A prospective study showing poor prognosis in Japanese NASH patients with fibrosis stage F3-4

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ABSTRACT Nonalcoholic fatty liver disease (NAFLD) is one of the most prevalent of all chronic liver diseases in Japan. The course and prognosis of disease in Japanese patients with NAFLD remains unclear. For a period of 5.2±2.4 years, we followed 274 Japanese patients with NAFLD who had undergone liver biopsy. The patients were divided into two groups: patients with simple steatosis (SS) or nonalcoholic steatohepatitis (NASH) with fibrosis Stages 0-2 (mild-fibrosis group), and patients having NASH with fibrosis Stages 3-4 (advanced-fibrosis group). The course of hepatic disease and cerebro-cardiovascular events was evaluated in these two groups. Survival of the advanced-fibrosis group was lower than that of the mild-fibrosis group (p=0.001). In the advanced-fibrosis group, 7 patients (8.5%) experienced cerebro-cardiovascular events after diagnosis, and one died. Hepatic disease (9.8%) occurred in 8 patients: 4 with liver decompensation, 3 with hepatocellular carcinoma (HCC), and 1 with both liver decompensation and HCC. The 3 patients with liver decompensation died; one underwent liver transplantation. No patient in the mild-fibrosis group experienced any cerebro-cardiovascular events or hepatic disease, and none of these patients died. The advanced-fibrosis group had higher values than the mild-fibrosis group for fibrosis marker, HOMA-IR, hemoglobin A1c, leptin, hs-CRP and lower platelet count, and had a tendency toward a higher incidence of diabetes mellitus and hypertension. It is important to understand how to diagnosis advance fibrosis NASH among so many patients with NAFLD.

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Key words : **Nonalcoholic steatohepatitis (NASH), Simple steatosis, Prognosis, Advanced histological stage, Survival**

INTRODUCTION

Nonalcoholic fatty liver disease (NAFLD) is associated with metabolic syndrome (i.e., abdominal obesity, hypertension, atherogenic dyslipidemia, and dysglycemia). NAFLD may progress to cirrhosis, liver failure, or hepatocellular carcinoma (HCC).

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NAFLD is the most common cause of chronic liver disease in Western and Asian countries[1]. Based on histological disease activity, NAFLD is divided into simple steatosis (SS) and nonalcoholic steatohepatitis (NASH). SS has a relatively good prognosis, while NASH may progress to cirrhosis or HCC[2, 3]. In Japan, lifestyle-related diseases such as obesity, diabetes mellitus, hyperlipidemia, and hypertension are becoming more prevalent, along with NAFLD. There are possible racial differences with respect to NAFLD and NASH. Asian patients with NAFLD and NASH tend to have lower body mass index (BMI) than Western patients, but have relatively more severe insulin resistance despite their lower BMI[4]. The course and prognosis of disease in Asian patients with NAFLD remains unclear. With the dramatically increasing number of patients with NAFLD in Japan, it is important to investigate the disease, focusing on identifying patients with poorer prognosis to proactively provide thorough examination and treatment to those patients. The aim of our study was to prospectively evaluate the course and prognosis of disease in patients with NAFLD by comparing the causes of NAFLD and survival between patients with mild fibrosis (SS and NASH Stages 0-2) and patients with advanced fibrosis (NASH Stages 3-4). The present report provides data from the prospective study on the course and prognosis of patients with NASH at a single tertiary care hospital.

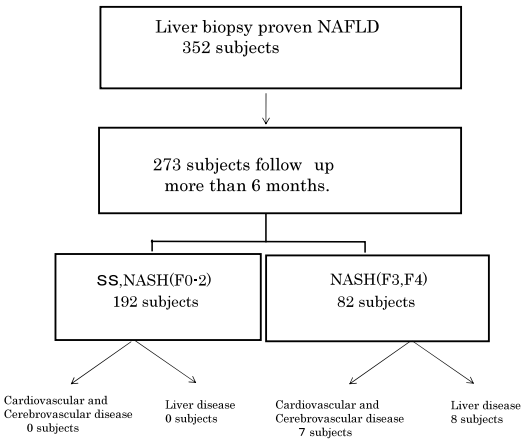

Fig. 1. Out of the total (n = 353) patients with liver biopsy-proven NAFLD, 274 patients were followed up for more than 6 months, of whom 192 patients had mild-fibrosis group , and 82 patients had advanced- fibrosis group.
Among the patients in the mild-fibrosis group, there were no occurrences of cardiovascular or cerebrovascular disease or liver disease, but in the advanced-fibrosis group, cardiovascular or cerebrovascular disease was found in 7 patients, and liver disease was found 8 patients after diagnosis.

