6drinks（週約420g）以上の飲酒者で高血圧の
発症率が高いことを示した[4]。米国Nurse
Health Studyにおける4年間の追跡では、非飲
酒者に比べ1日20〜34gアルコール摂取で1.4倍、
1日35g以上で1.9倍の高血圧発症リスクが上昇し
ていた[7]。田主丸研究での12年の追跡では1日
46g以上の多量飲酒者では非飲酒者に比べて高
血圧発症リスクが2.1倍であった[8]。

著者らは企業従業員男性の7年間の追跡にお
いて、多変量解析にて加齢や体重増加などの
影響を調整後の収縮期血圧上昇度が、週300g
以上のアルコール摂取者では非飲酒者に比べて
1年当たり0.33mmHg高かったことを明らかにし
た[4]。多量飲酒者は非飲酒者に比べて10年で
3.3mmHg余計に血圧上昇する計算である。

Ⅳ. 飲酒量減少による
血圧低下のエビデンス

では、習慣的な飲酒者が飲酒量を減量したり
飲酒を中止したりすると血圧が低下するのだろ
うか。これを確認するための無作為化比較試験
も国内外において行われており、わが国では上
島らの研究が有名である（図4）[9]。この研究で
は日本酒にして1日2合程度飲酒していた30〜59
歳の軽症高血圧男性54名を対象として、1日1合
程度になるように節酒してもらった。その結果、
節酒群は通常飲酒のコントロール群に比し、2週
間前後で収縮期血圧が約5mmHg大きく低下す
ることが確認された。

Xinらは2001年にこれまでの質の高い15の無
作為化比較試験をメタアナリシスした結果を報

図4　節酒による血圧低下確認のための無作為化比較試験（軽症高血圧の30〜59歳男性54名）
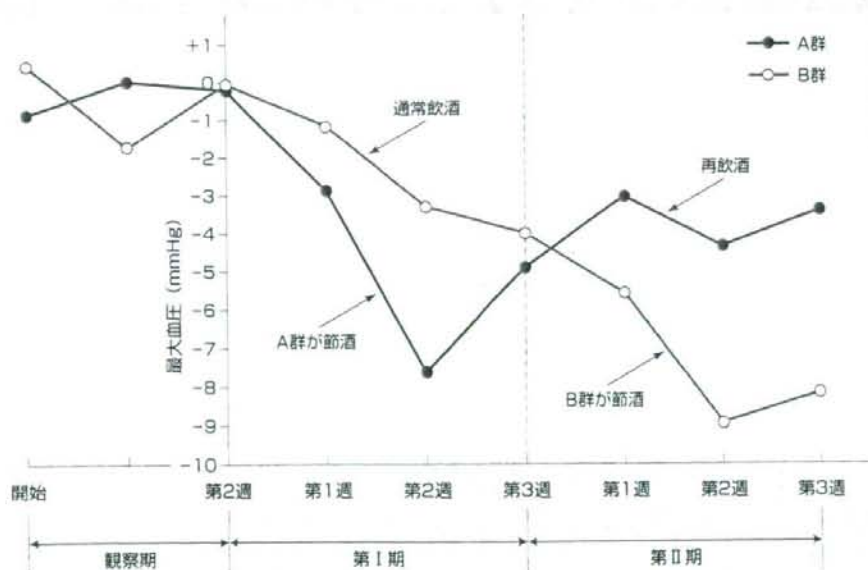
告している[10]。飲酒量の減量割合は中央値76%、介入期間の中央値は8週間で、全体として収縮期血圧が3.3mmHg、拡張期血圧が2.0mmHg低下していた。**表1**のサブグループ分析で示されるとおり、高血圧者でも非高血圧者でも一定の血圧低下が得られている。血圧低下の幅はアルコール減量の大きさと有意に関連し、アルコール減量10%あたり収縮期血圧1.0mmHg、拡張期血圧0.8mmHg低下した。アルコール減量が50%なら収縮期血圧5mmHg、100%なら10mmHgの収縮期血圧低下が期待できることになる。また、介入前の血圧値が高いほど、節酒による血圧低下も大きい。

無作為化比較試験での節酒による血圧低下効果は、飲酒と血圧上昇との間の因果関係の最も強い証拠となる。しかし、習慣的飲酒による血圧上昇のメカニズムについては、血管の収縮反応の亢進、交感神経の作用、腎臓からのカルシウムやマグネシウムの排泄が高まることなどが指摘されているが、現時点では十分には解明されていない。

Ⅴ. 高血圧の予防と治療のための飲酒に関するガイドラインの現状

これまでの疫学調査で示されている飲酒量と血圧との関係はほぼ直線的、段階的であり、非飲酒者の血圧が最も低く、飲酒量が増加するとともに血圧は上昇し、高血圧リスクは高まる。したがって高血圧予防のためにはできるだけ飲酒をしないのが理想である。しかし、飲酒量と心臓病発症との関連については、非飲酒者よりも1日1合程度の飲酒者で最もリスクが低くなる報告が多い。これは適度の飲酒によるHDLコレステロール上昇などの影響と考えられている。そこで各国の高血圧ガイドラインでは高血圧者における飲酒量は「適量」に抑えるように勧告されており、禁酒の勧告は行っていない。例えば、米国高血圧ガイドライン（JNC 7）では高

表1　アルコール減量による血圧低下の無作為化比較試験のメタアナリシス
　　　（サブグループ分析）

	平均の収縮期血圧低下 （mmHg）	平均の拡張期血圧低下 （mmHg）
介入期間		
4週間以下	−2.4	−1.7
6〜8週間	−3.9	−2.1
12週間以上	−3.2	−2.2
介入方法		
代替物使用	−3.9	−2.1
節酒カウンセリング	−2.5	−1.9
高血圧		
あり	−3.9	−2.4
なし	−3.6	−1.8
降圧剤使用		
あり	−4.0	−2.6
なし	−3.1	−1.8

（文献10より引用）

血圧管理のための生活習慣修正に関し1日当たりアルコール換算で男性30mL以下、女性15mL以下[11]、欧州高血圧学会と欧州心臓病学会による高血圧ガイドライン（2007ESH-ESC）では1日当たりアルコール換算で男性20～30g（週140～210g）未満、女性10～20g（週70～140g）未満にアルコール摂取量を制限するように指導すべきであるとしている[12]。日本高血圧学会ガイドラインでもアルコール換算で男性は20～30mL/日以下、女性は10～20mL/日以下にすべきとしており[13]、これらは日本酒換算で男性ではほぼ1日1合以下、女性では1日半合以下に該当する。高血圧の予防と治療のための適切な飲酒量は男性では日本酒換算で週7合以内（アルコール換算週160g以下）、女性では週3.5合以内（週80g以下）が妥当なところであろう。

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Original Article

Association between changes in body composition and the increasing prevalence of fatty liver in Japanese men

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Aim: Prevalence of fatty liver is increasing. In this study, to elucidate the factor that contributes most to recent increases in prevalence of fatty liver, we determined the independent predictors for the onset of fatty liver and compared these predictors between 2000 and 2005.

Methods: Japanese persons, aged 30–74 years, who participated in regular health checks at Kagoshima Kouseiren Medical Health Care Center (10 336 persons in 2000 and 11 011 persons in 2005) were enrolled in the study. Diagnosis of fatty liver was performed by ultrasonography. Body fat percentage (BFP) was determined using a bipedal bioimpedance instrument.

Results: The prevalence of fatty liver has increased between 2000 and 2005 in men (33.3 vs 38.5% in 2000 vs 2005, respectively, $P < 0.0001$), but not in women (21.3 vs 21.0%, $P = 0.8101$). Logistic regression analysis revealed that both

body mass index (BMI) and BFP are independent predictors of fatty liver in both men and women. BMI did not change in either men (23.4 ± 2.9 vs 23.8 ± 3.0 kg/m², $P = 0.0528$) or women (22.8 ± 3.1 vs 22.8 ± 3.3 kg/m², $P = 0.9862$) during the survey period. In contrast, BFP increased in men (20.6 ± 4.7 vs 22.3 ± 5.0 kg/m², $P = 0.0003$), but not in women (27.4 ± 5.5 vs 28.4 ± 5.9 kg/m², $P = 0.3993$). There was no significant change in triglycerides and glucose levels.

