Keywords Iron overload · Hepatic fibrosis · Nonalcoholic steatohepatitis

Introduction

Nonalcoholic fatty liver disease (NAFLD) is the most common chronic liver disease (CLD) in many developed countries and is a serious public health problem worldwide. NAFLD includes a wide spectrum of liver diseases that range from simple steatosis, which is usually a benign and nonprogressive condition, to nonalcoholic steatohepatitis (NASH), which can progress to liver cirrhosis (LC) and hepatocellular carcinoma (HCC) despite the absence of significant alcohol consumption [1–5]. Liver biopsy remains a reliable tool for the diagnosis of NASH [1, 6], and the most sensitive and specific method of providing prognostic information. Practically speaking, however, it is difficult to perform liver biopsy for every patient with NAFLD to ascertain the presence of NASH [7]. Moreover, biopsy itself has significant limitations such as pain, risk of severe complications, sampling error [8, 9], cost, and patient unwillingness to undergo invasive testing. Therefore, there is an urgent need to develop and validate simple, reproducible, noninvasive tests that accurately distinguish NASH from NAFLD and determine the stage of the disease [7]. Noninvasive approaches for this purpose have included a combination of clinical features and routine laboratory investigations, as well as some readily available serum markers of fibrosis [6, 7, 10, 11]. Most of these noninvasive approaches have consisted of small sample sizes and have lacked rigorous external validation.

The purposes of this study were (1) to develop a simple noninvasive scoring system aimed at differentiating NASH from NAFLD patients by using easily available clinical and biochemical variables and (2) to validate the results in a separate cohort of patients.

Methods

Patients

A total of 177 patients with well-characterized and liver-biopsy-confirmed NAFLD were included in this study to establish a simple method to detect NASH. They were consecutively biopsied patients who were seen at the Center for Digestive and Liver Diseases, Nara City Hospital from 2002 to 2008. To validate the model, 442 patients with biopsy-proven NAFLD from 2002 to 2008 were enrolled from eight Hepatology Centers in Japan: Division of Gastroenterology, Yokohama City University Graduate School of Medicine; Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University; Department of Gastroenterology and Hepatology, Kochi Medical School; Department of Internal Medicine, Saga Medical School, Saga University; Department of Hepatology, Graduate School of Medicine, Osaka City University; Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine; Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa Medical College; and Hepatology Center, Saiseikai Suita Hospital.

The diagnosis of NAFLD was based on the following criteria: (1) liver biopsy showing steatosis in at least 5% of hepatocytes [12] and (2) appropriate exclusion of liver diseases of other etiology including viral hepatitis, autoimmune hepatitis, drug-induced liver disease, primary biliary cirrhosis, biliary obstruction, hemochromatosis, Wilson's disease, and α-1-antitrypsin-deficiency-associated liver disease. Patients who consumed >20 g alcohol per day and patients with evidence of decompensated LC or HCC were excluded. Diabetic patients treated with exogenous insulin or insulin sensitizers (metformin or pioglitazone) were also excluded. Written informed consent was obtained from all patients at the time of their liver biopsy, and the study was conducted in accordance with the Helsinki Declaration.

Anthropometric and laboratory evaluation

Venous blood samples were taken in the morning after a 12-h overnight fast. The laboratory evaluation in all patients included a blood cell count and the measurement of aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyl transpeptidase, cholinesterase (ChE), total cholesterol, triglyceride, albumin, fasting plasma glucose (FPG), immunoreactive insulin (IRI), ferritin, hyaluronic acid (HA), and type IV collagen 7S. These parameters were measured using the standard techniques of clinical chemistry laboratories. Body mass index (BMI) was also calculated. Obesity was defined as BMI >25, according to the criteria of the Japan Society for the Study of Obesity [13]. Patients were assigned a diagnosis of diabetes mellitus (DM) if they had documented use of oral hypoglycemic medication, a random glucose level >200 mg/dl, or FPG >126 mg/dl [14]. Dyslipidemia was diagnosed if the cholesterol level was >220 mg/dl and/or triglyceride level was >160 mg/dl. Hypertension was diagnosed if the patient was taking antihypertensive medication and/or had a resting recumbent blood pressure ≥140/90 mmHg on at least two occasions.

The HAIR score [15] was calculated by summation of the scores of hypertension (1 point), ALT >40 IU/l (1 point), and insulin resistance (IR) index >5 (1 point). IR

index was calculated using the formula: 1/quantitative insulin sensitivity check index (QUICKI) [16] = log fasting IRI (μU/ml) + log FPG (mg/dl). Palekar's score [17] was calculated by summing the risk factor of age ≥50 years, female sex, AST ≥45 IU/l, BMI ≥30 kg/m^2, AST/ALT ratio (AAR) ≥0.80, and HA ≥55 ng/ml. Gholam's score [18] was calculated by the formula: 2.627 × ln AST + 2.13 for DM. The BAAT score [19] was calculated by summing the risk factor of BMI ≥28, age ≥50 years, ALT levels measuring twice normal or higher, and triglyceride level ≥1.7 mmol/l (150 mg/dl). The BARD score [20] is a weighted sum of three easily available variables [BMI ≥28 kg/m^2 (1 point), AAR ≥0.8 (2 points), and DM (1 point)]. Modified scores (with cutoff values of BMI changed to 25 kg/m^2) of Palekar's, BAAT, and BARD were also calculated. The NAFLD fibrosis score (NFS) [21] was calculated according to the following formula: −1.675 + 0.037 × age (years) + 0.094 × BMI + 1.13 × impaired fasting glycemia (IFG)/DM (yes = 1, no = 0) + 0.99 × AAR − 0.013 × platelet (×10^9/l) − 0.66 × albumin (g/dl). The N (Nippon) score [22] was calculated as the total number of the following risk factors: female sex, older age (>60 years), type 2 DM (T2DM), and hypertension.

Histological evaluation

All patients enrolled in this study underwent a percutaneous liver biopsy under ultrasonic guidance. The liver specimens were embedded in paraffin and stained with hematoxylin and eosin, Masson-trichrome, and reticulin silver stain. Two hepatopathologists (T.O. and Y.S.) who were blinded to the clinical data reviewed the liver biopsy specimens. Adequate liver biopsy samples were defined as >1.5 cm long and/or having more than six portal tracts. NASH was defined as steatosis with lobular inflammation and ballooning degeneration, with or without Mallory-Denk body or fibrosis [2, 3]. Patients whose liver biopsy specimens showed simple steatosis or steatosis with nonspecific inflammation were identified as the nonNASH cohort [2, 3]. The presence or absence of hepatocyte ballooning degeneration is influenced by the variability in pathologists' interpretation. The NAFLD Activity Score (NAS) proposed by Kleiner et al. [12] was the unweighted sum of the scores for steatosis (0–3), lobular inflammation (0–3), and ballooning degeneration (0–2). If liver histology was too atypical to make a judgment, cases with an NAS of ≥5 were considered to be NASH. The severity of hepatic fibrosis (stage) was defined as follows: stage 1, zone 3 perisinusoidal fibrosis; stage 2, zone 3 perisinusoidal fibrosis with portal fibrosis; stage 3, zone 3 perisinusoidal fibrosis and portal fibrosis with bridging fibrosis; and stage 4, cirrhosis [23].

Statistical analysis

Results are presented as the means and standard deviation (SD) for quantitative data, or as numbers with percentages in parentheses for qualitative data. Statistical differences in quantitative data were determined using the t test. Fisher's exact probability test or χ^2 analysis was used for qualitative data. Multivariate analysis was performed by logistic regression analysis to identify variables independently associated with the presence of NASH. Those variables with $P < 0.05$ by multivariate analysis were used to construct a scoring system to predict NASH. The scoring system was a weighted sum of significant variables on the basis of odds ratio (OR) obtained from logistic regression analysis. To assess the accuracy of the clinical scoring system in differentiating NASH from NAFLD, we calculated the sensitivity (Se) and specificity (Sp) for each value of each test, and then constructed receiver operating characteristic (ROC) curves by plotting the Se against (1 − Sp) at each value. The diagnostic performance of the scoring systems was assessed by analysis of ROC curves. The most commonly used index of accuracy was the area under the ROC curve (AUROC), with values close to 1.0 indicating high diagnostic accuracy. To evaluate the overall accuracy of our score and NFS in detecting significant or advanced fibrosis, the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated. Differences were considered statistically significant at $P < 0.05$.

Results

Characteristics of the patient population in the estimation group

Table 1 summarizes the clinical, laboratory, and liver biopsy data of the patient population in the estimation group. Eighty-six (49%) patients were female, and 120 (68%) were obese. Of 177 NAFLD patients involved in this estimation group, 98 (55%) were histologically diagnosed with NASH, and 79 (45%) had nonNASH NAFLD. NASH patients were significantly older, predominantly female, heavier, hypertensive, and more likely to have T2DM; had lower hemoglobin (Hb), platelet count and ChE; and had higher levels of AST, ALT, AAR, ferritin, FPG, IRI, HA, and type IV collagen 7S.