Materials and Methods

PATIENTS

Of 352 patients with NAFLD who underwent liver biopsy at Kawasaki Hospital, Kawasaki Medical School College in a 15-year period between April 1996 and June 2011, 274 were followed up for more than 6 months and enrolled in this study. These patients were divided into the mild-fibrosis group (SS and NASH Stages 0-2; n = 192) and the advanced-fibrosis group (NASH Stages 3-4; n = 82) (Fig. 1).

Diagnostic criteria

A diagnosis of NAFLD was made according to the following criteria (i) alcohol intake of ≤ 20 g/week (ii) hepatitis B surface (HBs) antigen negative, hepatitis C virus (HCV) RNA negative, with exclusion of autoimmune liver disease, drug-induced hepatic disorder, and metabolic liver disease (e.g., Wilson's disease, hemochromatosis) and (iii) presence of steatosis (>30%) or steatohepatitis. The pathological classification proposed by Matteoni *et al.*[5] was used to diagnose histological types 1 and 2 as SS and types 3 and 4 as NASH. Fibrosis was graded from Stage 0 to Stage 4 according to the staging system proposed by Brunt *et al.*[6], and the study patients were divided into two groups: the mild-fibrosis group (SS, NASH Stages 0-2) and the advanced-fibrosis group (Stages 3-4). Liver inflammation was graded from Grade 0 to Grade 3, also according to the grading system proposed by Brunt *et al.*[6].

The demographic information collected and clinical data included age, sex, BMI, history of diabetes, hyperlipidemia, hypertension, hyperuricemia, and number of complications (obesity [BMI ≥ 25], type2 diabetes mellitus, hypertension, dyslipidemia and/or hyperuricemia). Diabetes mellitus was diagnosed based on the following criteria proposed by The Japan Diabetic Society: (i) fasting blood glucose ≥ 126 mg/dL; (ii) 2-h post-75 g oral glucose tolerance test result ≥ 200 mg/dL; (iii) casual blood glucose ≥ 200 mg/dL; and (iv) hemoglobin A1c (HbA1c) ≥ 6.1 or treatment with one or more antidiabetics. Dyslipidemia was defined as triglyceride >150 and/or low-density lipoprotein (LDL) cholesterol level>140 or treatment with one or more lipid-lowering drugs. Hypertension was defined as systolic/diastolic blood pressure ≥ 140/90 mmHg or treatment with one or more antihypertensives. Hyperuricemia was defined as serum uric acid ≥ 7.0 mg/dL or pharmacotherapy for hyperuricemia. The number of complications (obesity [BMI ≥ 25], diabetes mellitus, hypertension, dyslipidemia and/or hyperuricemia) was determined (0 to 5 complications). All patients underwent laboratory tests at the time of liver biopsy: alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ -glutamyl transpeptidase (γ -GTP), total bilirubin (T-Bil), platelet count, fasting glucose, hemoglobin A1c, homeostasis model assessment-insulin resistance (HOMA-IR), leptin, adiponectin, hyaluronic acid, type IV collagen 7S, TIMP-1 and procollagen peptide, type III (P-III-P). In addition, the patients with NAFLD were separated according to sex, and baseline variables were compared between the mild-fibrosis group and the advanced-fibrosis group. The patients were followed up by blood laboratory examination and body weight measurements every one to three months and by abdominal computed tomography (CT) scan, magnetic resonance imaging (MRI) and abdominal ultrasonography every three to twelve months. The patients were examined for cerebro-cardiovascular events and hepatic disease to evaluate their course and prognosis.

Cerebro-cardiovascular events included cerebral infarction, cerebral hemorrhage, and coronary artery disease. Hepatic disease included HCC and liver decompensation at Child-Pugh class C.