Conclusion: These results suggest that altered body composition, particularly increased BFP without an increase in BMI, has developed in men and is strongly associated with the increasing prevalence of fatty live amongst Japanese men.

Key words: fatty liver, body fat percentage, body mass index, body composition, life-style, metabolic syndrome

1. INTRODUCTION

FATTY LIVER HAS become a significant problem on a worldwide scale, including in Japan, because the prevalence of fatty liver is increasing.[1-3] Although body mass index (BMI) is considered to be a major risk factor for fatty liver, BMI is only slightly increased amongst Japanese men, and is slightly decreased in women according to national surveys.[4] Although the increase in the prevalence of fatty liver cannot be explained simply

by the increase in the prevalence of obesity, the underlying factors have yet to be fully clarified.

In this study, to elucidate the factors that contribute to the recent increase in the prevalence of fatty liver, we determined the predictors of fatty liver in participants who underwent health checks and compared these predictors between the participants in 2000 and 2005.

2. METHODS AND MATERIALS

THE SUBJECTS IN this study were Japanese persons aged 30–74 years, who participated in regular health checks at Kagoshima Kouseiren Medical Health Care Center: 10336 persons (6484 men, 3852 women) from April 2000 to March 2001 (2000 group) and 11011

Table 1 Comparison of variables between participants with and without fatty liver

Fatty liver	Men			Women		
	With	Without	P value	With	Without	P value
Age (years)	51 ± 9	54 ± 10	<0.0001	56 ± 9	54 ± 10	0.0015
BMI (kg/m²)	25.6 ± 2.8	22.6 ± 2.5	<0.0001*	25.9 ± 3.4	21.9 ± 2.7	<0.0001*
BFP (%)	25.4 ± 4.4	20.3 ± 4.3	<0.0001*	33.9 ± 5.5	26.9 ± 5.0	<0.0001*
ALT (IU/L)	38 ± 24	24 ± 29	<0.0001*	28 ± 19	18 ± 8	<0.0001*
γ-GTP (IU/L)	58 ± 60	40 ± 49	0.0012*	24 ± 21	16 ± 14	<0.0001*
TC (mg/dL)	218 ± 36	206 ± 33	<0.0001*	224 ± 35	215 ± 33	<0.0001*
TG (mg/dL)	179 ± 148	118 ± 106	<0.0001*	221 ± 62	81 ± 41	<0.0001*
HDL-C (mg/dL)	51 ± 12	59 ± 15	<0.0001*	57 ± 12	67 ± 14	<0.0001*
BG (mg/dL)	113 ± 26	105 ± 19	0.0002*	108 ± 27	97 ± 11	0.0004*
S-BP (mmHg)	125 ± 17	121 ± 17	0.0003*	122 ± 17	114 ± 18	<0.0001*
D-BP (mmHg)	82 ± 11	77 ± 11	<0.0001*	77 ± 10	72 ± 10	<0.0001*

Data are expressed as mean ± SD. ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; S-BP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; γ-GTP, γ-glutamyl transpeptidase. *P value after adjusting for age (ANCOVA).

persons (6829 men and 4182 women) from April 2005 to March 2006 (2005 group).

Diagnosis of fatty liver was carried out using ultrasonography (SSA-250A and SSA-750A, Toshiba, Japan; Logic 400, GE Yokogawa, Japan), which was based on the findings of bright liver (increased echogenity) with liver-kidney contrast (increased echo level of the liver compared with the right kidney). BMI was calculated as follows: body weight (kg)/height² (m²). Body fat percentage (BFP) was determined using a bipedal bio-impedance instrument (Model TBF-220; Tanita, Japan). Venous blood samples were taken from all subjects before 09.00 hours after overnight fasting and analyzed immediately. Alanine aminotransferase, γ-glutamyl transpeptidase, total cholesterol, triglycerides and glucose were measured by standard laboratory procedures. High-density lipoprotein (HDL) cholesterol level was determined by direct homogeneous assay of the serum using detergents (Daiichi Chemicals, Japan). History of alcohol intake was determined by questionnaire in which subjects reported a rough approximation of their daily intake.

Differences between groups were examined for statistical significance using the χ^2 test test, unpaired *t*-test and analysis of covariance (ANCOVA). Multivariate analysis was performed using logistic regression analysis. Correlations were examined by linear regression analysis using the coefficient of correlation. All data analyses were performed using Statview software version J-5.0 (Abacus Concepts, CA, USA). A P-value less than 0.05 was considered significant.

3. RESULTS

3.1. Independent predictors of fatty liver in the 2005 group

TO IDENTIFY FACTORS that associated with the pathogenesis of fatty liver, we compared the clinical and laboratory features between persons with and without fatty liver (Table 1) and performed logistic regression analysis (Table 2).

In both men and women, there was a significant difference in age between the fatty liver and non-fatty liver groups. In men, the age of the fatty liver group was lower than that of the non-fatty liver group; in contrast, the age of women in the fatty liver group was higher. This may reflect the gender difference in incidence of fatty liver, which has been reported elsewhere.[3] Only in men was age found to be an independent predictor.

Markers of obesity, BMI and BFP, were significantly higher in the fatty liver group. In addition, both BMI and BFP were found to be independent predictors of fatty liver in both men and women.

Triglycerides, HDL-cholesterol, glucose and ALT were also independent predictors in both sexes. Total cholesterol and diastolic blood pressure were independent predictors only in men.

As for alcohol, we compared the proportion of persons who drink more than 20 g/day between those with and without fatty liver, and found no difference between the groups (37.4 vs. 39.8%, $P = 0.0569$ in men; 1.1 vs. 1.8%, $P = 0.1463$ in women).

Table 2 Independent predictors of fatty liver by logistic regression analysis

		Regression Coefficient	Standard	Error	P value	Odds ratio	95% CI
Men	Age	−0.011	0.003	<0.0001	0.989	0.983–0.995
	BMI	0.207	0.019	<0.0001	1.227	1.183–1.273
	BFP	0.126	0.011	<0.0001	1.135	1.110–1.160
	ALT	0.017	0.002	<0.0001	1.017	1.017–1.021
	TC	0.006	0.001	<0.0001	1.006	1.004–1.008
	TG	0.001	0.000	0.0035	1.001	1.000–1.008
	HDL-C	−0.026	0.003	<0.0001	0.974	0.968–0.999
	FBS	0.011	0.001	<0.0001	1.011	1.008–1.014
	D-BP	0.009	0.003	0.0015	1.009	1.003–1.015
Women	BMI	0.161	0.034	<0.0001	1.175	1.098–1.256
	BFP	0.136	0.020	<0.0001	1.146	1.102–1.191
	ALT	0.049	0.005	<0.0001	1.051	1.041–1.061
	TG	0.009	0.001	<0.0001	1.009	1.007–1.011
	HDL-C	−0.022	0.004	<0.0001	0.978	0.971–0.987
	FBS	0.025	0.003	<0.0001	1.025	1.019–1.031

ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; CI, confidence interval; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides.

3.2. Comparison between 2000 and 2005 groups (Table 3)

The prevalence of fatty liver increased between 2000 and 2005 in men (from 33.3 to 38.5%), but not in women (from 21.3 to 21.0%).

Age was significantly higher in the male 2005 group. Age might not be involved in the higher prevalence of fatty liver in 2005 group, because the age of men was lower in the fatty liver group (Table 1).

There was no significant difference in BMI of both sexes. BFP increased significantly in men, but not in women.

Total cholesterol level was significantly elevated in both men and women. HDL-cholesterol levels decreased significantly in men. There was no significant difference in triglycerides and glucose levels. There was a significant difference in men's diastolic blood pressure.

