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Title: Risk factors for histological progression of nonalcoholic steatohepatitis analyzed from repeated biopsy cases

Short title: Factors for therapeutic response in nonalcoholic steatohepatitis.

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Abbreviations:

NASH: nonalcoholic steatohepatitis

NAFLD: nonalcoholic fatty liver disease

NAFL: nonalcoholic fatty liver

LC: liver cirrhosis

HCC: hepatocellular carcinoma

GWAS: genomic-wide association studies

TNF: tumor necrosis factor

Abstract

Background and Aim

The most important prognostic factor for non-alcoholic steatohepatitis (NASH) is liver fibrosis. The aim of this study is to examine clinical parameters involved in pathological progression in NASH patients who underwent repeated liver biopsy and to analyze the response to treatment with respect to NASH-related single nucleotide polymorphisms (SNPs).

We performed longitudinal analysis of genetic and clinical factors associated with progression of NASH.

Methods

Eighty NASH patients who had undergone serial liver biopsies were enrolled in this retrospective cohort study. Histological exacerbation was determined based on NAFLD activity score (NAS) and liver fibrosis.

Results

22.5% had progression of fibrosis, 22.5% had improvement of fibrosis, and 55.0% had no change. NAS increased in 12.5%, decreased in 61.3%, and remained stable in the remaining 26.3%. We examined factors associated with histological progression versus non-progression. Poor response of ALT levels, increase in HbA1c levels, and presence of the TNF risk allele in the rs1799964 SNP were identified as independent risk factors contributing to histological progression in NASH patients. In addition, we found that the histological progression rate varies with ALT response, HbA1c levels, and rs1799964 genotype.

Conclusions

In this study, we clarified the serum ALT level and the clinical significance of HbA1c to evaluate the progression of fibrosis in Japanese NASH patients. Furthermore, the TNF SNP was more likely to be involved in the response than PNPLA3 SNP. By simultaneously evaluating three factors, it is possible to estimate the risk of histological progression more accurately.

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Keywords: non-alcoholic fatty liver disease (NAFLD), NASH(nonalcoholic steatohepatitis), repeated liver biopsy

Introduction

Nonalcoholic fatty liver disease (NAFLD) is one of the most commonly encountered chronic liver diseases in the world. According to Japanese annual health check reports, 9-30% of Japanese adults suffer from NAFLD [1 2 3] . Since it is now known that almost 10-20% of individuals with NAFLD have nonalcoholic steatohepatitis (NASH), the prevalence of NASH is estimated to be 1-3% of the adult Japanese population, similar to the prevalence reported in Western countries.

NAFLD includes a wide spectrum of liver diseases, ranging from nonalcoholic fatty liver (NAFL), a benign and non-progressive condition, to NASH, which can progress to liver cirrhosis (LC) and hepatocellular carcinoma (HCC), even in the absence of a history of significant alcohol consumption [4 5 6 7] .

Patients with NASH, particularly those with advanced fibrosis, are at high risk for HCC and death from liver-related causes. [8]

The most important contributing factor to the prognosis of NASH is liver fibrosis. [9] Liver fibrosis is strongly associated with abnormalities in glucose metabolism. [10]

Risk factors for the progression of NASH have been analyzed in many cross-sectional studies, but adequate longitudinal studies have not been performed.

Genome-wide association studies (GWAS) have identified single nucleotide polymorphisms (SNP) that are associated with increased hepatic fat or elevated liver enzymes, presumably reflecting NASH. [11]

Many studies have shown that polymorphisms within the tumor necrosis factor (TNF) locus are associated with pathogenesis of NASH. [12]

Recent genome-wide association studies have revealed that a SNP (rs738409; I148M) within the Patatin-like phospholipase domain-containing 3 (PNPLA3) locus influence NAFLD risk and plasma levels of liver enzymes. [13]

Although TNF and PNPLA3 SNP genotypes correlate strongly in all stages of NASH, including as inflammation, fibrosis, and carcinogenesis, it is unclear to what extent they are associated with response to treatment.

The aim of this study is to examine clinical parameters such as physical and biochemical findings involved in pathological progression in NASH patients who underwent repeated liver biopsies, as well as to analyze the response to treatment with respect to NASH-related SNPs.

Method

Patients

A total of 80 NASH patients who had undergone repeated biopsies between 2004 and 2018 at our hospital were enrolled in this study. Patients with other liver diseases, including viral hepatitis, autoimmune hepatitis, primary biliary cirrhosis, biliary obstruction, hemochromatosis, Wilson's disease and drug-induced liver injury were excluded. Patients consuming more than 20g/day alcohol and those with evidence of decompensated LC or HCC were also excluded.

