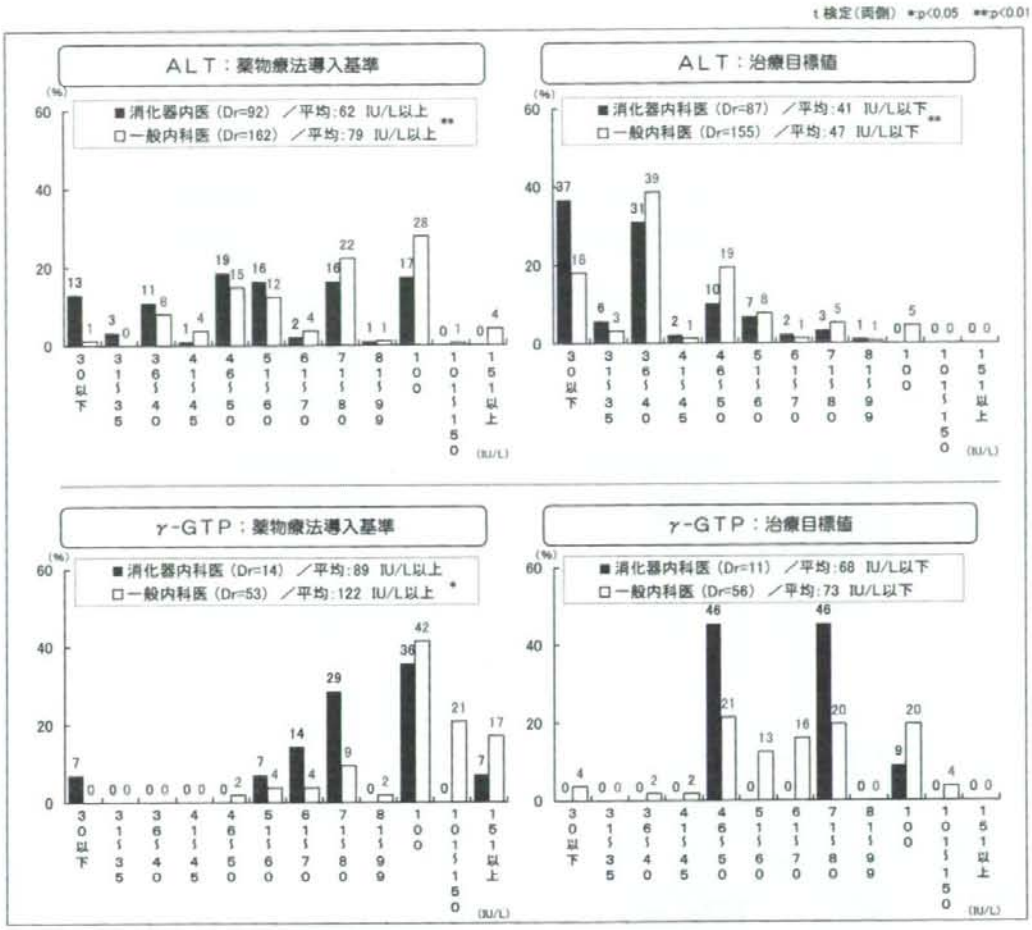

Figure 4. C型肝炎：ALT・γGTPの治療導入基準値（問6）

0.01）．血清ALP値では消化器内科医79%，一般内科医54%で両者間に有意差を認めた（P<0.01）．

IV C型肝炎

C型肝炎治療の目安や病態把握の指標としてどの検査値が活用されているかの調査結果をFigure 3に示す．血清ALT値は，消化器内科医の92%，一般内科医の85%が薬物療法導入の基準として活用しており，両者間に有意差を認めなかった．次いで活用度の高い指標は，血清AST値やHCV-RNA量であった．血小板数を肝線維化の指標として活用しているのは，一般内科医，消化器内科医ともに約半数であった．γGTP値や血清ALP値は，肝臓の炎症や線維化マーカーとしては，ほとんど活用されていなかった．

次いで治療導入に際しての血清ALT，血清γGTP値についての調査結果をFigure 4に示す．C型肝炎の薬物療法導入基準として考慮する血清ALTの平均値は，消化器内科医で62IU/L（30～100IU/L）以上，一般内科医で79IU/L（30～200IU/L）以上であり両者間に有意差を認め（P<0.01），一般内科医の33%が100IU/L以上，56%が70IU/L以上と回答した．消化器内科医でも約半数が50IU/L以上が治療導入の目安であると回答した．同様に血清γGTP値から見た薬物療法導入の平均値は消化器内科医で89IU/L（30～200IU/L）以上，一般内科医で122IU/L（50～200IU/L）以上であり両者間に有意差を認め（P<

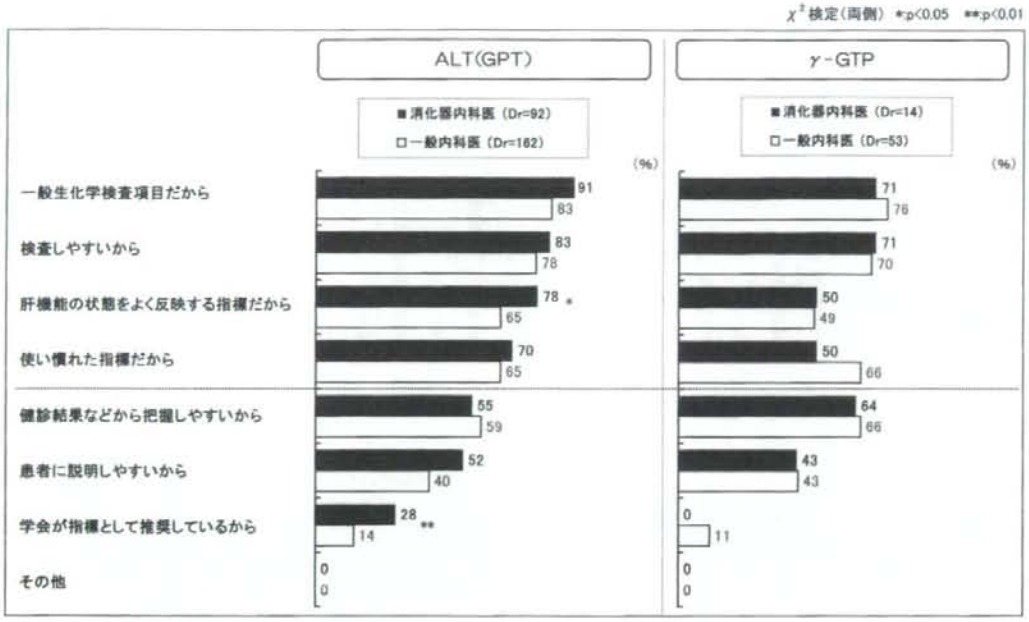

Figure 5. C型肝炎：ALT・γGTPが薬物療法基準となる理由（問7）

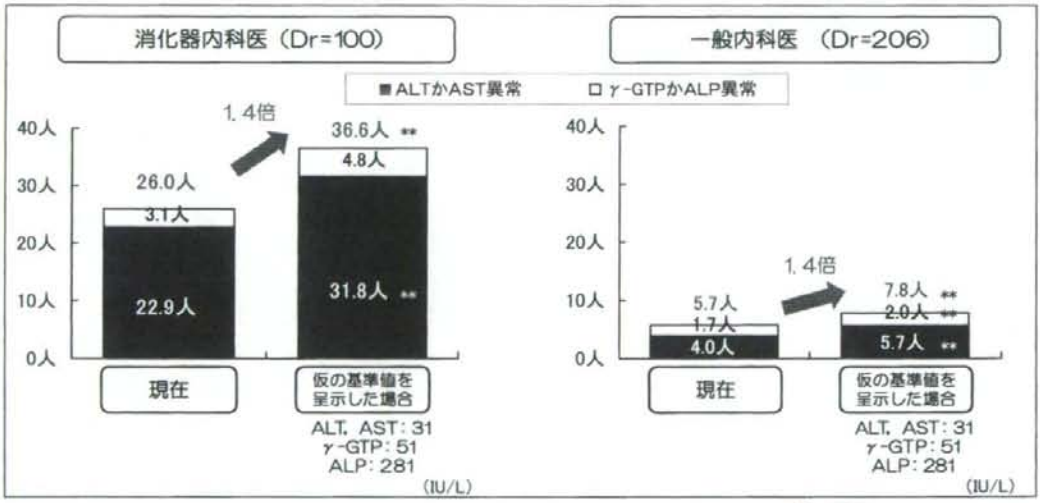

Figure 6. C型肝炎：薬物療法導入基準からみた医師1人あたりの平均患者数（1カ月）（問8・9）

0.01），消化器内科医の43%，一般内科医の80%が100IU/L以上であった．治療目標としての血清ALTの平均値は消化器内科医で41IU/L（20～99IU/L）以下，一般内科医で47IU/L（10～100IU/L）以下で両者間に有意差を認めた（P<0.01），血清γGTP値は，消化器内科医で68U/L（50～100IU/L）以下，一般内科医で73IU/L（30～150IU/L）以下で有意差を認めなかった．