Statistical analysis

The patients were followed for cerebro-cardiovascular events and hepatic disease to evaluate their course and prognosis adjusted for sex and age. Comparisons between groups were performed using Student's *t*-test or the Wilcoxon rank sum test or the $\chi 2$-test. Cumulative survival was calculated using the Kaplan-Meier method, and the differences between the groups were analyzed with the log-rank test. Univariate and multivariate analyses of predictors of survival were assessed using the Cox proportional hazards model. A p value less than 0.05 was considered to be significant. All analyses described above were performed using JMP Ver. 9.0.1 software (SAS Institute Japan, Tokyo, Japan).

RESULTS

The enrolled patients consisted of 142 men and 132 women with a mean age of 53.5 ± 14.8 years. Among the 274 patients followed for at least 6 months, the mean follow-up period was 5.2 ± 2.8 years for the 192 patients in the mild-fibrosis group and 4.5 ± 2.3 years for the 82 patients in the advanced-fibrosis group. The demographic and basic characteristics of the enrolled patients are shown in Table 1. The mean age was significantly higher in the advanced-fibrosis group (55.7 ± 14.2 years) than in the mild-fibrosis group (48.2 ± 15.4 years). There was no significant difference between groups in the male/female ratio. The prevalence of type 2 diabetes mellitus and hypertension were significantly higher in advance-fibrosis group. Table2a and Table2b shows comparison

of laboratory date and fibrosis maeker. Platelet counts were significantly lower and HOMA-IR, hemoglobin A1c, and leptin, hs-CRP were higher in the advance-fibrosis group. Hyaluronic acid , TIMP-1, type 4 collagen 7S, P-III-P as fibrosis markers were higher in the advanced-fibrosis group.

The course and prognosis of the patients in the mild-fibrosis group and advanced-fibrosis group are shown in Figs. 1 and 2, respectively. Among the 82 patients with advanced fibrosis, 7 patients

Table 1. Comparison of clinicopathological features between mild-fibrosis group and advanced- fibrosis group.

	SS,NASH(F0-F2) n=192	NASH(F3,F4) n=82	Univariate P-value
Stage	(SS/F0/F1/F2) 30/10/100/29	(F3/F4) 72/10	*
Median follow up (years)	5.2 ± 2.8 (0.6-15.3)	4.5 ± 2.3 (0.6-12)	0.1021
Age(years)	48.2 ± 15.4	55.7 ± 14.2	0.0002
Sex(female)	44.2%	57%	0.1083
Body mass index(Kg/m2)	27.2 ± 3.8	28.9 ± 5.4	0.0454
Diabete	29%	43%	0.0208
Dyslipidemia	78%	73%	0.3510
Hypertension	24%	45%	0.0013
Hyperuricemia	24%	19%	0.3179
Number of complication (0/1/2/3/4/5)	8/34/72/53/21/4	0/11/27/29/13/2	0.1320

Table 2a. Comparison of laboratory date between mild- fibrosis group and advanced-fibrosis group.

	SS,NASH(F0-F2) n=192	NASH(F3,F4) n=82	Univariate P-value
AST（IU/L）	54.8 ± 39.9	62.8 ± 29.5	<0.0001
ALT（IU/L）	90.1 ± 69.2	90.7 ± 51.4	0.2824
GGTP（IU/L）	80.1 ± 67.2	76.9 ± 66.5	0.5187
Total bilirunin(mg/dl)	0.8 ± 0.3	0.9 ± 0.5	0.0290
TotalCholesterol(mg/dl)	210.8 ± 37.9	208.7 ± 35.1	0.5791
Platelet count($\times 10^4$)	22.4 ± 5.9	19.5 ± 6.5	0.0002
Hemoglobin A1c(%)	5.6 ± 1.2	6.0 ± 1.3	0.0131
Fasting glucose(mg/dl)	107.8 ± 34	110.1 ± 22.2	0.0354
HOMA-IR(%)	3.8 ± 3.8	4.5 ± 3.4	0.0035
Leptin(ng/dl)	10.9 ± 8.2	14.1 ± 7.8	0.0002
Iron(ug/dl)	118.5 ± 38.8	123.3 ± 37.1	0.6437
Ferritin(ng/ml)	187.6 ± 150.1	236.8 ± 192.9	0.0880
high sensivity-CRP(ng/dl)	148.3 ± 101.9	173.4 ± 106.7	0.0505
Thioredoxin(ng/dl)	46.3 ± 37.7	49.3 ± 44.7	0.6447
Adiponectin(Male)(ug/dl)	5.1 ± 2.1	5.4 ± 4.1	0.4778
Adiponectin(Female)(ug/dl)	7.6 ± 4.1	7.4 ± 5.9	0.0678

AST,aspartate aminotransferase; ALT, alanine aminotransferase; GGTP, gamma-glutamyl transpeptidase; HOMA-IR,homeostasis model assessment-insulin resistance

Table 2b. Comparison of fibrosis markers between mild-fibrosis group and advanced-fibrosis group.