Predictors of NASH

Table 2 shows the univariate comparison and the results of the multivariate analysis performed in the 177 patients in the estimation group. Univariate analysis showed that age

Table 1 Clinical characteristics of patients in the estimation and validation groups

Clinical parameter	Estimation group ($n = 177$)			Validation group ($n = 442$)		
	NASH ($n = 98$)	NonNASH ($n = 79$)	P value	NASH ($n = 244$)	NonNASH ($n = 198$)	P value
Age (years)	57.7 ± 15.2	47.8 ± 16.5	0.0001	54.2 ± 15.2	48.2 ± 14.3	<0.0001
Gender (female)	58 (59%)	28 (35%)	0.0024	127 (52%)	77 (39%)	0.0072
BMI (kg/m^2)	28.5 ± 5.0	26.6 ± 4.7	0.0075	28.4 ± 5.0	27.4 ± 4.7	0.0207
Obesity (BMI >25)	74 (76%)	46 (58%)	0.0311	187 (77%)	139 (70%)	0.1548
Dyslipidemia	57 (58%)	39 (49%)	0.2886	173 (71%)	149 (75%)	0.3339
Hypertension (yes)	33 (34%)	12 (15%)	0.0055	100 (41%)	47 (24%)	0.0002
Type 2 diabetes (yes)	50 (51%)	14 (18%)	<0.0001	121 (50%)	69 (35%)	0.0027
Hemoglobin (g/dl)	14.1 ± 1.6 ($n = 97$)	14.8 ± 1.5 ($n = 78$)	0.0029	14.7 ± 2.5 ($n = 239$)	14.7 ± 1.5 ($n = 195$)	0.9758
Platelet count (×10^4/μl)	21.3 ± 6.4 ($n = 97$)	24.8 ± 8.1 ($n = 78$)	0.0018	21.2 ± 6.7 ($n = 240$)	24.1 ± 5.7 ($n = 194$)	<0.0001
AST (IU/l)	70 ± 30	44 ± 25	<0.0001	88 ± 387	38 ± 22	0.0694
ALT (IU/l)	102 ± 53	79 ± 54	0.0002	111 ± 217	65 ± 43	0.0001
AST/ALT ratio	0.77 ± 0.32	0.63 ± 0.23	0.0022	0.75 ± 0.32	0.65 ± 0.21	0.0001
GGT (IU/l)	105 ± 128	86 ± 66 ($n = 78$)	0.1211	85 ± 73 ($n = 242$)	85 ± 102 ($n = 197$)	0.9852
Cholinesterase (IU/l)	365 ± 83 ($n = 93$)	390 ± 83 ($n = 78$)	0.0317	364 ± 89 ($n = 224$)	387 ± 85 ($n = 176$)	0.0091
Total cholesterol (mg/dl)	206 ± 43 ($n = 97$)	214 ± 42 ($n = 77$)	0.2431	207 ± 43 ($n = 195$)	210 ± 39 ($n = 125$)	0.5121
Triglyceride (mg/dl)	189 ± 106 ($n = 93$)	167 ± 81 ($n = 73$)	0.1365	172 ± 106 ($n = 241$)	173 ± 86	0.9038
Ferritin (ng/ml)	270.7 ± 231	160 ± 158	0.0011	346 ± 989	183 ± 159	0.0221
FPG (mg/dl)	108 ± 45	96 ± 17.0	0.0301	113 ± 63	105 ± 39	0.1081
IRI (μU/ml)	18.5 ± 14.7	9.6 ± 6.3	<0.0001	16.8 ± 12.9	11.9 ± 8.3	<0.0001
Hyaluronic acid (ng/ml)	95 ± 134	29 ± 30	<0.0001	67 ± 74 ($n = 211$)	34 ± 37 ($n = 181$)	<0.0001
Type IV collagen 7S (ng/ml)	5.4 ± 1.7	3.9 ± 0.7	<0.0001	5.2 ± 2.1	3.9 ± 0.8	<0.0001
Histological fibrosis						
0–1	47 (48%)			143 (59%)		
2	29 (30%)			56 (23%)		
3	18 (18%)			30 (12%)		
4	4 (4%)			15 (6%)		

Results are presented as numbers with percentages *in parenthesis* for qualitative data or as means ± SD for quantitative data

BMI Body mass index, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin

P values were calculated by *t* test or χ^2 analysis

(≥55 years), female sex, obesity, Hb ≤14.5 g/dl, presence of T2DM, platelet count ≤22 × 10^4/μl, AST ≥60 IU/l, ALT ≥90 IU/l, AAR ≥0.8, ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male), FPG ≥100 mg/dl, IRI ≥10.0 μU/ml, HA ≥50 ng/ml, and type IV collagen 7S ≥5.0 ng/ml were significant variables. By multivariate analysis, three variables remained significant, including ferritin, IRI, and type IV collagen 7S. Thus, these three variables, ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male), IRI ≥10.0 μU/ml, and type IV collagen 7S ≥5.0 ng/ml, were combined to form the NAFIC score. The score was weighted based on OR obtained from logistic regression analysis (Table 2). Ferritin was given 1 point, IRI 1 point, and type IV collagen 7S 2 points. A score ranging from 0 to 4, defined by the presence of laboratory parameters, was calculated. The score for NASH patients ($n = 98$, 2.36 ± 1.28) was significantly higher than that for nonNASH patients ($n = 79$, 0.66 ± 0.82, $P < 0.0001$). The percentage of NASH in NAFLD with an NAFIC score of 0, 1, 2, 3, and 4 was 14% (6/44), 44% (34/61), 74% (14/19), 100% (28/28), and 92% (23/25), respectively (Fig. 1). The score was significantly higher even in NASH patients without significant fibrosis (stage 0 or 1) ($n = 47$, 1.83 ± 1.15) than in nonNASH patients ($P < 0.0001$). Figure 2 shows the ROC curve for NAFIC score to differentiate NASH from NAFLD. This scoring system had an AUROC of 0.851. At a cutoff value of NAFIC score 2, the sensitivity, specificity, PPV, and NPV were 66, 91, 90, and 67%, respectively. At a cutoff value of NAFIC score 1, the sensitivity, specificity, PPV, and NPV were 94, 48, 31, and 86%, respectively.

Table 2 Results of univariate and multivariate analysis: independent predictors of NASH and assigned score values in the estimation group ($n = 177$)

Variables	Unadjusted (univariate)			Adjusted (multivariate)			Score value
	OR	95%CI	*P* value	OR	95%CI	*P* value	
Age ≥55 years	2.28	1.24–4.18	0.0077				
Gender (female)	2.64	1.43–4.87	0.0019				
Obesity (BMI ≥25)	2.10	1.09–4.04	0.0268				
Hemoglobin ≤14.5 g/dl	1.94	1.06–3.56	0.0312				
Hypertension	2.83	1.35–5.96	0.0060				
Type 2 diabetes	4.84	2.40–9.74	<0.0001				
Platelet count $\leq 22 \times 10^4/\mu l$	2.66	1.43–4.91	0.0019				
AST ≥60 IU/l	5.74	2.81–11.73	<0.0001				
ALT ≥90 IU/l	2.04	1.10–3.77	0.0230				
AST/ALT ratio ≥0.8	1.98	1.18–4.76	0.0153				
Cholinesterase ≤380 IU/l	1.55	0.83–2.90	0.1689				
Ferritin ≥200 ng/ml (female) or ≥300 ng/ml (male)	5.08	2.48–10.37	<0.0001	4.01	1.07–15.02	0.0396	1
FPG ≥100 mg/dl	2.25	1.19–4.26	0.0127				
IRI ≥10 μU/ml	5.33	2.78–10.22	<0.0001	5.59	1.71–18.31	0.0045	1
Hyaluronic acid ≥50 ng/ml	4.94	2.38–10.26	<0.0001				
Type IV collagen 7S ≥5.0 ng/ml	21.20	7.19–62.49	<0.0001	15.54	1.49–162.39	0.0219	2

OR Odds ratio, *CI* confidence interval, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *GGT* gamma glutamyl transpeptidase, *FPG* fasting plasma glucose, *IRI* immunoreactive insulin

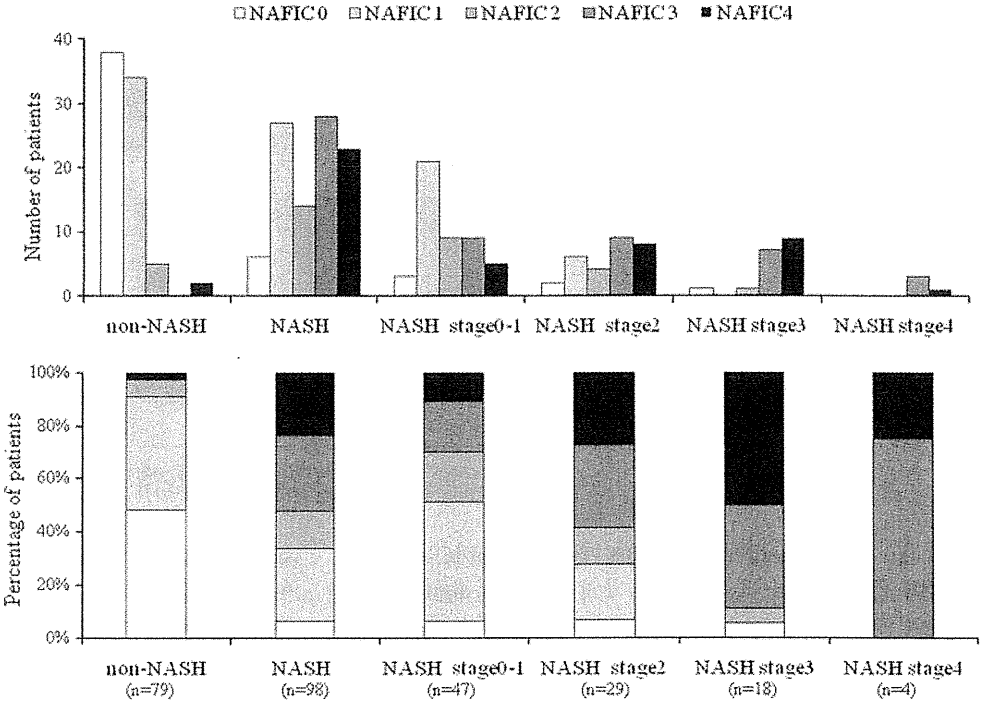

Fig. 1 Distribution of NAFIC scores in patients with NASH and nonNASH in the estimation group ($n = 177$)

Validation results

The diagnostic accuracy of the scoring system in separating patients with and without NASH was validated in 442 patients. Table 1 summarizes the clinical, laboratory, and liver biopsy data of the patient population in the validation group. Two hundred and two patients (46%) were female, and 326 (74%) patients were obese. Patients with NASH were significantly older, predominantly female, heavier, hypertensive, and more likely to have T2DM; had lower platelet count and ChE level; and had higher levels of AST, ALT, AAR, ferritin, IRI, HA, and type IV collagen 7S,