血清ALT，γGTP値が薬物療法開始基準の指標となる理由についてのアンケート結果を示す

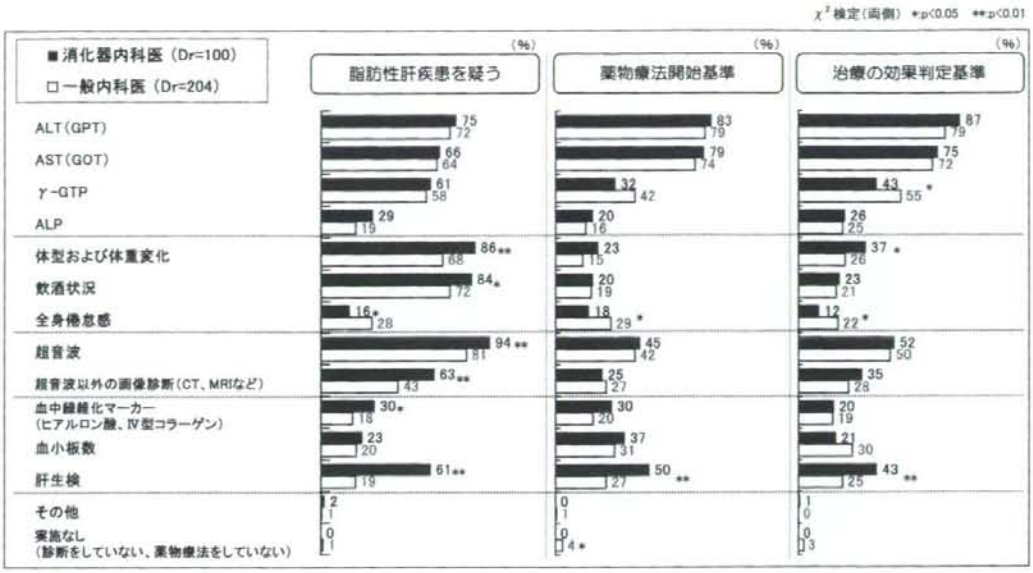

Figure 7. 脂肪性肝疾患：基準となる所見・検査（問 10）

(Figure 5)．血清 ALT，γ-GTP 値ともに「一般生化学検査項目だから」「検査しやすいから」といった項目が上位にあげられた．「肝機能の状態をよく反映する指標だから」も血清 ALT 値で消化器内科医，一般内科医ともに多くあげたが，血清γ-GTP 値について「肝機能の状態をよく反映する指標だから」と回答した割合は消化器内科医，一般内科医ともに約半数程度であった．

最後に仮に薬物療法導入の基準を「血清 ALT，AST 値が 31IU/L 以上，血清γ-GTP 値が 51IU/L 以上，ALP が 281IU/L 以上」とした場合，1 カ月の診療の中で患者数がどれくらい増える可能性があるかとの質問では，Figure 6 に示すように消化器内科医で現在の基準値では 1 人あたり平均 26.0 人であった C 型肝炎患者数は 1.4 倍の 36.6 人と有意に増加した（$P<0.01$），一般内科医でも同様に，現在の基準値では平均 5.7 人であった患者数が 1.4 倍の 7.8 人と有意に増加した（$P<0.01$）．

V 脂肪性肝疾患

脂肪性肝疾患の治療や病態把握の指標としてどの検査値が活用されているかの調査結果を Figure 7 に示す．活用度の高い指標は血清 ALT や AST 値であった．血清 ALT 値は消化器内科医・一般内科医ともに約 80% が薬物療法導入や効果判定基準として活用しており両者間に有意差を認めなかった．肝線維化を反映する血小板数を薬物療法開始基準として考えている消化器内科医は 37%，一般内科医は 31% であった．同様に，血清γ-GTP 値を薬物療法導入の資料として考慮している消化器内科医は 32%，一般内科医は 42% であった．

続いて，血清 ALT，γ-GTP 値の治療導入における基準値についての調査結果を Figure 8 に示す．血清 ALT 値を脂肪性肝疾患の薬物療法導入基準として活用している医師での血清 ALT 値の平均値は，消化器内科医で 93IU/L（30～200IU/L）以上，一般内科医で 90IU/L（30～600IU/L）以上であり両者間に有意差を認めず，消化器内科医，一般内科医ともに約 50% の医師が 100IU/L 以上と回答した．同様に血清γ-GTP 値の薬物療法導入基準平均値は，消化器内科医で 140IU/L 以上（50～300IU/L），一般内科医で 134IU/L（50～500IU/L）以上であり差を認めなかった．治療目標平均血清 ALT 値は消化器内科医で 45IU/L（20～150IU/L）以下，一般内科医で 46IU/L（10～100IU/L）以下で差を認めなかった．血清γ-GTP

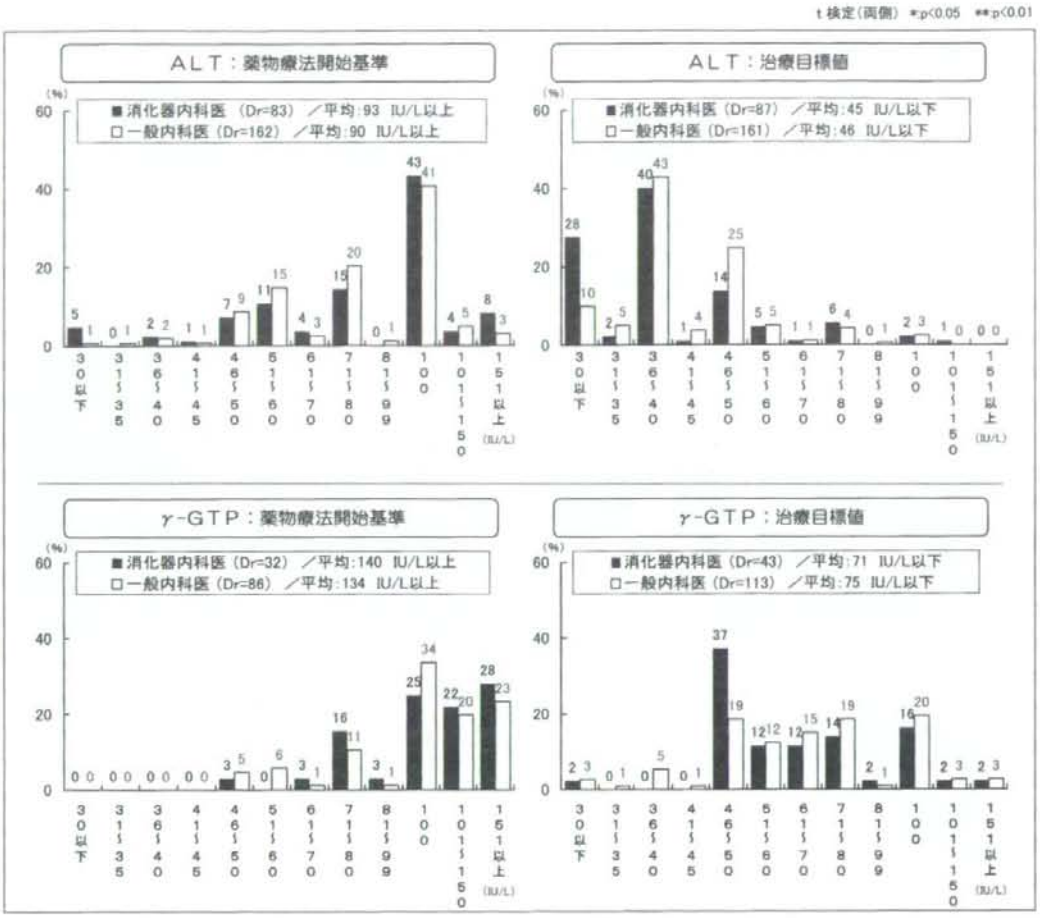

Figure 8. 脂肪性肝疾患：ALT・γGTP の治療導入基準値（問 11）

値においては，消化器内科医で 71IU/L（30～200 IU/L）以下，一般内科医で 75IU/L（20～200IU/L）以下であった（有意差なし）．

血清 ALT，γGTP 値が薬物療法導入基準の指標となる理由に関する調査結果を Figure 9 に示す．血清 ALT，γGTP 値ともに「一般生化学検査項目だから」「検査しやすいから」「使い慣れた指標だから」といった項目が上位にあげられた．「肝機能の状態をよく反映する指標だから」も血清 ALT 値で消化器内科医，一般内科医ともに多くあげていたが，血清 γGTP 値が「肝機能の状態をよく反映する指標だから」と回答したのは，消化器内科医 75%，一般内科医 40% と有意差を認めた（P<0.01）．

