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***Original Article***

# Impact of *PNPLA3* polymorphisms on the development of hepatocellular carcinoma in patients with chronic hepatitis C virus infection

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*Aim:* The *PNPLA3* rs738409 C>G polymorphism (encoding for I148M) has recently been identified as a susceptibility factor for steatosis-mediated liver damage. We evaluated the influence of this polymorphism on hepatocarcinogenesis in patients with chronic hepatitis C (CHC) virus infection.

*Methods:* We genotyped the rs738409 single nucleotide polymorphism in 358 hepatitis C-associated hepatocellular carcinoma (HCC) patients and correlated the age at onset of HCC and the interval between hepatitis C virus (HCV) infection and the development of HCC in patients with each genotype.

*Results:* The frequencies of CC, CG and GG genotypes were 27.9% (100/358), 49.2% (176/358) and 22.9% (82/358), respectively, and were in Hardy–Weinberg equilibrium. The median age at onset of HCC for the GG genotype was significantly younger compared to for non-GG genotypes (67.81 vs 69.87 years, $P < 0.001$), and the median interval between HCV infection and the development of HCC was significantly shorter in patients with the GG genotype (39.96 vs 40.85 years, $P = 0.008$). *PNPLA3* GG genotype was also associated with a higher aspartate aminotransferase level (69.5 vs 59.0 IU/L, $P = 0.02$), lower prothrombin time (73.0% vs 78.0%, $P = 0.008$) and a higher prevalence of histological steatosis (40.0% *vs.* 22.2%, $P = 0.01$) at the time of HCC onset.

*Conclusion:* The *PNPLA3* genotype GG may be associated with accelerated hepatocarcinogenesis in CHC patients through increased steatosis in the liver.

**Key words:** fibrosis, hepatocarcinogenesis, risk allele, rs738409, steatosis

## INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is a major health burden, with 130–170 million people infected, representing nearly 3% of the world's population.[1] HCV infection is one of the major causes of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma (HCC).[2]

In epidemiological studies of chronic HCV infection (CHC), age, duration of infection, alcohol consumption, co-infection with HIV, low CD4 count, male sex and HCV genotype 3 have been shown to be associated with histological activity.[3–8] We also reported higher body mass index (BMI) as an independent risk factor for HCC development in CHC patients.[9] Although these factors explain part of the extreme variability seen in fibrosis progression among HCV-infected patients, they do not completely account for the differences. Genetic host factors have long been suspected to play a role in CHC.[10–12] Recently, two genome-wide association studies (GWAS) carried out in Japan reported genetic factors, MICA locus (rs2596542) and DEPDC5 locus (rs1012068), associated with HCV-related HCC.[13,14]

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Because of the global epidemic of obesity, non-alcoholic fatty liver disease (NAFLD) is rapidly becoming the most common liver disorder worldwide.[15–18] Liver steatosis also has gained increasing attention as a modifier of CHC progression. In fact, hepatic steatosis is a common histological feature of CHC, seen in more than half of patients, and has been associated with fibrosis progression and increased risk of HCC via overproduction of reactive oxygen species.[19–21]

Adiponutrin encoded by *PNPLA3* has been reported to have both lipolytic and lipogenic properties.[22] Recently, independent GWAS identified a single nucleotide polymorphism (SNP; rs738409 C>G) in the *PNPLA3* gene on chromosome 22, encoding an isoleucine to methionine substitution (p.I148M) of patatin-like phospholipase A3 as a genetic determinant of liver fat content or disease severity.[23,24] A recent meta-analysis showed that this polymorphism has been related, in NAFLD, to inflammatory activity and progression of fibrosis.[25] The previous basic research showed that the *PNPLA3* I148M impairs hydrolytic activity against triacylglycerol *in vitro* and is thought to lead to accumulation of triacylglycerol.[26] Other studies using mice showed that the inactivation of *PNPLA3* has no effect on hepatic fat accumulation,[27] but the overexpression of *PNPLA3* I148M causes an increase in hepatic triacylglycerol content.[28] The rs738409 polymorphism was also found to be associated not only with elevated liver enzymes or prevalence of fatty liver histology in healthy subjects,[29,30] but also with disease severity and fibrosis in NAFLD,[25,31,32] alcoholic liver disease[33,34] and CHC.[35,36] However, the influence of *PNPLA3* (rs738409 C>G) polymorphism on HCV-related HCC still remains controversial.[34,36,37] In the present study, we focused on the association between the rs738409 SNP and the age at onset of HCC and the interval between HCV infection and the development of HCC to evaluate the influence of the *PNPLA3* polymorphism on hepatocarcinogenesis in CHC patients.

## METHODS

### Patients

THIS RESEARCH PROJECT was approved by the ethics committees of the University of Tokyo (no. 400). The patients analyzed in the present study were derived from a HCV study cohort of the University of Tokyo Hospital. All patients visited the liver clinic at our institution between August 1997 and August 2009 and agreed to provide blood samples for human genome studies along with written informed consent according with the Declaration of Helsinki. We enrolled patients who had developed HCC and received initial therapy for HCC at our institution by 31 January 2010, and with samples available for genotyping. Exclusion criteria were positivity for hepatitis B surface antigen and presence of biliary disease. We also excluded patients without information on BMI, daily alcohol intake, HCV genotype and HCV viral load. Finally, 358 patients were enrolled, and all subjects were Japanese. We analyzed the association of rs738409 C>G polymorphism with the age at onset of HCC and the interval between HCV infection and the development of HCC. Because we lacked knowledge of the exact date of hepatitis C seroconversion, the duration of HCV infection was estimated indirectly, based on the year of the first transfusion.

### Diagnosis of HCC

Hepatocellular carcinoma was diagnosed by dynamic computed tomography, and hyperattenuation in the arterial phase with washout in the late phase was considered a definite sign of HCC. When the diagnosis of HCC was ambiguous, an ultrasound-guided tumor biopsy was performed, and a pathological diagnosis was made based on the Edmondson and Steiner criteria.[38]

### Genotyping

Human genomic DNA was extracted from the whole blood of each patient. Genotyping for the *PNPLA3* rs738409 C/G polymorphism was performed by polymerase chain reaction (PCR) using the TaqMan predesigned SNP Genotyping Assay (Applied Biosystems, Foster City, CA), as recommended by the manufacturer. Allele-specific primers were labeled with fluorescent dye (6-carboxyfluorescein or hexachloro-6-carboxyfluorescein) and used in the PCR reaction. Aliquots of the PCR products were genotyped using an allele-specific probe of the SNP on a real-time PCR thermocycler (MX3000P; Stratagene, La Jolla, CA, USA). Samples were subjected to 45 cycles of denaturation for 15 s at 95°C, annealing of primers for 30 s at 60°C and elongation for 30 s at 60°C.

### Study end-point

We analyzed the relationship between host factors, including *PNPLA3* (rs738409 C>G) polymorphisms, sex, BMI, alcohol consumption and HCV genotype, and the age at onset of HCC or the interval between HCV infection and the development of HCC (the primary end-points of this study). We also examined the relationship between rs738409 polymorphisms and clinical

findings at the onset of HCC (the secondary end-point), such as biochemical markers and histological findings. The histological grade of disease activity and the histological stage of fibrosis were assessed using the reproducible METAVIR scoring system as follows: grades A1 to A3 for the degree of necroinflammatory activity (A1 = mild to A3 = marked), and stages F0 to F4 for the degree of fibrosis (F0 = no fibrosis to F4 = cirrhosis).[39,40] The presence of steatosis was studied as a qualitative (<5% vs ≥5%) variable.

## Statistical analysis

Continuous variables are presented as medians with 1st and 3rd quartiles, whereas categorical variables are expressed as frequencies (%). Categorical data were analyzed using the $\chi^2$-test, and stepwise logistic regression analyses were used to adjust the influence of the *PNPLA3* genotype by other covariates such as sex, BMI (<25 or not) and alcohol consumption (<50 g/day or not). For continuous data, the univariate associations were evaluated using Student's *t*-test or the non-parametric Wilcoxon rank sum test as appropriate. Because the age at onset of HCC and the length of time between HCV infection and the development of HCC (the primary end-points of this study) satisfied the assumption of normal distribution (Kolmogorov–Smirnov test, $P > 0.05$), we used stepwise regression analysis for multivariate analyses. We evaluated the association between the rs738409 mutant G allele and each outcome using a recessive model of inheritance, comparing G allele homozygotes (GG genotype) with patients carrying one copy or no copies of the G allele (CG or CC genotypes) because this was suggested to be the most appropriate one by studies of the impact of rs738409 on CHC liver damage.[36,41] The Jonckheere–Terpstra trend test for continuous variables and the Cochran–Armitage trend test for categorical variables were used to evaluate the increasing or decreasing tendency of the findings across rs738409 CC, CG and GG genotypes. All statistical analyses were two-sided, and the threshold of the reported *P*-values for significance was less than 0.05. All statistical analyses were performed using the R version 2.13.1 software (http://www.r-project.org).