4. DISCUSSION

FATTY LIVER IS an increasingly recognized condition, linked to the metabolic syndrome associated with obesity and insulin resistance. BMI has been considered to be the most important risk factor for fatty liver.[3,5]

Table 3 Comparison between the 2000 and 2005 groups

	Men			Women		
	2000	2005	P value	2000	2005	P value
Fatty liver (%)	33.3	38.5	<0.0001*	21.3	21.0	NS*
Age (years)	52 ± 10	53 ± 10	<0.0001**	54 ± 10	55 ± 10	0.0243**
BMI (kg/m²)	23.4 ± 2.9	23.8 ± 3.0	NS***	22.8 ± 3.1	22.8 ± 3.3	NS***
BFP (%)	20.6 ± 4.7	22.3 ± 5.0	0.0003***	27.4 ± 5.5	28.4 ± 5.9	NS***
ALT (IU/L)	28 ± 28	29 ± 28	NS***	19 ± 13	20 ± 12	NS***
TC (mg/dl)	204 ± 33	211 ± 35	0.0058***	209 ± 34	217 ± 34	<0.0001***
TG (mg/dl)	151 ± 126	142 ± 127	NS***	100 ± 57	89 ± 42	NS***
HDL-C (mg/dl)	60 ± 16	56 ± 14	0.0230***	69 ± 16	65 ± 14	NS***
BG (mg/dl)	109 ± 21	108 ± 23	NS***	102 ± 16	97 ± 17	NS***
D-BP (mmHg)	78 ± 11	79 ± 23	<0.0001***	73 ± 16	73 ± 16	NS***

Data except fatty liver prevalence were expressed as mean ± SD. ALT, alanine aminotransferase; BFP, body fat percentage; BG, blood glucose; BMI, body mass index; D-BP, diastolic blood pressure; HDL-C, high density lipoprotein-cholesterol; TC, total cholesterol; TG, triglycerides. *χ^2 test; **unpaired *t*-test; ***analysis of covariance (ANCOVA, adjusted for age).

However, the recent increase in prevalence has not necessarily involved increasing BMI;[3] factors other than an increase in BMI are concerned. In the present study, as shown in Table 3, the increasing prevalence of fatty liver amongst men was associated with changes in BFP, and total cholesterol and HDL-cholesterol levels. It is significant that the prevalence of fatty liver increased with increasing BFP, even without an increase in BMI.

The increase in BFP without a corresponding increase in BMI may indicate changes in body composition; that is, an increase in body fat deposits and corresponding decrease in the fat-free component. As for fat deposits, Eguchi *et al.* report that the severity of fatty liver is positively correlated with visceral fat accumulation regardless of BMI.[6] It is possible that the increase in BFP corresponds to an increased accumulation of visceral fat. On the other hand, Caprosto *et al.* report that the resting metabolic rate (RMR) is lower in patients with non-alcoholic steatohepatitis than in controls.[7] Because of the correlation between RMR and muscle mass, lower RMR possibly reflects a decrease in muscle mass. Thus, the decrease in the fat-free component may correspond to a decrease in muscle mass. The literature suggests that Asian populations have a high level of abdominal fat at a lower BMI relative to Caucasians.[8,9] Therefore, Asians are at a higher risk for obesity-associated disorders even without obesity, and this is the rationale behind the World Health Organization's Regional Office for the Western Pacific criteria (overweight at risk) for Asians.[9] It is considered that the characteristic body composition of the Asian population has been further impacted upon by the present-day lifestyle in Japan, resulting in an increased prevalence of fatty liver without an increase in BMI. Since visceral fat obesity strongly associates with the metabolic syndrome, our study underscores the importance of monitoring visceral fat accumulation using representative indicators such as waist circumference or waist/hip ratio, or monitoring visceral fat volume by CT scan, during regular health checks. Table 3 shows that the prevalence of fatty liver has increased with increases in total cholesterol and decreases in HDL-cholesterol, possibly suggesting an association with insulin resistance.[10] Additional studies are required to further clarify these associations.

The changes in body composition in the present study should be distinguished from obesity. Obesity may bring about increases in both BFP and BMI. We consider that inadequate dieting (and rebound), irregular eating habits (e.g. fasting at breakfast) and a lack of exercise are the probable causes of the reported changes in body composition. As described above, it seems difficult to prevent the increasing prevalence of fatty liver only by weight (BMI) control. Thus, we must emphasize the need for a new strategy to reduce risk of fatty liver, in which relevant nutritional support and exercise are employed to reduce body fat deposits and develop muscle mass.

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Association of a genetic polymorphism in ectonucleotide pyrophosphatase/phosphodiesterase 1 with hepatitis C virus infection and hepatitis C virus core antigen levels in subjects in a hyperendemic area of Japan

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Background. The clinical course of chronic hepatitis C virus (HCV) infection is strongly associated with insulin resistance and obesity. The K121Q polymorphism in the ectonucleotide pyrophosphatase/phosphodiesterase (ENPP)-1 gene and the rs7566605 genotype located near insulin-induced gene 2 have been shown to be associated with insulin resistance and obesity. This study examined whether the K121Q polymorphism in *ENPP1* or the rs7566605 genotype is associated with the clinical course of HCV infection. **Methods.** The relationships between the clinical characteristics of 469 anti-HCV antibody-seropositive subjects (353 were positive for HCV core antigen or RNA, whereas 116 were negative for HCV RNA) and the polymorphisms were analyzed. **Results.** No significant differences in body mass index, plasma glucose level, serum insulin level, and other biochemical markers were observed between subgroups of subjects with different genotypes at the K121Q polymorphism or rs7566605. The frequency of the homozygous wild-type genotype at K121Q in HCV carriers, however, was significantly higher than that in subjects who were negative for HCV RNA (84.5% vs. 75.9%; *P* < 0.05). Moreover, in HCV carriers, HCV core antigen levels in subjects homozygous for the wild-type genotype at K121Q were significantly higher than in heterozygous carriers of K121Q (5358 fmol/l vs. 4002 fmol/l; *P* = 0.04). In contrast, the rs7566605 genotype was not associated with hepatitis C viremia or with the HCV core antigen level. **Conclusions.** The K121Q variant of *ENPP1* may be associated with hepatitis C viremia and core antigen levels in HCV carriers.

Introduction

Hepatitis C virus (HCV) infection, a major cause of chronic hepatitis, may progress to cirrhosis or hepatocellular carcinoma (HCC). Persistent HCV infection can be detected in the sera of 50%–80% of subjects positive for anti-HCV antibodies; in contrast, 20%–50% of those subjects are consistently negative for HCV RNA, suggesting that they have successfully eliminated the HCV infection.[1] Factors such as ethnicity, icteric clinical presentation, absence of human immunodeficiency virus (HIV) infection, and specific HLA type II alleles have been shown to be associated with viral clearance.[2-4] Even in the absence of these factors, however, viral clearance may occur, suggesting the presence of other unidentified cofactors.

Being overweight or obese is an independent risk factor for hepatic steatosis, which accelerates the activity and progression of chronic hepatitis C (CHC).[5] Another risk factor for steatosis is insulin resistance, which is associated with advanced fibrosis and hyporesponsiveness to antiviral therapy.[6] Although obesity and insulin resistance are known to be caused by a combination of genetic and environmental factors, the impact of genetic factors on the clinical course of HCV infection or the severity of liver disease has not been fully elucidated.

A number of reports indicate that single nucleotide polymorphisms (SNPs) in the gene encoding the K121Q variant of ectonucleotide pyrophosphatase/phosphodi-

esterase 1 (*ENPP1*, also known as PC-1) influence insulin resistance, type 2 diabetes, and obesity.[7-11] Recently, the rs7566605 genotype, which is located near the gene encoding insulin-induced gene 2 (*INSIG2*), was also shown to be strongly associated with insulin resistance.[12] Other studies, however, have reported no significant associations between the K121Q variant and insulin resistance or type 2 diabetes,[13-15] and the association between the K121Q variant or rs7566605 genotype and the clinical features of patients with chronic HCV infection has not been fully evaluated.

We examined the natural history of HCV infections in an adult Japanese community-based population in an HCV hyperendemic area beginning in 1994.[16,17] Because movement of the residents in or out of this region is rare, this area provided an appropriate setting to investigate the effects of a genetic background on HCV infections. In this study, we sought to determine the prevalence of the rs7566605 genotype and polymorphisms of the *ENPP1* gene encoding the K121Q variant and to assess their relationship with body mass index (BMI), insulin resistance, and the clinical characteristics of subjects positive for anti-HCV antibodies in an HCV hyperendemic area in Japan.