最後に，仮に薬物療法開始基準を「血清 ALT，AST 値が 31IU/L 以上，血清 γGTP 値が 51IU/L 以上，血清 ALP 値が 281IU/L 以上」とした場合，1 カ月間の診療の中で患者数がどのくらい増える可能性があるか検討した（Figure 10）．消化器内科医が認識している現在の基準値では，1 人あたり平均 12.9 人だった脂肪性肝疾患患者数は，仮の基準値では 1.5 倍の 19.4 人に有意に増加し（P>0.01），一般内科医では，現在の基準値で平均 10.7 人だった患者数が 1.6 倍の 17.4 人に有意に増加した（P<0.01）．

VI 考　察

C 型肝炎，脂肪性肝疾患の治療導入の目安となる肝機能検査値や治療目標値などについて，医師

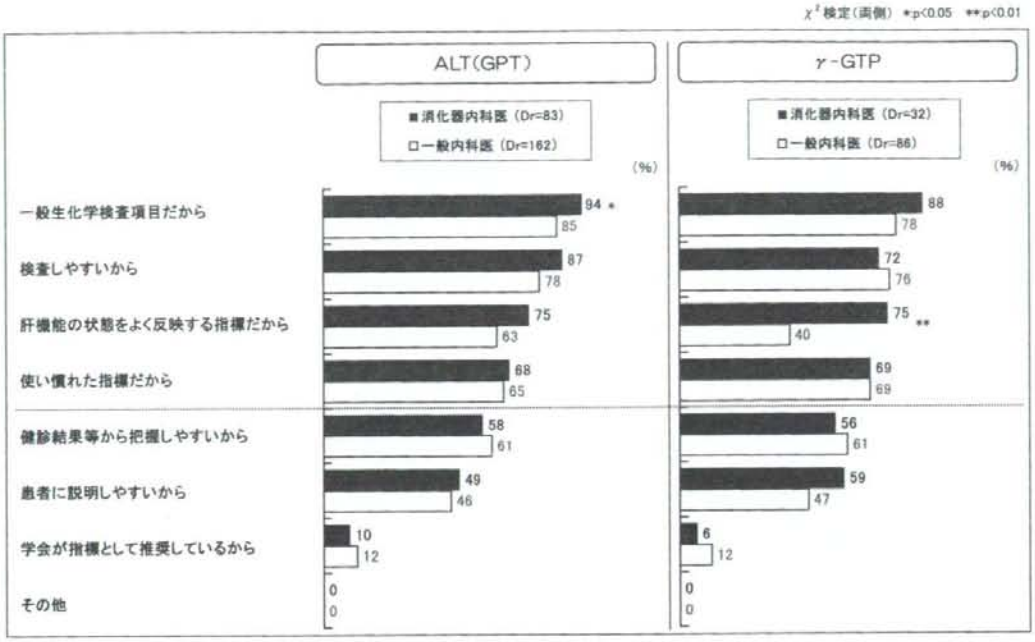

Figure 9. 脂肪性肝疾患：ALT・γGTPが薬物療法基準となる理由（問12）

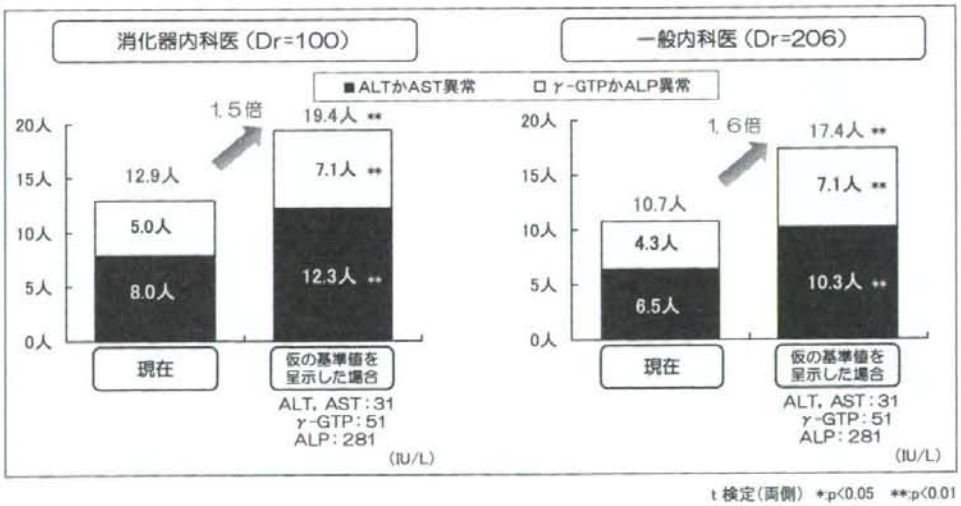

Figure 10. 脂肪性肝疾患：薬物療法導入基準からみた医師1人あたりの平均患者数（1カ月）（問13・14）

がどのように認識しているかを知る目的でアンケート調査を実施したが，消化器内科医と一般内科医との間で肝機能検査の臨床的位置づけが大きく異なっていた．

一方，近年血清ALT正常値は30IU/L以下とする考えが強く，肝機能正常C型慢性肝炎患者に対する治療ガイドラインの中でも，血清ALT値31～40IU/Lかつ血小板数15万/μL未満の症例では，病期進展例が多く慢性肝炎治療に準じた治療を行うとされている．本調査結果から考慮す

ると，本来治療が必要とされる多くの患者が未治療のままフォローされている可能性が高い．

血清γ-GTP値については胆道系障害の指標としての役割だけでなく，2型糖尿病や心血管系疾患，メタボリックシンドローム発症の独立した危険因子であることが近年報告されている[5)~7)]．血清γ-GTP値上昇がこれらの疾患の病態とどのように関与しているのか，発症のマーカーの1つであるのか，現時点では充分解明されていない．しかし，生理的な役割として抗酸化物質の1つであるグルタチオンの分解酵素という側面もあり[8)]，血中に上昇した血清γ-GTP値が酸化ストレス障害の増悪因子の1つであると考えるならば，血清γ-GTP値についても肝疾患における臨床的意義や治療導入や目標の基準値の認識を改める必要がある．

また，平成20年4月からは「標準的な健診・保健指導プログラム」に基づき健診者への保健指導が開始された．メタボリックシンドロームに関連する検査に加え，肝機能検査として血清ALT，AST，γ-GTP値の保健指導判定値，受診勧奨判定値が決定されたことにより，国民は生活習慣病としての肝機能異常を今後意識していくことになる．

以上より，臨床医は血清ALT，AST値や血清γ-GTP値などの上昇を生命予後などに悪影響を及ぼすイベント発生の予測因子として扱うことと同時に，肝細胞・胆管細胞障害や機能障害が発生している状態の証であるということを再認識し，肝機能異常の程度やその異常値の持続期間にかかわらず，数値が上昇する臨床的意義を考慮し治療介入を検討する必要性がある．肝機能検査値については基準値や治療基準が未統一であり，また診療する医師の間に肝障害に対する認識の大きな差があることが大きな問題である．