# RESULTS

## Patient characteristics

PATIENT CHARACTERISTICS ARE shown in Table 1. Frequencies of the rs738409 CC, CG and GG genotypes were 27.9% (100/358), 49.2% (176/358)

**Table 1** Clinical characteristics and genotype distributions of the subjects ($n = 358$)

| Parameter | Values |
|---|---|
| Median age at onset of HCC, years | 69.76 (63.88–75.35) |
| Male sex | 200 (55.9%) |
| BMI >25 | 67 (18.7%) |
| Alcohol consumption (>50 g/day) | 75 (20.9%) |
| *PNPLA3* genotype | |
| CC | 100 (27.9%) |
| CG | 176 (49.2%) |
| GG | 82 (22.9%) |
| G allele frequency | 0.47 |
| HCV genotype | |
| Genotype 1 | 271 (75.7%) |
| Genotype 2 | 87 (24.3%) |

Continuous variables are presented as medians with 1st and 3rd quartiles, and categorical variables as numbers and frequency (%).
BMI, body mass index; HCC, hepatocellular carcinoma; HCV, hepatitis C virus.

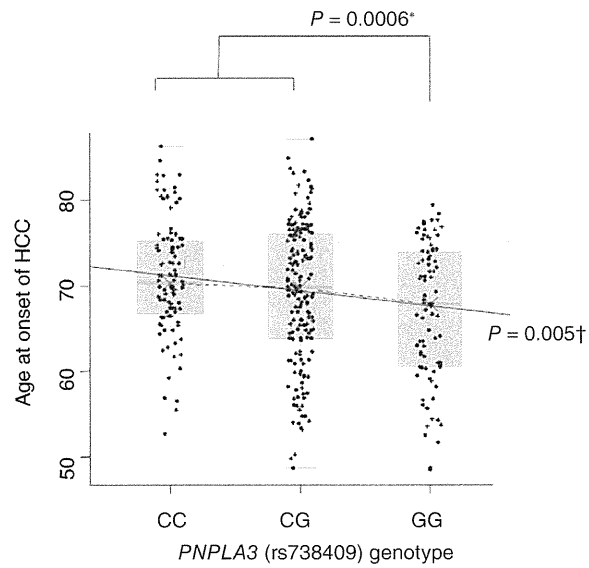


**Figure 1** Box and whisker and dot plot: distributions of the age at onset of hepatocellular carcinoma (HCC) in each genotype. The dashed line connects the median value of each genotype, and the solid line shows the linear regression. The Jonckheere–Terpstra trend test showed a significant trend across the CC, CG and GG alleles ($P = 0.005$). **P*-values after adjustment for sex, body mass index and alcohol consumption. †*P*-value by the Jonckheere–Terpstra trend test.

and 22.9% (82/358), respectively. The SNP genotype distribution was in Hardy–Weinberg equilibrium (*P*-value was non-significant). The median age at onset of the HCC patients was 69.76 years, and approximately 55% were male.

## Primary end-point

Table 2 shows the age at onset of patients with HCC and the associations among rs738409 genotypes, sex, BMI, alcohol consumption, HCV genotype and HCV viral load. The median ages (1st–3rd quartile) at onset in patients with HCC for the rs738409 GG and non-GG (CC/CG) genotypes were 67.8 years (range, 60.6–74.0) and 69.9 years (range, 65.2–75.6), respectively. The median age was significantly younger in patients with the rs738409 GG genotype than in those with non-GG genotype ($P = 0.004$). In multivariate analysis, early age at onset of HCC was independently associated with rs738409 GG genotype ($P < 0.001$), male sex ($P = 0.004$) and higher BMI ($P = 0.03$). The median ages at onset of patients with HCC for the CC and CG genotypes were 70.3 and 69.7 years, respectively. The Jonckheere–Terpstra trend test showed a significant trend across the GG, CG and CC alleles ($P = 0.005$; Fig. 1). One hundred and sixty-six patients had histories of blood transfusion. The median (1st–3rd quartile) intervals between blood transfusion and the onset of HCC in patients with rs738409 GG and non-GG (CC/CG) genotypes were 39.96 (range, 33.43–45.84) and 40.85 years (range, 33.52–46.76), respectively. In multivariate analysis, the median interval between blood transfusion and the onset of HCC was significantly shorter in patients with rs738409 GG genotype ($P = 0.008$) and male sex ($P < 0.001$) (Table 3).

## Secondary end-point

Table 4 shows the clinical findings and associations between the rs738409 genotypes at the time of HCC onset. The rs738409 GG genotype was significantly associated with a higher aspartate aminotransferase (AST) level (69.5 vs 59.0 IU/L, $P = 0.02$), a lower prothrombin time (72.95% vs 78.00%, $P = 0.008$) and a higher prevalence of histological steatosis (40.00% *vs.* 22.16%, $P = 0.01$) compared to the non-GG genotype after adjustment for sex, BMI and alcohol consumption. There were no significant associations between rs738409 genotype and histological stage of fibrosis or histological grade of disease activity. Figure 2 shows the

**Table 2** Factors associated with the age at onset of HCC ($n = 358$)

| Variable | Median | 1st–3rd quartile | *P*-value | |
|---|---|---|---|---|
| | | | Univariate | Multivariate† |
| *PNPLA3* genotype | | | 0.004 | <0.001 |
| GG | 67.81 | 60.58–73.97 | | |
| CC/CG | 69.87 | 65.20–75.62 | | |
| Sex | | | <0.001 | 0.004 |
| Male | 68.59 | 62.09–74.20 | | |
| Female | 71.81 | 65.98–76.26 | | |
| BMI | | | 0.07 | 0.03 |
| >25 | 68.95 | 63.05–73.50 | | |
| ≤25 | 70.49 | 64.32–75.57 | | |
| Alcohol consumption | | | 0.02 | 0.11 |
| >50 g/day | 68.25 | 59.75–73.35 | | |
| ≤50 g/day | 70.12 | 64.80–75.47 | | |
| HCV genotype | | | 0.2 | |
| Genotype 1 | 69.87 | 64.35–75.53 | | |
| Genotype 2 | 68.65 | 63.50–74.17 | | |
| Viral load | | | 0.09 | 0.06 |
| High‡ | 70.57 | 65.08–75.82 | | |
| Low§ | 68.89 | 63.75–74.59 | | |

†Stepwise regression analysis for the age at onset of hepatocellular carcinoma (HCC; the dependent variable) using *PNPLA3* genotype, sex, body mass index (BMI), alcohol consumption, hepatitis C virus (HCV) genotype and HCV viral load as independent variables.
‡At or above the median value.
§Below the median value.

**Table 3** Factors associated with the time between HCV infection and the development of HCC ($n = 166$)

| Variable | Median | 1st–3rd Quartile | *P*-value | |
|---|---|---|---|---|
| | | | Univariate | Multivariate† |
| *PNPLA3* genotype | | | 0.47 | 0.008 |
| GG ($n = 40$) | 39.96 | 33.43–45.84 | | |
| CC/CG ($n = 126$) | 40.85 | 33.52–46.76 | | |
| Sex | | | 0.04 | <0.001 |
| Male | 38.54 | 31.95–44.93 | | |
| Female | 42.45 | 35.67–47.25 | | |
| BMI | | | 0.75 | – |
| >25 kg/m$^2$ | 37.94 | 32.91–45.60 | | |
| ≤25 kg/m$^2$ | 40.85 | 33.70–46.87 | | |
| Alcohol consumption | | | 0.26 | – |
| >50 g/day | 40.13 | 28.55–45.33 | | |
| ≤50 g/day | 40.87 | 33.79–46.76 | | |
| HCV genotype | | | 0.09 | – |
| Genotype 1 | 41.46 | 34.20–46.92 | | |
| Genotype 2 | 37.80 | 28.70–45.44 | | |
| Viral load | | | 0.008 | 0.11 |
| High‡ | 41.81 | 35.18–48.28 | | |
| Low§ | 38.53 | 30.79–45.12 | | |

†Stepwise regression analysis of age at onset of hepatocellular carcinoma (HCC; the dependent variable) using *PNPLA3* genotype, sex, body mass index (BMI), alcohol consumption, hepatitis C virus (HCV) genotype, HCV viral load and the age at blood transfusion as independent variables.
‡At or above the median value.
§Below the median value.

histological findings for CC, CG and GG genotypes. The increment in the G allele was significantly associated with a higher prevalence of steatosis, as demonstrated by the Cochran–Armitage trend test (CC 13.11% vs CG 28.45% vs GG 40.00%, respectively; $P = 0.004$).

