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...
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 second-generation enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan) and were negative for hepatitis diseases. We obtained written informed consent for DNA genotyping from all subjects. We also obtained approval for this study from the institutional ethics committee.

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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, 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'-gggctegacatactatatattagcgt-3') and R1 (Ref. 13; 5'-gggatatctagacgctgtatagtggcctagc-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'-accatggaagctgc-3'), S129R (5'-acattagcaccag-3'), S208F (5'-ctgctctctctctctg-3'), and S208R (5'-tggaaatagggc-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 presence 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):

\[
 n = \frac{(Z_{\alpha/2} + Z_{1-P})^2}{P(1-P)B^2(1-R^2)}
\]

(A)

where \( Z_{\alpha/2} \) is the upper \( \alpha/2 \) percentile of the standard normal distribution; \( P \) is the proportion of patients with HCC when \( X = 1 \); \( B^2 \) 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 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
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 χ² test. The association between the genotype of each locus and the presence of HCC was evaluated using the χ² 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 equilibrium in both the patients and healthy individuals (P > 0.05). The associations between the genotype and HCC (Table 2) were calculated using the χ² test and *Mann–Whitney U test.

### RESULTS

**Patient Characteristics.** There were no significant differences in alcohol intake, HCV genotype, 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).

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

<sup>a</sup> 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).

<sup>b</sup> HCC, hepatocellular carcinoma; HCV, hepatitis C virus

<sup>c</sup>–<sup>d</sup> Ps were calculated by χ² test and *Mann–Whitney U test.

<sup>3</sup> ftp://linkage.rockefeller.edu/software.

<sup>4</sup> www.r-project.org.
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 alleles, 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 $IL-1\beta$ Genetic Polymorphisms and HCC.** Consistent with our previous report, polymorphisms in the $IL-1\beta$ gene were associated with HCC. The $IL-1\beta$−31 and −511 loci were in complete linkage disequilibrium ($D' = 1$). The $IL-1\beta$−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 $IL-1\beta$−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 $IL-1\beta$ (2q41) 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 $IL-1\beta$ 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 $IL-1\beta$ 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 $IL-1\beta$−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 <125 × 10^9/l ($P < 0.001$). Stepwise multivariate logistic regression analysis was performed with these 11 variables. Six variables ($UGT1A7$ $L/L$ and $IL-1\beta$−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
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 −31−511/T-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 (−511/C/C) genotype, might be used as markers of host factors associated with higher risk of HCC in Japanese patients with chronic HCV infection.

### REFERENCES

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