All subjects gave written informed consent to participate in the study in accordance with the Declaration of Helsinki.

Study design

In all patients, a complete physical examination had been performed within 1 month prior to the initial liver biopsy. The body mass index (BMI) was calculated as the weight (kg) divided by the square of the patient's height (m). Obesity was defined as having a BMI greater than 25, according to the criteria of the Japan Society for the Study of Obesity. Computed tomography (CT) was used to determine the visceral fat area at the level of the umbilicus. [14]

Dyslipidemia was diagnosed based on serum cholesterol levels higher than 220 mg/dl, high density lipoprotein cholesterol levels lower than 40mg/dl, or triglyceride levels over 150 mg/dl. Hypertension was diagnosed if the patient was on antihypertensive medication and/or had a resting recumbent blood pressure of ≥ 130/85 mmHg on at least two occasions. Hyperuricemia was diagnosed based on serum uric acid levels higher than 7.0 mg/dl. Diabetes mellitus was diagnosed according to the 2006 World Health Organization (WHO) criteria.

Venous blood samples were taken in the morning following overnight fasting for 12 h. Laboratory evaluation in all patients included periodic measurement of serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), gamma glutamyl transpeptidase (GGT), total bilirubin, albumin, total cholesterol, triglycerides, high density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), fasting blood sugar (FBS), hemoglobin A1c (HbA1c), ferritin, and uric acid using standard clinical chemistry laboratory techniques.

Liver histology

All enrolled patients underwent a percutaneous liver biopsy under ultrasonic guidance after providing informed consent. Formalin-fixed, paraffin-embedded liver sections were stained routinely with hematoxylin-eosin, silver reticulin, and Masson trichrome. All specimens were examined by an experienced pathologist blinded to the clinical and biochemical data of the patients. Histological diagnosis of NASH was performed according to the methods of Matteoni et al [6]. Grading and staging were classified according to Brunt et al. and Kleiner et al., as previously reported [15] [16]. In brief, steatosis was graded as follows: grade 1 (5-33% of hepatocytes affected), grade 2 (34-66% of hepatocytes affected), or grade 3 (>66% of hepatocytes affected). Necroinflammation was graded from grade 0 (absent) to grade 3 (1:

octagonal ballooned hepatocytes and no or very mild inflammation; 2: ballooning of hepatocytes and mild-to-moderate portal inflammation; 3: intra-acinar inflammation and portal inflammation). Fibrosis was staged from grade 0 (absent) to grade 4 (1: perisinusoidal/pericellular fibrosis; 2: periportal fibrosis; 3: bridging fibrosis; 4; cirrhosis).

Histological progression was defined as change in fibrosis stage or exacerbation of NAS (NAFLD activity score).

We examined the association between various clinical parameters and cases of histological progression versus non-progression.

Responses

Response of biochemical markers, such as ALT, AST, and GGT, were defined as ≥30% decrease from baseline, in accordance with a proposal by the NASH Clinical Research Network (NASH CRN) [17]. HbA1c(NGSP) increase was defined as ≥0.2% increase from baseline or reaching 6.5% from ≤6.4% at baseline. This definition was found to have the highest area under the receiver-operator curve (AUROC) for predicting histological progression in NASH patients.

Single Nucleotide Polymorphisms

Genomic DNA was extracted from peripheral blood leukocytes collected from each subject. SNP genotyping was carried out by multiplex-PCR-based Invader assays (Third Wave Technologies, Madison, WI, USA) as described previously [18].

In this study, we selected and genotyped 6 SNPs that have been reported to be associated with NASH/NAFLD. [19] [20]

Measurement of serum cytokines

Serum sample were stored at -80°C. IL-1β, IL-6, TNF-α, and TNF-β/Lympotoxin-α were measured by the multiplex MAP Human Cytokine/Chemokine Panel (EMD Millipore Corp.) and Bio-Plex 200 System using the Bio-Plex Manager Software program (Bio-Rad Laboratories Inc.)

Statistical analysis

The data were analyzed using R software, version 3.0.0. Continuous variables are reported as mean ± standard deviation (SD) or median (range), and categorical data are expressed as counts with percentages shown in parentheses. Statistically significant differences in quantitative data were determined using the t-test, Mann-Whitney U-test or Jonckheere-Terpstra test, as appropriate. Multivariate analysis was carried out using logistic regression. Differences were considered to be statistically significant at P values of less than 0.05.