Materials and methods

Study population

We evaluated 459 anti-HCV antibody-seropositive subjects. Among these subjects, 343 were positive for HCV RNA or HCV core antigen (HCV carrier group), and 116 were negative for both HCV RNA and HCV core antigen (HCV RNA-negative group). All the subjects were Japanese and lived in an HCV hyperendemic area (Town C).[16-18] The Town C HCV study is a cohort study examining the natural course of HCV infections in adult residents of a community in Miyazaki Prefecture, Japan. Residents who were identified as anti-HCV antibody positive at general health examinations were invited to participate in annual examinations for liver disease. No one in this study population had received interferon therapy or was positive for hepatitis B surface antigen. Informed consent was obtained from all participants at the time of enrollment. This study was approved by the human subjects committees of the University of Miyazaki (Faculty of Medicine, Japan), the Harvard School of Public Health, and the Boston University School of Public Health.

Blood tests for hepatic fibrosis markers, anti-HCV antibodies, and HCV core antigen levels

Serum anti-HCV antibodies were detected using chemiluminescence enzyme immunoassays and a third-generation kit (Lumipulse Ortho II; Ortho-Clinical Diagnostics, Tokyo, Japan) at least once for each subject between 2001 and 2003. Additionally, 301 subjects in the HCV carrier group and 100 subjects in the HCV RNA-negative group were known to be positive for anti-HCV antibodies before 1996 as a result of second-generation enzyme immunoassay testing (Immunocheck F-HCV Ab; International Reagents, Kobe, Japan).[16-19] The presence of serum HCV RNA was determined using qualitative reverse transcription-polymerase chain reaction (RT-PCR) (Amplicore HCV; Nippon Roche, Tokyo, Japan). HCV core antigen levels were measured using immunoradiometric assays and a cutoff value for a positive result of 20 fmol/l (Ortho HCV Ag IRMA test; Ortho-Clinical Diagnostic). The levels of plasma glucose (normal range, 70–109 mg/dl), serum insulin (≤17 mU/ml), aspartate aminotransferase (AST) (10–40 IU/l), alanine aminotransferase (ALT) (5–40 IU/l), γ-glutamyl transpeptidase (GTP) (female: 7–30 IU/l; male: 7–70 IU/l), ferritin (female: 7–110 mg/dl; male: 24–286 mg/dl), and the platelet count (12.0–34.0 × 10⁴ cells/µl) were examined in each patient. The HCV serotype of each subject was determined before 2001. If the HCV serotype was not determined, the HCV genotype was examined (HCV Core Genotype; SRL, Tokyo, Japan). HCV genotype 1b was considered to be serotype I and genotypes 2a and 2b were considered to be serotype II. No other HCV genotype was detected in this study. Insulin resistance was assessed using a homeostasis model assessment of insulin resistance (HOMA-IR). HOMA-IR values were calculated as follows: plasma glucose (mg/dl) × serum insulin (mU/ml)/405. Hyaluronic acid and type IV collagen 7S, which are known to be hepatic fibrosis markers, were examined using a latex bead agglutination assay (LPIA-ACE HA; Mitsubishi Kagaku Iatron, Tokyo, Japan; normal range: ≤50 ng/ml) and a radioimmunoassay (Type IV collagen 7S kit; Mitsubishi Kagaku Iatron; normal range: ≤6.0 ng/ml), respectively.

DNA extraction and real-time PCR allelic discrimination assays

DNA extraction and real-time PCR allelic discrimination assays were carried out as described previously.[19] Briefly, 10 µl whole blood was drawn into an ethylenediaminetetraacetic acid (EDTA)-containing Vacutainer by venipuncture. Genomic DNA was extracted from the buffy coat fraction, which was separated from the blood by centrifugation at 3000 rpm using Mag-Extractor System MFX-2000 (Toyobo, Osaka, Japan) according to the manufacturer's protocol. The *ENPP1* K121Q SNP was examined using PCR and sequence-specific primers. Real-time PCR allelic discrimination assays were designed using TaqMan SNP genotyping

assays (Applied Biosystems, Foster City, CA, USA). Assays were performed to genotype the A→C SNP corresponding to *ENPPI* K121Q using commercially available primers (dbSNP ID: rs1044498; TaqMan SNP genotyping assays ID: C_1207994_20). We also evaluated the rs7566605 genotype located near the *INSIG2* gene.[12] Genotyping of the G→C SNP (rs7566605) was performed with the primers rs7566605-F (AGTAGGGTGAGGAAACCAAATTCTC) and rs7566605-R (CATGACCCCTACCGTCTCTATTTT), and the probes rs7566605-VIC (ACAGAGATGTTA CATCAC labeled with the dye VIC) and rs7566605-FAM (CACAGAGATATTACATCAC labeled with the dye FAM) in a custom TaqMan genomic assay. Briefly, 5 ng DNA was mixed with TaqMan Universal PCR master mix (Applied Biosystems) and allelic discrimination assay mix (900 nM each primer and 200 nM each FAM or VIC-labeled probe). PCRs were carried out in a total volume of 6 or 10 μl in 96-well PCR plates. The PCR conditions were as follows: 50°C for 2 min for contamination control with AmpErase uracil-*N*-glycosylase and 95°C for 10 min to activate the Ampli-Taq Gold enzyme, followed by 40 cycles of 92°C for 15 s and 60°C for 1 min. Genotypes were assessed using the TaqMan allele-specific assay method and an ABI Prism 7000 sequence detection system according to the manufacturer's protocol (Applied Biosystems). All genotypes were scored using the allelic discrimination program from the ABI software.

Statistical evaluation

The differences in mean values were assessed using Mann–Whitney U tests. Fisher's exact tests and χ^2 tests were used where appropriate. Univariate and multivariate logistic regression analyses were also used to determine the factors that significantly associated with viral clearance or viral load. All statistical analyses were performed using STATVIEW 4.5 software (Abacus Concepts, Berkeley, CA, USA) or SPSS version 11.01 statistical analysis software (SPSS, Chicago, IL, USA). P values less than 0.05 were considered statistically significant.

Results

Characteristics of the subjects

The clinical characteristics of the study population are shown in Table 1. In this study, 343 subjects were positive for anti-HCV antibodies and the presence of HCV RNA and/or HCV core antigen (HCV carrier group), whereas 116 subjects were positive for anti-HCV antibodies but were negative for both HCV RNA and HCV core antigen (HCV RNA-negative group). The mean age of the subjects was 70 years (range, 42–97 years old), and the mean BMI of the subjects positive for anti-HCV antibodies was 23 kg/m² (range, 15.6–33.5 kg/m²). Although there were no differences in the distribu-

Table 1. Clinical characteristics of subjects positive for antihepatitis C virus (HCV), according to the presence of hepatitis C viremia

Characteristics	HCV carrier[a] ($n = 343$)	HCV RNA-negative[b] ($n = 116$)	P value[c]
Age (years)	70.7 ± 9.7	69.6 ± 11.2	0.67
Sex (male/female)	117/226	37/79	0.66
History of alcohol consumption (daily/occasionally/none)[d]	110/23/174	35/7/63	0.83
Past history of BT (yes/no)[d]	50/273	25/83	0.07
HCV core antigen	4871.6 ± 4869.4 (325)	–	–
HCV serotype (I/II)[e]	225/118	–	–
Body mass index	23.1/1/3.0 (286)	23.1 ± 3.3 (93)	0.73
AST (IU/l)	49.4 ± 32.9	26.4 ± 8.6	<0.001
ALT (IU/l)	44.9 ± 38.2	20 ± 10.1	<0.001
γ-GTP (IU/l)	35.0 ± 52.3 (248)	21.6 ± 26.4 (91)	<0.001
PLT (×10⁴)	19.1 ± 6.2 (342)	23.8 ± 5.6	<0.001
Tryglyceride (mg/dl)	110.2 ± 57.2 (248)	123.2 ± 59.4 (93)	0.02
Total cholesterol (mg/dl)	170.3 ± 34.7 (248)	193.1 ± 30.8 (93)	<0.001
HbA1c (%)	5.3 ± 0.7 (248)	5.4 ± 1.0 (91)	0.12
Glucose (mg/dl)	97.3 ± 34.4 (273)	95.6 ± 23.6 (88)	0.86
Insulin (μU/ml)	11.4 ± 11.4 (273)	9.3 ± 13.7 (88)	<0.001

