A7 allele (estimated haplotype)				
**1*	127 (52%)	208 (66%)	1.00	83 (69%)
**2*	46 (19%)	44 (14%)	1.71 (1.07–2.74)	18 (15%)
**3*	69 (28%)	54 (17%)	2.09 (1.38–3.18)	19 (16%)
**4*	2 (1%)	10 (3%)	0.33 (0.07–1.52)	0 (0%)
*IL-1*β/−31 genotype				
C/C	15 (12%)	38 (24%)	1.00	17 (28%)
T/C	62 (51%)	80 (51%)	1.96 (0.99–3.89)	30 (50%)
T/T	45 (37%)	40 (25%)	2.85 (1.37–5.94)	13 (22%)
*IL-1*β/−31 allele				
C	92 (38%)	156 (49%)	1.00	64 (53%)
T	152 (62%)	160 (51%)	1.61 (1.15–2.26)	56 (47%)

[a] UGT, UDP-glucuronosyltransferase; IL, interleukin; HCC, hepatocellular carcinoma; HCV, hepatitis C virus, OR, odds ratio; CI, confidence interval.

[b] The OR of each polymorphism for the presence of HCC was calculated compared with the first row of each category.

[c] H, higher activity allele of *UGT1A7* (**1*); L, lower activity alleles of *UGT1A7* (**2, *3, *4*).

er-activity alleles) were higher in patients with HCC (25 and 45%, respectively) than in patients without HCC (15 and 39%, respectively) with ORs of 2.73 (95% CI, 1.40–5.35) and 1.80 (95% CI, 1.05–3.09) compared with *UGT1A7 H/H* (genotype, homozygous for the higher-activity allele **1*). This suggests a positive correlation between the *L* alleles and the presence of HCC (Table 2). Among patients with *L/L*, *H/L*, and *H/H* alleles, 57, 47, and 33%, respectively, had HCC. When we tested for trends, the *L* allele (haplotype) showed a slightly increased risk for the presence of HCC ($P = 0.0019$).

Association between *IL-1*β Genetic Polymorphisms and HCC. Consistent with our previous report, polymorphisms in the *IL-1*β gene were associated with HCC. The *IL-1*β −31 and −511 loci were in complete linkage disequilibrium ($D' = 1$). The *IL-1*β/−31T/T (*i.e.*, −511C/C) genotype showed a significant association with the presence of HCC compared with the C/C genotype, consistent with our previous report (OR, 2.85; 95% CI, 1.37–5.94). The *IL-1*β/−31 T allele also showed a significant association with the presence of HCC (OR, 1.61; 95% CI, 1.15–2.26; Table 2).

Although the *IL-1*β (2q14) and the *UGT1A7* (2q37) genes are located on the same arm of chromosome 2, our analyses found no significant linkage disequilibrium between them ($D' < 0.2$). We tried combining *UGT1A7* and *IL-1*β polymorphisms with clinical information to improve the diagnosis of the risk for HCC in HCV-infected patients but could not find an SNP map that predicted high susceptibility to HCC. It therefore appears that the genetic polymorphisms of *UGT1A7* and *IL-1*β are independently associated with the presence of HCC.

Factors Associated with Presence of HCC in HCV-Infected Patients. The following factors were significantly associated with the presence of HCC according to univariate analyses: the *UGT1A7 L/L* alleles ($P = 0.0225$); the *IL-1*β/−31T/T(−511C/C) genotype ($P = 0.0368$); age >60 years ($P = 0.007$); male sex ($P = 0.002$); the presence of cirrhosis ($P < 0.001$); serum albumin <3.9 g/dl ($P < 0.001$); total bilirubin >0.7 mg/dl ($P = 0.04$); ALT >80 units/l ($P = 0.043$); AFP >20 ng/ml ($P < 0.001$); prothrombin time <70% ($P < 0.001$); and platelet count $<12.5 \times 10^4/\mu l$ ($P < 0.001$). Stepwise multivariate logistic regression analysis was performed with these 11 variables. Six variables (*UGT1A7 L/L* and *IL-1*β/−31T/T genotypes, presence of cirrhosis, age >60 years, male sex, and serum AFP >20 μg/l) were included in the final model with ORs of 2.33 (compared with *H/H*), 2.67 (compared with C/C), 4.20, 3.12, 3.09, and 2.90, with 95% CIs of 1.32–6.99, 1.22–4.76, 3.21–6.59, 1.42–6.37, 1.69–4.72, and 1.30–4.32, respectively (Table 3).