## DISCUSSION

IN THIS STUDY, we found that the risk allele of *PNPLA3*, which was strongly correlated with significant liver steatosis, also may be a risk factor for hepatocarcinogenesis in CHC patients. Median age at onset of HCC was significantly younger ($P < 0.001$), and the median interval between blood transfusion and the onset of HCC was significantly shorter ($P = 0.008$) in patients with the rs738409 GG genotype than in those with non-GG genotypes after adjustment for sex, BMI, alcohol consumption, HCV genotype and HCV viral load.

Earlier age at HCC onset or shorter time between HCV infection and the development of HCC in the GG genotype was thought to be caused by the acceleration of liver fibrosis. The patients with the rs738409 GG genotype may reach the stage of advanced cirrhosis and develop HCC in their early age or shorter time after HCV infection. Previous studies reported hepatic steatosis as a risk factor for progressed fibrosis and HCC in CHC patients.[4,42] The *PNPLA3* polymorphism was originally reported as a determinant of liver fat content,[23] and a significant association between rs738409 SNP and histological evidence of steatosis (≥5%) was identified in the present study. The *PNPLA3* polymorphism was thought to affect the susceptibility to HCC in CHC patients via alteration of lipid accumulation in the liver.

Although this was not confirmed histologically, the *PNPLA3* GG genotype was also significantly associated with higher AST level and tended to be associated with a higher prevalence of progressed histological fibrosis compared to the non-GG genotypes (74.0% vs 60.5%, $P = 0.11$) at the time of HCC onset. Moreover, the GG genotype was associated with a lower prothrombin time, which suggests depressed liver function. Increased lipid accumulation in the *PNPLA3* GG genotype may enhance the risks of hepatic inflammation, fibrosis and impairment of liver function in CHC patients.

**Table 4** Associations between *PNPLA3* genotype and clinical findings at the time of HCC onset ($n = 358$)

| Variable | Median/number (1st–3rd quartile) | | *P*-values | |
|---|---|---|---|---|
| | GG | Non-GG | *P*-value | Adjusted *P*-value† |
| Platelet count ($\times 10^4/\mu L$) | 10.05 (7.73–12.78) | 10.30 (7.68–13.35) | 0.53 | – |
| AST (IU/L) | 69.5 (49.0–88.5) | 59.0 (43.0–83.5) | 0.048 | 0.02§ |
| ALT (IU/L) | 59.0 (42.0–93.3) | 55.0 (37.0–86.3) | 0.29 | – |
| TB (mg/dL) | 0.8 (0.6–1.1) | 0.8 (0.6–1.1) | 0.85 | – |
| Albumin (g/dL) | 3.7 (3.3–3.9) | 3.7 (3.4–3.9) | 0.41 | – |
| PT (%) | 73.0 (67.3–79.0) | 78.0 (69.0–90.0) | 0.004 | 0.008§ |
| Viral load (log IU/mL) | 4.73 (4.51–4.94) | 4.75 (4.35–5.20) | 0.90 | – |
| LDL cholesterol (mg/dL) | 77.2 (63.1–90.3) | 74.7 (57.6–93.6) | 0.77 | – |
| Triglyceride (mg/dL) | 82.0 (59.0–108.0) | 87.0 (66.0–114.0) | 0.32 | – |
| Fasting plasma glucose (mg/dL) | 100.0 (88.5–116.0) | 103.0 (91.3–121.8) | 0.20 | – |
| Plasma insulin (μg/mL) | 12.0 (8.0–18.0) | 12.0 (9.0–19.0) | 0.67 | – |
| Histological findings ($n = 235$) | | | | |
| Fibrosis | | | | |
| F0–3 | 13 | 73 | 0.11 | – |
| F4 | 37 | 112 | | |
| Activity | | | | |
| A0–1 | 30 | 112 | 0.93 | – |
| A2–3 | 20 | 73 | | |
| Steatosis‡ | | | | |
| <5% | 30 | 144 | 0.02 | 0.01¶ |
| ≥5% | 20 | 41 | | |

†Adjusted for sex, BMI and alcohol consumption (independent variables). The dependent variables of each *P*-value are the items in the leftmost fields of the corresponding row (e.g. platelet count, AST, ALT).
‡Odds ratio (95% CI) for the GG allele was 2.43 (1.24–4.77), and the 95% CI of each proportion is shown in parentheses for this outcome.
§*P*-value by stepwise regression analysis.
¶*P*-value by stepwise logistic regression analysis.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; CI, confidence interval; HCC, hepatocellular carcinoma; LDL, low-density lipoprotein; PT, prothrombin time; TB, total bilirubin.

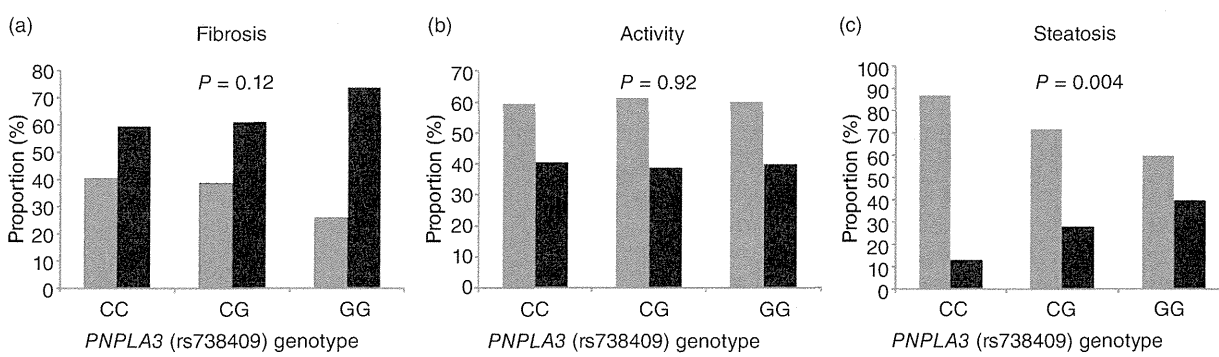


**Figure 2** Bar plot: prevalence of fibrosis (F1–3 vs F4, a), necroinflammation (A1 vs A2–3, b) and steatosis (<5% vs ≥5%, c) in 235 patients with chronic hepatitis C. The proportions are shown on the Y axis. *P*-values of the frequency distributions are shown (Cochran–Armitage trend test). ■, F1–3; ■, F4; ■, A1; ■, A2–3; ■, <5%; ■, ≥5%.

One study investigated the impact of the *PNPLA3* polymorphism on liver steatosis and fibrosis in CHC patients.[36] In this study, the cumulative incidence of HCC during the follow-up period was significantly higher in patients with the GG genotype.[36] The *PNPLA3* polymorphism is also associated with susceptibility to HCC in patients with other causes of hepatitis.[34,43] Our data suggest that the *PNPLA3* rs738409 polymorphism may provide important information that will assist identification of patients at particular risk for HCC.

In the present study, early age at onset of HCC was also independently associated with male sex and higher BMI, and the median interval between blood transfusion and the onset of HCC was significantly associated with male sex. These results are consistent with previous reports of male sex and higher BMI as independent risk factors for HCC development in CHC patients.[9,44,45]

A limitation of the present study is its retrospective design. The histology samples at the time of initial treatment were obtained via ultrasound-guided aspiration at the time of percutaneous tumor ablation or surgical resection. To minimize the risk of bleeding, ultrasound-guided aspiration was not performed for patients with a platelet count of less than 6 ($\times 10^4/\mu L$). Therefore, the histological samples were collected from a biased group of patients. Another limitation is the cross-sectional study design and the lack of controls without HCC. We are unable to confirm whether the age at onset of HCC (primary outcome of the present study) is an adequate indicator of susceptibility to HCC from the current study alone. Further prospective study is needed to validate the current results.

In conclusion, the *PNPLA3* rs738409 C>G polymorphism may play a significant role in hepatocarcinogenesis in CHC patients. Thus, this genetic factor should be taken into consideration when determining a treatment strategy intended to prevent the future development of HCC in CHC patients.

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HEALTH AND QUALITY OF LIFE OUTCOMES

RESEARCH Open Access

# Validation of the Japanese version of the EORTC hepatocellular carcinoma-specific quality of life questionnaire module (QLQ-HCC18)

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## Abstract

**Background:** This study examined the measurement properties of the Japanese version of the European Organisation for Research and Treatment of Cancer (EORTC) Hepatocellular Carcinoma-Specific Quality of Life Questionnaire (QLQ-HCC18).

