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Correlation of Histopathological Features and Genetic Variations with Prognosis of Japanese Patients with Nonalcoholic Fatty Liver Disease

Norio Akuta[1], Yusuke Kawamura[1], Fumitaka Suzuki[1], Satoshi Saitoh[1], Yasuji Arase[1], Hideo Kunimoto[1], Yushi Sorin[1], Shunichiro Fujiyama[1], Hitomi Sezaki[1], Tetsuya Hosaka[1], Masahiro Kobayashi[1], Yoshiyuki Suzuki[1], Mariko Kobayashi[2], Kenji Ikeda[1], and Hiromitsu Kumada[1]

1 Department of Hepatology, Toranomon Hospital, and Okinaka Memorial Institute for Medical Research, Tokyo, Japan

2 Liver Research Laboratory, Toranomon Hospital, Tokyo, Japan

Corresponding author:
Norio Akuta

Department of Hepatology, Toranomon Hospital, and Okinaka Memorial Institute for Medical Research, Tokyo, Japan

akuta-gi@umin.ac.jp

Tel: +81 (44) 877-5111

Fax: +81 (44) 860-1623

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Abstract

Background/Aims: Nonalcoholic fatty liver disease (NAFLD) is common worldwide. The aims of this study were to determine the long-term prognosis of NAFLD, including survival and development of hepatocellular carcinoma (HCC), and the effects of histopathological features and genetic variations on prognosis.

Methods: Potential factors that affect survival and HCC developmentafter up to 35 years of follow-up, were investigated in 284 Japanese patients with histopathologically-confirmed NAFLD (including 37 patients with history of malignancy). Especially, we analyzed the impact of *PNPLA3* rs738409, *TM6SF2* rs58542926, *A2BP1* rs3785233, and *IL28B* rs8099917 genetic variations on prognosis.

Results: The cancer development rate (per 1,000 person years) was 12.8, and that of HCC was 3.05. Multivariate analysis identified *TM6SF2* (non-CC type) and fibrosis stage (stage ≥3) as significant determinants of HCC at diagnosis of NAFLD. In 247 patients free of cancers at diagnosis, multivariate analysis identified fibrosis stage 4 and old age (≥60 years) as significant determinants of cancer development Univariate, but not multivariate, analysis showed a significant relation between *PNPLA3* GG type and cancer development. In 262 patients free of HCC at diagnosis of NAFLD, cumulative HCC and survival rates varied significantly according to severity of fibrosis, but not by genetic variation.

Conclusions: Genetic variants and histopathological features affect, at least in part, the prognosis of Japanese patients with NAFLD, including survival and cancerdevelopment. Further prospective studies are needed to determine the impact of clinical parameters on prognosis.

Keywords: Nonalcoholic Fatty Liver Disease; Nonalcoholic Steatohepatitis; Hepatocellular Carcinoma; Malignancy; Survival; Patatin-like Phospholipase domain containing 3; Transmembrane 6 Superfamily Member 2; Ataxin-2 Binding Protein 1; Interleukin 28B; Fibrosis Stage

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Introduction

Non-alcoholic fatty liver disease (NAFLD) is currently the most common liver disease worldwide across different ethnicities [1-6], and results in serious health-care issues. NAFLD includes a wide spectrum of liver pathologies ranging from non-alcoholic fatty liver, which is usually benign, to non-alcoholic steatohepatitis (NASH), which may lead to liver cirrhosis, hepatocellular carcinoma (HCC), and liver failure without excessive alcohol intake [7]. Vitamin E and Farnesoid X nuclear receptor ligand obeticholic acid had practically improved the histopathological features [8,9].NASH can only be diagnosed by histopathology by the presence of steatosis, lobular inflammation, ballooning, and fibrosis. Hence, there is a need to assess the prognostic value of liver histology in predicting disease outcome. The NAFLD activity score (NAS), consisting of the above-mentioned changes with the exception of fibrosis, has gained wide acceptance in defining NASH [10]. However, fibrosis stage was suggested to provide a

more reliable prediction of liver-specific mortality than NAS [11]. In fact, a recent report indicated that fibrosis stage, but not other histopathological features of steatohepatitis, is an independent and significant predictor of overall mortality, liver transplantation, and liver-related events [12]. Considered together, it is important to investigate the impact of histopathological features on long-term prognosis of histopathologically-confirmed NAFLD.