Result

Patient characteristics

Table 1 summarizes the baseline clinical, laboratory, and histological data of study patients before they began treatment. The treatment was comprehensive, including diet and exercise therapy, treatment of complications, vitamin E supplementation, etc.

Out of 80 patients, 45 patients were men and 35 were women. The median age was 61 years. The average observation period (i.e., the mean time interval between first and last biopsy) was 931 days. 54 patients had mild fibrosis (stage 1-2), and 26 patients had advanced fibrosis (stage 3). 27 patients had impaired glucose tolerance, and 39 patients had diabetes mellitus. 42 patients were diagnosed with hypertension, 71 with dyslipidemia, and 23 with

hyperuricemia.

Histological changes

After the observation period, 18 patients (22.5%) showed progression of fibrosis, 18 patients (22.5%) had improvement of fibrosis, and 44 patients (55.0%) showed no change. NAS was increased in 10 patients (12.5%), decreased in 49 patients (61.3%), and unchanged in 21 patients (26.3%) (Figure 1).

There were many cases in which fibrosis and NAS improved or remained unchanged (Supplement table 1)

Comparison of histological progression cases and non-progression cases

Patients were divided into two groups based on changes in liver fibrosis score and NAS score: histological progression and non-progression (see Methods and Table 2a). BMI, VAT (visceral adipose tissue), and L/S ratio (liver/spleen ratio) showed no significant differences between groups. SAT (subcutaneous adipose tissue) decreased in the non-progression group but increased in the progression group, but the difference was not significant (Figure 2a).

Furthermore, we compared both groups with respect to biochemical data (Figure 2b). AST and ALT decreased in both groups, but the percent improvement was poor in histologic progression cases. HbA1c and FBS levels increased with histological progression, suggesting that exacerbation of glucose metabolism might conspicuously influence histological progression.

Factors associated with histological progression were examined (Table 2b). We divided the data into before and after the treatment as well as the response to treatment, and analyzed biochemical parameters associated with histological progression. Multiple logistic

regression analysis identified ALT non-responder and HbA1c increase as risk factors for histological progression.

SNPs and serum cytokines involved in histological progression.

Supplement table2 shows results of analysis of SNP genotypes with respect to treatment response.

Although genome-wide association studies have reported various candidate genes associated with NAFLD / NASH, in this study we examined SNPs backed by multiple reports [21] [22] [23] [24] [25] [26]. There was no significant association between the risk allele of each SNP and these histological findings (fibrosis, inflammation, steatosis, and ballooning). We did not observe significant associations between histological progression in NASH and SNPs in the PNPLA3 or TN6SF2 loci. Only the TNFα-related SNP, rs1799964 was significantly associated with histological progression.

Then, we analyzed the relationship between histopathology and rs1799964 genotype (Supplement figure1). The percentage of histological progression increased in order of TT, CT, CC, from which we determined that C is the risk allele for NASH progression after treatment intervention. CT and CC genotypes occurred more frequently in the progression group, while more than 80% of patients in the non-progression group had genotype TT (TT vs. non-TT: $p = 0.002$; OR = 5.3).

However, rs1799964 genotype was not associated with serum TNFα level, although IL-1β, IL-6, TNF-α, and TNF-β/Lympotoxin-α levels were associated with histologic exacerbation of the liver.

Multi-variable analysis

We performed a multiple logistic regression analysis to identify factors associated with histologic progression (Table 3). Having the rs1799964 risk allele, poor ALT response, and HbA1c increase were identified as independent risk factors contributing to histological progression in NASH patients.

Figure 3 shows the relationship between these three factors and the rate of histological progression. The histologic progression rate varies with respect to ALT response, HbA1c increase, and SNP genotype. The tissue deterioration rate increased as the number of risk factors increased.

Receiver Operating Characteristic Curve in the study

We examined the ability to predict tissue progression in this study (Figure 4) and achieved relatively good results, with an AUROC of 0.884. We attempted to verify these results using cross validation, and obtained a relatively high of AUROC 0.827, although the accuracy was lower.

Discussion

In this study, we clarified the clinical significance of serum ALT level and HbA1c to estimate histological progression in Japanese NASH patients. We also found that a SNP in the TNF locus (rs1799964) but not the PNPLA3 locus (rs738409) was most strongly associated with therapeutic response.