Data are shown as means ± SD (number of subjects examined)
BT, blood transfusion; AST, aspartate aminotransferase; ALT, alanine transferase; GTP, guanosine triphosphatase; PLT, platelet count
[a] Positive for HCV RNA or HCV core antigen
[b] Negative for HCV RNA and HCV core antigen
[c] Data were evaluated by χ^2 test, Fischer's exact test, or Mann–Whitney test, as appropriate
[d] Excluding subjects whose history was not available
[e] Including subjects whose HCV genotype was determined even if serotype was undetermined

Table 2. Prevalence of *ENPPI* K121Q genotype or rs7566605 genotype in subjects with positive for anti-HCV, according to the presence of hepatitis C viremia

	HCV carrier[a]	HCV RNA-negative[b]	P value[c]
K121Q genotype	$n = 342$	$n = 116$	
AA	289 (84.5%)	88 (75.9%)	
AC	53 (15.5%)	26 (22.4%)	
CC	0	2 (1.7%)	0.01[d]
rs 7566605 genotype	$n = 341$	$n = 116$	
GG	159 (46.6%)	52 (44.8%)	
GC	141 (41.3%)	52 (44.8%)	
CC	41 (12.0%)	12 (10.3%)	0.75

[a] Positive for HCV RNA or HCV core antigen
[b] Negative for HCV RNA and HCV core antigen
[c] Data were analyzed by χ^2 test
[d] P value was 0.048 evaluated by subclasses of AA or AC + CC genotype

Table 3. Prevalence of *ENPPI* K121Q genotypes or rs7566605 genotype in HCV carriers, according to the body mass index (BMI)

	Normal weight (BMI <25)	Overweight (BMI ≥25 and <30)	Obesity (BMI ≥30)	P value[a]
K121Q genotype	$n = 216$	$n = 76$	$n = 4$ (%)	
AA	182 (84.3%)	66 (86.8%)	3 (75.0%)	
AC	34 (15.7%)	10 (13.2%)	1 (25.0%)	0.75[b]
CC	0	0	0	
rs 7566605 genotype	$n = 216$	$n = 75$	$n = 4$	
GG	107 (49.5%)	30 (40.0%)	2 (50.0%)	
GC	83 (38.4%)	35 (46.7%)	2 (50.0%)	
CC	26 (12.0%)	10 (13.3%)	0	0.36

[a] Data were evaluated by χ^2 test
[b] Data were analyzed excluding CC genotype

tions of age, sex, history of alcohol consumption, BMI, plasma glucose levels, and HbA1c levels between the groups, AST, ALT, γ-GTP, and insulin levels were significantly higher and triglycerides, total cholesterol, and platelet counts were significantly lower in the HCV carrier group than in the HCV RNA-negative group.

Differential distributions of the ENPP1 *K121Q SNP or rs7566605 genotypes and the clinical characteristics*

We successfully genotyped 458 and 457 subjects for the *ENPPI* K121Q SNP and rs7566605, respectively. The *ENPPI* K121Q SNP was differentially distributed between the HCV carrier group and the HCV RNA-negative groups ($P < 0.01$), whereas the rs7566605 genotype was not (Table 2). In univariate analysis, the *ENPPI* K121Q genotypes AC and CC were significantly more prevalent in the HCV RNA-negative group than in the HCV carrier group [odds ratio (OR), 1.74; 95% confidence interval (CI), 1.04–2.91; $P = 0.04$]. No other factors, including age, sex, BMI, history of alcohol consumption, past history of blood transfusion, and the rs7566605 genotype, were significantly different between the groups (data not shown). In multivariate analysis

using four factors (age, sex, *ENPPI* K121Q genotype, and rs7566605 genotype), only the *ENPPI* K121Q genotypes AC and CC were associated with being negative for HCV RNA (OR, 1.78; 95% CI, 1.05–2.99; $P = 0.03$).

Relationships between the ENPP1 *K121Q or rs7566605 genotypes and BMI or insulin resistance*

We examined the relationships between the SNPs and available BMI values in HCV carriers: the subjects were classified as overweight (BMI ≥25 and <30 kg/m²), obese (BMI ≥30 kg/m²), or normal (BMI <25 kg/m²). The distributions of the *ENPPI* K121Q and rs7566605 genotypes were similar in all three BMI subgroups (Table 3). In addition, there was no association between these two SNPs and fasting plasma glucose levels greater than 126 mg/dl or a history of diabetes (data not shown). Then, subjects with fasting plasma glucose levels less than 126 mg/dl were selected, and the relationship between the SNPs and insulin resistance was studied after classifying the subjects as insulin resistant (HOMA-IR value ≥2) or not (HOMA-IR value <2). The distributions of the *ENPPI* K121Q and rs7566605

Table 4. Prevalence of *ENPP1* genotypes or rs7566605 genotypes in HCV carriers, according to insulin resistance

	Lower HOMA-IR index (<2)	High HOMA-IR index (≥2)	P value[a]
K121Q genotype	n = 130	n = 106	
AA	106 (81.5%)	94 (88.7%)	
AC	24 (18.5%)	12 (11.3%)	0.13[b]
CC	0	0	
rs 7566605 genotype	n = 131	n = 105	
GG	68 (51.9%)	48 (45.7%)	
GC	47 (35.9%)	48 (45.7%)	
CC	16 (12.2%)	9 (8.6%)	0.27

HOMA, homeostasis model assessment of insulin resistance
[a] Data were valuated by χ^2 test
[b] Data were analyzed excluding CC genotype

Table 5. Clinical and virological characteristics in individuals who are HCV carriers, according to the *ENPP1* K121Q genotype

Characteristics	*ENPP1* K121Q genotype[a]		P value[b]
	AA (n = 289)	AC (n = 53)	
Age (years)	70.9 ± 9.5	69.7 ± 10.5	0.43
Sex (male/female)	101/188	15/38	0.35
Body mass index	23.1 ± 3.0 (251)	22.8 ± 3.1 (45)	0.44
Alcohol consumption (daily/occasionally/none)[c]	100/22/157	18/4/30	0.98
Past history of blood transfusion (yes/no)[c]	39/234	11/38	0.15
HCV core antigen (fmol/l)[d]	5358.3 ± 4906.7 (272)	4001.8 ± 4526.4 (53)	0.04
HCV core antigen (<1000/≥1000)[e]	73/216	18/35	0.19
HCV serotype (I/II)[f]	182/107	42/11	0.02
AST (IU/l)	49.9 ± 34.4	46.7 ± 23.4	0.83
ALT (IU/l)	45.9 ± 40.5	40.2 ± 21.7	0.86
γ-GTP (IU/l)	36.2 ± 55.0 (210)	28.1 ± 32.5 (38)	0.75
PLT (×10⁴)	19 ± 6.1 (288)	20.0 ± 6.7	0.30
TG (mg/dl)	110.1 ± 57.1 (210)	110.6 ± 58.6 (38)	0.92
Total cholesterol (mg/dl)	170.0 ± 35.0 (210)	172.3 ± 33.2 (38)	0.66
HbA1c (%)	5.3 ± 0.7 (210)	5.4 ± 0.9 (38)	0.67
Glucose (mg/dl)	98.0 ± 35.4 (230)	93.7 ± 28.9 (42)	0.20
Insulin (μU/ml)	11.6 ± 11.7 (230)	10.9 ± 10.2 (42)	0.59
Ferritin (mg/dl)	151.0 ± 215.5	138.5 ± 182.3	0.33
HA (ng/ml)	196.9 ± 365.9 (287)	236.4 ± 391.8	0.58
Type IV collagen 7S (ng/ml)	5.0 ± 1.8 (287)	5.0 ± 2.0	0.39

Data are shown as means ± SD (number of subjects examined)
[a] There was no subject with CC genotype in persistent HCV infection group
[b] Data were evaluated by χ^2 test, Fischer's exact test, or Mann–Whitney test, as appropriate
[c] Excluding subjects whose history was not available
[d] Excluding subjects whose HCV core antigen level was below the cutoff value
[e] Including subjects whose HCV core antigen level was below the cutoff values
[f] Including subjects whose HCV genotype was determined even if serotype was undetermined

genotypes were also similar in the HOMA-IR subgroups (Table 4).