**Methods:** EORTC quality of life (QOL) translation guidelines were followed to create a Japanese version of the EORTC QLQ-HCC18. This was then administered to 192 patients with hepatocellular carcinoma along with the EORTC QLQ-C30 and FACT-Hep questionnaires. Tests for reliability and validity were conducted including comparison of scores between the EORTC and FACT questionnaire and detailed assessment of the new scales and items in clinically distinct groups of patients.

**Results:** Multi-trait scaling analysis confirmed three putative scales in the QLQ-HCC18, fatigue, fever and nutrition. Cronbach's alpha for these scales were between 0.68 and 0.78. The QLQ-HCC18 scales correlated with scales measuring similar items in the FACT-Hep and the questionnaire was stable over time with an intra-class correlation score of 0.70 for almost all scales. The questionnaire had the ability to distinguish between patients with different Karnofsky Performance Status, and Child-Pugh liver function class.

**Conclusions:** The Japanese version of EORTC QLQ-HCC18 is a reliable supplementary measure to use with EORTC QLQ-C30 to measure QOL in Japanese patients with hepatocellular carcinoma.

**Keywords:** EORTC QLQ-HCC18, FACT-Hep, Hepatocellular carcinoma, Quality of life, Questionnaire

## Background

Hepatocellular carcinoma (HCC) is the most common malignancy in the world, accounting for more than half a million new cases annually [1,2]. The highest incidence rates are in eastern and south-eastern Asia, western and central Africa [2]. The incidence is low in most developed countries, however, Japan has a very high prevalence of HCC, and 70% are caused by hepatitis C viruses [3]. Although the 5-year survival rates of up to 60 to 70% can be achieved in well-selected patients, the recurrence rate remains very high [4,5]. The 5-year recurrence rate after potentially curative liver resection is up to 80% [4-6]. In countries such as Japan, where cadaveric donor organs are scarce, application of liver transplantation is limited [7,8]. Thus, most patients with HCC undergo repeated non-transplant treatments such as surgical resection, percutaneous radiofrequency ablation and embolization. Although survival data and information about the side effects of treatment are widely available, much less is known about how treatment for HCC impacts upon the patients' quality of life (QOL). Given the time course of the disease, and the burden of repeated treatment, there are increasing concerns about QOL associated with HCC. When deciding upon treatment, consideration of QOL outcomes could be as important as survival. However, there are no HCC-specific QOL questionnaires in Japan.

At present, there are two disease-specific QOL questionnaires for evaluating the QOL of patients with HCC. One is

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the European Organization for Research and Treatment of Cancer (EORTC) Quality of Life Group questionnaire, the QLQ-HCC18, and the other is the Functional Assessment of Cancer Therapy (FACT) Hepatobiliary (FACT-Hep) questionnaire [9,10]. As they are disease-specific, they are combined with generic questionnaires such as the QLQ-C30 and FACT generic questionnaires, respectively, to produce a generic and a specific QOL assessment [11,12]. The major difference between FACT-Hep and EORTC QLQ-HCC18 is that FACT-Hep targets not only patients with HCC but also patients with pancreatic, biliary and metastatic liver cancer, whereas the QLQ-HCC18 is designed specifically for patients with HCC. Currently there is a lack of published data demonstrating the measurement properties of EORTC QLQ-HCC18.

The objective of this study, therefore, was to develop a Japanese version of EORTC QLQ-HCC18, and to validate its measurement properties in patients with HCC.

## Methods

### Translation of the Japanese version of EORTC QLQ-HCC18

The EORTC guidelines for translation of the QLQ-HCC18 was followed and authorized by the EORTC [13]. This included a forward/backward translation of EORTC QLQ-HCC18. The original English version was translated into Japanese by two independent translators who were native Japanese speakers with proficiency in English. The research coordinator compared the two forward translations and checked them for any discrepancies. The discrepancies between the two translations were discussed with the translators until we agreed on one provisional forward translation. This forward translation was then back translated into English by two independent translators who were native speakers of English with proficiency in Japanese. The English back translations and the original English version were compared to assure that there were no differences in the meaning of the questions in the questionnaires. The provisional Japanese version was pilot tested on 10 patients diagnosed with HCC who had satisfied the following eligibility criteria: (1) age > 20 years; (2) ability to communicate in Japanese; (3) ability to participate in this study, as judged by an attending doctor; (4) confirmation of medical diagnosis; (5) no other concurrent malignancy; and (6) consent to participate in this study. The pilot test was conducted according to the manual provided by EORTC [13] as of June 2008. The average time necessary for completing the QLQ-HCC18 was less than 5 minutes and the questionnaire was well understandable and acceptable in most patients. Results of the translation and the pilot study were reviewed by the EORTC translation coordinator and the original author of QLQ-HCC18, to ensure the content and applicability was maintained, and the EORTC QLQ-HCC18 Japanese version was authorized by the EORTC Quality of Life Group. The Japanese version of EORTC QLQ-HCC18 was used in this validation study.

### Data collection

This study recruited 200 patients diagnosed with HCC at The University of Tokyo Hospital, one of the largest referral centers for treatment of HCC in Japan, and written consent was obtained. Patients were recruited between July 2008 and November 2008. The eligibility criteria were the same as for pilot testing. Patients completed each of the three questionnaires: EORTC QLQ-C30, QLQ-HCC18, and FACT-Hep, and a questionnaire about demographic characteristics. To confirm test-retest reliability of the Japanese version of QLQ-HCC18, patients with stable disease were invited to complete QLQ-HCC18 for a second time after two weeks. Medical data were collected by review of medical care records. The researcher checked for absent responses after receiving the questionnaire and wherever possible asked the patients to respond to the missing items. This study was conducted with the approval of the ethics committee of The University of Tokyo.

### Measurements

The EORTC QLQ-C30 core questionnaire (version 3.0) is a generic QOL measure for cancer patients, and comprises a global health status/QOL scale, five multi-item functional scales, three multi-item symptom scales and single items for the assessment of symptoms and the financial impact of disease and treatment [11]. The reliability and validity of the Japanese version of the EORTC QLQ-C30 has been demonstrated [14].

EORTC QLQ-HCC18 is an 18-item HCC-specific supplemental module developed to augment QLQ-C30 and to enhance the sensitivity and specificity of HCC-related QOL issues [9]. EORTC QLQ-HCC18 was developed in four stages on the basis of the EORTC guidelines for scale development [9]. Briefly, items were created during phase one after conducting a literature review and interviewing 32 patients with HCC from four different countries as well as 10 health professionals. In phase two, a preliminary questionnaire was constructed using the EORTC item bank as a reference. In phase three, a pretest was administered to 158 patients with HCC from three countries to examine receptivity and relevance. The original questionnaire is from the end of phase three. The hypothesized scale structure and single items address aspects of chronic liver disease (nutrition, jaundice, fever, abdominal swelling), as well as QOL issues specific to the primary tumor and its treatment (fatigue, body image, pain).

The original English version contains six multi-item scales addressing fatigue, body image, jaundice, nutrition, pain and fever, as well as two single items addressing sexual life and abdominal swelling. The scales and items are linearly transformed to a 0 to 100 score, where 100

represents the worst status. An international field test (the phase 4 part of questionnaire development) is currently being conducted to examine the validity and reliability of the scores in several countries.

The reliability and validity of the original version of FACT-Hep, another hepatobiliary cancer-specific scale, has been demonstrated [10]. FACT-Hep is a 45-item self-report instrument that comprises 27 FACT General (FACT-G) items and an 18-item hepatobiliary subscale. The Japanese version of the 18-item hepatobiliary subscale was used in this study as a comparison instrument. All items are scored from 0 to 4, with higher scores indicating better QOL.

### Data analysis

Multi-trait scaling analyses [15] evaluated the scale structures of QLQ-HCC18. This technique is used to test for item convergent and discriminant validity, and is based on the examination of item-scale correlations. The Pearson correlations of an item with its own scale (corrected for overlap) and other scales were calculated. Evidence of item convergent validity was defined as a correlation above 0.40 with its own scale. Evidence of item discriminant validity was based on a comparison of correlation of an item with its own scale and with other scales. Scaling success for any scale is defined as the number of convergent correlation coefficients significantly higher than the discriminant correlation coefficient divided by the total number of correlations. The mean scale and item scores were also calculated, and a frequency analysis was performed.

The following psychometric aspects were assessed: reliability, i.e., internal consistency and test-retest reliability; validity: known group comparison, and correlation analyses with the FACT-Hep.