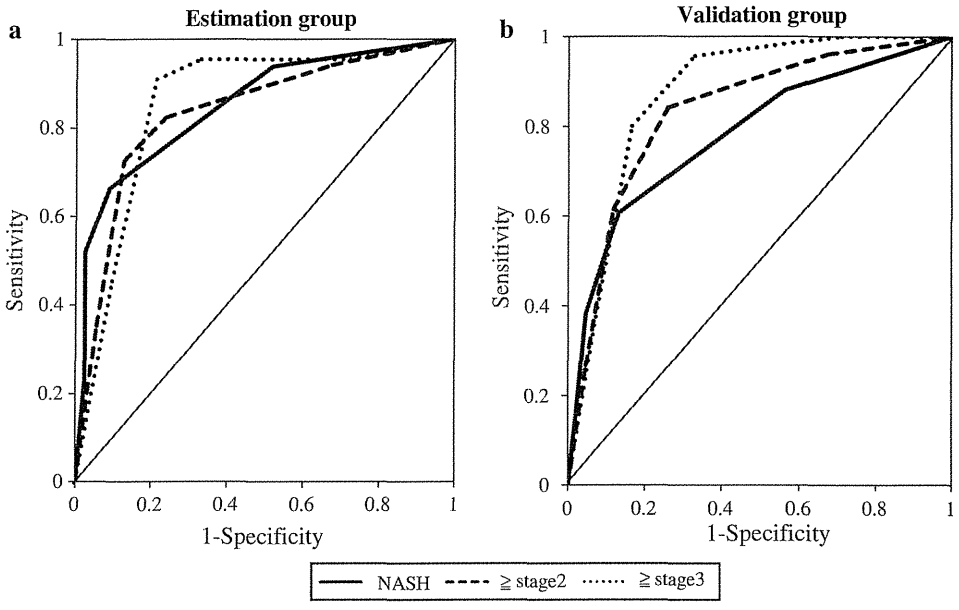

Fig. 2 ROC curves for the NAFIC score in the estimation (**a**) and validation (**b**) groups

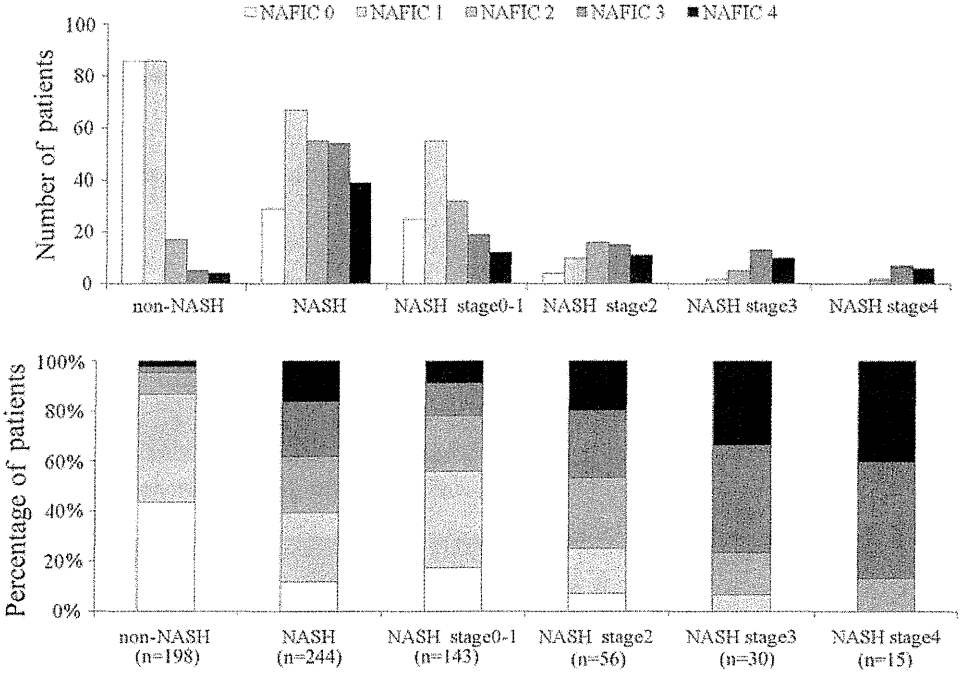

Fig. 3 Distribution of NAFIC score in patients with NASH and nonNASH in the validation group ($n = 442$)

than those with nonNASH NAFLD. The NAFIC score of NASH patients ($n = 244$, 2.03 ± 1.27) was significantly higher than that of nonNASH patients ($n = 198$, 0.76 ± 0.87, $p < 0.0001$). The percentage of NASH in NAFLD with an NAFIC score of 0, 1, 2, 3, and 4 was 25% (29/115), 44% (67/153), 76% (55/72), 92% (54/59), and 91% (39/43), respectively (Fig. 3). The score was significantly higher even in NASH patients without significant fibrosis (stage 0 or 1) ($n = 143$, 1.57 ± 1.17) than non-NASH patients ($p < 0.0001$). The AUROC remained relatively high in the validation set (0.782, Fig. 2; Table 3). At a cutoff value of NAFIC score 2, the sensitivity, specificity, PPV, and NPV were 60, 87, 85 and 64%, respectively. At a cutoff value of NAFIC score 1, the sensitivity, specificity, PPV, and NPV were 88, 43, 66, and 75%, respectively.

Comparing the NAFIC score to several previously established scoring systems

The AUROCs of various scoring systems that have been reported to differentiate NASH from NAFLD, or advanced from mild fibrosis, are shown in Table 3. To differentiate NASH from NAFLD, the AUROC in the validation group

Table 3 AUROC of NAFIC score and various scoring systems previously reported in the estimation and validation groups

Score	Group	AUROC for NASH	AUROC for significant fibrosis	AUROC for advanced fibrosis
NAFIC	Estimation ($n = 177$)	0.851	0.835	0.856
	Validation ($n = 442$)	0.782	0.833	0.874
	Total ($n = 619$)	0.803	0.834	0.869
HAIR [15]	Estimation ($n = 177$)	0.632	0.549	0.448
	Validation ($n = 432$)	0.636	0.620	0.631
	Total ($n = 609$)	0.631	0.593	0.566
Palekar et al. [17]	Estimation ($n = 173$)	0.784	0.794	0.847
	Validation ($n = 390$)	0.711	0.798	0.826
	Total ($n = 563$)	0.733	0.799	0.835
Modified Palekar et al.	Estimation ($n = 173$)	0.780	0.801	0.843
	Validation ($n = 390$)	0.709	0.810	0.830
	Total ($n = 563$)	0.730	0.808	0.837
Gholam et al. [18]	Estimation ($n = 177$)	0.829	0.784	0.713
	Validation ($n = 442$)	0.758	0.787	0.739
	Total ($n = 619$)	0.777	0.786	0.729
BAAT [19]	Estimation ($n = 164$)	0.672	0.533	0.473
	Validation ($n = 440$)	0.633	0.560	0.498
	Total ($n = 604$)	0.647	0.585	0.526
Modified BAAT	Estimation ($n = 164$)	0.741	0.615	0.566
	Validation ($n = 440$)	0.666	0.654	0.576
	Total ($n = 604$)	0.687	0.641	0.573
BARD [20]	Estimation ($n = 164$)	0.646	0.686	0.745
	Validation ($n = 440$)	0.621	0.689	0.731
	Total ($n = 604$)	0.627	0.688	0.734
Modified BARD	Estimation ($n = 164$)	0.647	0.709	0.734
	Validation ($n = 440$)	0.603	0.689	0.730
	Total ($n = 604$)	0.614	0.695	0.730
NAFLD fibrosis score [21]	Estimation ($n = 168$)	0.735	0.843	0.834
	Validation ($n = 420$)	0.663	0.805	0.862
	Total ($n = 588$)	0.685	0.817	0.853
N score (Nippon) [22]	Estimation ($n = 177$)	0.733	0.739	0.728
	Validation ($n = 408$)	0.642	0.715	0.698
	Total ($n = 585$)	0.668	0.720	0.704

was greatest for NAFIC (0.782, Fig. 2), then Gholam's score (0.758), followed by Palekar's score (0.711), modified Palekar's score (0.709), modified BAAT (0.666), NFS (0.663), *N* score (0.642), HAIR (0.636), BAAT (0.633), BARD score (0.621), and modified BARD score (0.603). Based on an evaluation of AUROC, NAFIC score outperformed other scoring systems in the estimation and validation groups to differentiate NASH from NAFLD. To differentiate NASH with significant fibrosis from NAFLD, the AUROC in the total cohort was greatest for NAFIC score (0.834), then NFS (0.817), followed by modified Palekar's score (0.808), Palekar's score (0.799), Gholam's score (0.786), *N* score (0.720), modified BARD (0.695), BARD (0.688), modified BAAT score (0.641), HAIR (0.593), and BAAT score (0.585). To differentiate NASH with advanced fibrosis from NAFLD, the AUROC in the total cohort was greatest for NAFIC score (0.869), then NFS (0.853), followed by modified Palekar's score (0.837), Palekar's score (0.835), BARD (0.734), modified BARD (0.730), Gholam's score (0.729), *N* score (0.704), modified BAAT (0.573), HAIR (0.566), and BAAT score (0.526). Among these prediction models, NAFIC score was superior to others in not only detecting NASH, but also predicting fibrosis stage.

We compared the diagnostic accuracy of NAFIC score to that of NFS in detecting advanced fibrosis (stage 3–4)

Table 4 Accuracy of the NAFIC score and NAFLD fibrosis score (NFS) in predicting advanced fibrosis (stage 3–4) and significant fibrosis (stage 2–4) in the total cohort

	NAFIC score			NAFLD fibrosis score		
	0–1	2	3–4	Low cutoff point (<−1.455)	Indeterminate (−1.455 to 0.676)	High cutoff point (>0.676)
Predicting advanced fibrosis (stage 3–4)						
N (%)	374 (60%)	90 (15%)	155 (25%)	330 (56%)	209 (36%)	49 (8%)
Stage 0–2	371	82	99	325	171	28
Stage 3–4	3	8	56	5	38	21
Se	96%		84%	92%		33%
Sp	67%		82%	62%		95%
PPV	26%		36%	23%		43%
NPV	99%		98%	98%		92%
LR (+)	2.913		4.660	2.427		6.141
LR (−)	0.067		0.200	0.126		0.710
Interpretation	Absence of advanced fibrosis (99% certainty)		Presence of advanced fibrosis (36% certainty)	Absence of advanced fibrosis (98% certainty)		Presence of advanced fibrosis (43% certainty)
	NAFIC score			NAFLD fibrosis score		
	0	1	2–4	Low cutoff point (<−1.455)	Indeterminate (−1.455 to 0.676)	High cutoff point (>0.676)
Predicting significant fibrosis (stage 2–4)						
N (%)	160 (26%)	214 (35%)	245 (40%)	330 (56%)	209 (36%)	49 (8%)
Stage 0–1	153	196	118	305	122	16
Stage 2–4	7	18	127	25	87	33
Se	95%		84%	86%		23%
Sp	33%		74%	69%		96%
PPV	32%		52%	47%		67%
NPV	96%		93%	92%		79%
LR (+)	1.416		3.266	2.657		6.301
LR (−)	0.141		0.070	0.250		0.801
Interpretation	Absence of significant fibrosis (96% certainty)		Presence of significant fibrosis (52% certainty)	Absence of significant fibrosis (92% certainty)		Presence of significant fibrosis (67% certainty)

Se Sensitivity, *Sp* specificity, *PPV* positive predictive value, *NPV* negative predictive value, *LR* likelihood ratio

and significant fibrosis (stage 2–4) (Table 4). To exclude advanced fibrosis using the low cutoff point (NFS < −1.455), 325 of 330 (98%) patients were correctly staged, whereas only 5 (2%) were understaged. The NPV of this cutoff for advanced fibrosis was 98%. Using the low cutoff point (NAFIC ≤1), 371 of 374 (99%) patients were correctly staged, whereas only 3 (1%) were understaged. The NPV of this cutoff for advanced fibrosis was 99%, which was equal to that of NFS. Using the high cutoff point (NFS >0.676), 21 of 49 (43%) patients were correctly staged, whereas 28 (57%) were overstaged. The PPV of this cutoff for advanced fibrosis was 43%. Using the high cutoff point (NAFIC ≥3), 56 of 155 (36%) patients were correctly staged, whereas 99 (64%) were overstaged. The PPV of this cutoff for advanced fibrosis was 36%, which was lower than that of NFS. The percentage of the undetermined range was much lower for the NAFIC score (15%) than for NFS (36%) (Table 4).