Clinical and biochemical characteristics of the HCV carriers classified based on the ENPP1 K121Q or rs7566605 genotype

In the HCV carrier group, biochemical markers from the subjects with AA and AC genotypes at the *ENPP1*

K121Q SNP were compared (Table 5). We did not identify any subjects in the HCV carrier group with a CC genotype at this locus. The levels of HCV core antigen in subjects with an AA genotype were higher than in subjects with an AC genotype. The frequency of serotype II was also higher in subjects with an AA genotype than in subjects with an AC genotype. No other clinical or biochemical characteristics were different between the subjects with the different K121Q genotypes.
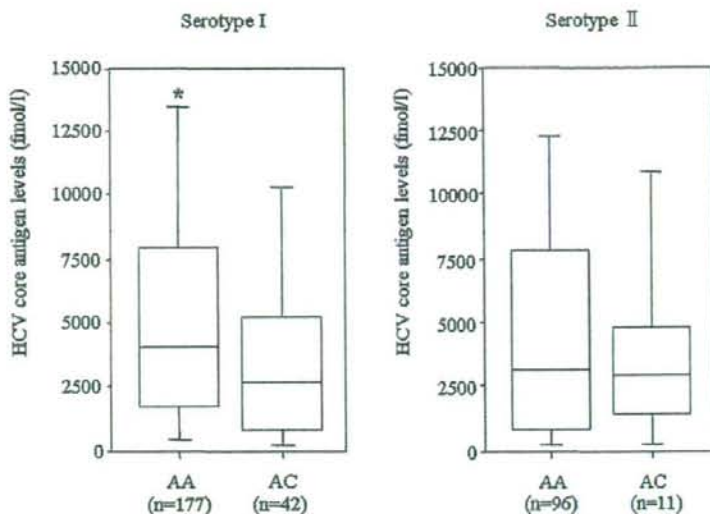
Fig. 1. The association between the K121Q genotype in *ENPP1* and the hepatitis C viral (HCV) load. The box-and-whisker plot shows the HCV core antigen level in the HCV carrier group according to the genotypes. The *boxes* indicate the 25th, 50th (median), and 75th percentiles. The *whiskers* indicate the 10th and 90th percentiles. The *asterisk* refers to a statistically significant difference between the HCV core antigen levels in patients with the AA or AC genotype (Mann–Whitney *U* test, *$P = 0.04$

We then further analyzed the association between the *ENPP1* K121Q variant and HCV core antigen levels according to the HCV serotype (Fig. 1). In the subgroup of subjects classified as HCV serotype I, the hepatitis C viral load was significantly higher in the subjects with the AA genotype (the wild-type genotype) than in those with the AC genotype ($P = 0.04$). Five subjects with the AA genotype were not included in this comparison because their levels of HCV core antigen were below the threshold. In any case, the percentage of subjects with HCV core antigen levels below the cutoff value of 1000 fmol/l was lower in the AA genotype subgroup than in the AC genotype subgroup (23.0% vs. 61.5%, $P < 0.01$ calculated using Fisher's exact test; OR, 2.68; 95% CI, 1.30–5.54; $P < 0.01$). Although a past history of blood transfusion was also associated with HCV core antigen levels (OR, 2.75; 95% CI, 1.25–6.06; $P = 0.01$), no other factors were associated with this variable. In multivariate analysis using the *ENPP1* K121Q variant and past history of blood transfusion, these two factors were independently associated with low HCV core antigen levels (OR, 2.44; 95% CI, 1.12–5.32; $P = 0.03$ and OR, 2.56; 95% CI 1.14–5.72; $P = 0.02$, respectively). This correlation between the HCV core antigen levels and the K121Q genotype, however, was not observed in the subgroup of subjects classified as HCV serotype II (Fig. 1).

In addition, we compared the biochemical markers from the subjects with the GG, GC, and CC genotypes at rs7566605. There were no significant differences among the clinical or biochemical characteristics of the subjects from these three groups, including the viral load (data not shown).

Discussion

Obesity and insulin resistance, which are caused by a combination of genetic and environmental factors, affect the clinical course of CHC infection.[5,6] The K121Q polymorphisms in the *ENPP1* gene and the rs7566605 genotype have been shown to be significantly associated with obesity and insulin resistance.[7–12] Whether polymorphisms in genes associated with obesity or insulin resistance affect persistent HCV infection or HCV-induced liver injury, however, has yet to be determined. We sought to examine the relationship between polymorphisms in these types of genes and viremia or the clinical course of liver injury in subjects positive for anti-HCV antibodies in a community-based HCV hyperendemic area in Japan. Our study, which shows that polymorphisms associated with the K121Q variant and the rs7566605 genotype are prevalent in Japan, suggests that these genotypes are not associated with obesity or insulin resistance in the examined HCV hyperendemic area. In addition, these polymorphisms were not associated with HCV-induced liver injury. In contrast, the frequencies of the K121Q polymorphism in subjects with hepatitis C viremia and those without viremia were different. Moreover, the K121Q polymorphism was associated with HCV viral load in a subgroup of HCV carriers (serotype I).

ENPP1 is the best characterized of the five human ectoenzyme *ENPP* proteins. *ENPP1* is expressed in many tissues, including muscle, fat, and liver, and overexpression of *ENPP1* in various cell lines inhibits insulin receptor tyrosine kinase activity and causes insulin resistance.[20] It was also reported that the K121Q variant

of *ENPP1* is associated with insulin resistance.[21,22] Compared to the *ENPP1* K121 protein, the *ENPP1* Q121 variant interacts more strongly with the insulin receptor and more effectively inhibits insulin-stimulated insulin receptor autophosphorylation and insulin receptor substrate-1 phosphorylation in vitro.[23] In our study, however, there was no association between the *ENPP1* K121Q variant and insulin resistance in HCV carriers. Keshavarz et al. also failed to find evidence of an association between the *ENPP1* K121Q variant and type 2 diabetes in a Japanese population.[24] The overall frequency of the 121Q allele (9.1%; 83/916) in our study was similar to that in the Japanese population, as previously reported (10.5%; 375/3562).[24] These results indicate that our study population represented the rest of Japan and that the K121Q variant does not influence insulin resistance in Japanese subjects, in particular in subjects with HCV infections.

rs7566605 is upstream of the transcription start site of *INSIG2*, the protein product of which inhibits the synthesis of fatty acids and cholesterol.[25] Overexpression of *INSIG2* in the liver reduced plasma triglyceride levels in obese Zucker diabetic fatty rats, and linkage between this gene and obesity phenotypes was observed in the mice.[26,27] Association testing in nine cohorts produced evidence that individuals with the CC genotype at rs7566605 have higher BMI values and a higher risk of obesity than those with the GG or GC genotype.[28] More recently, however, no association was reported between this genotype and obesity.[29,30] In addition, the rs7566605 genotype was not associated with the clinical or biochemical characteristics of subjects positive for anti-HCV antibodies, obesity, or insulin resistance in our study. These conflicting results about the relationship between the rs7566605 genotype and BMI may have resulted from the heterogeneous population samples. Future studies should enroll a large number of patients with HCV infections and control subjects from throughout the Japanese population.