	SS,NASH(F0-F2) n=192	NASH(F3,F4) n=82	Univariate P-value
Hyaluronic acid(ng/ml)	38.3 ± 43.6	88.9 ± 113.9	<0.0001
P-Ⅲ-P(U/ml)	0.7 ± 0.2	0.8 ± 0.3	0.0126
TIMP-1(ng/ml)	180 ± 58.9	199 ± 49.8	0.0007
Type4 collagen7S(ng/ml)	3.9 ± 0.8	5.1 ± 2	<0.0001

experienced at cerebro-cardiovascular event during the post-diagnosis follow-up period (mean duration: 5.2 years). The reported events included cerebral infarction, or cerebral hemorrhage in 4 patients, and angina pectoris or myocardial infarction in 3 patients. Of these patients, the patient with myocardial infarction died. In addition, hepatic disease was found in 8 patients: 4 with liver decompensation, 3 with HCC, and 1 with both liver decompensation and HCC. Subsequently, the 3 patients with liver decompensation died, and the remaining 1 patient underwent living donor liver transplantation. None of the patients in the mild-fibrosis group experienced any cerebro-cardiovascular events or liver-related disease during the follow-up period (mean duration: 4.5 years). These results indicate that the patients with advanced fibrosis were significantly more likely

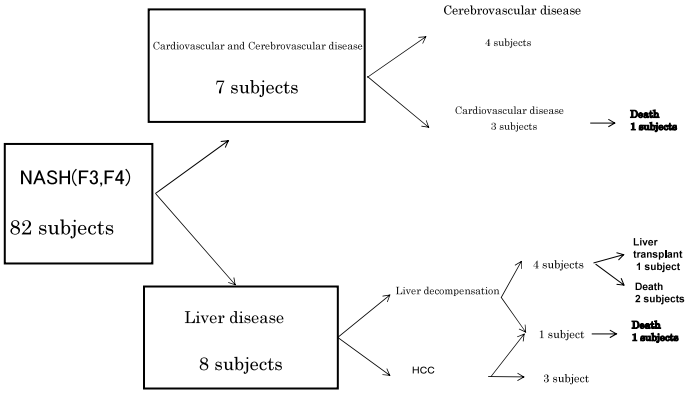

Fig. 2. Among the 82 patients with advanced fibrosis group, 7 patients experienced at cerebro-cardiovascular (cerebral infarction, or cerebral hemorrhage in 4 patients, and angina pectoris or myocardial infarction in 3 patients). Of these patients, one patient with myocardial infarction died .Hepatic disease was found in 8 patients: 4 with liver decompensation, 3 with HCC, and 1 with both liver decompensation and HCC. Subsequently, the 3 patients with liver decompensation died, and the remaining 1 patient underwent living donor liver transplantation.

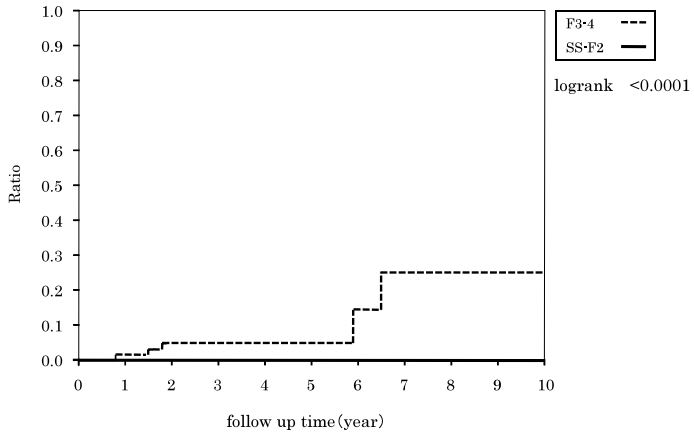

Fig. 3. Based on the log-rank test, the prevalence of cardiovascular and cerebrovascular disease among the patients with advanced-fibrosis group was significantly higher ($p < 0.0001$) than mild fibrosis group.

to experience cardiovascular events ($p < 0.0001$), hepatic disease ($p = 0.0016$), or all-cause mortality, and they had poorer prognosis ($p = 0.0012$) than the patients in the mild-fibrosis group adjusted for sex and age (Figs. 3 to 5).