文　献

1) Ikeda K, Arase Y, Saitoh S, et al : Anticarcinogenic impact of interferon on patients with chronic hepatitis C : a large-scale long-term study in a single center. Intervirology 49 : 82–90 : 2006
2) 岡上　武：肝機能正常C型肝炎患者に対する治療ガイドライン．厚生労働科学研究費補助金肝炎等克服緊急対策研究事業（肝炎分野）平成17年度共同研究報告書　13–18 : 2005
3) Okanoue T, Makiyama A, Nakayama M, et al : A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. J Hepatol 43 : 599–605 : 2005
4) Okanoue T, Itoh Y, Minami M, et al : Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts. Hepatol Res 38 : 27–36 : 2008
5) Doi Y, Kubo M, Yonemoto K, et al : Liver enzymes as a predictor for incident diabetes in a Japanese population : the Hisayama study. Obesity 15 : 1841–1850 : 2007
6) Nakanishi N, Suzuki K, Tatara K : Serum gamma-glutamyltransferase and risk of metabolic syndrome and type 2 diabetes in middle-aged Japanese men. Diabetes Care 27 : 1427–1432 : 2004
7) Ruttmann E, Brant LJ, Concin H, et al : Gamma-glutamyltransferase as a risk factor for cardiovascular disease mortality : an epidemiological investigation in a cohort of 163,944 Austrian adults. Circulation 112 : 2130–2137 : 2005
8) Whitfield JB : Gamma glutamyl transferase. Crit Rev Clin Lab Sci 38 : 263–355 : 2001

論文受領，平成20年4月21日
受理，平成20年7月25日

Management of hepatitis C and fatty liver : a consciousness survey among gastroenterologists and general internists

Takeshi OKANOUE, Toshihide SHIMA, Masayuki MIZUNO, Mika MATSUMOTO and Atsushi UMEMURA[1]

[1] *Division of Gastroenterology, Saiseikai Suita Hospital*

A questionnaire survey including hepatic function test values was performed to elucidate the medical treatment of hepatic diseases by gastroenterologists and general internists. Serum ALT level was considered to be an index of destruction of hepatic cells in 94 and 80% of gastroenterologists and general internists, respectively. Serum γ-GTP values were used as an index of bile stasis and destruction of the bile duct cells by 93% and 70% of the gastroenterologists, respectively and in 61% and 49% of the internists, respectively. In addition, for hepatitis C, gastroenterologists considered the mean serum ALT values (standard values for drug therapy introduction) as ≧62IU/L, while general internists considered it as ≧79IU/L. In the case of fatty liver, the mean serum ALT values considered by gastroenterologists and general internists were ≧93IU/L and ≧90IU/L, respectively. These survey results suggest that there is a need for research-based clarification of hepatic function test values regulating therapy and unification of guidelines for standard values in medical treatment.

Hepatology Research 2008; **38**: 27–36 doi: 10.1111/j.1872-034X.2007.00217.x

Special Report

Guidelines for the antiviral therapy of hepatitis C virus carriers with normal serum aminotransferase based on platelet counts

Takeshi Okanoue,[1] Yoshito Itoh,[1] Masahito Minami,[1] Hiroaki Hashimoto,[1] Kohichiro Yasui,[1] Hiroshi Yotsuyanagi,[2] Tetsuo Takehara,[3] Takashi Kumada,[4] Eiji Tanaka,[5] Shuhei Nishiguchi,[6] Namiki Izumi,[7] Michio Sata,[8] Morikazu Onji,[9] Gotaro Yamada,[10] Kiwamu Okita[11] and Hiromitsu Kumada[12]

[1]Molecular Gastroenterology and Hepatology, Kyoto Prefectural University of Medicine, Kyoto, [2]Department of Infectious Diseases, University of Tokyo, Tokyo, [3]Department of Gastroenterology and Hepatology, Osaka University, Osaka, [4]Department of Gastroenterology, Ogaki Municipal Hospital, Gifu, [5]Department of Internal Medicine, Shinshu University, Matsumoto, [6]Department of Internal Medicine, Hyogo College of Medicine, Hyogo, [7]Department of Gastroenterology and Hepatology, Musashino Red-Cross Hospital, Musashino, [8]Second Department of Internal Medicine, Kurume University, Kurume, [9]Department of Gastroenterology and Metabology, Ehime University, Matsuyama, [10]Department of Gastroenterology and Metabology, Kawasaki Hospital, Okayama, [11]Center of Liver Disease, Social Insurance Alliance Shimonoseki Hospital, and [12]Department of Hepatology, Toranomon Hospital, Tokyo, Japan

Aim: We aimed to identify the candidates for antiviral therapy, among patients who are hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT), focused on the inhibition of hepatocellular carcinoma (HCC).

Methods: Four hundred and sixty-four HCV carriers with normal serum ALT and 129 HCV carriers with persistently normal ALT (PNALT) and platelet (PLT) counts ≥150 000/μL who received liver biopsies were enrolled. HCV carriers with normal serum ALT were divided into four groups according to their ALT levels (≤30 U/L or 31–40 U/L) and PLT counts (≥150 000/μL or <150 000/μL).

Results: In 129 HCV carriers with PNALT, the rate of progression of fibrosis stage was 0.05/year and no HCC was detected during the follow up for 10 years. Approximately 20% of patients with ALT ≤40 U/L and PLT counts ≥150 000/μL were at stage F2–3; however, approximately 50% of patients with ALT ≤ 40 U/L and PLT counts <150 000/μL were at stage F2–4. An algorithm for the management of HCV carriers with normal serum ALT was advocated based on ALT and PLT counts.

Conclusion: The combination of ALT and PLT counts is useful for evaluating the fibrosis stage in HCV carriers with normal serum ALT. Most patients with PLT counts <150 000/μL are candidates for antiviral therapy, especially those with ALT levels ≥31 U/L when we focus on the inhibition of the development of HCC.

Key words: antiviral therapy, chronic hepatitis C, hepatitis C virus carriers, normal serum aminotransferase, platelet count

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) caused by hepatitis C virus (HCV) infection usually develops in patients with advanced chronic hepatitis (CH) or liver cirrhosis. The antiviral treatment for chronic hepatitis C (CH-C) is useful for inhibiting hepatic inflammation and progression of hepatic fibrosis, and consequently the development of HCC.[1–6]

Serum aminotransferase (ALT) levels are within the normal ranges in 20–40% of patients with chronic HCV infection,[7–11] defining the upper limit of normal serum ALT as ≤40 U/L. Significant hepatic fibrosis (≥F2 by the METAVIR classification) has been demonstrated in 5–30% of such patients.[9,12–16] We reported previously

Correspondence: Dr Yoshito Itoh, Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kamigyo-ku, Kyoto 602-8566, Japan. Email: yitoh@koto.kpu-m.ac.jp
Received 6 March 2007; revision 22 May 2007; accepted 14 June 2007.

that HCV carriers with persistently normal ALT (PNALT) had histological features ranging from normal to minimal CH[17,18]; they showed slow progression of liver fibrosis and were at very low risk of developing HCC.[18]

The National Institute of Health Consensus Development Conference reported that HCV carriers with normal serum ALT are candidates for antiviral therapy.[19] A controlled study for the treatment of HCV carriers with PNALT with pegylated interferon alpha and ribavirin (PEG-IFN/Riba) for 48 weeks led to the eradication of HCV RNA in 40% of patients with genotype 1 and high viral load,[20] which is similar to the results of CH-C patients with elevated ALT levels.[21,22] However, it remains controversial whether these patients are candidates for antiviral therapy because of the limited efficacy of treatment, post-treatment flare-up, various side-effects, high cost of treatment, and their good prognoses.

In many Western countries, the upper limits of normal serum ALT are below 40 U/L;[23] however, a recent report from Italy demonstrated that the upper limit in healthy individuals was less than 30 U/L for men and 19 U/L for women.[24] We attempted to draft therapeutic guidelines for the treatment of HCV carriers with normal serum ALT. The biochemical and histological analyses were performed in HCV carriers with serum ALT levels below 40 U/L. These patients were divided into two groups based on ALT levels and then further divided into two subgroups according to their platelet (PLT) counts. We proposed an algorithm for the treatment of HCV carriers with normal serum ALT, taking into consideration the risk of progression to cirrhosis and the development of HCC. The present study demonstrated that the ranges of serum ALT and PLT counts are useful for deciding the indication of antiviral therapy for HCV carriers with normal serum ALT.