The internal consistency reliability of the multi-item questionnaire scales was assessed by Cronbach's alpha coefficient. Preferable reliability was indicated by coefficient greater than 0.70. The test-retest reliability of the scales and single items was assessed by the intra-class correlation coefficient. Scale discriminant validity (clinical validity) was tested by known group comparisons to assess whether the questionnaire scores were able to discriminate between subgroups of patients differing in clinical status by using the Student t-test. The Karnofsky Performance Status (KPS) and Child-Pugh grade for clinical parameters were employed to form mutually exclusive patient subgroups. Higher scores in KPS signify better performance status. Liver function becomes worse in alphabetical order of Child-Pugh grade A, B, C. We hypothesized that scores of QLQ-HCC18 are low in patients with better performance status (KPS 80–100) and better liver function (Child-Pugh class A). Convergent validity was tested first by multi-trait analyses, and we then conducted another convergent validity test by correlation analyses with FACT-Hep. Pearson's correlation coefficient was used to examine the correlation between similar items in FACT-Hep and QLQ-HCC18. We hypothesized that if Pearson's correlation coefficients were more than 0.40 between scales, they were conceptually related. $P < 0.05$ was considered as statistically significant. Statistical analyses were performed using SAS software (SAS for Windows, release 9.1; SAS Institute Inc., Cary NC, USA).

## Results

### Participants

Responses were obtained from 192 patients (eight non responders), and 139 completed the test-retest questionnaire two weeks after the first assessment.

Socio-demographic and clinical characteristics at baseline are shown in Table 1. Most patients were male (64.1%), had good performance status (86.5%) and had good liver function (66.2%).

**Table 1 Socio-demographic and clinical characteristics of the study subjects (n = 192)**

| | |
|---|---|
| **Male gender** | **122 (63.5)** |
| Age, y* | 68.1 (8.5) |
| Employed full time or part-time | 77 (40.1) |
| Post compulsory education or above | 155 (80.7) |
| Married or living with partner | 151 (78.6) |
| Karnofsky Performance status | |
| 80-100 | 166 (86.5) |
| Comorbid liver disease | |
| Hepatitis C virus | 126 (65.6) |
| Hepatitis B virus | 38 (19.8) |
| Other or unknown | 28 (14.6) |
| Child-Pugh class | |
| A | 127 (66.1) |
| B | 53 (27.6) |
| C | 12 (6.3) |
| Cancer stage | |
| Stage I / II | 161 (83.9) |
| Stage III / IV | 31 (16.1) |
| Time since diagnosis, month* | 39.6 (34.5) |
| Past medical history† | |
| Hepatectomy | 48 (25.0) |
| Percutaneous ablation | 137 (71.4) |
| Chemoembolization | 64 (33.3) |
| Systemic therapy | 1 (0.5) |
| No medical history | 13 (6.8) |

Values are numbers (%) otherwise specified. * Data was expressed as mean (standard deviation). † Some patients underwent multiple treatments.

### Psychometric testing

We initially performed multi-trait scaling analyses for the putative scale structure, and the results showed that the original two-item scale of body image and jaundice had low convergent and discriminant validity. After discussion with the original author of QLQ-HCC18 (JMB) we decided to split the scale into single items. The tests were then performed on the remaining scales and four single items. Results of the multi-trait scaling analyses are shown in Table 2. A summary of the multi-trait scaling analysis and internal consistency is shown in Table 3. The convergent correlation coefficient of the scales for fatigue, nutrition and fever varied from 0.23 to 0.75, and the scaling success rate ranged from 87% to 100%. Cronbach's alpha coefficient of these scales was satisfactory, ranging from 0.68 to 0.78. The convergent correlation coefficient of the scales for pain was 0.25, and the scaling success rate was 50%. Cronbach's alpha coefficient of this scale was 0.37.

The results of the descriptive statistics of the putative scales/single items and test-retest reliability on the questionnaire are shown in Table 4. The intra-class correlation coefficients of the scales varied between 0.67 and 0.88. Ninety-four percent of the patients answered 'not at all' to item 36, which asked patients whether they were concerned by their skin or eyes being yellow. Responses to item 48, which asked about sexual function, were missing in seven patients (3.6%).

Results of the known group comparisons are shown in Table 5. Patients with poorer performance status (KPS of 70 or lower) reported significantly higher (worse) scores for all scales except for abdominal swelling and sexual interest than those with better performance status (KPS of 80–100). Patients with worse liver disease (Child-Pugh classes B and C) reported significantly higher (worse) scores for all scales except for body image and pain than those with better liver function (Child-Pugh class A).

Results of convergent validity are shown in Table 6. The QLQ-HCC18 Japanese version scales had an acceptable correlation (coefficient value over 0.40) with similar items in FACT-Hep except for items of weight loss, appetite and activity.

## Discussion

This study describes psychometric testing of the Japanese version of the QLQ-HCC18 questionnaire, which is an HCC-specific module of EORTC QLQ-C30. The overall results show that this questionnaire is reliable and has acceptable measurement properties for use with the QLQ-C30 to assess health-related QOL in Japanese patients with HCC.

Assessment of QOL in cancer patients is optimally performed with a combination of a generic questionnaire and a disease-specific questionnaire to ensure that common problems are uniformly detected and reported as well as specific issues related to disease site and treatment. This framework for QOL assessment has been adopted and popularized by the EORTC Quality of Life Group and the Functional Assessment of Chronic Illness Therapy

**Table 2 Item-scale correlations for multi-trait scaling analyses of the EORTC QLQ-HCC18**

| Item | | Hypothesized scales of the EORTC QLQ-HCC18† | | | |
|---|---|---|---|---|---|
| | | Fatigue | Nutrition | Pain | Fever |
| **Fatigue** | | | | | |
| Item46 | Have you been less active than you would like to be? | 0.68† | 0.57 | 0.29 | 0.32 |
| Item45 | Have you found it difficult to keep going or to finish things you started? | 0.75† | 0.55 | 0.32 | 0.35 |
| Item47 | Have you needed to sleep during the day? | 0.44† | 0.29 | 0.24 | 0.14 |
| **Nutrition** | | | | | |
| Item31 | Did you feel thirsty? | 0.42 | 0.50† | 0.30 | 0.23 |
| Item32 | Have you had problems with your sense of taste? | 0.30 | 0.50† | 0.18 | 0.18 |
| Item42 | Have you worried about getting enough nourishment? | 0.45 | 0.54† | 0.31 | 0.51 |
| Item43 | Have you felt full up too quickly after beginning to eat? | 0.48 | 0.44† | 0.30 | 0.27 |
| Item44 | Have you worried about your weight being too low? | 0.21 | 0.23† | 0.04 | 0.28 |
| **Pain** | | | | | |
| Item38 | Have you had pain in your shoulder? | 0.20 | 0.15 | 0.25† | 0.11 |
| Item39 | Have you had abdominal pain? | 0.39 | 0.48 | 0.25† | 0.36 |
| **Fever** | | | | | |
| Item40 | Have you had fevers? | 0.34 | 0.46 | 0.27 | 0.52† |
| Item41 | Have you had chills? | 0.23 | 0.31 | 0.19 | 0.52† |

Correlations marked † were corrected for overlap.

**Table 3 Convergent and discriminant validity and internal consistency reliability for the EORTC QLQ-HCC18**

| Scale | No. of items per scale | Convergent validity (range of correlations) | Discriminative validity (range of correlations) | Scaling success† | Scaling success rate ‡ | Internal consistency reliability (Cronbach's α) |
|---|---|---|---|---|---|---|
| Fatigue | 3 | 0.44-0.75 | 0.14-0.57 | 9/9 | 100 | 0.78 |
| Nutrition | 5 | 0.23-0.54 | 0.04-0.51 | 13/15 | 87 | 0.68 |
| Pain | 2 | 0.25 | 0.11-0.48 | 3/6 | 50 | 0.37 |
| Fever | 2 | 0.52 | 0.15-0.46 | 6/6 | 100 | 0.68 |

† Number of convergent correlations significantly higher than discriminant correlations divided by total number of correlations.
‡ Scaling success rate is the previous column as a percentage.