While the exact pathogenic mechanism of NAFLD is not clear, the severity and progression of NAFLD is known to be influenced by genetic variations [13]. Especially, one of the most significant genetic risk factor for NAFLD is a variant located in the *PNPLA3* (patatin-like phospholipase domain-containing 3) gene. The *PNPLA3* genotype is associated an increased hepatocyte fat content and natural course of NAFLD, including advanced fibrosis and HCC [13-18]. *TM6SF2* (transmembrane 6 superfamily member 2) genotype also affects hepatocyte triglyceride content and NAFLD disease severity [19-21]. Recently, the *IL28B* (interleukin 28B) genotype of the strongest host factor associated with hepatitis C viral clearance after interferon-based therapy [22-24], was also reported to be a significant predictor of tissue inflammation and fibrosis in patients with NAFLD [25]. Furthermore, *A2BP1* (Ataxin-2 binding protein 1) genotype in relation to type 2 diabetes mellitus as risk factors of NAFLD, might affect NAFLD disease severity [26,27]. Thus, the impact of these genetic variations on the prognosis of histopathologically-confirmed NAFLD is still unclear.

The present study included 284 patients with histopathological proven NAFLD. The first aim of this study was to determine the long-term prognosis of NAFLD in Japan, including survival and development of HCC.

The second aim was to determine the impact of clinical parameters, including genetic variations and histopathological features, on prognosis.

Materials and Methods

Patients

This is a retrospective cohort study of patients with histopathologically-confirmed NAFLD. NAFLD was diagnosed histopathologically in 284 Japanese patients at our hospital between 1980 and 2015. The median follow-up time, from the histopathological diagnosis to death or last visit, was 3.1 years (range, 0.3-35.3 years). We included in this study all of 284 patients who showed histopathological changes of steatosis in at least 5% of hepatocytes and whose daily alcohol intake was <20 g/day. We excluded patients with 1) underlying liver disease such as viral hepatitis, autoimmune hepatitis, drug-induced liver disease, or primary biliary cirrhosis; 2) underlying systemic autoimmune diseases, such as systemic lupus erythematosus and rheumatoid arthritis; and 3) underlying metabolic diseases, such as hemochromatosis, α-1-antitrypsin deficiency, or Wilson disease. The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board at Toranomon Hospital. All patients provided written informed consent for liver biopsy samples.

Liver Histopathology

Liver specimens were obtained using a 14-gauge modified Vim Silverman needle (Tohoku University style, Kakinuma Factory, Tokyo, Japan), a 16-gauge core tissue biopsy needle (Bard Peripheral Vascular Inc., Tempe, AZ) or surgical resection. The specimen was fixed in 10% formalin, and the prepared sections were stained with hematoxylin-eosin, Masson trichrome, silver impregnation, or periodic acid-Schiff after diastase digestion. The specimens were evaluated by five pathologists (M.I., K.K., F.K., T.F., and T.F.) who were blinded to the clinical findings. An adequate liver biopsy sample was defined as a specimen longer than 1.5 cm and/or containing more than 11 portal tracts.

Specimens with steatosis of <5%, ≥5 to <33, ≥33 to <66%, and ≥66% were scored as grade 0, 1, 2, and 3, respectively. Lobular inflammation with no foci, <2 foci, 2-4 foci, and ≥4 foci per 200× field was scored as 0, 1, 2, and 3, respectively. Hepatocyte ballooning of none, few cells, and many cells was scored as 0, 1, and 2, respectively. NAFLD activity score was the sum of steatosis, lobular inflammation, and hepatocyte ballooning scores (range, 0–8 points; 5–8 points as definition of NASH). Fibrosis stage of none, zone 3 peri-sinusoidal fibrosis (stage 1), zone 3 peri-sinusoidal fibrosis with portal fibrosis (stage 2), zone 3 peri-sinusoidal fibrosis and portal fibrosis with bridging fibrosis (stage 3), and cirrhosis (stage 4) was scored as 0, 1, 2, 3, and 4, respectively [10,28]. Patients were also classified into four categories according to the histopathological findings, based on the classification of Matteoni et al. [29] as follows; type 1: fatty liver alone, type 2: fat accumulation and lobular inflammation, type 3: fat accumulation and ballooning degeneration, type 4: fat accumulation, ballooning degeneration, and either Mallory-Denk body or fibrosis (type 3 or 4 as definition of NASH).