When the NFS low cutoff (NFS <−1.455) was applied to predict significant fibrosis, 305 of 330 (92%) patients were correctly staged, whereas 25 of 330 (8%) patients were understaged. The NPV for significant fibrosis was 92%. When their high cutoff (NFS >0.676) was applied to predict significant fibrosis, 33 of 49 (67%) patients were correctly staged, whereas 16 of 49 (33%) patients were overstaged. The PPV for significant fibrosis was 67%. When our low cutoff point (NAFIC = 0) was applied to exclude significant fibrosis, 153 of 160 (96%) patients were correctly staged, whereas only seven (4%) were understaged. The NPV of this cutoff for significant fibrosis was 96%, which

was slightly higher than NFS. Using our high cutoff point (NAFIC ≥2), 127 of 245 (52%) patients were correctly staged, whereas 118 (48%) were overstaged. The PPV of this cutoff for significant fibrosis was 52%, which was lower than that of NFS (67%) (Table 4).

Discussion

In this study, we developed and validated a simple scoring system to differentiate NASH from NAFLD. Our scoring system with the three variables ferritin, IRI, and type IV collagen 7S had an AUROC of 0.851 and 0.782 in the estimation and validation groups, respectively. Elevation of serum ferritin levels, a marker of iron storage, is associated with NASH [24, 25]. We previously reported high frequencies of hyperferritinemia and increased hepatic iron stores in Japanese NASH patients [11]. Yoneda and colleagues [26], our collaborative research group, also have reported that measurement of serum ferritin is useful to distinguish NASH from NAFLD. Their optimal cutoff value was 196 ng/ml, and their results for sensitivity, specificity, PPV, and NPV were 64, 77, 89, and 43%, respectively. Serum ferritin levels have been found to be a significant independent predictor of severe fibrosis in 167 Italian NAFLD subjects [27], but this has not been confirmed by other studies [28]. In Western countries, mildly increased serum ferritin does not necessarily indicate coexisting iron overload. However, it is well known that serum ferritin is closely associated with IR and can be considered a marker for metabolic syndrome [29].

Hyperinsulinemia (IRI ≥10.0 μU/ml) was also selected as an independent predictor of NASH. Hyperinsulinemia and increased IR could have important roles in the pathogenesis of NASH in both Western and Asian countries [30–33]. Hyperinsulinemia in NASH patients is attributable to increased insulin secretion, which compensates for reduced insulin sensitivity, and is not the consequence of decreased hepatic extraction of insulin, which occurs in all forms of CLD at the stage of advanced fibrosis or cirrhosis [30, 31]. The homeostasis model assessment (HOMA) has been validated and widely used for determining the degree of IR, and strongly predicts the development of T2DM [34]. Patients with NASH have a higher HOMA index compared with those with nonNASH NAFLD [30, 35]. Similarly, another study has reported the QUICKI model as being useful for predicting NASH [15]. However, appropriate cutoff values of these models have never been established. In contrast with these parameters that are mathematical transformations of FPG and IRI levels, fasting IRI, which has the advantage of being easily determined without calculations, was only applied to multivariate analysis in our study.

Type IV collagen is one of the extracellular matrices that are produced by hepatic fibroblasts. The 7S domain in the N-terminus of type IV collagen is inserted in tissues and released into the blood by turnover in connective tissues. Therefore, the serum 7S domain level increases in parallel with the amount of fibrosis and in synthesis from stellate cells and myofibroblasts following increased liver fibrosis [36]. In Japan, type IV collagen 7S is now widely used for assessing the extent of hepatic fibrosis in CLD because the test is covered by public health insurance. According to two reports [37, 38], a cutoff point of 5.0 or 4.25 ng/ml provided high NPV to exclude advanced fibrosis in Japanese NAFLD patients. Shimada et al. [39] have demonstrated that a cutoff point of 5.0 ng/ml provided sensitivity and specificity of 41 and 95%, respectively, to detect early-stage NASH. Serum HA levels are elevated during accelerated deposition of collagen in the extracellular space due to upregulation of HA production by activated stellate cells and myofibroblasts, and downregulation of its clearance by sinusoidal endothelial cells. Serum HA appears to be a relatively accurate predictor of advanced fibrosis stage in NAFLD, but less for distinguishing between minor degrees of fibrosis in NASH and nonNASH NAFLD [40, 41]. In our study, serum HA level was not an independent predictor of NASH by multivariate analysis. Moreover, HA increases in systematic inflammatory conditions, which might produce false-positive results. We believe that type IV collagen 7S is superior to HA in predicting the extent of fibrosis in NAFLD patients.

Currently, the NAFLD biomarkers have been evaluated for (1) distinguishing NASH from NAFLD and/or (2) diagnosing advanced fibrosis or cirrhosis. HAIR [15], Palekar's [17], and Gholam's [18] scores were derived for distinguishing NASH from NAFLD, and the others are for detecting significant or advanced fibrosis. The present study clearly demonstrated that NAFIC score was more useful than other scoring systems for detection of NASH and for prediction of fibrosis (Table 3). The HAIR score uses a combination of presence of hypertension, elevated ALT (>40 U/L), and IR, with at least two parameters that indicate NASH with high sensitivity and specificity [15]. None had an IR index >5 in our overall cohort (data not shown), and AUROCs were 0.632 and 0.634 in the estimation and validation groups, respectively. The score does not appear useful in a less obese population because it was described in a group of severely obese patients who were undergoing gastric bypass surgery. In Palekar's study [17], the presence of at least three out of six factors (age ≥50 years, female sex, AST ≥45 IU/l, BMI ≥30 kg/m^2, AAR ≥0.80, and HA ≥55 ng/ml) had a sensitivity and specificity for NASH diagnosis of 74 and 66%, respectively. In our estimation group, the presence of at least three of these factors had a sensitivity and specificity for

NASH diagnosis of 68 and 71%, respectively. In our validation group, the presence of at least three of these factors had a sensitivity and specificity for NASH diagnosis of 63 and 64%, respectively. AUROCs were 0.784 and 0.711 in the estimation and validation groups, respectively. Therefore, Palekar's score was not superior to NAFIC score for predicting NASH (Table 3).

Gholam's score [18], which consists of only two variables (AST and the presence of DM), is very simple and equally useful as NAFIC score for detection of NASH, but it was not superior to our score for predicting severe fibrosis in our cohort. Gholam et al. [18] have constructed other models that consist of ALT and HbA1c to detect the presence of fibrosis. We could not evaluate these models because HbA1c was only measured in a limited number of patients. Angulo et al. [21] have shown that the NFS, which consists of six variables (age, BMI, AAR, IFG/DM, platelet count, and albumin), can reliably predict advanced fibrosis. In ROC analysis, NFS is shown to be useful for prediction of advanced or significant fibrosis. The low cutoff point (NFS <-1.455) showed higher NPV (98%) than that in the estimation (93%) and validation (88%) cohort reported by Angulo et al. The low cutoff point in NAFIC score (≤ 1) had equally high NPV (99%). In contrast, the high cutoff point (NFS >0.676) showed lower PPV (43%) than that in the estimation (98%) and validation (80%) cohort reported by Angulo et al. The high cutoff point in NAFIC score (≥ 3) had lower PPV (36%). By applying the low cutoff score (NFS <-1.455, NAFIC ≤ 1), advanced fibrosis could be excluded with high accuracy. By applying the high cutoff score (NFS >0.676, NAFIC ≥ 3), the presence of advanced fibrosis could not be diagnosed with high accuracy. Consistent with our results, a separate validation study of NFS in 162 Chinese patients found that the NPV for excluding advanced fibrosis was 91%, but the PPV for predicting advanced fibrosis was 0% [42]. It is suggested that this low PPV might be due to lower prevalence of advanced fibrosis in the study of Wong et al. (11%) [42] than in that by Angulo et al. (27%) [21]. Similarly, the prevalence of advanced fibrosis was low (11%) in our study.