The rs738409 SNP in the PNPLA3 gene and the rs58542926 SNP in the transmembrane 6 superfamily member 2 (TM6SF2) gene have been reported to be associated with NASH progression, but promising results have been obtained with many other SNPs. [27]

However, we should rely on longitudinal rather than cross-sectional studies and focus on liver

pathological findings, which is more directly relevant to prognosis. Furthermore, we should jointly examine genetic and clinical data.

Although NAS is important for evaluating treatment responsiveness, the most important prognostic factor associated with NASH is fibrosis. Liver fibrosis is associated with increased overall and liver-related mortality and increased likelihood of developing liver-related complications. Furthermore, liver fibrosis increases the risk of cardiovascular diseases and cancers other than that of the liver [9]. Therefore, it is important to identify and address risk factors involved in hepatic fibrosis in patients with NASH / NAFLD.

ALT levels appear to be correlated with visceral fat, steatosis, inflammation, and fibrosis in NASH/NAFLD patients [28] [29]. However, we previously demonstrated that advanced fibrosis (stage 3-4) was observed in 16.1% of Japanese NASH patients who had normal ALT levels [30]. The result of these cross-sectional studies suggests that it is difficult to predict liver fibrosis with only serum ALT levels in NASH patients.

In vitro, hyperglycemia and hyperinsulinemia, which are frequently observed in NASH patients, stimulate expression of connective tissue growth factors, which is known as one of the most important mechanisms involved in the progression of hepatic fibrosis. Furthermore, cirrhosis is suspected to facilitate the development of hyperinsulinemia and hyperglycemia via deteriorated liver function [10].

While abnormal glucose metabolism is an important feature of liver fibrosis, it is both a cause and a consequence. It was confirmed in a longitudinal study that exacerbation of glucose tolerance is an independent risk factor of liver fibrosis.

This study shows that the response of ALT and HbA1c are risk factors predicting histological progression, independently of other metabolic factors, including BMI. The histological changes and predictive factors of disease progression in patients with NASH have been widely investigated in repeated biopsy studies [31] [32] [33]. Obesity [34] [35], age [36], insulin resistance [35],

type 2 DM [33], ALT [8 17], steatosis, inflammation and fibrosis at initial biopsy [31 32 36] were reported to be risk factors for disease progression.

Potential explanations for the differences between the present results and those of other studies may be the use of different inclusion criteria (NASH or NAFLD), medications (content of treatment), and/or intervals between biopsies (short or long term).

Furthermore, we investigated the relationship between histological progression and NASH-related SNPs. As a result, a TNFα-related SNP (rs1799964) was recognized as having a significant association with histological progression as an independent factor. TNFα has been noted as an especially important cytokine in the development of many forms of liver injury and is known to promote genetic predisposition to the progression of inflammation and fibrosis in NASH [33, 34 35]. The TNF polymorphism was reported to serve as a surrogate marker for distinguishing NASH from NAFL. [12]

In this study, there was no association between PNPLA3 gene polymorphism and progress of hepatic fibrosis and therapeutic response. A recent genome-wide association study revealed that two SNPs in the PNPLA3 gene, rs738409 (I148M) and rs6006460 (S453I), were associated with NAFLD [37]. Similarly, a GWAS conducted in Japan confirmed a significant association with rs738409 [38]. It seems likely that PNPLA3 polymorphisms are deeply involved in the onset and progression of NASH/NAFLD.

Wang JZ et al. reported that NAFLD patients with the PNPLA3 G allele may benefit more from lifestyle modifications, DPP-4 inhibitors, and bariatric surgery, which are characterized by weight loss and improved insulin resistance [39].

Response to treatment may also differ according to the presence of the PNPLA3 G allele. Previously, we demonstrated that response to treatment for DM is more favorable in patients with the PNPLA3 G allele [40]. Therefore, it is possible that the presence or absence of the G allele does not affect the histological progress in NASH cases treated appropriately.

This study has several limitations. A small number of patients were examined, all of whom were Japanese. This is a retrospective cohort study based on a single hospital, which carries a risk of selection bias. Furthermore, no specific protocol, such as performing a second biopsy at a fixed time interval, was followed. Their treatment was carried out in combination nutritional exercise therapy, and the drug treatment for such as hypertension, diabetes and dyslipidemia. Although we performed appropriate treatment for each case, the content of treatment intervention is not uniform.

For that reason, this result should be confirmed prospectively in a larger number of patients.