Determination of PNPLA3, TM6SF2, A2BP1, and IL28B genotype

PNPLA3 rs738409, *TM6SF2* rs58542926, *A2BP1* rs3785233, and *IL28B* rs8099917 were genotyped by the TaqMan SNP genotyping assay (Applied Biosystems, Foster City, CA).

Clinical Parameters

We investigated various clinicopathological parameters that could affect the prognosis of NAFLD. At our hospital, the normal range of aspartate aminotransferase (AST) was 13-33 IU/l, alanine aminotransferase (ALT) 8-42 IU/l for males and 6-27 IU/l for females. Obesity was defined as a body mass index (BMI) of >25.0 kg/m^2. Cardiovascular disease (CVD) risk was investigated using total cholesterol (TC) / high-density lipoprotein cholesterol (HDL-C) ratio [21,30]. CVD included arteriosclerosis, coronary artery disease, heart valve disease, arrhythmia, heart failure, hypertension, orthostatic hypotension, shock, endocarditis, diseases of the aorta and its branches, disorders of the peripheral vascular system, and congenital heart disease.

Follow-up and Diagnosis of HCC

All patients were followed up at least twice a year by monitoring hematological and biochemical data after the time of histopathological diagnosis of NAFLD. Imaging studies were

performed at least once a year with ultrasonography, computed tomography, or magnetic resonance imaging. During this time, liver-related death, which included HCC and liver failure, was also evaluated.

Statistical Analysis

The relations between potential predictive factors and prognosis of NAFLD were analyzed. Non-parametric tests (Chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly affected HCC development at the time of histopathological diagnosis of NAFLD. The cumulative cancer rate and cumulative survival rate were calculated using the Kaplan-Meier technique and differences between the curves were tested by the Log-rank test. Statistical analyses of cancer and survival rates according to groups, were calculated using the period from the time of histopathological diagnosis of NAFLD till the last visit or occurrence of event. Stepwise Cox regression analysis was used to determine independent predictive factors that were associated with cancer development. The hazard ratio (HR) and 95% confidence interval were also calculated. Variables that achieved statistical significance (P<0.05) on univariate analysis were tested by multivariate analysis to identify significant independent factors. Statistical comparisons were performed using the SPSS software (SPSS Inc., Chicago, IL). All *p* values <0.05 by the two-tailed test were considered significant.

Results

Predictors of HCC at Histopathological Diagnosis of NAFLD

Table 1 summarizes the clinicopathological characteristics of the patients. All 284 patients were evaluated for predictors of HCC at the time of histopathological diagnosis of NAFLD. In this regard, 22 of the 284 patients (7.7%) were found to have existing HCC at the time of diagnosis of NAFLD **(Table 1)**.

Univariate analysis identified 14 parameters that significantly correlated with HCC at the time of histopathological diagnosis of NAFLD. These included steatosis (≥66%;P<0.001), fibrosis stage (stage ≥3; P<0.001), old age (≥60 years; P<0.001), thrombocytopenia (platelet count <150×10^3/mm^3; P<0.001), alanine aminotransferase (<2×upper limit of normal [ULN]; P<0.001), fasting plasma glucose (≥110 mg/dl; P<0.001), hyaluronic acid (≥51 μg/l; P<0.001), presence of diabetes mellitus (P=0.002), aspartate aminotransferase (<2×ULN; P=0.002), *TM6SF2* rs58542926 (non-CC type; P=0.011), albumin (<4.1 g/dl; P=0.022), total cholesterol (<245 mg/dl; P=0.032), low-density lipoprotein cholesterol (<136 mg/dl; P=0.035), and NAFLD activity score (<5; P=0.043). These factors were entered into multivariate analysis, which then identified *TM6SF2* rs58542926 (non CC type; HR 35.7, P=0.001) and fibrosis stage (stage ≥3; HR 9.97, P=0.015) as significant and independent predictors of HCC at the time of histopathological diagnosis of NAFLD **(Table 2)**.