In Asian patients, steatohepatitis and other metabolic complications tend to develop at a lower BMI, which is one of the factors in the equation of the NFS. Therefore, NFS and NAFIC score were applicable to exclusion rather than detection of significant or advanced fibrosis. NFS can be easily obtained in clinical practice, but this scoring system can be cumbersome and difficult to apply in every practice. The easily determined NAFIC score is at least equivalent to the more complex NFS. Our results suggest that liver biopsies can be avoided in NAFLD patients with a NAFIC score of 0 or 1 because they are likely to have NAFLD without advanced fibrosis. In contrast, liver biopsies should be recommended in NAFLD patients with an NAFIC score of ≥ 2 to assess the extent of hepatic fibrosis and predict prognosis. The BARD score developed by Harrison et al. is a weighted sum of three easily available variables [BMI ≥ 28 kg/m^2 (1 point), AAR ≥ 0.8 (2 points), and DM (1 point)], and the authors have shown that a score of 2–4 was associated with an OR of 17 for predicting advanced fibrosis [20]. Although the BARD score is simple to calculate, our validation study did not reveal an advantage of this score over others. In our cohort, when a BARD score of ≥ 2 was found, the sensitivity, specificity, PPV, and NPV for detecting advanced fibrosis were 73, 65, 19, and 95%, respectively. According to a study of 122 Japanese NAFLD patients by Fujii and colleagues [43], our collaborative research group, when a BARD score of ≥ 2 was used, the AUROC was 0.73 with an OR of 4.9 for detection of advanced fibrosis. It has been concluded that BARD score is less predictive of advanced fibrosis in Japanese NAFLD patients because they are not as obese as those in Western countries. Disappointingly, modified scores of Palekar's score, BAAT, and BARD could not improve the diagnostic accuracy for NASH or advanced fibrosis. The *N* score (the total number of the following risk factors: female sex, age >60 years, T2DM, and hypertension), which was established on the basis of data collection from 182 Japanese NAFLD patients in multiple centers in Nagasaki [22], is very simple, without the need for detailed laboratory tests. However, it was not superior to other scoring systems in our validation study.

Our study had several limitations. The fact that we excluded diabetic patients treated with exogenous insulin or insulin sensitizers (metformin or pioglitazone) from the analysis was a major limitation. In the future, we must find better scoring systems that are applicable to these patients. Other limitations include the largely retrospective study design and lack of complete data in many subjects. We included patients from different hepatology centers in Japan that have a particular interest in studying NAFLD, and thus, some referral bias could not be ruled out. Patient selection bias could also have existed because liver biopsy might have been considered for NAFLD patients who were likely to have NASH. We acknowledge that pathological diagnosis was mainly determined using liver tissues derived from percutaneous liver biopsy, which is prone to sampling error or interobserver variability [8, 9]. In fact, 11 patients of our total cohort were diagnosed as nonNASH in spite of an NAFIC score of 3 or 4. Although the exact reason was unknown, sampling error could have led to this misdiagnosis. These patients need follow-up care or repeat liver biopsies. It should be emphasized that we had a central pathology review by two hepatopathologists to prevent interobserver variability, although we were not able to quantify the effect on our results of some

intraobserver variability. Because all participants were Japanese, there is a possibility that our results might not be adaptable for NAFLD patients of other races. Due to these limitations, the present results need to be validated in independent populations by other investigators.

In conclusion, NAFIC score can predict NASH in Japanese NAFLD patients with sufficient accuracy and simplicity to be considered for clinical use, thus identifying a very high-risk group in whom liver biopsy would be very likely to detect NASH, as well as a low-risk group in whom liver biopsy can be safely delayed or avoided.

Acknowledgments The authors thank the following individuals for assistance in preparation of this manuscript: Atsushi Nakajima, M.D., Ph.D., Division of Gastroenterology, Yokohama City University Graduate School of Medicine; Kyoko Sakai, M.D., Yutaka Inada, M.D., Akitoshi Douhara, M.D., Tasuku Hara, M.D., Center for Digestive and Liver Diseases, Nara City Hospital; Tomokazu Ishitobi, M.D., Department of Medicine and Molecular Science, Graduate School of Biomedical Sciences, Hiroshima University; Yoshihiro Kamada, M.D., Ph.D., Department of Gastroenterology and Hepatology, Osaka University Graduate School of Medicine; Takaaki Ohtake, M.D., Ph.D., Division of Gastroenterology and Hematology/Oncology, Department of Medicine, Asahikawa; Yoshito Itoh, M.D., Ph.D., Toshikazu Yoshikawa, M.D., Ph.D., Department of Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine.

Conflicts of interest The authors have no conflicts of interest to disclose.

References

1. Ludwig J, Viggiano TR, McGill DB, Ott BJ. Non-alcoholic steatohepatitis. Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc. 1980;55:434–8.
2. Matteoni CA, Younossi ZM, Gramlich T, Boparai N, Liu YC, McCullough AJ. Nonalcoholic fatty liver diseases: a spectrum of clinical and pathological severity. Gastroenterology. 1999;116:1413–9.
3. Rafiq N, Bai C, Fang Y, Srishord M, McCullough A, Gramlich T, et al. Long-term follow-up of patients with nonalcoholic fatty liver. Clin Gastroenterol Hepatol. 2009;7:234–8.
4. Saibara T. Nonalcoholic steatohepatitis in Asia-Oceania. Hepatol Res. 2005;33:64–7.
5. Hamaguchi M, Kojima T, Takeda N, Nakagawa T, Taniguchi H, Fujii K, et al. The metabolic syndrome as a predictor of nonalcoholic fatty liver disease. Ann Intern Med. 2005;143:722–8.
6. Vuppalanchi R, Chalasani N. Nonalcoholic fatty liver disease and nonalcoholic steatohepatitis: selected practical issues in their evaluation and management. Hepatology. 2009;49:306–17.
7. Wieckowska A, McCullough AJ, Feldstein AE. Noninvasive diagnosis and monitoring of nonalcoholic steatohepatitis: present and future. Hepatology. 2007;46:582–9.
8. Ratziu V, Charlotte F, Heurtier A, Gombert S, Giral P, Bruckert E, et al. Sampling variability of liver biopsy in nonalcoholic fatty liver disease. Gastroenterology. 2005;128:1898–906.
9. Merriman RB, Ferrell LD, Patti MG, Weston SR, Pabst MS, Aouizerat BE, et al. Correlation of paired liver biopsies in morbidly obese patients with suspected nonalcoholic fatty liver disease. Hepatology. 2006;44:874–80.
10. Pagadala M, Zein CO, McCullough AJ. Predictors and advanced fibrosis in non-alcoholic fatty liver disease. Clin Liver Dis. 2009;13:591–606.
11. Sumida Y, Nakashima T, Yoh T, Furutani M, Hirohama A, Kakisaka Y, et al. Serum thioredoxin levels as a predictor of steatohepatitis in patients with nonalcoholic liver disease. J Hepatol. 2003;38:32–8.
12. Kleiner DE, Brunt EM, Van Natta M, Behling C, Contos MJ, Cummings OW, et al. Design and validation of a histological scoring system for nonalcoholic fatty liver disease. Hepatology. 2005;41:1313–21.
13. Japanese Society for the Study of Obesity. New criteria of obesity (in Japanese). J Jpn Soc Study Obes. 2000;6:18–28.
14. American Diabetes Association. Report of the expert committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 1997;20:1183–97.
15. Dixon JB, Bhathal PS, O'Brien PE. Nonalcoholic fatty liver disease: predictors of nonalcoholic steatohepatitis and liver fibrosis in the severely obese. Gastroenterology. 2001;121:91–100.
16. Katz A, Nambi SS, Mather K, Baron AD, Follmann DA, Sullivan G, et al. Quantitative insulin sensitivity check index: a simple, accurate method for assessing insulin sensitivity in humans. J Clin Endocrinol Metab. 2000;85:2402–10.
17. Palekar NA, Naus R, Larson SP, Ward J, Harrison SA. Clinical model for distinguishing nonalcoholic steatohepatitis from simple steatosis in patients with nonalcoholic fatty liver disease. Liver Int. 2006;26:151–6.
18. Gholam PM, Flancbaum L, Machan JT, Charney DA, Kotler DP. Nonalcoholic fatty liver disease in severely obese subjects. Am J Gastroenterol. 2007;102:399–408.
19. Ratziu V, Giral P, Charlotte F, Bruckert E, Thibault V, Theodorou I, et al. Liver fibrosis in overweight patients. Gastroenterology. 1999;118:1117–23.
20. Harrison SM, Oliver D, Arnold HLM, Gogia SM, Neuschwander-Tetri BAM. Development and validation of a simple NAFLD clinical scoring system for identifying patients without advanced disease. Gut. 2008;57:1441–7.
21. Angulo P, Hui JM, Marchesini G, Bugianesi E, George J, Farrell GC, et al. The NAFLD fibrosis score: a noninvasive system that identifies liver fibrosis in patients with NAFLD. Hepatology. 2007;45:846–54.
22. Miyaaki H, Ichikawa T, Nakao K, Yatsuhashi H, Furukawa R, Ohba K, et al. Clinicopathological study of nonalcoholic fatty liver disease in Japan: the risk factors for fibrosis. Liver Int. 2008;28:519–24.
23. Brunt EM, Janney CG, Di Bisceglie AM, Neuschwander-Tetri BA, Bacon BR. Non-alcoholic steatohepatitis: a proposal for grading and staging the histological lesions. Am J Gastroenterol. 1999;94:2467–74.
24. Bonkovsky HL, Jawaid Q, Tortorelli K, LeClair P, Cobb J, Lambrecht RW, et al. Non-alcoholic steatohepatitis and iron: increased prevalence of mutations of the HFE gene in non-alcoholic steatohepatitis. J Hepatol. 1999;31:421–9.
25. Bugianesi E, Manzini P, D'Antico S, Vanni E, Longo F, Leone N, et al. Relative contribution of iron burden, HFE mutations, and insulin resistance to fibrosis in nonalcoholic fatty liver. Hepatology. 2004;39:179–87.
26. Yoneda M, Nozaki Y, Endo H, Mawatari H, Iida H, Fujita K, et al. Serum ferritin is a clinical biomarker in Japanese patients with nonalcoholic steatohepatitis (NASH) independent of HFE gene mutation. Dig Dis Sci. 2010;55:808–14.
27. Chitturi S, Weltman M, Farrell GC, McDonald D, Kench J, Liddle C, et al. HFE mutations, hepatic iron, and fibrosis: ethnic-specific association of NASH and C282Y but not with fibrotic severity. Hepatology. 2002;36:142–9.