DISCUSSION

We assessed the prognosis and outcome of NASH patients stratified by the stage of fibrosis. NAFLD is the most common cause of chronic liver disease in Western and Asian countries[1]. But the course and prognosis of the Japanese NASH patients remains unclear. In Western country, various reports have been published on the outcomes of NASH and NAFLD. Regarding the progression from SS to NASH, Ekstedt *et al.*[7] conducted a controlled cohort study and found that during a 13.7-year follow-up period, patients with SS had a mortality rate similar to the general population, but patients with NASH had a significantly higher mortality rate. Teli *et al.*[2] and Dam-Larsen *et al.*[3] reported that patients who had NAFLD without fibrosis had

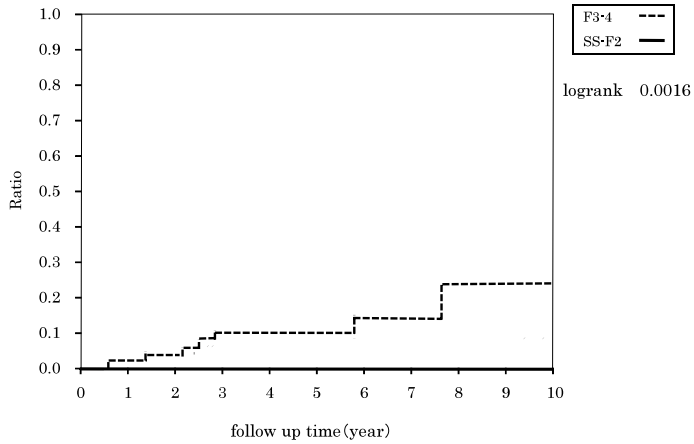

Fig. 4. Based on the log-rank test, the prevalence of liver disease among patients with advanced-fibrosis group, was significantly higher ($p < 0.0016$) than in the patients with mild-fibrosis group.

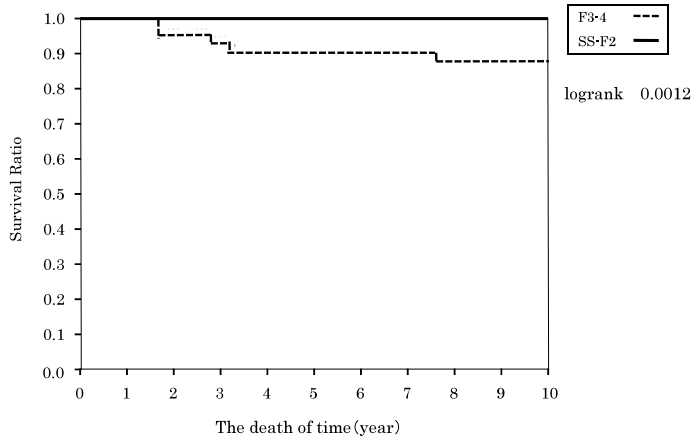

Fig. 5. Based on the log-rank test, the survival ratio among the patients with mild-fibrosis group was significantly higher ($p < 0.0012$) compared with advanced-fibrosis group.

a benign course. However, in a previous study[8], in which 44 patients with biopsy-proven NAFLD had their liver biopsies repeated at month 36, among 13 patients with SS at first biopsy, 8 (61%) had developed borderline NASH or NASH by the time of the second biopsy. Argo *et al.*[9] reported on their systematic analysis of 221 cases of biopsy-proven NASH. In total, 38% of the study patients had progressive fibrosis, 21% improved, and 41% remained stable over a mean follow-up period of 5.3 years. The study of Angulo *et al.*[10], who observed the outcomes of SS in 342 patients for more than 5 years, revealed that SS progressed to cirrhosis in only 0.7% of the patients, and NASH progressed to cirrhosis in 10.8% of the patients (mean follow-up duration: 15.5 years) [7-8,10-13]. In fact, almost all patients with SS have a benign course, but some cases of NASH may progress to cirrhosis or HCC.