**Table 4 Descriptive statistics of the EORTC QLQ-C30 and the QLQ-HCC18 and test-retest reliability**

| Scale<br>The QLQ-HCC18 | Score* | Intraclass correlation coefficient† |
|---|---|---|
| Scales‡ | | |
| Fatigue | 25.6 ± 22.2 | 0.82 |
| Nutrition | 12.7 ± 14.1 | 0.88 |
| Pain | 13.5 ± 17.1 | 0.80 |
| Fever | 5.3 ± 12.9 | 0.67 |
| Single items | | |
| Body Image 1 (item33) | 34.0 ± 31.9 | 0.73 |
| Body Image 2 (item35) | 21.1 ± 27.7 | 0.70 |
| Jaundice 1 (item36) | 2.77 ± 2.45 | 0.79 |
| Jaundice 2 (item37) | 22.2 ± 27.4 | 0.82 |
| Abdominal Swelling | 15.8 ± 23.6 | 0.78 |
| Sexual Interest | 12.6 ± 25.2§ | 0.77‖ |
| The QLQ-C30 | | |
| Scales | | |
| Physical¶ | 84.9 ± 16.9 | - |
| Role¶ | 84.1 ± 22.3 | - |
| Cognitive¶ | 84.2 ± 17.0 | - |
| Emotional¶ | 79.6 ± 20.5 | - |
| Social¶ | 86.1 ± 20.6 | - |
| Global QOL¶ | 66.5 ± 21.63 | - |
| Fatigue‡ | 30.9 ± 21.5 | - |
| Nausea/Vomiting‡ | 1.74 ± 6.4 | - |
| Pain‡ | 12.8 ± 20.6 | - |
| Single items‡ | | |
| Dyspnea | 15.8 ± 21.6 | - |
| Sleep Disturbance | 21.5 ± 27.5 | - |
| Appetite Loss | 12.7 ± 22.5 | - |
| Constipation | 14.2 ± 23.9 | - |
| Diarrhea | 7.81 ± 16.4 | - |
| Financial Impact | 14.6 ± 23.3 | - |

Data were expressed as mean ± standard deviation. * Score range 0 to 100. † Data were assessed in 130 patients. ‡Higher score indicates lower QOL. § Data were assessed in 185 patients. ‖ Data were assessed in 127 patients. ¶ Higher score indicates higher QOL.

(FACIT) Organization. For patients with primary and secondary liver tumors, cholangiocarcinoma or pancreatic cancer, the FACIT system has developed a single hepatobiliary-pancreatic module [10]. The EORTC QOL Group has, however, focused in more depth on the specific clinical experiences within each disease site and therefore developed separate modules for pancreatic, primary and secondary liver cancer. The separate modules may be clinically more sensitive than a single questionnaire, although this has not yet been formally examined. A second advantage of the EORTC QLQ-HCC18 is that it provides subscale scores for different domains of functioning. FACT-Hep generates only a total score, which may obscure findings in particular problem areas. EORTC QLQ-HCC18 possesses a multi-dimensional QOL assessment that may be more useful for clinicians to direct therapy. A final advantage of the EORTC QLQ module is that it was specifically developed for use in international trials; a large database will soon be available to facilitate comparisons across studies, and there is some assurance of cross-cultural suitability.

In this study, we tested the reliability and validity, including internal consistency reliability, test-retest reliability, convergent and discriminant validity, known group comparison, of the Japanese version of QLQ-HCC18. In the descriptive statistics and frequency analyses, the item assessing problems related to jaundice showed low scores. This was because few patients were jaundiced at the time of the data collection. In addition, because the Japanese belong to a race with a yellowish skin complexion, jaundice tends to be masked. The results of multi-trait scaling analyses (convergent and discriminant validity), had a good scaling success rate and acceptable Cronbach's alpha (internal consistency reliability) except for the scale for pain, which had a low scaling success rate and a low Cronbach's alpha. One reason for this may be because shoulder and abdominal pain are not necessarily related symptoms that occur simultaneously. Furthermore, although pain scales have been created in anticipation of pain caused by cancer treatment and progression, few patients had advanced cancer. The nutrition scale had a high rate of success. However, the convergent validity of the item termed "concern about low weight" was below the standard value. The nutrition scale was assumed to involve problems caused by impaired liver

**Table 5 Known group comparison of differences in mean scores of scales and items**

| | Karnofsky Performance status score | | | Child-Pugh grade | | |
|---|---|---|---|---|---|---|
| | 100-80 n = 166 | <80 n = 26 | t-test p value | A n = 127 | B and C n = 65 | t-test p value |
| Scales† | | | | | | |
| Fatigue | 21.0 ± 17.8 | 54.7 ± 25.9 | < 0.001 | 22.0 ± 20.4 | 32.7 ± 24.1 | 0.001 |
| Nutrition | 10.8 ± 11.4 | 24.9 ± 22.0 | < 0.001 | 10.9 ± 12.0 | 16.2 ± 17.1 | 0.02 |
| Pain | 11.6 ± 15.7 | 25.6 ± 20.7 | 0.003 | 12.3 ± 17.2 | 15.9 ± 16.8 | 0.17 |
| Fever | 4.3 ± 10.2 | 11.5 ± 23.0 | 0.003 | 3.5 ± 9.1 | 8.7 ± 17.7 | 0.02 |
| Single items† | | | | | | |
| Body Image 1(item33) | 30.7 ± 29.6 | 55.1 ± 38.8 | 0.004 | 29.7 ± 30.3 | 42.6 ± 33.6 | 0.01 |
| Body Image 2 (item35) | 19.5 ± 26.5 | 32.1 ± 33.3 | 0.03 | 19.7 ± 25.3 | 24.1 ± 32.0 | 0.34 |
| Jaundice 1 (item36) | 1.2 ± 6.2 | 12.8 ± 28.4 | 0.04 | 0.8 ± 5.1 | 6.7 ± 19.7 | 0.02 |
| Jaundice 2 (item37) | 17.7 ± 23.4 | 51.3 ± 33.0 | < 0.001 | 16.5 ± 23.7 | 33.3 ± 30.6 | 0.002 |
| Abdominal swelling | 14.1 ± 21.2 | 27.0 ± 34.0 | 0.07 | 13.1 ± 21.5 | 21.0 ± 26.7 | 0.04 |
| Sexual interest | 12.0 ± 24.1‡ | 16.7 ± 31.6 | 0.47 | 9.3 ± 21.1§ | 19.0 ± 30.9‖ | 0.03 |

Data were expressed as mean ± standard deviation unless otherwise specified. * $p < 0.05$, †Higher score indicates worse QOL
‡ missing in 7, § missing in 5, ‖ missing in 2.

function, but items regarding weight loss may also have been affected by cancer progression. Patients included in the original article and patients in this study had almost identical liver function, but the extent of cancer progression differed, and many of our patients had cancer that was detected at an earlier stage. The results of test-retest reliability showed good intra-class correlation coefficients for most scales. Results of known group comparisons showed that the module had the ability to assess differences between groups with different clinical characteristics in almost all of the scales, showing the module has clinical validity. We confirmed good correlations between the groups for most scales/single items in the two questionnaires (QLQ-HCC18 and FACT-Hep). However, correlations between items of weight loss, appetite, and activity in FACT-Hep and corresponding scales in QLQ-HCC18 were low. This may have occurred because of the reverse scoring used in appetite and activity items in FACT-Hep which may have led to confusion.

While the results show the Japanese version of EORTC QLQ-HCC18 is a reliable instrument, some caution is necessary. First, these results on the QLQ-HCC18 are

**Table 6 Pearson's correlation coefficients between scales in the QLQ-HCC18 and the FACT-Hep**

| | The FACT-Hep | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| The QLQ-HCC18 | Abdominal Swelling | Weight Loss | Appetite† | Appearance | Fatigue | Activity† | Jaundice | Fever | Itching | Taste | Chill | Thirsty | Abdominal Pain |
| Fatigue | 0.33 | 0.26 | 0.2 | 0.42* | **0.59*** | **0.26** | 0.26 | 0.3 | 0.43* | 0.32 | 0.17 | 0.37 | 0.42* |
| Body Image 1 (item33) | 0.31 | **0.32** | −1.7 | **0.46*** | −0.08 | 0.17 | 0.3 | 0.3 | 0.25 | 0.25 | 0.23 | 0.45 | 0.38 |
| Body Image 2 (item35) | **0.39** | 0.01 | −0.05 | **0.4*** | 0.1 | 0.07 | 0.18 | 0.31 | 0.2 | 0.3 | 0.12 | 0.23 | 0.47 |
| Jaundice 1 (item36) | 0.25 | 0.19 | −0.07 | 0.2 | −0.13 | 0.73 | **0.53*** | 0.3 | 0.12 | 0.18 | 0.56 | 0.28 | 0.3 |
| Jaundice 2 (item37) | 0.25 | 0.05 | −0.05 | 0.31 | 0.45 | −0.01 | 0.18 | 0.28 | **0.83*** | 0.33 | 0.26 | 0.35 | 0.37 |
| Nutrition | 0.32 | **0.34** | **0.31** | 0.32 | 0.38 | 0.23 | 0.31 | 0.28 | 0.26 | **0.44*** | 0.23 | **0.68*** | 0.33 |
| Pain | **0.32** | 0.11 | 0.14 | 0.28 | 0.32 | 0.14 | 0.21 | 0.26 | 0.34 | 0.15 | 0.2 | 0.3 | **0.40*** |
| Fever | 0.25 | 0.26 | 0.13 | 0.18 | 0.25 | 0.09 | 0.29 | **0.72*** | 0.35 | 0.18 | **0.61*** | 0.22 | 0.27 |
| Abdominal Swelling | **0.43*** | 0.07 | 0.03 | 0.16 | 0.29 | 0.06 | 0.23 | 0.3 | 0.3 | 0.15 | 0.28 | 0.24 | 0.42* |
| Sexual Interest ‡ | 0.26 | 0.14 | −0.06 | 0.25 | 0.24 | 0.08 | 0.06 | 0.12 | 0.12 | 0.06 | 0.12 | 0.13 | 0.24 |