28. Sumida Y, Yoshikawa T, Okanoue T. Role of hepatic iron in non-alcoholic steatohepatitis. Hepatol Res. 2009;39:213–22.
29. Jehn M, Clark JM, Guallar E. Serum ferritin level and risk of the metabolic syndrome in U.S. adults. Diabetes Care. 2004;27: 2422–8.
30. Chitturi S, Abeygunasekera S, Farrell GC, Holmes-Walker J, Hui JM, Fung C, et al. NASH and insulin resistance: insulin hypersecretion and specific association with the insulin resistance syndrome. Hepatology. 2002;35:373–9.
31. Pagano G, Pacini G, Musso G, Gambino R, Mecca F, Depetris N, et al. Nonalcoholic steatohepatitis, insulin resistance, and metabolic syndrome: further evidence for an etiologic association. Hepatology. 2002;35:367–72.
32. Marcheisini G, Bugianesi E, Forlani G, Cerrelli F, Lenzi M, Manini R, et al. Nonalcoholic fatty liver, steatohepatitis, and the metabolic syndrome. Hepatology. 2003;37:917–23.
33. Ono M, Saibara T. Clinical features of nonalcoholic steatohepatitis in Japan: evidence from literature. J Gastroenterol. 2006;41:725–32.
34. Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia. 1985;28:412–9.
35. Boza C, Riquelme A, Ibañez L, Duarte I, Norero E, Viviani P, et al. Predictors of non-alcoholic steatohepatitis (NASH) in obese patients undergoing gastric bypass. Obes Surg. 2005;15:1148–53.
36. Murawaki Y, Ikuta K, Koda M, Kawasaki H. Serum type III procollagen peptide, type IV collagen 7S domain, central triple-helix of type IV collagen and tissue inhibitor of metalloproteinases in patients with chronic viral liver disease: relationship to liver histology. Hepatology. 1994;20:780–7.
37. Sakugawa H, Nakayoshi T, Kobashigawa K, Yamashiro T, Maeshiro T, Miyagi S, et al. Clinical usefulness of biochemical markers of liver fibrosis in patients with nonalcoholic fatty liver disease. World J Gastroenterol. 2005;11:255–9.
38. Yoneda M, Mawatari H, Fujita K, Yonemitsu K, Kato S, Takahashi H, et al. Type IV collagen 7s domain is an independent clinical marker of the severity of fibrosis in patients with non-alcoholic steatohepatitis before the cirrhotic stage. J Gastroenterol. 2007;42:375–81.
39. Shimada M, Kawahara H, Ozaki K, Fukura M, Yano H, Tsuchishima M, et al. Usefulness of a combined evaluation of the serum adiponectin level, HOMA-IR, type IV collagen 7S level to predict the early stage of nonalcoholic steatohepatitis. Am J Gastroenterol. 2007;102:1931–8.
40. Kaneda H, Hashimoto E, Yatsuji S, Tokushige K, Shiratori K. Hyaluronic acid levels can predict severe fibrosis and platelet counts can predict cirrhosis in patients with nonalcoholic fatty liver disease. J Gastroenterol Hepatol. 2006;21:1459–65.
41. Suzuki A, Angulo P, Lymp J, Li D, Satomura S, Lindor K. Hyaluronic acid, an accurate serum marker for severe fibrosis in patients with non-alcoholic fatty liver disease. Liver Int. 2005;25:779–86.
42. Wong VW, Wong GL, Chim AM, Tse AM, Tsang SW, Hui AY, et al. Validation of the NAFLD fibrosis score in a Chinese population with low prevalence of advanced fibrosis. Am J Gastroenterol. 2008;103:1682–8.
43. Fujii H, Enomoto M, Fukushima W, Tamori A, Sakaguchi H, Kawada N. Applicability of BARD score to Japanese patients with NAFLD. Gut. 2009;58:1566–7.

NASHに関係するリポ蛋白遺伝子

宗景玄祐* 小野正文* 西原利治*

索引用語：肥満，脂肪肝，インスリン感受性，酸化ストレス，adiponutrin

1 はじめに

'80年代初頭に肝機能異常を示した者は検診受診者の8％にすぎなかったが，その後，NAFLD（非アルコール性脂肪性肝疾患）と呼ばれる非飲酒者に生じた肥満を誘因とする原因不明の慢性肝疾患が急増した．このため，疾病構造の変化に関する日本病院会の検討によれば，検診受診者の23％が今日では異常を示すようになった[1]．そのような症例の肝臓の組織学的変化は多様であるが，中～大滴性の脂肪滴を有する肝細胞が小葉中心性集簇し，肝細胞のアポトーシス・壊死像に近接して炎症性細胞の浸潤を認めることが多い．このような症例の中には，肝細胞の風船様変性や肝細胞周囲性の線維化，Mallory体など従来はアルコール性肝炎に特徴的とされてきた肝病変を伴う症例が含まれている．肝病変の進行性が強いという臨床的な予後を勘案し，このような症例は特にNASH（非アルコール性脂肪肝炎）と呼ばれ，その他の単純性脂肪肝症例と区別されている（図1, 図2）[2～4]．

脂肪肝の発症については肥満が主な誘因（図3）とされ，運動不足や過食などの環境要因に加えてβ3 adrenergic receptorなどの倹約遺伝子の関与が示されている．また，単純性脂肪肝からNASHへの進展や肝線維化の進行に際しては，遺伝子多型により規定される遺伝的素因の関与がgenome wide association study（GWAS）により強く示唆されている．加えて，炎症惹起性遺伝子の機能性遺伝子多型，酸化ストレスに対するミトコンドリアの脆弱性を規定する機能性遺伝子多型，肝細胞からの中性脂肪放出能を規定する遺伝子群における機能的遺伝子多型，PPAR-αを介した脂肪酸β酸化誘導能を規定する機能的遺伝子多型などすでに報告されたものだけでも多岐にわたり，NASHの疾患感受性を規定する多様な遺伝的背景が示されている（図4）[5,6]．

しかし，NASHに対する疾患感受性を規定する機能性遺伝子多型やNASHにおける肝病変の進展に寄与すると想定される機能性遺伝子の検索をいくら続けても，予防や治療という観点からみて真に最も重要なス

Genyu MUNEKAGE et al: Risk factors involed in the development of non-alcoholic steatohepatitis
*高知大学医学部附属病院消化器内科［〒783-8505 高知県南国市岡豊町小蓮］

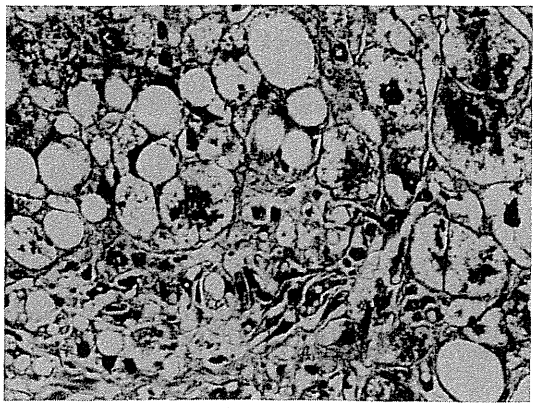

図1　NASHの肝組織像
中～大滴性の脂肪滴の沈着，細胞質の淡明化を伴う肝細胞の腫大，マロリー小体の出現，線維化を伴う炎症性細胞浸潤などが観察される

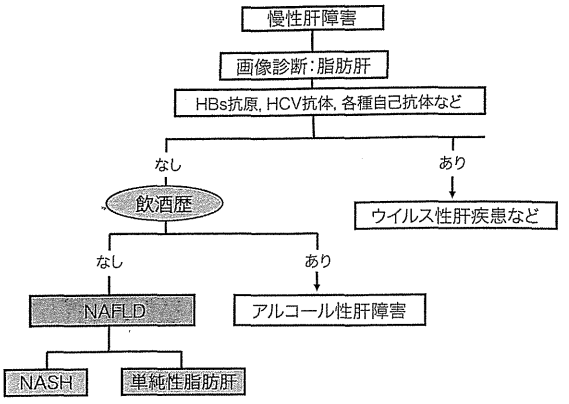

図2　NASHにおけるインスリン抵抗性と耐糖能の異常

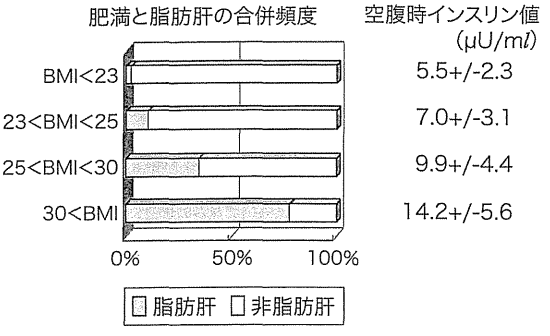

図3　NASHにおける血中レジスチン値と遺伝子多型

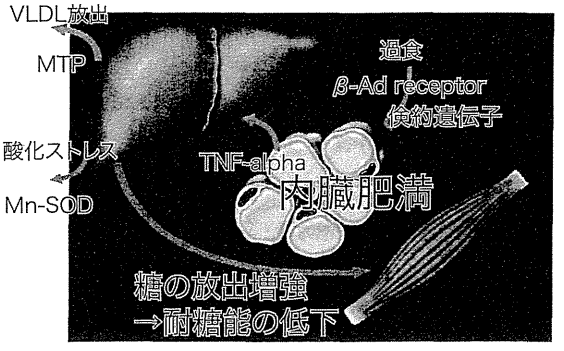

図4　機能性SNPの関与

テップを規定する因子が何であるのかという点を明らかにすることはできない．新たな観点からのアプローチが必要である．GWASはその有力な候補の一つであるが，NASHのように多様な遺伝的素因により感受性が規定される疾患における有用性は限定的である．

本稿ではNASHの病態を紹介した上で，GWASから得られた成績の持つ意味について考察したい．

2　節約遺伝子と肥満

欧米ではBMIが30を超えた場合に初めて肥満と呼ばれる．しかし，本邦では糖尿病の罹患率などを考慮し，BMIが25を超える症例を肥満と呼び，BMIが30を超えた症例は高度肥満と呼ぶ．高度肥満者ではNASH発症の相対危険率が非肥満者(BMI<25)の30～50倍と極めて高値である．上述のように日本人では倹約遺伝子として有名な変異型β3-adrenergic receptor（Trp64Arg)の保有率が約3割と，世界的にみても高頻度である[7]．