In addition, no significant correlation between the TNFα-related SNP risk allele and blood TNFα concentration was observed and will require further study.

In conclusion, this study suggests that serum ALT and HbA1c should be monitored in NASH patients to prevent histological progression. Furthermore, rs1799964 genotype may serve as a potential biomarker in developing personalized treatment for NASH.

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Figure 1. Histological changes (a) Fibrosis (b) NAS

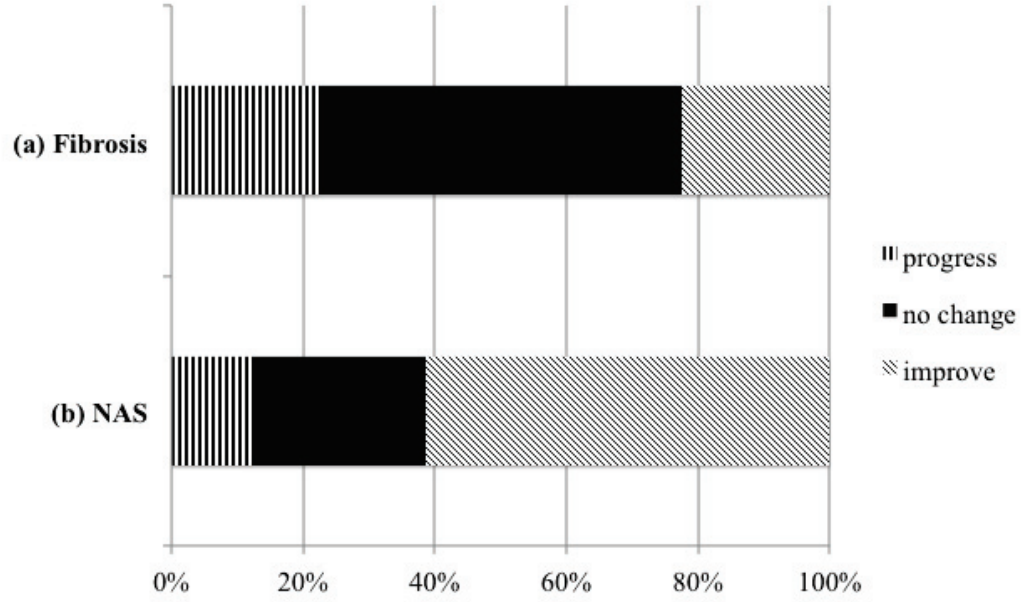


Figure 1. Histological changes between the first and second biopsies. The horizontal axis shows the percentage of progress, improve or no change. The striped bars denote progression cases, the filled bars denote no change, and the diagonal bars denote improvement. (a) Fibrosis, (b) NAFLD activity score

Figure 2. Comparison of histological progression and non-progression cases.

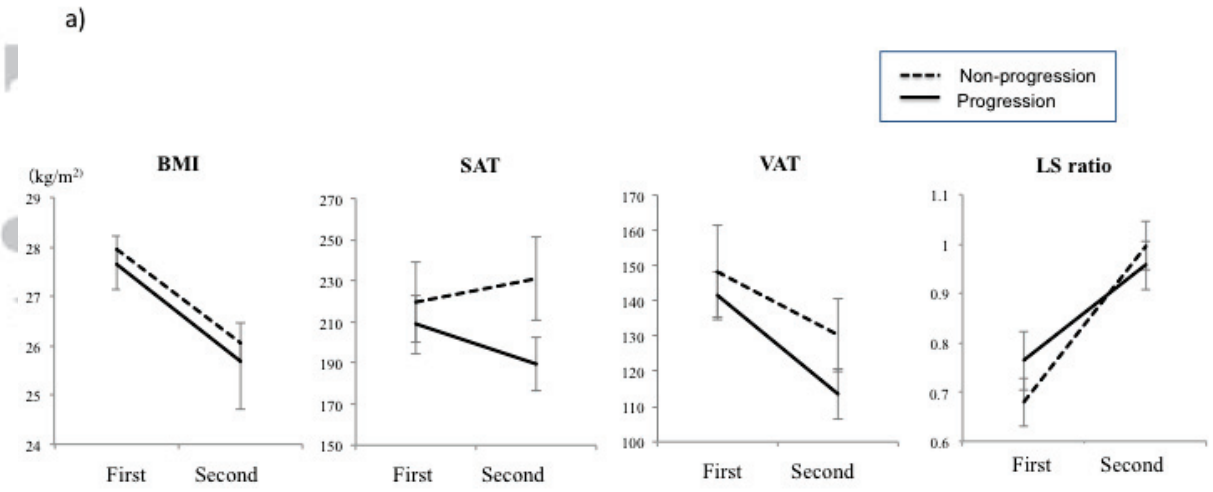


Figure 2 Comparison of histological progression and non-progression cases (a) Body mass index (BMI), subcutaneous adipose tissue (SAT), visceral adipose tissue (VAT), and liver/spleen ratio (LS ratio) in computed tomography.