In Japanese patients with NASH, only a few reports have been published. Yastuji *et al.*[15] and Hashimoto *et al.*[15,16] have reported on 68 cases of cirrhotic NASH in Japanese patients, with a mean follow-up period of 41 months. The outcome of patients having NASH with cirrhosis was a 5-year survival rate of 75.2%. Multivariate analysis showed that HCC and a high Child-Pugh score were significant risk factors for mortality in patients with NASH who developed cirrhosis.

Additionally, with respect to the cause of death in a community-based cohort of 420 patients with NAFLD who were followed for a mean period of 7.6 years (range, 0.1 to 23.5 years), the rate of death from the most common cause of cardiovascular disease was revealed to be higher among patients with NASH or NASH cirrhosis than among the general population[1]. In another study[8], cardiovascular disease was found to be the most frequent cause of death in 173 patients with biopsy-proven NAFLD who were followed for 13 years. Soderberg *et al.*[11] confirmed that NASH was associated with increased mortality from all causes and from cardiovascular disease and liver-related causes among patients with NAFLD who were followed for a mean period of 21 years[11].

A systematic review and meta-analysis of seven cross-sectional studies (3497 subjects) confirmed that NAFLD diagnosed on ultrasonography is strongly associated with increased carotid-artery intima-medial thickness (carotid IMT) and an increased prevalence of carotid atherosclerotic plaque[17]. Carotid IMT has been independently associated with future risk for ischemic coronary events and stroke in middle-aged and older individuals[18]. Targher *et al.*[19] found that carotid IMT was greatest in patients with NASH, intermediate in those with SS, and lowest in healthy controls matched for age, sex, and BMI. In addition, the histologic severity of NASH was associated with the degree of IMT, independent of classic cardiovascular risk factors, insulin resistance, and metabolic syndrome.

In the present study, the advanced-fibrosis group was associated with a higher incidence of cerebro-cardiovascular events, and hepatic disease, and a higher mortality rate. In contrast, no cerebro-cardiovascular events or hepatic disease were reported during the course of the study among the 192 patients in the mild-fibrosis group. For this reason, it is important that we find the biomarkers that can predict the NASH with advanced fibrosis among NAFLD. In this study, the hallmark of disease progression to advance stage NASH patients was increasing fibrosis marker, as reflected by the disease stage. In addition we have previously reported that high-sensitivity C-reactive protein (hs-CRP), adiponectin (in men) and leptin (in women) seem to be good non-invasive markers of NASH; these markers can be used to detect up to 90% of patients with NASH[20]. Moreover, a number of studies[19,21] have reported that circulation levels of several inflammatory markers (e.g., hs-CRP, TNF-α, plasminogen activator inhibitor-1 (PAI-1),

fibrinogen), adipocytocin (e.g., adiponectin, leptin), and oxidative stress markers (e.g., thioredoxin, nitrotyrosine) are highest in patients with NASH and are associated with cardiovascular disease. Targher *et al.*[19] showed that the severity of liver histology found in NASH was strongly associated with increasing plasma concentrations of hs-CRP, PAI-1, and fibrinogen, and with decreasing plasma concentrations of adiponectin. Additionally, NASH and visceral adiposity have been used as cardiovascular risk biomarkers independent of potential confounders. In adult men, NASH has been found to predict a tendency toward atherogenic risk in a manner that is partly independent of the contribution of visceral adiposity[21].

In conclusion, NASH with advanced fibrosis may induce cardiovascular events and hepatic disease, and is associated with increased mortality. For these reasons, it is essential to identify high-risk patients and proactively provide thorough examination and treatment. Patients with NASH who have advanced fibrosis should be followed, not only to monitor their liver condition, but also to provide systemic surveillance to reduce the incidence of cerebro-cardiovascular events in this population. It is important to understand how to diagnosis advance fibrosis NASH among so many patients with NAFLD. For this purpose, it is necessary to find biomarkers that can predict the development of NASH with advanced fibrosis in Japanese patients with NAFLD. Further research is needed to investigate the process of disease progression in NASH.

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