Overall Survival Rates in NAFLD

Absence of HCC was confirmed in 262 of 284 patients (92.3%) at the time of histopathological diagnosis of NAFLD. Data of these patients were evaluated for overall survival rate. During the follow-up period, 7 patients (2.8%) died; 3 of the 7 patients (42.9%) died of liver-related causes, 1 (14.3%) of lung cancer, 1 (14.3%) of hemangiopericytoma, 1 (14.3%) interstitial pneumonia, and 1 (14.3%) to infectious endocarditis. In 3 patients of liver-related causes, 2 (66.7%) and one (33.3%) died due to HCC and liver failure, respectively. The cumulative survival rate was 91.8, 91.8, and 91.8% at the end of 10, 20, and 30 years, respectively. The median interval between the histopathological diagnosis of NAFLD and death was 7.0 years (range, 0.1-8.8 years).

Cancer-related Death Rate and Survival Rate in NAFLD

The 247 patients who were confirmed to have no malignancies at the time of histopathological diagnosis of NAFLD were evaluated for cancer-related deaths and survival rates. During the follow-up period, cancer was diagnosed in 16 patients (6.5%). The cumulative cancer rate was 7.8, 33.2, and 57.1% at the end of 10, 20, and

28 years, respectively (**Figure 1**). The median interval between diagnosis of NAFLD and cancer detection was 9.7 years (range, 0.2-23.7 years). During the follow-up period, 3 patients (1.2%) died due to cancer-related causes. 2 of 3 patients (66.7%) and one (33.3%) developed HCC and lung cancer, respectively. The cumulative survival rates for cancer-related deaths were 95.4, 95.4, and 95.4% at the end of 10, 20, and 30 years, respectively. The median interval between diagnosis of NAFLD and cancer-related death was 4.2 years (range, 2.9-5.9 years).

Predictors of Cancer Development in NAFLD

The 247 patients who were confirmed to have no malignancies at the time of histopathological diagnosis of NAFLD were evaluated for prediction of cancer development. Univariate analysis identified 5 parameters that significantly correlated with cancer development. These included fibrosis stage (stage

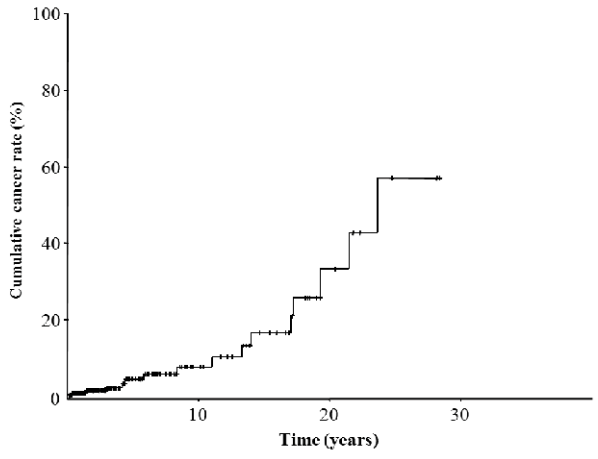

Figure 1 In 247 patients free of cancers at the time of histopathological diagnosis of NAFLD, the cumulative cancer rates were 7.6, 22.1, and 49.9% at the end of 10, 20, and 28 years, respectively.

Table 1 Patients' characteristics at the time of histopathological diagnosis of NAFLD.