METHODS

Eligibility and definition

TWELVE HEPATOLOGISTS BELONGING to the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis, supported by the Ministry of Health, Labour and Welfare of Japan, which was settled on April 2004, participated in the study. Hiromitsu Kumada (Toranomon Hospital, Tokyo, Japan) serves as a chief and Takeshi Okanoue served as a researcher responsible for drafting the guidelines for the treatment of HCV carriers with normal serum ALT. In the present study, we tentatively defined the upper limit of the normal serum ALT as ≤40 U/L.

Patients with hepatitis B virus surface antigen, previous IFN treatment, history of heavy alcohol abuse, antinuclear antibody or antismooth muscle antibody, overt diabetes mellitus, or obesity (body mass index; ≥25 kg/m^2) were excluded from the study.

All of the patients underwent liver biopsy (≥2.0 cm in length) within 6 months prior to antiviral therapy, at which time their serum ALT levels were ≤40 U/L. Informed consent was obtained from every patient prior to liver biopsy and antiviral therapy.

Another study was conducted from January 1990 to August 2004 at Kyoto Prefectural University of Medicine (Kyoto, Japan). HCV carriers with PNALT were defined by serum ALT levels ≤30 U/L on at least three different occasions over a 12-month period and PLT counts ≥150 000/μL as reported previously.[18]

Study design

Among the 580 HCV carriers with normal serum ALT (≤40 U/L), 116 patients were excluded from the study because of insufficient data. Thus, 464 patients who received antiviral therapy from 1995 to 2004 were enrolled in this study (Table 1). Formalin-fixed liver specimens were stained with hematoxylin–eosin, and with Masson's trichrome. The liver specimens ($n = 262$) were also stained with Perls' Prussian blue to study hepatic iron loading. The histological findings were scored according to the classification proposed by Desmet *et al.*[25] and Ishak *et al.*[26] Steatosis was defined as fat droplets in >10% of hepatocytes. The degree of iron loading was assessed using a Perls' score of 0–4+, based on the scoring system of MacSween *et al.*[27]

The serum ALT, blood glucose level, immunoreactive insulin (IRI), serum ferritin, PLT count, serum hyaluronic acid, amount of serum HCV RNA, and the HCV genotype were examined. The homeostasis model assessment-insulin resistance was calculated as follows: plasma fasting glucose (mg/dL) × IRI (ng/mL) ÷ 405. The serum HCV RNA levels were determined using an Amplicor GT HCV monitor (Roche Diagnostic Systems, Tokyo, Japan). HCV genotype 1 (G1) and 2 (G2) were determined by a serologic genotyping assay.[28] G1 and G2 in this assay correspond to genotype 1 (1a, 1b) and 2 (2a, 2b) proposed by Simmonds *et al.*[29]

All the patients received IFN monotherapy or IFN/Riba combination therapy for 12–36 weeks. The average

Table 1 Baseline of hepatitis C virus patients with normal serum aminotransferase (ALT) received antiviral therapy

	ALT ≤ 30 U/L (group A)	ALT 31–40 U/L (group B)	*P*-value
No. patients	255	209	
Age	51.6 ± 13.0	53.5 ± 13.2	0.548*
Sex (male/female)	112/143	117/92	0.01**
BMI (kg/m^2)	21.6 ± 2.9	22.8 ± 3.0	<0.001*
HOMA-IR	2.5 ± 3.2	5.2 ± 6.5	0.093*
Genotype: 1/2/others	127/127/1	112/96/1	0.881**
Viral load: low/high	138/117	99/110	0.203**
G1 (low/high)	114/125		
G2 (low/high)	161/62		
Histology			
F stage (0/1/2/3/4)	29/166/48/11/1	22/122/57/6/2	0.169**
Grade (0/1/2/3)	25/187/41/2	7/159/43/0	0.046**
Fatty change† 0–1/2–4	232/23	161/48	0.033**
Iron load‡ 0/1–4	101/15	97/19	0.458**
Ferritin (ng/mL)	83.9 ± 103.7	118.8 ± 135.3	0.006*
PLT count (/μL)	19.2 ± 5.4	18.4 ± 6.1	0.059*
≥150 000/<150 000	204/51	141/68	0.002**
Hyaluronate (ng/mL)	60.8 ± 73.7	69.1 ± 73.0	0.249*
Duration of antiviral therapy (weeks)	25.6 ± 12.0	26.1 ± 12.1	0.297*
Effects of therapy			
SVR/non-SVR	142/113	99/110	0.075**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in sex distribution ($P = 0.01$), BMI ($P = 0.01$), frequency of steatosis ($P = 0.033$), serum ferritin level ($P = 0.006$), grade of hepatic inflammation ($P = 0.046$), incidence of fatty change ($P = 0.033$), serum ferritin level ($P = 0.006$), and the incidence of low PLT counts ($P = 0.002$) between groups A and B. Values are expressed as mean ± SD.

ALT, alanine aminotransferase; BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; PLT, platelet; SVR, sustained viral responders.

duration of therapy between 1995 and 2003 was 26 weeks for IFN monotherapy and 24 weeks for IFN/Riba combination therapy. In principle, 6–10 MU IFN was administered daily for 2 weeks and three times per week subsequently. The daily dosage of ribavirin was 600–1000 mg depending on body weight. Sustained viral responders (SVR) were defined as patients who were negative for serum HCV RNA 6 months after the completion of antiviral therapy.

All of the patients were divided into two groups (group A: ALT ≤ 30 U/L, group B: 31 U/L ≤ ALT ≤ 40 U/L) which were further divided into two subgroups based on PLT counts: group A-1 and B-1 (PLT counts ≥150 000/μL) and groups A-2 and B-2 (PLT counts <150 000/μL).

One hundred and twenty-nine HCV carriers with PNALT were enrolled to determine their long-term prognosis. These patients showed normal serum ALT levels (≤30 U/L) over a 12-month period on least three different occasions (PLT counts ≥150 000/μL, and body mass index [BMI] <25 kg/m^2). Thirty-nine patients received serial liver biopsies. The mean follow-up period of the 129 patients was 7.2 ± 3.2 years on 15 November 2006.

Statistical analyses

Data are expressed as mean ± SD. We compared continuous variables using the Mann–Whitney *U*-test. A frequency analysis and comparison between the groups were performed using the χ^2-test or Fisher's exact test and the Mann–Whitney *U*-test. ANOVA and Tukey's HSD procedure was used to determine the difference between multiple groups. All tests were two-tailed and *P*-values of less than 0.05 were considered significant. All statistical analyses were performed using Statistical Package of Services Solutions software, version 11.0 (SPSS, Chicago, IL, USA).

Table 2 Baseline of hepatitis C virus patients with less than 30 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group A-1)	PLT < 150 000/μL (group A-2)	*P*-value
No. patients	204	51	
Age	48.4 ± 12.7	58.7 ± 7.5	<0.001*
Sex (male/female)	90/114	22/29	1.000**
BMI (kg/m^2)	21.6 ± 3.0	21.3 ± 2.4	0.514*
HOMA-IR	2.8 ± 3.5	1.2 ± 0.8	0.598*
Genotype: 1/2/others	101/101/2	25/26/0	0.952**
Viral load: low/high	112/92	26/25	0.574**
Histology			
F stage (0/1/2/3/4)	29/142/27/6/0	1/25/21/3/1	<0.001**
Grade (0–1/2,3)	179/25	33/18	<0.001**
Fatty change† 0–1/2–4	188/16	44/7	0.582**
Iron load‡ 0/1–4	82/12	17/3	0.762**
Ferritin (ng/mL)	86.0 ± 112.1	73.9 ± 46.6	0.204*
PLT count (/μL)	21.0 ± 4.4	12.1 ± 2.5	<0.001*
Hyaluronate (ng/mL)	41.8 ± 56.1	112.5 ± 109.9	<0.001*
Duration of antiviral therapy (weeks)	25.7 ± 10.3	27.0 ± 9.9	0.503*
Effects of therapy			
SVR/non-SVR	115/89	27/24	0.66**

**P*-values were calculated by Mann–Whitney-*U*-test. ***Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. There were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), grade of inflammatory activity ($P < 0.001$), PLT count ($P < 0.001$), and serum-hyaluronic acid ($P < 0.001$) between groups A-1 and A-2. Frequency of F2–4 patients was 16.2% in group A-1 and 51.6% in group A-2. Values are expressed as mean ± SD.

BMI, body mass index; HOMA-IR, homeostasis model assessment–insulin resistance; PLT, platelet counts; SVR, sustained viral responders.