False-positive results for the HCV antibody test may have occurred in the HCV RNA-negative group in our study. Several studies have shown that samples with readings just slightly above the cutoff value of the anti-HCV test have a greater likelihood to be false-positives compared with those with higher values.[31,32] HCV-positive patients may also show reactivity to nuclear and smooth muscle antigens.[33,34] There was, however, no difference in the distributions of the *ENPP1* K121Q genotypes (AA, AC, or CC) among patients with low titers (≥1 and <5), intermediate titers (≥5 and <30), and high titers (≥30) of anti-HCV antibodies in our study (data not shown). In addition, although there was no evidence of spontaneous clearance of HCV infection in this study, Micallef et al. systematically reviewed 31 longitudinal studies with a total of 675 subjects and reported that

spontaneous viral clearance occurs in approximately one in four people with acute hepatitis C, which was similar to the size of the HCV RNA-negative group (25%).[35] Although autoantibody data and evidence of spontaneous HCV clearance in the clinical courses are not available, these results indicate that many subjects in the HCV RNA-negative group in our study population may have cleared their HCV infection spontaneously without false-positive results for the HCV antibody test.

Spontaneous HCV clearance typically occurs within the first 6 months after acute infection,[36] and spontaneous elimination of HCV in subjects with chronic HCV infection is rare.[16] These results suggest that *ENPP1* may influence the spontaneous clearance of HCV during the acute phase of infection in our population. Furthermore, sex is known to be an important factor for HCV clearance,[37–39] although a sex-based difference was not observed in our study (see Table 1). Studies based on polymorphisms have been widely used to identify host genetic factors that influence disease occurrence, progression, and outcome.[40] However, it is unclear whether *ENPP1* and sex are associated in HCV clearance. Another potential confounding variable is alcohol use, which is known to be negatively associated with HCV clearance.[41] Alcohol use, however, is limited in this community, and thus was unlikely to be a confounder. Further studies are needed to clarify the associations between host factors and *ENPP1* and their roles in HCV clearance.

Analysis of the *ENPP1* gene in 6147 subjects showed an association between a three-allele risk haplotype (K121Q, IVS20delT-11, and A→G+1044TGA) and obesity and type 2 diabetes.[42] In that report, it was shown that the presence of at least one copy each of the Gln121(121Q), IVS20delT-11, and G+1044TGA variants was associated with a significant increase in serum *ENPP1* protein levels. In addition, serum levels of osteopontin were lower in *ENPP1*-deficient mice than in wild-type mice, suggesting that *ENPP1* affects osteopontin expression.[43] Osteopontin-deficient mice also suffered from prolonged rotavirus-induced diarrhea.[44] SNPs in the promoter region of the osteopontin gene have been identified as markers that predict the efficacy of interferon-based therapies in patients with CHC.[45] Although our studies do not directly identify increased serum levels of *ENPP1* or osteopontin, *ENPP1* may induce nonproductive binding of HCV to cells, blockade of HCV attachment, or inhibition of penetration into cells through osteopontin expression.

The precise roles that host factors play in HCV replication have not been well characterized. Although Woitas et al. reported that anti-HCV-antibody-seropositive patients who were homozygous for the HIV-protective CC chemokine receptor (CCR) 5-Δ32

showed a markedly increased viral load compared with CCR5 wild-type or CCR5-Δ32 heterozygous patients,[46] the authors did not show results based on the HCV genotype or serotype. Hepatitis C viral load was found to be significantly higher in patients infected with HCV genotype 1 compared to patients infected with HCV genotype 2 or 3.[47] Our study indicates that the AC genotype at the K121Q SNP of *ENPP1* is linked to lower HCV core antigen levels, which correlated with hepatitis C viral load in the HCV serotype I subgroup, but not in the serotype II subgroup. The mechanisms contributing to the relationship between the K121Q polymorphism and the hepatitis C viral load are unclear. HCV replication in the cytoplasm, however, is highly dependent on the functions of nonstructural HCV proteins together with those of host factors.[48,49] Thus, functional studies about the molecular mechanisms underlying *ENPP1* signaling in HCV replication should be conducted in the future.

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臨床

NASH の治療

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要 旨

NASH は今後日本で増加することが予想されるが，NASH に対する薬物療法に関してはエビデンスレベルの高い報告は少なく，日本人を対象とした検討も少ない．NASH は内臓脂肪蓄積を基盤とするメタボリックシンドロームを背景に，インスリン抵抗性・耐糖能異常，糖尿病，脂質異常症，高血圧などを合併することが多く，その対策が NASH 治療の基本である．つまり，日常生活・生活習慣の是正や肥満の改善に加え，合併する疾患に対する治療が NASH の病態改善にも有効と考えられる．

はじめに

非アルコール性脂肪肝炎（NASH）は，非アルコール性脂肪性肝疾患（NAFLD）の中で，肝細胞の変性・壊死に伴う炎症や線維化を伴う進行性の疾患である．NASH の成因機序として，Day らの提唱した "two hit theory" が広く知られている．すなわち，NASH の発生には，正常肝から単純性脂肪肝（simple steatosis）となる "1st hit" と，単純性脂肪肝から NASH へ進行する "2nd hit" が必要と言われている[1]．"1st hit" には，肥満，脂質異常症，糖尿病などの生活習慣病や遺伝子多型，薬剤などが原因として考えられ，"2nd hit" には，高インスリン血症，TNFα などのサイトカイン，鉄沈着などに起因する酸化ストレスなどが重要な因子として挙げられる．このことから，NASH の治療は肥満，糖尿病，脂質異常症，高血圧といった生活習慣病に対する食事・運動療法を主体とした生活習慣の改善が中心となる．しかし，NASH に対する薬物治療に関してはエビデンスレベルの高い報告は少なく，推奨度の高い治療法は十分確立していない[2]．本稿では，NASH の治療に関して最近の知見を含めて紹介する．

日常生活指導

1．食事・運動療法

NASH は，肝における生活習慣病やメタボリックシンドロームの表現型と言われており，生活習慣の改善が必須である．そのため，NASH ではメタボリックシンドロームの治療に則した食事・運動療法を行うことが重要である[3]．食事指導の目安としては，1 日当たりのエネルギーは標準体重当たり 25～35 kcal/kg 程度，タンパク質は 1.0～1.5g/kg，

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表1　NASH に対する薬物治療

治療薬	対象患者		結果（文献 No.）
インスリン抵抗性改善薬			
ピオグリタゾン	NASH	55 例	ALT 値低下，組織学的改善（6）
ロシグリタゾン	NASH	63 例	ALT 値低下，組織学的改善，アディポネクチン上昇（7）
メトホルミン	NAFLD	110 例	ALT 値低下，評価可能患者の組織学的改善（8）
糖尿病治療薬			
ナテグリニド	NASH	10 例	ALT 値低下，組織学的改善（9）
抗酸化薬			
ビタミン E	NASH	12 例	ALT 値低下，組織学的改善（10）
ビタミン E＋C	NASH	49 例	ALT 値変化なし，組織学的線維化改善（11）
ベタイン	NASH	10 例	ALT 値低下，組織学的改善（12）
UDCA	NASH	166 例	ALT 値低下あるもコントロール群と有意差なし（13）
UDCA＋ビタミン E	NASH	48 例	ALT 値低下，組織学的改善（14）
脂質異常症治療薬			
ゲムフィブロジル	NASH	46 例	ALT 値低下（16）
アトルバスタチン	NASH	27 例	ALT 値低下（17）
プロブコール	NASH	30 例	ALT 値低下（18）
高血圧薬			
ロサルタン	NASH	7 例	ALT 値低下，組織学的改善（22）

略語：巻末の「今月の略語」参照

脂肪は総エネルギーの 20％ 以下とする[4]．また，散歩などの有酸素運動により筋肉や脂肪細胞における代謝が改善し，脂肪細胞より分泌される TNFα が減少し，インスリン抵抗性の改善やトリグリセリド（TG），VLDL の減少，HDL コレステロール（HDL–C）の増加など脂質代謝も改善する[5]．この際，体重は 1 週間に 1.6kg 未満の割合で緩徐に減量することが肝要である[2]．

薬物療法

NASH を含めた NAFLD に肥満，糖尿病，脂質異常症，高血圧などの生活習慣病を合併する場合には，これらの合併症に対する薬物治療がまず必要である．NASH の病態進展にはインスリン抵抗性や糖代謝異常，酸化ストレス，脂質代謝異常，高血圧などが重要な因子と考えられ，それぞれを分子標的とした薬物治療が行われている（表1）．