* indicates Pearson's correlation coefficient larger than 0.4, _Underline indicates a pair of scales that should correlate theoretically, † Reverse scoring, ‡ Data were assessed in 185 patients.

preliminary as this study was performed in a single institution using the Japanese version, few patients with severe cirrhosis or advanced disease were recruited, and no patient had undergone liver transplantation, which may limit the generalizability of the findings.

Second, this study did not address longitudinal construct validity and responsiveness for clinical validity. In future work, the Japanese version of EORTC QLQ-HCC18 should be performed in multicenter facilities to confirm the generalizability of the findings and to increase the number of liver transplantation groups and more severely ill patients. Furthermore, testing the sensitivity of the instrument to changes over time is needed to evaluate treatment effects.

There are currently a variety of treatment options for patients with HCC. Molecular targeted therapy for HCC has recently been introduced [16], and this will lead to increased demand for evaluating the QOL in more detail. In addition, Japanese patients with HCC are older than in other countries, which make the Japanese version of QLQ-HCC18 particularly valuable because treatment effects on QOL are more important in older patients.

## Conclusion

This study showed that the Japanese version of the EORTC QLQ-HCC18 demonstrated evidence for the measurement properties of the questionnaire. These results suggest that it would be a reliable instrument for measuring QOL in patients with HCC in Japan.

### Abbreviations

QOL: Quality of Life; EORTC: European Organisation for Research and Treatment of Cancer; QLQ: Quality of life questionnaire; HCC: Hepatocellular carcinoma; KPS: The Karnofsky Performance Status; RFA: Radio frequency ablation.

### Competing interests

The authors declare that they have no competing interests.

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### Authors' contributions

NM, TS, MT, RT, JMB, NK and KK conceptualized the rationale and design of the study. NM, TS, MT, RT, JMB and KK conducted scale development. NM and TS presented this study to patients and collected data. NM, TS, MT, RT, JMB and KK conducted statistical analyses and interpreted the data. NM, RT, JMB drafted the manuscript. All authors read and approved the final manuscript.

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*Open*

# CT With Hepatic Arterioportography as a Pretreatment Examination for Hepatocellular Carcinoma Patients: A Randomized Controlled Trial



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OBJECTIVES: **The combination of computed tomography with hepatic arteriography and arterial portography (CTHA/CTAP) can detect additional hepatocellular carcinoma (HCC) nodules undetected by conventional dynamic CT.**

METHODS: **In this single-center, randomized, open-label, controlled trial, we randomly assigned 280 patients who were diagnosed as having HCC by conventional dynamic CT, and eligible for radiofrequency ablation (RFA), to undergo CTHA/CTAP before treatment, or to the control group. Newly detected HCC nodules by CTHA/CTAP were intended to be ablated completely. The primary end point was recurrence-free survival and the key secondary end point was overall survival. The analysis was conducted on an intention-to-treat basis. Those with nonablated nodules were treated as for recurrence.**

RESULTS: **A total of 75 nodules were newly diagnosed as HCC by CTHA/CTAP in 45 patients. Three patients (one in the CTHA/CTAP group and two in the control group) who refused treatment were excluded from all analyses. The cumulative recurrence-free survival rates at 1, 2, and 3 years were 60.1, 29.0, and 18.9% in the CTHA/CTAP group and 52.2, 29.7, and 23.1% in the control group, respectively ($P$=0.66 by log-rank test; hazard ratio, 0.94 for CTHA/CTAP vs. control; 95% confidence interval (CI), 0.73–1.22). The cumulative overall survival rates at 3 and 5 years were 79.7 and 56.4% in the CTHA/CTAP group and 86.8 and 60.1% in the control group, respectively ($P$=0.50; hazard ratio, 1.15, 95% CI, 0.77–1.71).**

CONCLUSIONS: **CTHA/CTAP may detect recurrent lesions earlier. However, CTHA/CTAP before RFA did not improve cumulative recurrence-free survival or overall survival.**

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## INTRODUCTION

Hepatocellular carcinoma (HCC) ranks as the fifth most common cancer worldwide (1). In Japan, ~35,000 patients die from HCC every year (2), and the main cause of HCC is hepatitis C virus infection. In chronic hepatitis patients, screening of HCC is usually performed by ultrasonography, and the diagnosis is confirmed by contrast-enhanced dynamic computed tomography (CT). Hyperattenuation in the arterial phase and hypoattenuation in the equilibrium phase are considered to be definitive signs of HCC (3–7). Hyperattenuation in the arterial phase is more emphasized when contrast material is injected from the hepatic artery through a catheter, because dilution of contrast material in the systemic circulation is avoided, thus keeping a high concentration of contrast material in the liver. This technique is called CT during hepatic arteriography (CTHA) (6,8–10). Similarly, hypoattenuation in the equilibrium phase is accentuated after injection of contrast material into the superior mesenteric artery, which is referred to as CT during arterial portography (CTAP) (11–14). The combination of CTHA and CTAP gives higher sensitivity and specificity for HCC detection than conventional dynamic enhanced CT (8).

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If new HCC nodules are detected with CTHA/CTAP, in addition to those detected with dynamic CT, the treatment of choice may be changed (15,16). For example, surgical resection and liver transplantation are usually contraindicated for multinodular HCC; that is, exceeding three nodules. Percutaneous tumor ablation methods, such as ethanol injection and microwave coagulation, have played an important role as nonsurgical treatments that can achieve high local cure rates without affecting background liver function (17–20). Radiofrequency ablation (RFA) is currently considered to be the most effective first-line percutaneous ablation protocol because of its greater efficacy in terms of local cure as compared with ethanol injection (21–24). However, even after complete ablation, patients frequently encounter intrahepatic tumor recurrence at a rate of 50% in 2 years, the majority of which occurs at locations distant from the primary ablated site (25). Considering the tumor doubling time, many nodules diagnosed as recurrent within 2 years were probably present at the time of first ablation. If nodules that are undetectable by conventional dynamic CT could be detected and ablated, the recurrence rate would be decreased.

Although CTHA/CTAP is one of the most sensitive techniques available for detection of small HCC, its disadvantages include invasiveness, high cost, and a high false-positive rate (26). The indication for CTHA/CTAP can be justified only when the expected benefits exceed the risk and cost of the procedure. We conducted a single-center, randomized, open-label, controlled trial to assess the utility of CTHA/CTAP before RFA in patients with early-stage HCC by comparing recurrence-free and overall survival.

## METHODS

### Patients

The study population consisted of patients with early-stage HCC with an indication for RFA. Those who met the following criteria were enrolled between September 2004 and February 2009: (i) diagnosis of typical HCC on dynamic CT performed within 2 weeks, i.e., hyperattenuation during the arterial phase and hypoattenuation during the equilibrium phase (5,6); (ii) tumor size ≤3.0 cm and no more than three tumor nodules; (iii) Child–Pugh class A liver function; and (iv) age >20 years. Exclusion criteria were: allergy to contrast media; portal or hepatic vein tumor thrombosis; extrahepatic metastasis; diffuse and infiltrative tumors; renal failure (serum creatinine >2.0 mg/dl, or serum urea nitrogen >30 mg/dl); impaired coagulation (e.g., platelet count $<50\times10^3/\mu l$, or prothrombin activity <50%); pregnancy; or past history of choledochojejunostomy. We included those with previous treatments as well as treatment-naive cases provided that there was no local recurrence at enrollment. These inclusion criteria and the study design did not change till the study completely ended. The study design conformed to the Declaration of Helsinki Principles and was approved by the ethics committee of our institution. The study was registered at the University Hospital Medical Information Network (UMIN) Clinical Trial Registry (UMIN-CTR000000070). Written informed consent was obtained from each patient. This study complied with the CONSORT guidelines for reporting of clinical trials (27).