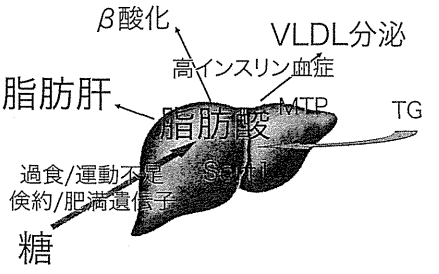

図5　肝臓における脂肪代謝

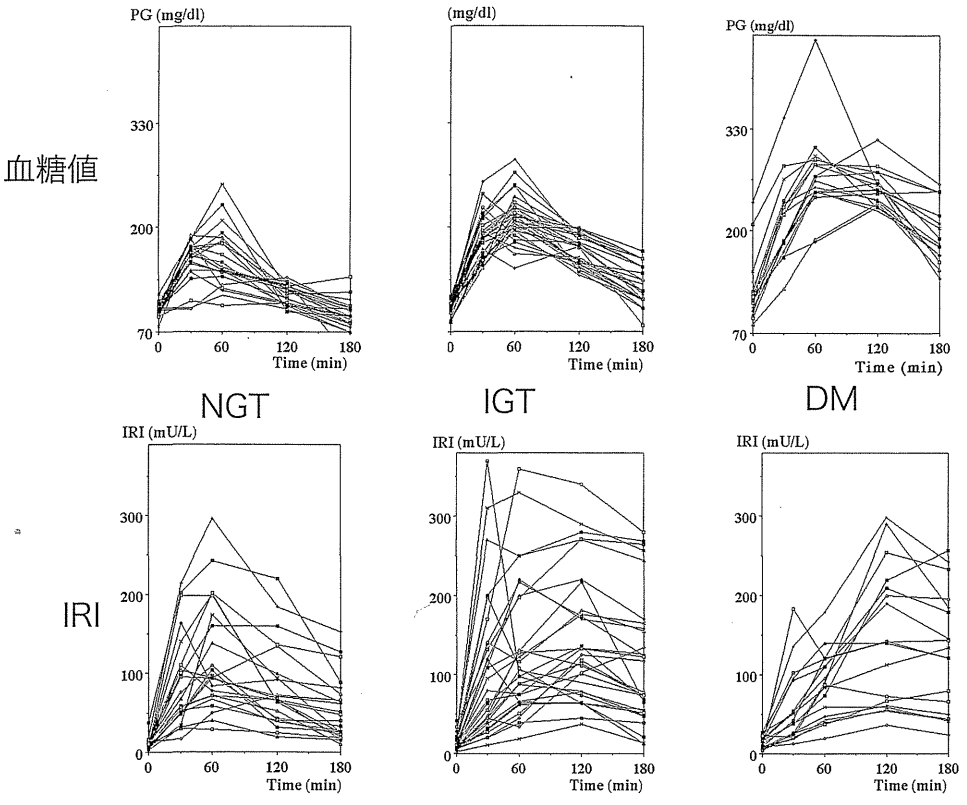

図6　75 g経口糖負荷試験

このTrp64Arg変異を持つと基礎代謝量が少なくて済むため，飢餓には抵抗性が高まる．しかし，基礎代謝量が少ないということは同時に，一旦肥満となると減量が困難となることをも意味する[8]．実際，本邦のNASH症例の5割はこの変異を有しており，このTrp64Arg変異を有するとNASHに2.4倍罹患しやすくなると推計される[6]．また，一旦Trp64Arg変異保有者がNASHを発症すると，「肥満の持続⇒NASHの遷延⇒肝線維化進展」という状況に陥りやすいことが予想され，変異非保有者のNASHに比してより強い治療抵

抗性を獲得する可能性がある．

3 高インスリン血症

高度肥満者はしばしば糖尿病を合併するため，NASHを経て，肝硬変に至る可能性が指摘されている[9,10]．このような症例の多くは高度のインスリン抵抗性を呈するので，肝臓からの糖の放出を抑えて空腹時血糖を低値に維持するためには高濃度のインスリンが必要となる．食後血糖の上昇を抑制するためには，より一層高濃度のインスリンが必要である[11]．

このような症例の肝臓における中性脂肪沈着機構を図5に示した．過食・肥満により肝臓における脂肪酸合成が亢進する一方で，高度のインスリン抵抗性により惹起された高インスリン血症のためにVLDLの分泌や脂肪酸のβ酸化が抑制され，肝細胞に中性脂肪が蓄積して脂肪肝が形成される．さらには，酸化ストレスを初めとする未知の因子の関与によりNASHを発症するに至り，線維化が伸展すると想定される．

日本人NASH症例における75 g経口ブドウ糖負荷試験の成績を図6に示した．健常日本人のインスリンのピーク値は50 mU/L程度であるが，ほとんどのNASH症例ではより高値が観察されている．すなわち，高インスリン分泌者でありながら，高度のインスリン抵抗性のために耐糖能異常を呈することが分かる．糖尿病の成因と関連して低インスリン分泌の機構については詳細な研究が行われているが，このような高インスリン分泌者の日本人における遺伝的背景については，十分な解明がなされていない．

他方，日本人の多くはインスリン分泌予備力が乏しいため，多量のインスリンを長期にわたって分泌することは困難である．肥満が進展する過程で日本人の多くはインスリン分泌不足に陥り，空腹時血糖を正常に維持することができなくなり，糖尿病を発症すると考えられる．しかし，そのような症例でもインスリン分泌促進剤であるスルフォニルウレア系薬剤の処方を受ければ，高インスリン分泌を維持することが可能となり，VLDLの分泌や脂肪酸のβ酸化の抑制が遷延し，高度の脂肪肝から医原性NASHの発症につながる可能性がある．

4 肝細胞における中性脂肪消費

肝細胞における中性脂肪量は，門脈からの中性脂肪の流入量に肝臓における脂肪酸の産生量を加えた供給量と，肝細胞における脂肪酸β酸化能にVLDL（very low density lipoprotein）として分泌される中性脂肪量を加えた消費量とのバランスで規定される．肝臓における脂肪酸β酸化能は内因性のPPAR-α ligandやadiponectinによって制御されると考えられる[12,13]が，その詳細はPPAR-γの遺伝子多型などが明らかにされたのみでNASH発症に結びつく遺伝的背景の大部分はいまだ明らかではない．

これに対して，アポリポ蛋白Bに中性脂肪を付加してVLDLを形成し分泌する過程の解明は進んでおり，microsomal triglyceride transfer protein（MTP）の果たす役割の重要性が認識されている[14,15]．MTPのpromoter領域には機能性遺伝子多型(G-493T)が存在[16]し，健常日本人の6割，NASH症例の8割が中性脂肪量の少ないVLDLを放出する低機能性ホモ接合体である．低機能性ホモ接合体の場合，NASHに3.0倍罹患しやすくなると推計される[6]．

MTPの発現はインスリンをはじめとする種々の因子により制御されるため，遺伝子多

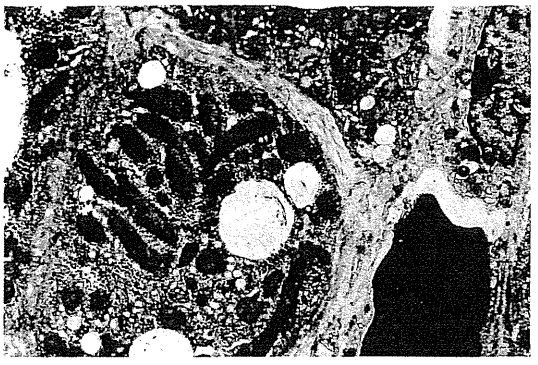

図7　NASH症例の肝組織像(電顕像)
肝細胞のミトコンドリアは腫大し，paracrystalline封入体を有する

型のみでMTP活性が決定されるわけではない．そこでNASH症例を対象にして，この遺伝子多型の関与する臨床的表現型が存在するか検討してみた．両遺伝子型間でBMIには差異を認めないのに，CT検査における肝/脾比が低機能性ホモ接合体ではヘテロ接合体より低値であった．これはMTPの発現が多因子により制御されるにもかかわらず，低機能性ホモ接合体では中性脂肪の分泌能が低値であり，肝細胞に中性脂肪がより貯留しやすいことを示す成績と考えられた．

5 肝細胞ミトコンドリアにおける酸化ストレス

NASH症例の肝細胞ミトコンドリアは強い酸化ストレスに曝されている．通常，このような酸化ストレスの大部分はミトコンドリアに局在するMn-superoxide dismutase(Mn-SOD)により消去される．しかし，適切に消去できない場合にはミトコンドリア膜に存在する不飽和脂肪酸が過酸化を受けて，4-hydroxy-2-noneal (4-HNE)の沈着を生じる．このような肝細胞のミトコンドリアはしばしば腫大し，paracrystalline封入体を有する(図7)．

Mn-SODにはT1183Cという機能性遺伝子多型が存在[5]し，健常日本人の7割，NASH症例の8割以上がMn-SOD量の少ない低機能性ホモ接合体である．このため，低機能性ホモ接合体の場合，NASHに2.4倍罹患しやすい．一旦NASHを発症すると，ヘテロ接合体に比し酸化ストレスの影響を強く受けやすく，ミトコンドリア傷害が高度になる可能性がある．

6 潜在的コリン欠乏症

PEMTはコリン合成に関与する酵素であり，Val175Met遺伝子多型が存在し，175Metは機能が低下する遺伝子型であることが知られている．コーカソイドではMet/Met型のホモ接合体が50％に到達する場合もあり，潜在的なコリン欠乏状態にある可能性が知られている[17]．このため，Met/Met型のホモ接合体はNASHの前駆病態であるNAFLD(nonalcoholic fatty liver disease，非アルコール性脂肪肝疾患)の危険因子であることがしられている．幸い日本人における175Metの頻度は2.3％程度であるため，Met/Metの頻度は0.05％程度と推定される．ところがNASHではMet/Metのホモ接合体が3％強存在することから補充が望ましい症例が存在することも明らかになった．

7 易炎症惹起性

NASHでは肝細胞の脂肪変性に加えて，肝小葉内への炎症細胞浸潤が組織学的特徴である．このような炎症細胞の浸潤には，上記のような肝細胞障害の亢進に加えて，腸管由来のエンドトキシンに対する感受性の亢進や高サイトカイン血症をきたしやすい素因が関与すると考えられる．

tumor necrosis factor-α（TNF-α）やinterleukin-1β（IL-1β）は代表的なproinflammatory cytokineであるが，これらにも脂肪肝発症に関連する機能性遺伝子多型が知られている．IL-1βのpromoter領域にも機能性遺伝子多型(T-511C)が存在し，健常日本人の3割，NASH症例の6割がIL-1β高産生性ホモ接合体である．このため，高産生性ホモ接合体はNASHに4.7倍罹患し易いと推計される[6]．