DISCUSSION

In this study, we evaluated the relationships between polymorphisms of the *UGT1A7* gene and the outcome of chronic

Table 3 Factors associated with presence of HCC[a] in HCV-infected patients in multivariate analysis

Factor	Category[b]	Odds ratio	95% CI
UGT1A7 alleles (genotype)	H/H	1.00	
	H/L	1.53	0.81–3.21
	L/L	2.33	1.32–6.99
IL-1β/-31-511 genotypes	C/C-T/T	1.00	
	T/C-T/C	1.32	0.93–3.39
	T/T-C/C	2.67	1.22–4.76
Cirrhosis	Presence	4.20	3.21–6.59
Age	>60 years	3.12	1.42–6.37
Sex	Male	3.09	1.69–4.72
α-Fetoprotein	>20 ng/ml	2.90	1.30–4.32

[a] HCC, hepatocellular carcinoma; HCV, hepatitis C virus; CI, confidence intervals; UGT, uridine UDP-glucuronosyltransferase; IL, interleukin.
[b] *H*, higher activity allele of *UGT1A7* (**1*); *L*, lower activity allele of *UGT1A7* (**2, *3, *4*)

HCV infection. The *UGT1A7 L* alleles, *i.e.*, alleles that confer lower activity, were found to be associated with HCC. Our multivariate model confirmed the association between *UGT1A7 L/L* alleles and the presence of HCC. Genetic polymorphisms in the *IL-1β* gene were also included in the final model.

*UGT1A7*3* was previously shown to be associated with HCC in a German population (13). Although our study focused only on HCV-infected patients, our results were similar to those of the German study, which indicates that endogenous or environmental carcinogens facilitate the development of HCC in patients with HCV infection. One difference between our results and those of the German study is the frequency of the *H/H* (**1/*1*) alleles, which was only 7% in German patients with HCC but was as high as 30% in Japanese patients with HCC. This may be explained in part by ethnic differences. It is also possible that factors other than *UGT1A7* polymorphisms contribute to HCC in HCV-infected patients.

The UGTs are expressed in the gastrointestinal tract and in the liver in a tissue-specific manner (32). UGT1A7 plays a critical role in the detoxification of carcinogens. Specifically, it was shown previously that polymorphisms in codons 129, 131, or 208 markedly decreased the carcinogen detoxification activity of UGT1A7 (23, 33). In our current study, patients harboring the *UGT1A7 L/L* alleles showed higher risk for HCC although the *UGT1A7* gene is known to be expressed predominantly in the lung and gastrointestinal tract and not in the liver and biliary epithelium. We speculate that the *UGT1A7* gene plays a critical role in the detoxification of hepatocarcinogens at the epithelia of the lung and gastrointestinal tract, which are thought to be entry sites for mutagens. Alternatively, there may be an unknown gene that is directly associated with HCC and that acts together with the *UGT1A7* polymorphisms, or perhaps an unknown function of *UGT1A7* affects the liver through actions at a different site.

We do not yet have a clear explanation for the fact that the *UGT1A7 *2/*3* alleles had a higher OR for HCC than did **2/*2* or **3/*3*. An evaluation of the functional impacts of the amino acid sequence differences may clarify one of the steps in the development of hepatocarcinogenesis.

To examine whether these *UGT1A7* alleles were related to cirrhosis, we calculated the allele frequencies in the presence and absence of liver cirrhosis and found no statistically significant relationship between the two conditions. Thus, the association between the *UGT1A7* polymorphisms and HCC was independent of cirrhosis. In our multivariate logistic regression model, the ORs of covariates other than polymorphisms in *UGT1A7* and *IL-1β*, the presence of cirrhosis, age, gender, and AFP were similar to those reported in previous studies (29–31). Our study population may have been biased toward patients with HCC or cirrhosis because many patients are referred to our hospital for treatment of HCC. However, our multivariate model included most of the previously reported risk factors for HCC with polymorphisms in the *UGT1A7* and *IL-1β* genes. This suggests that our results can be generalized to the Japanese population.

Recent reports of genetic polymorphisms associated with HCC in patients with chronic HCV infection include the −31−511T/C haplotype in the promoter region of the gene for the proinflammatory cytokine IL-1β in the Japanese population (11), polymorphisms in the microsomal epoxide hydrolase gene in Italians (14), and polymorphisms in the enzyme cytochrome P in Caucasians (12). Although we failed to find a SNP map that predicted high susceptibility to HCC in this study, accumulating information on HCC-related SNPs may facilitate the identification of patients with high susceptibility to HCC in the future.

Despite the limitations of a case–control study, our analyses showed a prominent effect of the *UGT1A7* lower-activity alleles on the risk of developing HCC. The uncertainty of the ORs that arises from the study design might be resolved in a subsequent controlled trial or in a large-scale screening association study. The *UGT1A7 L/L* alleles, as well as the *IL-1β*/−31T/T (−511C/C) genotype, might be used as markers of host factors associated with higher risk of HCC in Japanese patients with chronic HCV infection.

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