RESULTS

Demographic, clinical, and histological features of 464 HCV carriers with normal serum ALT

THE CHARACTERISTICS OF the 464 HCV carriers with normal serum ALT are shown in Table 1. There were significant differences in sex, frequency of steatosis, serum ferritin levels, BMI, and the incidence of low PLT counts (<150 000/μL) between groups A and B.

There were significant differences in age, fibrosis (F) stage, inflammatory activity, PLT counts, and serum hyaluronate between groups A-1 and A-2 (Table 2). The frequency of stage F2–4 patients was 16.2% in group A-1, and 49.0% in group A-2 (Table 2). In group B, there were significant differences in age, F stage, PLT counts, and serum hyaluronate between groups B-1 and B-2 (Table 3). There were no F4 patients in group A-1 and B-1, and the frequency of F3 patients was very low compared with those in groups A-2 and B-2 (2.6% *vs* 7.6%). The PLT counts decreased in proportion to the progression of liver fibrosis as follows; F0 ($n = 51$); $20.7 \pm 5.2 \times 10^4$/μL, F1 ($n = 288$); $19.8 \pm 5.6 \times 10^4$/μL, F2 ($n = 105$); $16.9 \pm 5.3 \times 10^4$/μL, F3 ($n = 17$); $15.9 \pm 4.6 \times 10^4$/μL, and F4 ($n = 3$); $11.3 \pm 3.8 \times 10^4$/μL.

Of the 464 patients, the frequency of the F0–1 stages was 80.1% and that of the F2–4 stages was 19.9% in patients with PLT counts ≥150 000/μL, and it was 50.4% and 49.6%, respectively, in patients with PLT counts <150 000/μL. In patients with PLT counts $\geq 17.0 \times 10^4$/μL, 80.8% were in stages F0–1 and 19.2% were in stages F2–4, and in patients with PLT counts $< 17.0 \times 10^4$/μL, 60.1% were in stages F0–1 and 39.9% were in stages F2–4.

The SVR rates of IFN therapy were 52.4% in F0–1 patients, 49.5% in F2–4 patients ($P = 0.896$ by Fisher's exact test), and 58.0% and 43.8% ($P = 0.592$) in IFN/Riba therapy, respectively.

In patients with genotype 1b and high viral load, the SVR rate was 12.5%. The SVR rate in genotype 2 patients was 60.4% in the IFN group and 67.7% in the IFN/Riba combination therapy group.

Table 3 Baseline of hepatitis C virus carriers with 31–40 U/L aminotransferase who received antiviral therapy

	PLT ≥ 150 000/μL (group B-1)	PLT < 150 000/μL (group B-2)	*P*-value
No. patients	141	68	
Age	48.2 ± 11.9	57.9 ± 7.5	<0.001*
Sex (male/female)	80/61	37/31	0.751**
BMI (kg/m^2)	22.9 ± 3.1	22.7 ± 2.6	0.08*
HOMA-IR	3.0 ± 2.0	8.2 ± 9.5	0.8.8*
Genotype: 1/2/others	82/58/1	30/38/0	0.095**
Viral load: low/high	64/77	35/33	0.542**
Histology			
F stage (0/1/2/3/4)	17/91/31/2/0	4/30/26/6/2	<0.001**
Grade (0–1/2,3)	116/25	50/18	0.114**
Fatty change† 0–1/2–4	111/30	50/18	0.10**
Iron load‡ 0/1–4	67/12	30/7	0.762**
Ferritin (ng/mL)	114.4 ± 116.1	127.2 ± 167.8	0.869*
PLT count (/μL)	21.5 ± 4.9	12.2 ± 2.1	<0.001*
Hyaluronate (ng/mL)	46.9 ± 35.4	100.7 ± 0.98.1	<0.001*
Administration of IFN (weeks)	26.1 ± 11.9	27.7 ± 11.4	0.983*
Effects of therapy			
SVR/non-SVR	64/77	35/33	0.409**

**P*-values were calculated by Mann–Whitney-*U*-test. **Fisher-exact-test. †0: no fatty change, 1: ≤10%, 2: 11–33%, 3: 34–66%, 4: ≥67% of hepatocyte; ‡no stain by 400×, 1: few stains by 250×, 2: stains by 100×, 3: stains by 25×, 4: stains by 10×. In group B, there were significant differences in age ($P < 0.001$), distribution of F stage ($P < 0.001$), PLT count ($P < 0.001$), and hyaluronic acid ($P < 0.001$) between B-1 and B-2. Frequency of F2–4 was 23.4% in B-1and 50.0% in B-2, respectively. Values are expressed as mean ± SD.
BMI, body mass index; HOMA-IR, homeostasis model assessment-insulin resistance; IFN, interferon; PLT, platelet counts; SVR, sustained viral responders.

Demographic, clinical, and histological features of 129 HCV carriers with PNALT

The demographic and clinical features of the 129 HCV carriers with PNALT who were followed up for 7.2 years are shown in Table 4. Normal liver histology was noted in 17 patients, 102 showed minimal to mild CH, and 10 had moderate CH. Steatosis was seen in 7% and iron loading was noted in 12%.[18]

Of the 78 patients followed longer than 7 years (mean follow-up period; 10.4 ± 3.1 years), 11 (14%) had continuously normal ALT (G-1), 43 (55%) showed a transient elevation of ALT (G-2), and 24 (31%) changed to CH with continuously elevated ALT (G-3).

Thirty-nine patients received repeated liver biopsies (2–4 times). Of the 39 patients, six were in G-1, 17 were in G-2, and 16 were in G-3. The intervals between the first biopsy and the last biopsy in these three groups were 7.1, 7.8, and 7.2 years, respectively. The progression of the F stage was noted in two of six in G-1, six of 17 in G-2, and seven of 16 in G-3. The median rates of fibrosis progression per year for these three groups were 0.05, 0.05, and 0.08 fibrosis unit. HCC was not detected in any patients during the follow-up periods.

Guidelines for the antiviral therapy of HCV carriers with normal serum ALT focused on the inhibition of the development of HCC

Considering the risk of progression to liver cirrhosis and the development of HCC, as well as the expected efficacy and various side-effects of antiviral therapy, an algorithm is needed for the management of HCV carriers with normal serum ALT. The progression rate of liver fibrosis stage was 0.05/year in HCV carriers with PNALT. The annual incidence of HCC in CH-C patients has been reported to be 0.5% at stages F0–F1, 1–2% at stage F2, 3–5% at stage F3, and 7% at stage F4.[4]

In principle, follow up without antiviral treatment is recommended for HCV carriers with PNALT (ALT ≤30 U/L) and PLT counts ≥150 000/μL, particularly in older patients (i.e. >65 years old), because over 90% show normal or minimal liver damage with good prognoses. However, antiviral therapy is not contraindicated for such patients since roughly 40% are infected with HCV genotype 2,[18] which suggests a high rate of SVR to the therapy with PEG-IFN/Riba.

As for the indication of antiviral therapy for HCV carriers with normal serum ALT (≤40 U/L), the PLT

Table 4 Characteristics of 129 HCV carriers with persistently normal ALT who received liver biopsy

	$n = 129$	Follow up over 5 years ($n = 78$)
Follow-up period (years)	7.2 ± 3.2	10.4 ± 3.1
Age (years)	48 (21–77)	45 (29–71)
Male ($n = 24$)	49.8 ± 16.4	42.3 ± 14.9
Female ($n = 105$)	47.2 ± 12.5	46.6 ± 11.6
Sex (male/female)	24/105	10/68
ALT (U/L)	8–30	9–30
Male ($n = 24$)	22.5 ± 5.7	21.1 ± 5.4
Female ($n = 105$)	21.6 ± 4.8	22.3 ± 5.1
PLT ($\times 10^4/\mu L$)	15–31	15–31
Ferritin (ng/mL)	5–225	5–225
Male ($n = 24$)	76.2 ± 53.5	84.6 ± 59.2
Female ($n = 105$)	60.0 ± 43.3	66.6 ± 52.5
HCV genotype	G1 ($n = 58$), G2 ($n = 45$) Mixed and unclassified ($n = 16$)	
BMI (kg/m^2)	16–27	16–27
Male	22.2 ± 1.7	21.9 ± 1.9
Female	21.3 ± 2.2	21.0 ± 2.4

Values are expressed as mean ± SD.
ALT, alanine aminotransferase; BMI, body mass index; HCV, hepatitis C virus; PLT, platelet.

count is a good indicator for discriminating as to whether or not they have minimal to mild fibrosis or moderate to advanced fibrosis. Serum hyaluronate levels were significantly higher in HCV carriers with 31–40 U/L ALT having less than 150 000/μL PLT (Table 3). Advanced hepatic F stage, an elevated ALT level, old age (>65 years old), and sex (male) are important risk factors for the development of HCC.[6,18,30] We advocated an algorithm for such patients (Fig. 1) taking into consideration the risk of the progression to cirrhosis and the development of HCC. Therapy with PEG-IFN/Riba is the first-line treatment; therapy for 48 weeks is recommended for genotype 1 patients with high viral load and 12–24 weeks therapy for genotypes 2 and 1 with low viral load.