1．インスリン抵抗性改善薬

NASH 患者の 8 割に認められるインスリン抵抗性は NASH の基本病態と考えられ，まずその治療が NASH の治療ターゲットとして挙げられる．インスリン抵抗性は，脂肪細胞からのアディポサイトカイン分泌異常によって引き起こされると考えられており，それらの受容体や細胞内シグナル伝達に作用する薬剤が，治療薬として用いられている．PPARγ はインスリン感受性の作用増強にかかわる核内受容体であり，脂肪細胞の分化，脂質代謝調節にもかかわっている．チアゾリジン系誘導体は PPARγ のリガンドとして作用する薬剤であり，全身のインスリン抵抗性改善や，肝局所において微小炎症の改善や線維化進展抑制などの作用を有することも報告されており，最近，ピオグリタゾンとロシグリタゾンはそれぞれ二重盲検試験で NASH に対する有効性が報告されている[6,7]．本邦では，ピオグリタゾンはインスリン抵抗性改善

薬としてすでに臨床応用されており，インスリン抵抗性を呈する糖尿病を合併しているNASHには試みるべき治療法である．その他，ビグアナイド系薬剤であるメトホルミンもインスリン抵抗性改善薬であり，肝での糖新生を抑制し，肝での糖取り込みを亢進させることによって血糖を調節する．腎機能障害や呼吸循環不全の患者には禁忌であるが，糖尿病患者に有効であるだけでなくNASHに対しても，トランスアミナーゼの改善，肝内脂肪化，線維化の改善作用を有することが示されている[8]．また，ナテグリニドはインスリン分泌能がある程度保たれた2型糖尿病に用いられるが，NASHに対する有効性も報告されている[9]．

2．抗酸化療法

酸化ストレスとは，生体内の酸化還元状態を維持する機構が破綻し，種々の活性酸素種（ROS）が過剰となった状態である．NASHでは，過剰の脂肪酸負荷に対しROSが過剰産生状態となっており，NF-κBを活性化し，TNFαやインターロイキンの転写亢進が認められ，肝細胞死が誘導される．また酸化ストレスは肝星細胞の増殖促進，活性化作用も有しているため，肝線維化も進展させ，NASHの増悪に促進的に作用する．ビタミンE，ビタミンC，ベタイン，ウルソデオキシコール酸（UDCA）などは抗酸化作用を有し，NASHの治療薬の候補である．ビタミンE単独投与やビタミンCとの併用により，NASHにおけるトランスアミナーゼや肝線維化の改善が報告されている[10)11]．コリン代謝産物であるベタインはメチオニンの代謝に関与し，グルタチオンなどの抗酸化物質の供給に重要であり，小胞体のストレスを軽減する可能性があり，NASHのトランスアミナーゼや肝組織の炎症，線維化を改善する[12]．

UDCAは抗酸化作用のほか，利胆作用，肝細胞膜保護作用，免疫調節作用などさまざまな機能を有しており，NASHに対するUDCAの有効性も期待されている．しかしLindorらが行った二重盲検試験では，UDCA投与により肝機能検査値，肝組織所見の改善は認められたが，プラセボ投与群と有意差はなかった[13]．一方Dufourらは無作為比較試験で，UDCAとビタミンEの併用はUDCA単独やコントロールと比較して有意にALTの低下および組織学的改善を認めたと報告している[14]．このような結果から，NASHに対するUDCAの効果は十分ではなく，ビタミンEなどの併用がより効果的であると考えられる．また，肝内に沈着した鉄はフェントン反応を介して酸化力の強いヒドロキシルラジカルを生成し，酸化ストレスをもたらしている．NASH患者では肝組織中の鉄沈着や血清フェリチンの上昇が高頻度に認められ，瀉血療法による生体からの適切な鉄の除去は抗酸化療法の1つと考えられる[15]．

3．脂質異常症治療薬

NAFLD，特にNASHにはメタボリックシンドロームを伴うことが多い．メタボリックシンドロームの診断基準の1つである高TG血症と低HDL-C血症などの脂質代謝異常に対する治療は，NASHの治療にも有用である可能性がある．PPARαアゴニストであるフィブラート系薬剤は，肝臓や骨格筋に作用して脂肪酸燃焼を促進し，組織内脂肪量を低下させ，血中TGは低下，HDL-Cは上昇させ，インスリン抵抗性も改善するため，肝脂肪化に対して有効な薬剤と期待されている[16]．またスタチン製剤は，コレステロール生合成の律速酵素であるHMG-CoA還元酵素を阻害することにより，肝でのコレステロール合成を抑制する．さらに，血中コレステロール，血中TGの低下作用を有しているだけでなく，レニン・アンジオテンシン系抑制，抗炎症作

用やマトリックスメタロプロテアーゼ阻害作
用など多彩な作用を有しており，NASH の
治療に有用と期待されている．脂質異常症を
伴った NASH 患者にスタチン製剤の 1 つで
あるアトルバスタチンを，脂質異常症のない
NASH 患者には UDCA を投与した Kiyici
らの前向き試験では，アトルバスタチン群に
おいて肝の脂肪化が有意に改善している[17]．
一方，プロブコールは肝でのコレステロール
合成抑制作用とともに抗酸化作用を有するこ
とが知られているが，NASH に対する ALT
低下作用も報告されている[18]．脂質異常症治
療薬のトランスアミナーゼや肝脂肪化に対す
る作用機序は不明な点も多く，NASH に対
する治療に関しては今後の検討が待たれる．

4．高血圧治療薬

　NAFLD の約 40% 近くに高血圧が認めら
れ[19]，血圧調整をつかさどる種々の因子が
NASH の発症・増悪に関与し，特にレニン・
アンジオテンシン系の活性亢進やアディポサ
イトカイン分泌異常の関与が重要と考えられ
ている．脂肪組織由来のアディポサイトカイ
ンにはアディポネクチン，TNFα などが含ま
れ，インスリン抵抗性を介して血圧上昇に関
連している．アンジオテンシン II はレニン・
アンジオテンシン系の主要な因子の 1 つであ
り，昇圧反応や血管リモデリングにも重要な
役割を担っている．肝星細胞の表面にはアン
ジオテンシン II タイプ 1 受容体が発現してお
り，肝星細胞自体もアンジオテンシン II を産
生し，オートクリン的およびパラクリン的に
肝星細胞自身を活性化・増殖させ，肝線維化
の促進に重要な役割を果たしているが，アン
ジオテンシン II にはインスリン抵抗性，酸化
ストレスの増強作用もある[20][21]．実際，アン
ジオテンシン II タイプ 1 受容体拮抗薬（ARB）
には降圧効果，抗動脈硬化，インスリン抵抗
性改善などの多彩な作用があり，ARB は

NASH において抗線維化作用のみならずイ
ンスリン抵抗性改善，抗炎症作用も期待でき
る．つまり，アンジオテンシン II をターゲッ
トとした治療には，血圧管理，インスリン抵
抗性改善，肝線維化予防の効果が期待でき，
NASH の治療に有用である可能性がある[22]．

外科治療法

　NASH の病態進行・悪化には肥満が大き
く影響するが，体格指数（BMI）が 40kg/m²
以上の重症肥満で体重の自己コントロールが
困難な場合には，外科的治療法も考慮される．
外科的治療の基本的な戦略は摂取エネルギー
の抑制であり，術式として消化吸収能抑制術
と胃縮小手術に分けられる．消化吸収能抑制
術には，小腸バイパス術，胆膵バイパス術，
十二指腸転換術などが挙げられ，胃縮小手術
には，胃バイパス術，胃形成術，胃バンディ
ング術などがある[23]．胃バンディング術は胃
上部に巻きつけたバンドにより胃の容積を減
らして食事摂取量を減らす術式で，腹腔鏡下
にて治療が可能である．

おわりに

　本邦においてはメタボリックシンドローム
の罹患者の増加が危惧されており，NASH
の患者数も今後増加していくと予想される．
NASH は進行性の比較的予後不良な疾患で
あり，EBM に基づいた治療法の早期確立が
望まれる．

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