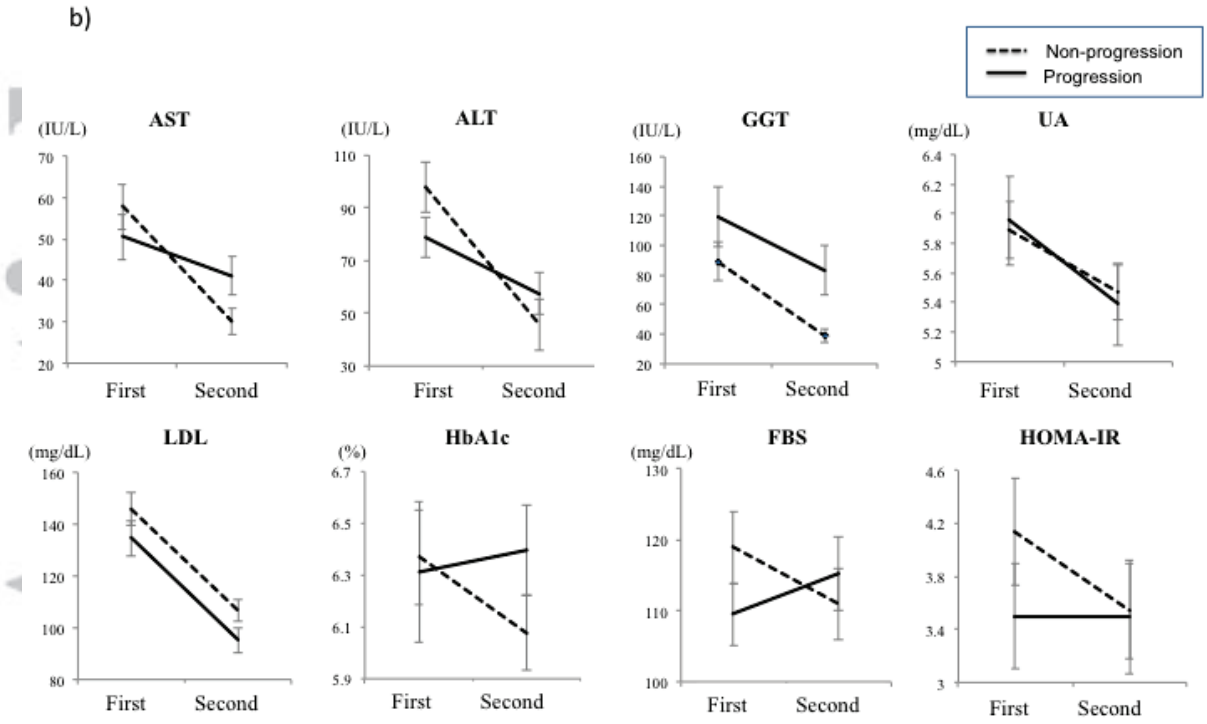


Figure 2 Comparison of histological progression and non-progression cases (b) Aspartate aminotransferase (AST), alanine aminotransferase (ALT), γ-glutamyltranspeptidase (GGT), uric acid (UA), LDL cholesterol, HbA1c, fasting blood sugar (FBS), and homeostasis model assessment ratio (HOMA-IR). In each figure, the horizontal axis shows the first and second biopsies and the longitudinal axis shows the means. The solid lines show progression cases and the dotted line shows non-progression cases.

Figure 3. Relationship between histological exacerbation and rs1799964 allele, ALT response, and HbA1c elevation.