8 GWASを用いた遺伝的素因の解析

白人におけるGWASではNASH発症に関与する因子として，PNPLA3遺伝子多型の関与が示された[18]．脂肪細胞や肝細胞に脂肪の蓄積が生じると，この遺伝子の産物であるadiponutrinの発現が増強される．lipase活性を有するこの蛋白の遺伝子多型は人種を超えて広く分布し，日本人の2/3はこの遺伝子多型を有する．日本人におけるGWASによる検討では，この遺伝子多型が日本人におけるNASH発症の最大の危険因子であり，日本人のNASHでこの遺伝子多型が3/4の症例で検出されることが明らかとなった[19]．さらに，その後，肝臓の線維化進展にも寄与することも明らかとなった．この遺伝子多型はadiponutrinにアミノ酸変異を生じる遺伝子多型であるため，adiponutrinの機能喪失がNASHの危険因子であるかadiponutrin欠損マウスを用いた検討がなされた[20]．しかし，脂肪酸負荷や果糖負荷をかけても，野生型マウスと同等のインスリン抵抗性や脂肪肝が生じるのみで，どのような機序によりNASHの危険因子となるのか依然明らかではなく，蛋白—蛋白相互作用によるdominant negative作用に可能性が示唆されるに留まっている．GWASでは検定される遺伝子多型が機能性遺伝子多型であるか否かを考慮せず危険因子の検出に用いられるため，連鎖不平衡にある他の遺伝子多型，あるいは近傍にある別の遺伝子の遺伝子多型が真の危険因子であることも稀ではないと考えられる．この点については今後の検討課題である．

では，その他のGWASによる研究，特にapolipoproteinに関する研究の現状はどのようなものであろうか．すでに述べたように肝細胞における中性脂肪量は，門脈からの中性脂肪の流入量に肝臓における脂肪酸の産生量を加えた供給量と，肝細胞における脂肪酸β酸化能にVLDL（very low density lipoprotein)として分泌される中性脂肪量を加えた消費量とのバランスで規定される．肝臓から放出されたVLDLに含まれる中性脂肪は末梢血管内皮に分布するlipoprotein lipaseにより分解され，末梢組織に脂肪酸を供給する．lipoprotein lipase活性を抑制するapolipoprotein C3は血中のVLDL濃度を上昇させ，動脈硬化に促進的に作用することが知られている．apolipoprotein C3遺伝子ではpromotor部位に遺伝子の転写を高める遺伝子多型が存在し，血中の中性脂肪濃度を増加させる．インド人男性を対象としたGWASで，この遺伝子多型がNASH発症の危険因子と同定された[21]．この遺伝子多型は黒人に多く認められるが日本人には存在せず，NASHに対する感受性の人種差を説明する因子と考えられる．

9 おわりに

日本の歴史が始まって以来初めて，日本人は肥満による淘汰を受けようとしている．飢餓を乗り切るうえで有用であったために淘汰により頻度が増加した遺伝子多型は，

今や，生活習慣病の危険因子として再認識されようとしている．GWASにより明らかとなったNASHを誘発しやすい遺伝的素因adiponutrin variant carrierがどのような機序によりNASHを誘発するかを明らかにすれば，それはNASH治療にも有用である可能性が高い．今後はNASHの発症予防に取り組むことができるように，インフラの整備が強く望まれる所以である．

文　献

1) Kojima S, Watanabe N, Numata M et al : Increase in the prevalence of fatty liver in Japan over the past 12 years: analysis of clinical background. J Gastroenterol 38 : 954–961, 2003
2) Ludwig J, Viggiano TR, McGill DB et al : Nonalcoholic steatohepatitis: Mayo Clinic experiences with a hitherto unnamed disease. Mayo Clin Proc 55 : 434–438, 1980
3) 日本肝臓学会編，NASH・NAFLDの診療ガイド，東京，文光堂，2010
4) Neuschwander-Tetri BA, Caldwell SH : Nonalcoholic steatohepatitis: summary of an AASLD Single Topic Conference. Hepatology 37 : 1202–1219, 2003
5) Namikawa C, Shu-Ping Z, Vyselaar JR et al : Polymorphisms in microsomal triglyceride transfer protein gene and manganese superoxide dismutase gene in Japanese patients with nonalcoholic steatohepatitis. J Hepatol 40 : 781–786, 2004
6) Nozaki Y, Saibara T, Nemoto Y et al : Polymorphisms of interleukin-1 beta and beta 3-adrenergic receptor in Japanese patients with nonalcoholic steatohepatitis.Alcohol Clin Exp Res 28（8 Suppl Proceedings）: 106S–110S, 2004
7) Fujisawa T, Ikegami H, Yamato E et al : Association of Trp64Arg mutation of the beta 3-adrenergic-receptor with NIDDM and body weight gain. Diabetologia 39 : 349–353, 1996
8) Pietri-Rouxel F, Manning BSJ, Gros J et al : The biochemical effect of the naturally occurring Trp64→Arg mutation on human b3-adrenoreceptor activity. Eur J Biochem 247 : 1174–1179, 1997
9) Kalk H : Uber die Fettleber. Munch Med Wschr 107 : 1141–1147, 1965
10) Robbers H, Strohfeldt P, Krüger C : Differential diagnosis of diabetic and alcoholic fatty liver. Studies on 171 diabetics and 100 patients with alcohol abuse. Dtsch Med Wochenschr 93 : 112–113, 1968
11) Valenti L, Fracanzani AL, Dongiovanni P et al : Tumor necrosis factor alpha promoter polymorphisms and insulin resistance in nonalcoholic fatty liver disease. Gastroenterology 122 : 274–280, 2002
12) Xu A, Wang Y, Keshaw H et al : The fat-derived hormone adiponectin alleviates alcoholic and nonalcoholic fatty liver disease in mice. J Clin Invest 112 : 91–100, 2003
13) Nemoto Y, Toda K, Ono M et al : Altered constitutive expression of fatty acid-metabolizing enzymes in aromatase-deficient（ArKO）mice. J Clin Invest 105 : 1819–1825, 2000
14) Shelness GS, Ingram MF, Huang XF et al : Apolipoprotein B in the rough endoplasmic reticulum: translation, translocation and the initiation of lipoprotein assembly. J Nutr 129 : 456S–462S, 1999
15) Letteron P, Sutton A, Mansouri A et al : Inhibition of microsomal triglyceride transfer protein: Another mechanism for drug-induced steatosis in mice. Hepatology 38 : 133–140, 2003
16) Karpe F, Lundahl B, Ehrenborg E et al : A common functional polymorphism in the promoter region of the microsomal triglyceride transfer protein gene influences plasma LDL levels. Arterioscler Thromb Vasc Biol 18 : 756–761, 1998
17) Song J, Ann da Costa K, Fischer LM et al : Polymorphism of the PEMT gene and susceptibility to nonalcoholic fatty liver disease（NAFLD）. FASEB J 19 : 1266–1271, 2005
18) Romeo S, Kozlitina J, Xing C et al : Genetic variation in PNPLA3 confers susceptibility to nonalcoholic fatty liver disease. Nat Genet 40 : 1461–1465, 2008
19) Hotta K, Yoneda M, Hyogo H et al : Association of the rs738409 polymorphism in PNPLA3 with liver damage and the development of nonalcoholic fatty liver disease. BMC Med Genet 22 : 172, 2011
20) Chen W, Chang B, Li L et al : Patatin-like

phospholipase domain-containing 3/adiponutrin deficiency in mice is not associated with fatty liver disease. Hepatology 52 : 1134–1142, 2010
21) Petersen KF, Dufour S, Hariri A et al : Apolipoprotein C3 gene variants idn nonalcoholic fatty liver disease. N Engl J Med 362 : 1082–1089, 2010

*　　*　　*

KeyWords
◎NAFLD
◎NASH
◎疫学
◎肥満
◎心血管イベント

疾患概念の変遷と国内外の疫学

Author 小野正文*，宗景玄祐*，越智経浩*，西原利治*
*高知大学医学部消化器内科学

Headline

1. 非アルコール性脂肪性肝疾患（NAFLD），非アルコール性脂肪肝炎（NASH）は肥満人口の増加の推移に伴っており，肥満との関連が特に強い慢性肝疾患である．
2. NAFLD，NASHは肥満人口の増加に伴って全世界的に増加傾向にあり，今後は一番頻度の高い慢性肝疾患になってくることが予想される．
3. 成人のみならず，小児肥満，小児のメタボリックシンドロームに伴ったNAFLD，NASHの増加も懸念されている．
4. NAFLD，NASHの発症と心血管イベントとの関連は明らかにされていないが，NAFLD，NASH患者には肥満を中心としたメタボリックシンドロームが多いため，死因の1位は心血管イベントとなっており，合併に注意が必要である．

はじめに

今や“メタボリックシンドローム”という言葉を知らない日本人はいないと思われるくらい健康に対する社会的関心が高まっており，メタボリックシンドロームの肝臓での表現型である非アルコール性脂肪性肝疾患（nonalcoholic fatty liver disease；NAFLD）や非アルコール性脂肪肝炎（nonalcoholic steatohepatitis；NASH）に対する認識は以前に比べると少しは浸透してきたように思われる．ただ，一般消化器内科医や一般医家にはNASHの重要性が十分に浸透しているとは言いがたいのが実情である．このため，メタボリックシンドロームの基本病態である肥満とNAFLDおよびNASHの発症頻度がいかに増加しているのか，またNAFLDの概念がどのようにして確立されるようになったのかなどについても概説する．

NAFLD，NASHの疾患概念の変遷

1980年，Ludwigは脂肪肝を背景病変とし，飲酒歴に乏しいにもかかわらずアルコール性肝障害に類似した組織変化を呈する症例をNASHとし，新たな疾患概念として提唱した[1]．これはアルコール性肝障害の肝組織像がしばしばsteatohepatitisと表現されることに基づくものであり，それ以後，NASHもアルコール性肝障害と同様に進行性慢性肝疾患であることが徐々に明らかになってきた．その頃，わが国では欧米に比して非B型ウイルスによる慢性肝疾患が多かったことや，肥満人口も問題となるほど多くなかったこともあり，NASHには注目されなかった．しかし，1990年に入ってhepatitis C virus（HCV）抗体がスクリーニング検査できるようになるとともに，肥満や糖尿病などの生活習慣病の増加に伴い，飲酒歴に乏しい人にも脂肪肝やsteatohepatitisを認めるケースが多く認められるようになり，わが国でもNASHの疾患概念が

0370-999X/11/¥100/頁/JCOPY