Demographic data	Numbers of patients	284
	Gender (male/female)	170/114
	Age (years)*	51 (20-85)
	Body mass index (kg/m2)*	25.9 (18.1-40.4)
	Diabetes mellitus (presence/absence/unknown)	67/180/37
	History of malignancy (none/hepatocellular carcinoma/other cancers)	247/22/15
Histopathological findings		
	Steatosis (≥5% to <33%/≥33% to <66%/≥66%)	108/109/63
	Lobular inflammation (no foci/<2 foci/2-4 foci/≥4 foci per 200× field)	26/166/77/11
	Ballooning (none/few cells/many cells)	36/160/84
	Stage (0/1/2/3/4)	40/123/32/65/24
	Matteoni classification (type 1/2/3/4)	17/17/14/232
	NAFLD activity score (≤2/3, 4/≥5)	36/120/128
Genetic variation		
	PNPLA3 rs738409 (CC/CG/GG/not determined)	35/99/87/63
	TM6SF2 rs58542926 (CC/CT/TT/not determined)	167/46/7/64
	A2BP1 rs3785233 (TT/TG/GG/not determined)	155/56/9/64
	IL28B rs8099917 (TT/TG/GG/not determined)	178/38/3/65
Laboratory data*		
	Serum aspartate aminotransferase (IU/l)	48 (12-378)
	Serum alanine aminotransferase (IU/l)	74 (15-783)
	Gamma-glutamyl transpeptidase (IU/l)	71 (11-649)
	Serum albumin (g/dl)	4.0 (2.6-5.8)
	Platelet count (×103/mm3)	213 (45-445)
	Fasting plasma glucose (mg/dl)	100 (65-273)
	Uric acid (mg/dl)	5.9 (1.8-10.6)
	Total cholesterol (mg/dl)	204 (101-370)
	Triglycerides (mg/dl)	137 (31-610)
	High-density lipoprotein cholesterol (mg/dl)	45 (14-85)
	Low-density lipoprotein cholesterol (mg/dl)	124 (28-243)
	Total cholesterol/High density lipoprotein cholesterol	4.5 (1.7-15.3)
	Serum ferritin (µg/l)	231 (<10-2,067)
	Hyaluronic acid (µg/l)	32 (1-814)

Data are number of patients, except those denoted by *, which represent the median (range) values.

Table 2 Predictive factors of hepatocellular carcinoma at the time of histopathological diagnosis of NAFLD in 284 patients.

Factors	Category	Hazard ratio	95% confidence interval	P
TM6SF2 rs58542926	CC	1		
	Non-CC	35.7	4.39-333	0.001
Fibrosis stage	≤2	1		
	≥3	9.97	1.57-63.5	0.015

Table 3 Predictive factors for cancer development in 247 patients with NAFLD who were free of cancers at the time of histopathological diagnosis.

Factors	Category	Hazard ratio	95% confidence interval	P
Fibrosis stage	≤3	1		
	4	7.76	1.38-43.6	0.020
Age (years)	<60	1		
	≥60	5.11	1.22-21.4	0.026

Table 4 Rate (per 1,000 person years) of development of each type of cancer in 247 patients with NAFLD.

Malignancy	n (%)	1,000 person years
Total	16 (100)	12.76
Hepatocellular carcinoma	4 (25.0)	3.05
Bladder cancer	2 (12.5)	1.50
Pancreatic cancer	2 (12.5)	1.49
Malignant lymphoma	2 (12.5)	1.49
Breast cancer	2 (12.5)	1.49
Lung cancer	1 (6.3)	0.74
Colon cancer	1 (6.3)	0.74
Uterine cancer	1 (6.3)	0.74
Prostate cancer	1 (6.3)	0.74

4; P<0.001), lobular inflammation (>4 foci 200× field; P=0.001), thrombocytopenia (platelet count <150×10^3/mm^3; P=0.001), old age (≥60 years; P=0.015), and *PNPLA3* rs738409 (GG type; P=0.028). These factors were entered into multivariate analysis, which identified two parameters that significantly and independently influenced cancer development; fibrosis stage (stage 4; HR 7.76, P=0.020) and old age (≥60 years; HR 5.11, P=0.026) **(Table 3)**. Thus, *PNPLA3* GG type was identified as a significant determinant of cancer development inunivariate but not multivariate analysis.

Rate of Development of Each Type of Cancer in NAFLD

The 247 patients who were confirmed to have no cancers at the time of histopathological diagnosis of NAFLD were evaluated for the rate of development of each type of cancer **(Table 4)**. During the follow-up, 16 patients developed various cancers, and the development rate per 1000 person years was 12.8%. The following cancers were diagnosed: HCC 4 cases (25.0%), bladder cancer 2 (12.5%), pancreatic cancer 2 (12.5%), malignant lymphoma 2 (12.5%), breast cancer 2 (12.5%), lung cancer 1 (6.3%), colon cancer 1 (6.3%), uterine cancer 1 (6.3%), and prostate cancer 1 (6.3%). HCC accounted for one quarter of malignancies, and the development rate per 1000 person years was 3.05.