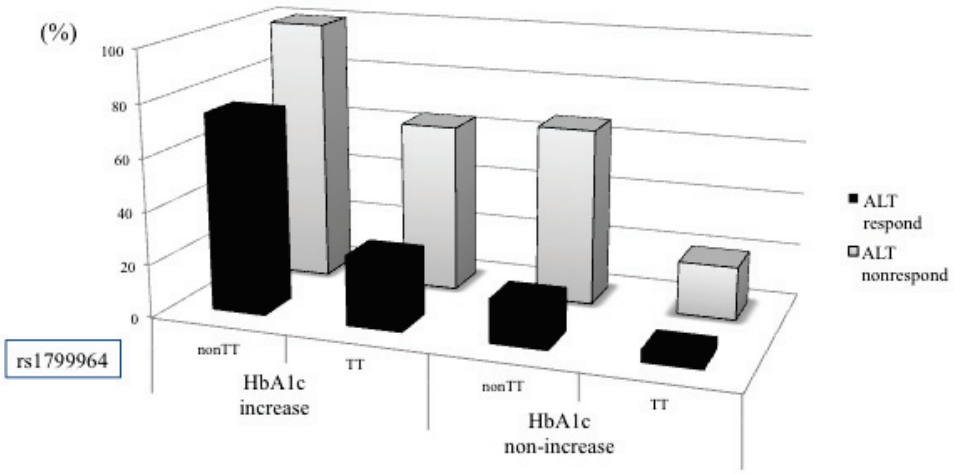
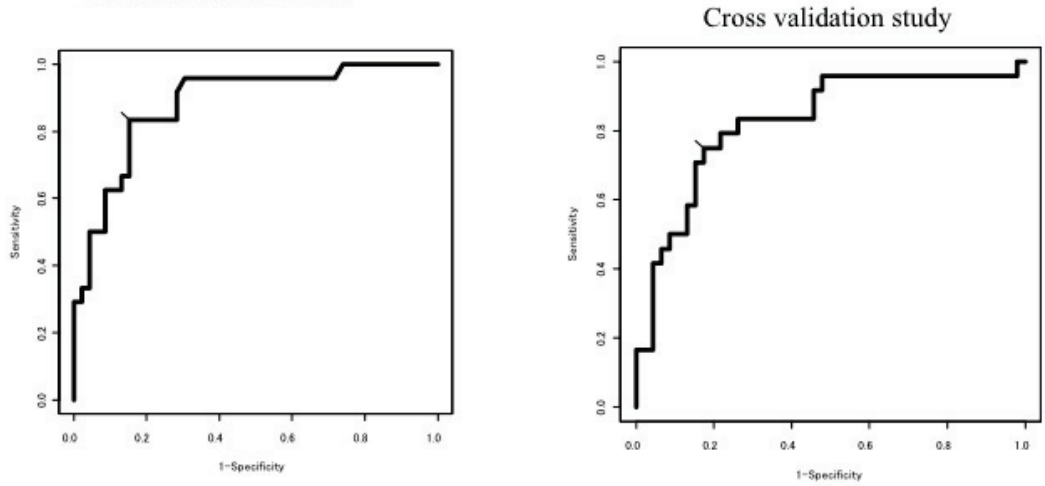


Figure 3. Relationship between histological exacerbation and rs1799964 allele, ALT response, and HbA1c elevation.

The horizontal axis shows HbA1c increase or non-increase and non-TT or TT in rs1799964. The dark bars show ALT responders and the light bars show ALT non-responders. The longitudinal axis shows percentage of patients who experienced histological exacerbation.

Figure 4. Risk estimation of NAFLD using genetic variation and biochemical markers.



	Study	Cross validation
AUROC	0.884	0.827
PPV (%)	74.1	69.2
NPV (%)	90.7	86.4
Correct diagnosis rate (%)	90.0	78.6

Figure 4. Risk estimation of NAFLD using genetic variation and biochemical markers. The receiver Operating Characteristic (ROC) curves shows the potential to predict histological exacerbation based on ALT response, HbA1c levels, and allele type of rs1799964. The probability for correct diagnosis rate is 90.0% with AUC=0.884. Using leave one out cross-validation, we obtained a probability for correct diagnosis rate of 78.6% with AUC=0.827.