### Study design

Before receiving RFA, patients were randomly assigned to undergo CTHA/CTAP or not in equal numbers. Patient registration and randomization were performed by computer-generated allocation at a web-based data center (Internet Data and Information Center for Medical Research) administered by UMIN. At the time of randomization, patients were stratified either as treatment naive, for whom RFA was planned as an initial treatment for HCC, or recurrent, for whom RFA was planned for recurrent HCC. The randomization was based on the Efron's biased-coin design (28). In principal, the assignment was not blinded to the investigators and the participants. The interval between random assignment and implementation of treatment for HCC was <4 weeks. CTHA/CTAP was performed on the assigned patients on the second day of admission, and RFA was performed 2 or 3 days later, given that the total number of HCC nodules remained <4. When ≥4 HCC nodules were detected on CTHA/CTAP, patients first received transarterial chemoembolization (TACE) immediately after CTHA/CTAP, followed later by RFA to achieve complete ablation of the tumor nodules.

### Radiographic procedures

For the diagnosis of HCC at study entry, intravenous contrast-enhanced dynamic CT was performed on an outpatient basis using an X-ray CT device with 4, 8, or 16 detector rows (Aquilion 4/16; Toshiba, Tokyo, Japan; LightSpeed Qx/I, LightSpeed Ultra; GE Healthcare, Milwaukee, WI). Images were obtained during the early arterial, late arterial, and equilibrium phases at 28, 40, and 120 s after starting the intravenous bolus injection of iopamidol (Iopamiron; Nihon Schering, Osaka, Japan) or iohexol (Omnipaque; Daiichi Sankyo, Tokyo, Japan) at a rate of 2.3–3.3 ml/s with a power injector. The total dose of iodine was 0.7 g/kg body weight, with an upper limit of 37 g iodine. The injection time for the contrast material was 30 s. Images were reconstructed with a section thickness of 2.5 mm and a reconstruction interval of 1.5 mm, and were reviewed by experienced radiologists.

CTHA/CTAP was performed on an inpatient basis. First, a 4-Fr modified Shepherd-hook catheter and a 4-Fr hepatic-curve catheter were placed in the celiac artery and superior mesenteric artery, respectively, through bilateral femoral arteries, according to Seldinger's method. Digital subtraction angiography was performed from the celiac artery to evaluate hepatic artery anatomy. A microcatheter was inserted through the 4-Fr catheter and placed in the proper or common hepatic artery for hepatic arteriography.

The CTAP catheter was placed in the superior mesenteric artery in all cases. In the case of a replaced or accessory right hepatic artery, the catheter was inserted well beyond the origin of the hepatic artery to prevent contrast medium overflow into the hepatic artery. Less than 30 ml of contrast agent, which was diluted to 100 mg I/ml, was used before the CTHA/CTAP study. First, CTAP was performed using 90 ml nonionic contrast medium diluted to 100 mg I/ml, and then CT scanning was performed 30 s after the start of the injection at a rate of 3.0 ml/s. Multidetector-row CT images were obtained during a single breath hold in a longitudinal direction with collimation of 1 mm, table speed of 30 mm/s, 120 kVp, and

300 mAs. CTHA was performed at least 5 min after CTAP, using the same parameters. CT scanning was performed at 10 and 45 s after the start of contrast medium injection into the microcatheter at a rate of 2.0–2.5 ml/s. A total of 30–50 ml contrast agent diluted to 100 mg I/ml was used. When the liver was perfused by two or more hepatic arteries such as a replaced right hepatic artery, accessory right hepatic artery, or left hepatic artery downstream of the left gastric artery, CTHA was performed from each of the respective arteries. A diagnosis of typical HCC on CTHA/CTAP was defined as a round hypervascular nodule on CTHA with a defect on CTAP, accompanied by corona enhancement during the second phase of CTHA or hypoattenuation during the equilibrium phase of prior dynamic CT (10,29).

TACE was additionally performed when ≥4 HCC nodules were detected on CTHA/CTAP, as evaluated at the time by the operating radiologist. The procedure used 3.0 ml contrast medium, 30 mg doxorubicin (Adriacin; Kyowahakko Kirin, Tokyo, Japan), and 3.0 ml iodized oil (Lipiodol Ultra-Fluid; Guerbet Japan, Tokyo, Japan). The amounts of contrast medium and iodized oil in this suspension were arbitrarily adjusted according to tumor size. This agent was injected into each feeder of the HCC, followed by infusion of 2-mm-diameter gelatin sponge particles (Gelpart; Nihonkayaku, Tokyo, Japan).

CTHA/CTAP images were scrutinized by two experienced radiologists, who made the final diagnosis. The radiologists were not blinded to information regarding the preceding conventional dynamic CT. Preceding intravenous contrast-enhanced dynamic CT was retrospectively reviewed for nodules newly diagnosed by CTHA/CTAP to determine whether the nodules could have been detected on dynamic CT.

### Radiofrequency ablation

RFA was performed on an inpatient basis. The precise procedure of RFA is described elsewhere (30). All RFA procedures were performed percutaneously under ultrasonographic guidance. We used a 17-gauge cooled-tip electrode (Cool-Tip; RF Ablation System, Covidien, Boulder, Colombia, CO) for RFA. Radiofrequency energy was delivered for 6–12 min for each application. For large tumors, the electrode was repeatedly inserted into different sites, such that the entire tumor could be enveloped by assumed necrotic volumes. A CT scan with a 5-mm section thickness was performed 1–3 days after RFA to evaluate technical effectiveness. Complete ablation was defined as hypoattenuation of the entire tumor. We intended to ablate not only the tumor but also some of the liver parenchyma surrounding it. When we suspected that some portion of tumor remained nonablated, RFA was repeated. We did not predefine the procedure number in a treatment: treatment was generally continued until CT imaging demonstrated necrosis of the entire tumor.

### Follow-up

The follow-up regimen after RFA consisted of blood tests and monitoring of tumor markers in an outpatient setting. Ultrasonography and dynamic CT were performed every 4 months. Tumor recurrence was defined as a newly developed lesion on a dynamic CT that showed hyperattenuation in the arterial phase with washout in the late phase. Recurrent site was categorized as intrahepatic recurrence distant from ablated nodules, local tumor progression defined as the appearance of viable cancer tissue touching the ablated nodules, and extrahepatic metastasis (31). The follow-up was censored in February 2011 when 2 years had passed after the enrollment of patient 280. No interim analysis was specified in the protocol.

### End points

The primary end point was recurrence-free survival, where both recurrence and death were treated as an event. We intended to ablate all detected nodules in both groups. When additional nodules were detected by CTHA/CTAP, the newly detected nodules were also ablated. When >3 nodules were diagnosed as HCC by CTHA/CTAP, we performed TACE and subsequently intended to ablate all of the nodules. When nonablated viable tumors were detected by CT for treatment evaluation, those cases were treated as an event 120 days after randomization. Even when newly detected nodules showed dense Lipiodol deposits after TACE, the nodules were considered as viable if the nodules were nonablated.

Secondary end points were the number of additional nodules detected by CTHA/CTAP, the proportion of patients with complete ablation, overall survival, and safety of CTHA/CTAP and RFA. Complications were defined according to the guidelines of the Society of Interventional Radiology (32). According to the guidelines, major complications were defined as those that required therapy or prolonged hospitalization, or left permanent adverse sequelae, or death.

### Statistical analysis

This study was designed to detect a 15% increase in 2-year recurrence-free survival in the CTHA/CTAP group from an anticipated 35% in the control group. To detect this difference with a power of 80% and type I error of 5% (two-sided test), we needed 280 patients (140 for each arm). Differences between groups for each characteristic were tested for significance with Fisher's exact test for categorical variables and *t*-test for continuous variables. All data necessary for analysis was corrected in the main computer server system of University of Tokyo, Department of Gastroenterology.

Recurrence-free survival and overall survival were calculated using the Kaplan–Meier method and were compared by the log-rank test. Cox proportional hazard regression was used to calculate hazard ratios with 95% confidence interval (CI) between the groups in univariate and multivariate settings. The primary end point was evaluated in subgroups according to the following characteristics: age, sex, body mass index, treatment naivety, hepatitis B surface antigen (HBsAg) positivity, hepatitis C virus antibody positivity, tumor size, tumor number, platelet count, tumor marker positivity for α-fetoprotein (AFP), lens culinaris agglutinin-reactive fraction of AFP, and des-γ-carboxy prothrombin. An adjusted hazard ratio comparing the groups was calculated using multivariate Cox regression with factors that showed significance