HCC Development Rateand HCC-related Survival Rate in NAFLD

The 262 patients who were confirmed to have no HCC at the time of histopathological diagnosis of NAFLD were evaluated for the HCC development rateand HCC-related survival rate. During the follow-up, 4 patients (1.5%)

developed HCC. The cumulative HCC rates were 3.4, 3.4, and 19.5% at the end of 10, 20, and 30 years, respectively. The median interval between diagnosis of NAFLD and detection of HCC was 5.1 years (range, 4.2-23.7 years). During the follow-up period, 3 patients (1.1%) died due to liver-related causes; 2 (66.7%) and one (33.3%) died due to HCC and liver failure, respectively. The cumulative survival rates for liver-related deaths were 95.9, 95.9, and 95.9% at the end of 10, 20, and 30 years, respectively. The median interval between the diagnosis of NAFLD and liver-related death was 8.5 years (range, 0.5-8.8 years).

Impact of Genetic Variations and Histopathological Features on HCC Development and Survival in NAFLD

The 206 of 262 patients (78.6%) patients who were confirmed to have no HCC at the time of histopathological diagnosis of NAFLD and evaluated for genetic variations were evaluated for the development of HCC and survival rate. The cumulative HCC rates were not significantly different among the *PNPLA3* rs738409 (P=0.106), *TM6SF2* rs58542926 (P=0.933), *A2BP1* rs3785233 (P=0.714), and *IL28B* rs8099917 (P=0.602) groups. The cumulative survival rates were also not significantly different among the *PNPLA3* rs738409 (P=0.790), *TM6SF2* rs58542926 (P=0.339), *A2BP1* rs3785233 (P=0.631), and *IL28B* rs8099917 (P=0.934) groups.We also evaluated all 262 patients who were free of HCC at the time of histopathological diagnosis of NAFLD for HCC developmentand survival rates, according to histopathological features. The cumulative HCC rates and survival rates were significantly influenced by the fibrosis stage (P<0.001, each). Especially, the HCC rate was significantly higher in stage 4 than in stage ≤3 (P<0.001), and the survival rate in stage 4 was significantly lower than in stage ≤3 (P<0.001). Furthermore, the cumulative survival rate was significantly influenced by the severity of steatosis (P=0.016). Especially, the survival rate in steatosis ≥66% was significantly lower than steatosis <66% (P=0.005). The cumulative HCC rate was not significantly influenced by the severity of steatosis (P=0.234), ballooning (P=0.448), or lobular inflammation (P=0.609). The cumulative survival rate was also not significantly influenced by ballooning (P=0.378) or lobular inflammation (P=0.794).

Discussion

The histopathological diagnosis of NASH is based on the presence of four changes: steatosis, lobular inflammation, ballooning, and fibrosis. However, the impact of each of these changes on long-term prognosis remains to be defined. Ekstedt et al. [31] showed that NAS was not a predictor of overall mortality, while the fibrosis stage was the strongest predictor of disease-specific mortality in NAFLD. Another recent study [12] concluded that fibrosis stage was the only independent feature that correlated with long-term overall mortality, outcome of liver transplantation,

and liver-related events. In the present study of 284 patients with histopathologically-confirmed NAFLD, fibrosis stage was a significant predictor of HCC development and survival after up to 35 years of follow-up. On the other hand, ballooning and lobular inflammation had no significant effect on the cumulative HCC and survival rates. Hence, the present findings add support to the conclusion that fibrosis stage can be used to predict liver-specific mortality more reliably than NAS [31]. One limitation of the present study is the lack of control subjects (steatosis in <5% of hepatocytes), who could not be included based on the histopathological definition of NAFLD. Another limitation is the small number of HCC cases, which could explain the lack of relation between NAS and long-term prognosis on univariate analysis (possible type II error). Further large-scale studies, based on multivariate analysis, should be performed to investigate the separate effect of each of the four histopathological features of NAFLD on long-term prognosis.