Table 1 Patient background

	median	min	max
Age, years	61	(32 -	90)
Sex, (Male/Female)	(45/35)		
observation period, days	931	(328 -	2640)
NAS (first, 2/3/4/5/6/7/8)	(2/29/21/9/7/10/2)		
Fibrosis stage(first, 1/2/3)	(17/37/26)		
BMI, kg/m^2	27.4	(19.6 -	38.3)
Platelet count, x10^4	21.9	(9.2 -	58.9)
AST, IU/L	45.5	(17 -	163)
ALT, IU/L	79	(23 -	324)
γ-GTP, IU/L	61	(18 -	481)
Total cholesterol, mg/dL	222	(139 -	372)
Triglyceride, mg/dL	142.5	(65 -	634)
HDL cholesterol, mg/dL	49.5	(29 -	100)
LDL cholesterol, mg/dL	141.5	(53 -	294)
Fasting blood sugar, mg/dL	108	(71 -	239)
HbA1c, %	5.9	(3.8 -	11.7)
UA, mg/dL	5.8	(2.9 -	8.9)
Ferritin, ng/mL	179.4	(8.2 -	1598.9)
SAT	199.6	(68.57 -	420.8)
VAT	136.5	(58.2 -	340.6)
waist	96.59	(79.27 -	124.20)
CTLSratio	0.70	(0.05 -	1.40)
HOMA-IR	3.158	(0.933 -	12.039)
NGT/IGT/DM	14/27/39		
Hyper tension	42/80		
Dyslipidemia	71/80		
Hyper uremia	23/80		

Values are median(range)

Table 2 (a) Comparison of histological progression cases and non-progression cases.

	Progression		Non-progression		
	mean	sd	mean	sd	*P*
age	63.38	(14.9)	60.06	(11.8)	0.329
sex (M/F)	13/14		34/21		0.212
BMI	27.95	(4.2)	27.64	(3.8)	0.890
BMI (second)	26.05	(6.8)	25.66	(5.2)	0.339
SAT	219.58	(98.9)	208.65	(95.4)	0.662
SAT (second)	231.16	(103.7)	189.15	(86.5)	0.113
VAT	148.03	(68.1)	141.63	(42.1)	0.924
VAT (second)	130.35	(53.1)	113.58	(48.3)	0.193
LSratio	0.76	(0.3)	0.68	(0.3)	0.304
LSratio_ (second)	0.96	(0.2)	1.00	(0.3)	0.231

date are expressed as mean values and standard deviation.

p-values associated with continuous variables via a Mann-whitney U-test.

p-values associated with categorical values were caculated with t-test.

Table 2 (b) Clinical parameters contribute to the histological progression.

	Progression		Non-progression			Multivariate analysis			
	mean	sd	mean	sd	p	OR	95%CI lo	95%CI hi	P
AST	50.4	(28.0)	57.7	(35.9)	0.538				
AST (second)	41.0	(23.8)	30.1	(21.5)	0.031	□			
AST_nonresponder	10/26		15/54		0.593				
ALT	78.8	(39.1)	97.8	(63.2)	0.361				
ALT_ (second)	57.3	(40.9)	45.6	(63.7)	0.041	□			
ALT_nonresponder	13/26		12/54		3.3.E-04	0.230	0.071	0.748	0.015
rGTP	119.3	(102.6)	89.0	(85.5)	0.070	□			
rGTP_ (second)	83.1	(84.1)	38.7	(29.8)	0.002	□			
rGTP nonresponder	8/26		10/54		0.010	1.014	1.000	1.028	0.053
LDL	134.5	(34.9)	146.0	(42.7)	0.423				
LDL (second)	95.3	(25.3)	106.7	(28.5)	0.083	□			
LDL increase	4/26		10/54		0.975				
FBS	109.5	(22.4)	118.9	(33.6)	0.314				
FBS_ (second)	115.1	(26.4)	110.9	(33.0)	0.700				
HbA1c	6.31	(1.4)	6.37	(1.2)	0.382				
HbA1c (second)	6.40	(0.9)	6.08	(0.9)	0.113				
HbA1c increase	18/26		18/54		0.005	4.651	1.453	14.883	0.010
UA	5.95	(1.5)	5.89	(1.3)	0.933				
UA_ (second)	5.39	(1.4)	5.47	(1.2)	0.890				
UA increase	10 / 26		19 /54		0.970				
HOMA_IR	3.50	(2.0)	4.14	(2.7)	0.420				
HOMA_IR_(second)	3.50	(2.2)	3.54	(2.4)	0.861				

responder ; decrease of more than 30% of the value, or decrease to the normal range

Table 3　Risk factors contributing to the histological progression.

	Univariate analysis	Multivariate analysis					
	P	*P*	OR		95%CI lo		95%CI hi
ALT_nonresponder	3.3.E-04	0.035	4.073	(	1.100	-	15.078)
γ – GTP_nonresponder	0.010	-					
HbA1c increase	0.005	0.003	11.902	(	2.264	-	62.555)
rs1799964(CC vs nonCC)	0.006	0.009	9.558	(	1.755	-	52.045)