DISCUSSION

OUR PREVIOUS STUDY in 129 HCV carriers with PNALT demonstrated a predominance of females, higher frequency of genotype 2, minimal to mild liver histology, and very slow progression of hepatic fibrosis.[18] However, over 30% of these patients advanced to CH-C with elevated ALT levels during the 7-year follow up.

There are many reports concerning the natural course of liver fibrosis in CH-C patients, including those who are HCV carriers with normal serum ALT.[19,31–39] More than half of CH-C patients show progression of F stage from F1 to F2–4 within 10 years, and it was reported that the progression of liver fibrosis in HCV carriers with normal serum ALT was more rapid than was observed in the present study.[23] The main reason for the discrepancy between the report by Puoti *et al.*[23] and our results might be due to the definitions used for the normal range of serum ALT. In our previous study, the patients were HCV carriers with PNALT (ALT ≤ 30 U/L) and PLT counts ≥150 000/μL. On the other hand, the patients in the study by Puoti *et al.* had ALT levels ≤40 U/L, irrespective of PLT counts, in which cirrhotic patients might be included.[23] However, recent studies have demonstrated that normal ALT levels are less than 30 U/L[24] or 25 U/L in men[40] and less than 19 U/L[24] or 22 U/L in women.[40]

The present study demonstrated that the different distribution of hepatic F stage became remarkable when the A and B groups were divided into two subgroups according to their PLT counts. In HCV carriers with ALT levels ≤30 U/L, the frequency of stages F2–3 was 16.2% among those with PLT counts ≥150 000/μL; however, the frequency of stages F2–3 was 49.0% in those with PLT counts <150 000/μL. Conversely, in HCV carriers with ALT levels between 31 and 40 U/L, the frequency of stages F2–4 was 23.4% among those with PLT counts ≥150 000/μL and 50.0% in those with PLT counts <150 000/μL. The PLT count is a useful marker in dis-

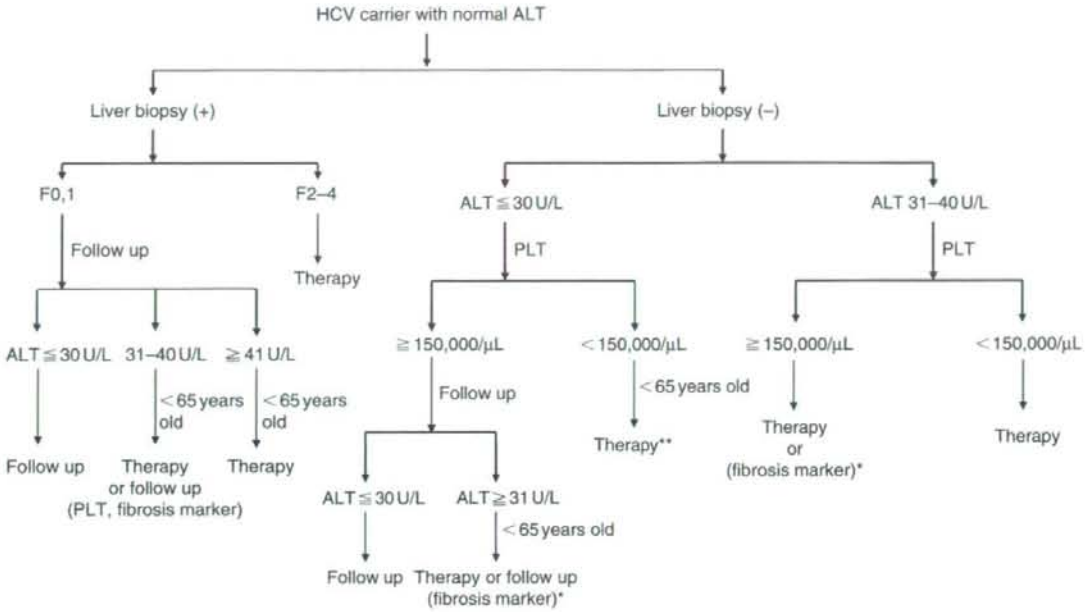

Figure 1 Algorithm for the management of hepatitis C virus (HCV) carriers with normal serum aminotransferase (ALT, ≤40 U/L) focused on the inhibition of the development of hepatocellular carcinoma. In patients who underwent liver biopsy, F0 and F1 patients younger than 65 years are candidates for antiviral therapy, especially those with genotype 2 after the elevation of serum ALT levels. In patients who did not undergo liver biopsy, ALT and platelet (PLT) levels are good indicators for determining candidates for antiviral therapy. Older patients (>65 years) and/or patients having uncontrolled hypertension, diabetes mellitus, or anemia should not be treated with pegylated interferon and ribavirin. Combination therapy with pegylated interferon and ribavirin for 48–72 weeks is recommended for patients with genotype 1 and high viral load, and 12–24 weeks therapy is suggested for patients with genotype 2 and genotype 1 with low viral load. *,**Serum fibrosis markers, such as hyaluronate, might be useful to decide whether patients are candidates for antiviral therapy or not.

criminating between stages F0–1 and F2–4 F in HCV carriers with normal serum ALT (≤40 U/L). In the present study, the mean PLT count in F2 and F3 patients was 16.9 ± 5.3 ($\times 10^4/\mu L$) and 15.9 ± 4.6 ($\times 10^4/\mu L$), respectively. The distribution of the F stage was not significantly different between patients with PLT counts $\geq 15 \times 10^4/\mu L$ versus $< 15 \times 10^4/\mu L$ and $\geq 17 \times 10^4/\mu L$ versus $< 17 \times 10^4/\mu L$.

The SVR rate for genotype 1 patients with high viral load treated with either IFN monotherapy or IFN/Riba were 12.5% and 37.7%, respectively. In genotype 2 patients with high viral load, the SVR rate in the present study was better than the data of Japanese CH-C patients with elevated ALT levels in our previous paper.[6] It was not reasonable to compare the SVR rates between HCV carriers with normal serum ALT and CH-C with elevated ALT in the present study, because the total dosage of IFN and the duration of treatment were significantly different.

The annual incidence of HCC is correlated with the progression of liver fibrosis, that is, the stage of liver disease.[2–4,8] Sustained low serum ALT levels are also associated with a lower incidence of HCC.[2,6,41] PEG-IFN/Riba therapy is expensive and induces various side-effects. The present results indicate that most HCV carriers with normal serum ALT (≤40 U/L) and PLT counts ≥150 000/μL have minimal to mild liver damage, indicating a low risk for the progression to cirrhosis and the development of HCC. This was more remarkable in patients with ALT levels ≤30 U/L and PLT counts ≥150 000/μL. However, nearly half of the patients with PLT count <150 000/μL have F2 or F3 F stages, indicating a certain risk for the progression to cirrhosis and the development of HCC. Fibrosis

progression is associated with age, baseline and follow-up ALT levels, inflammatory activity and steatosis in the initial liver biopsy, and alcohol consumption.[42] The present results indicate that most HCV carriers with PNALT have a good prognosis and a low risk of developing HCC.

Liver biopsy is a useful procedure for identifying the stage of liver fibrosis; however, it is invasive and may sometimes cause complications.[43,44] The error rate of predicting the F stage with this procedure can be estimated to be as high as 20%.[45] Recently introduced biochemical markers, such as FibroTest,[46] and FibroScan,[47–49] are excellent procedures for identifying liver fibrosis stage in CH-C patients.[50] The combined use of FibroScan and FibroTest is useful for accurately estimating moderate to severe liver fibrosis in most patients with CH-C, but not in F0 and F1 patients.[51]

Recently, Alberti proposed an individualized management algorithm for HCV carriers with PNALT with or without liver biopsy in which HCV genotype, patient age, motivation to receive antiviral therapy, and factors influencing side-effects were included.[52] The algorithm using a combination of serum ALT levels and PLT counts in the present study is simple, but it is useful because it focuses mainly on the inhibition of the progression to cirrhosis and the development of HCC.