To our knowledge, the present study is the first to analyze the effect of genetic variations (*PNPLA3*, *TM6SF2*, *A2BP1*, and *IL28B*) on long-term prognosis of NAFLD after up to 35 years of follow-up. Based on meta-analysis of 24 studies that included 9,915 patients, Singal et al. [18] reported that *PNPLA3* genotype was associated with increased risk of advanced fibrosis among patients with a variety of liver diseases and was an independent risk factor for HCC among patients with NASH. In the present study based on patients free of cancers at the time of histopathological diagnosis of NAFLD, univariate, but not multivariate, analysis showed a significant relation between *PNPLA3* genotype and cancer. Furthermore, in patients free of HCC at the time of diagnosis of NAFLD, the cumulative HCC rate was not significantly affected by the *PNPLA3* genotype. These discrepant results could be due one or more factors. One reason is that only 4 patients developed HCC during the follow-up (possible type II error). The other reason is that progression of fibrosis stage could not be evaluated.

The relation between *TM6SF2* genotype and long-term prognosis of NAFLD remains obscure. In this regard, Liu et al. [20] reported that *TM6SF2* genotype influenced necroinflammation score, but they could not replicate this result in their large-scale validation cohort. The same group also indicated that *TM6SF2* genotype significantly affected liver fibrosis stage in the two explored cohorts by adopting an additive model. Another study [21] showed that *TM6SF2* genotype was a low-frequency variant with a modest effect on NAFLD, suggesting that carriers of the NAFLD risk-allele are slightly more likely to accumulate fat in the liver and to develop NASH than those without. Our results indicated that *TM6SF2* non-CC type significantly influenced HCC rate in patients with NAFLD. However, in patients free of HCC at the time of diagnosis of NAFLD, the cumulative HCC rate was not significantly different among patients of the *TM6SF2* genotype. This is the first study to investigate the impact of TM6SF2 genotype on long-term prognosis of NAFLD. Indeed, NAFLD-HCC is often diagnosed later than HCC occurring in the setting of liver diseases due to other etiologies, which results in reduced chance of cure and survival [32-34]. Against this scenario, identification of genetic modifiers that could help the clinician in assessing the risk of hepatic and extra-hepatic manifestations and complications in individual patients is highly desirable [35]. Further large-scale studies are needed to investigate the impact of genetic variants on the progression of fibrosis stage and development of HCC.

Arase and colleagues [36] reported that the incidence rate of HCC among all cancers was 6.0%, and the HCC development rate per 1,000 person years was 0.78, in 1,600 patients with NAFLD diagnosed based on the presence of fatty liver by ultrasonography. However, the present study indicated that the incident rate of HCC among all cancers was 25.0%, and the HCC development rate per 1,000 person years was 3.05. In other words, HCC accounted for one quarter of cancers in our study, indicating a higher rate of HCC compared to the above studies. The discrepant results could reflect the patient selection bias; all patients had histopathologically-confirmed NAFLD, with high levels of AST and/or ALT. Interestingly, multivariate analysis identified fibrosis stage as a significant and independent determinant of HCC development. One reason for this result could be that HCC accounted for one quarter of malignancies. Further comprehensive studies should be performed to determine the molecular mechanisms behind the complex relationship between histopathological features including fibrosis stage and cancer development.

Another limitation of the present study is that we did not investigate the development of cardiovascular disease in our patients. It was recently reported that NAFLD patients are at increased risks of both HCC (HR 6.55, P=0.001) and cardiovascular diseases (HR 1.55, P=0.01), compared with the control [31]. Further studies should be performed to investigate the impact of cardiovascular disease on mortality in NAFLD.

In addition to the above-mentioned limitations, the present study was a hospital-based study and retrospective in nature and thus did not take lead time bias into account and might include selection bias. Furthermore, all participants were Japanese, and thus the results might not be applicable to patients of other races or ethnic groups. Also, the study failed to highlight the epidemiological burden and complexity of the natural history of NAFLD [37,38]. Identification of predictors of development of HCC in patients with NAFLD is a clinical priority due to the currently available suboptimal surveillance criteria [39,40].

In conclusions, the results of the present study demonstrated that genetic variants and histopathological features of NAFLD affect, at least in part, the prognosis of Japanese patients with NAFLD. Further large-scale prospective studies are needed to determine the impact of genetic variants and histopathological features on long-term prognosis of NAFLD.

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