ACKNOWLEDGMENTS

THIS PROJECT WAS supported in part by a grant-in-aid from the Ministry of Health, Labour and Welfare of Japan. Twelve hepatologists were from the Japanese Study Group of the Standard Antiviral Therapy for Viral Hepatitis (chief: Hiromitsu Kumada, Toranomon Hospital).

REFERENCES

1 Kasahara A, Hayashi N, Mochizuki K *et al.* Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology* 1998; **27**: 1394–402.
2 Okanoue T, Itoh Y, Minami M *et al.* Interferon therapy lowers the rate of progression to hepatocellular carcinoma in chronic hepatitis C but not significantly in advanced stage: a retrospective study in 1148 patients. *J Hepatol* 1999; **30**: 653–9.
3 Ikeda K, Saitoh S, Arase Y *et al.* Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology* 1999; **29**: 11–19.
4 Yoshida H, Shiratori Y, Moriyama M *et al.* Interferon therapy reduced the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. IHIT Study Group. Inhibition of hepatocarcinogenesis by interferon therapy. *Ann Inter Med* 1999; **131**: 174–81.
5 Tanaka H, Tsukuma H, Kasahara A *et al.* Effect of interferon therapy on the incidence of hepatocellular carcinoma and mortality of patients with chronic hepatitis C: retrospective cohort study of 738 patients. *Int J Cancer* 2000; **87**: 741–9.
6 Okanoue T, Itoh Y, Kirishima T *et al.* Transient biochemical response in interferon therapy decreases the development of hepatocellular carcinoma for five years and improves the long-term survival of chronic hepatitis C patients. *Hepatol Res* 2002; **23**: 62–77.
7 Serfaty L, Nousbaum JB, Elghouzzi MH, Giral P, Legendre C, Poupon R. Prevalence, severity, and risk factors of liver disease in blood donors positive in a second-generation anti-hepatitis C virus screening test. *Hepatology* 1995; **21**: 330–7.
8 Piton A, Poynard T, Imbert-Bismut F *et al.* Factors associated with serum alanine transaminase activity in healthy subjects: consequences for patients with chronic hepatitis C. *Hepatology* 1998; **27**: 1213–19.
9 Alberti A, Noventa F, Benvegnu L, Boccata S, Gatta A. Prevalence of liver disease in a population of asymptomatic individuals with hepatitis C virus infection. *Ann Intern Med* 2002; **137**: 961–4.
10 Tassopoulos NC. Treatment in patients with normal ALT levels. European Association for the Study of the Liver (EASL) International Conference on Hepatitis C, Paris, February 26–27, 1999. *J Hepatol* 1999; **30**: 956–61.
11 Marcellin P, Levy S, Erlinger S. Therapy of hepatitis C: patients with normal aminotransferase levels. *Hepatology* 1997; **26**: 133S–6.
12 Jamal MM, Soni A, Quinn PG, Wheeler DE, Arora S, Johnston DE. Clinical features of hepatitis C-infected patients with persistently normal alanine transaminase levels in the Southwestern United States. *Hepatology* 1999; **30**: 1307–11.
13 Nutt AK, Hassan HA, Lindsey J, Lamps LW, Raufman JP. Liver biopsy in the evaluation of patients with chronic hepatitis C who have repeatedly normal or near-normal serum alanine aminotransferase levels. *Ann Intern Med* 2000; **109**: 62–4.
14 Pradat P, Alberti A, Poynard T *et al.* Predictive value of ALT levels for histologic findings in chronic hepatitis C: a European collaborative study. *Hepatology* 2002; **36**: 973–7.
15 Hui CK, Monto A, Belaye T, Lau E, Wright TL. Outcomes of interferon alpha and ribavirin treatment for chronic hepatitis C in patients with normal serum aminotransferase. *Gut* 2003; **52**: 1644–8.
16 Renou C, Halfon P, Pol S *et al.* Histological features and HLA class II alleles in hepatitis C virus chronically infected

patients with persistently normal alanine aminotransferse levels. *Gut* 2002; **51**: 585–90.
17 Okanoue T, Yasui K, Sakamoto S *et al.* Circulating HCV RNA, HCV genotype, and liver histology in asymptomatic individuals reactive for anti-HCV antibody and their follow-up study. *Liver* 1996; **16**: 241–7.
18 Okanoue T, Makiyama A, Nakayama M *et al.* A follow-up study to determine the value of liver biopsy and need for antiviral therapy for hepatitis C virus carriers with persistently normal serum aminotransferase. *J Hepatol* 2005; **43**: 599–605.
19 Bacon BR. Treatment of patients with hepatitis C and normal serum aminotransferase levels. *Hepatology* 2002; **36**: S179–84.
20 Zeuzem S, Diago M, Gane E *et al.* Peginterferon alfa-2a (40 kilodaltons) and ribavirin in patients with chronic hepatitis C and normal aminotransferase levels. *Gastroenterology* 2004; **127**: 1724–32.
21 Manns MP, McHutchison JG, Gordon SC *et al.* Peginterferon alfa-2b plus ribavirin compared to interferon alfa-2b plus ribavirin for the treatment of chronic hepatitis C: a randomized controlled trial. *Lancet* 2001; **359**: 958–65.
22 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.
23 Puoti C, Magrini A, Stati T *et al.* Clinical, histological, and virological features of hepatitis C virus carriers with persistently normal or abnormal alanine aminotransferase levels. *Hepatology* 1997; **26**: 1393–8.
24 Prati D, Taidoli E, Zanela A *et al.* Updated definition of healthy ranges for serum alanine aminotransferase levels. *Ann Intern Med* 2002; **137**: 1–9.
25 Desmet VJ, Gerber M, Hoofnagle JH *et al.* Classification of chronic hepatitis: diagnosis, grading and staging. *Hepatology* 1994; **19**: 1513–20.
26 Ishak K, Baptista L, Bianchi L *et al.* Histological grading and staging of chronic hepatitis. *J Hepatol* 1995; **22**: 696–9.
27 MacSween RNM, Anthony PP, Sheuer PJ. *Pathology of the Liver*. Edinburgh: Churchill Livingstone, 1987.
28 Tsukiyama-Kohara K, Yamaguchi K, Maki N *et al.* Antigenicities of group 1 and 2 hepatitis C virus polypeptides: molecular basis of diagnosis. *Virology* 1993; **192**: 430–7.
29 Simmonds P, Alberti A, Alter HJ *et al.* A proposed system for the nomenclature of hepatitis C virus genotypes. *Hepatology* 1994; **19**: 1321–4.
30 Makiyama A, Itoh Y, Kasahara A *et al.* Characteristics of patients with chronic hepatitis C who develop hepatocellular carcinoma after sustained response to interferon. *Cancer* 2004; **101**: 1616–22.
31 Healey CJ, Chapman RWG, Fleming KA. Liver histology in hepatitis C virus infection: a comparison between patients with persistently normal or abnormal transaminase. *Gut* 1993; **37**: 274–8.
32 Ohkoshi S, Tawaraya H, Kuwana K *et al.* A retrospective study of hepatitis C virus carriers in a local endemic town in Japan. *Dig Dis Sci* 1995; **40**: 465–71.
33 Puoti C, Castellacci R, Montagness F *et al.* Histological and virological features and follow-up of HCV carriers with normal aminotransferase levels: the Italian Study of the Asymptomatic C Carriers (ISACC). *J Hepatol* 2002; **37**: 117–23.
34 Yano M, Kumada H, Kage M *et al.* The long-term pathological evolution of chronic hepatitis C. *Hepatology* 1996; **332**: 1463–6.
35 Poynard T, Bedossa P, Opolon P. Natural history of liver fibrosis progression in patients with chronic hepatitis C. The OBSVIRC, METAVIR, CLINVIR, and DOSVIRC. *Lancet* 1997; **346**: 825–32.
36 Takahashi M, Yamada G, Miyamoto R, Doi H, Endo H, Tsuji T. Natural course of chronic hepatitis C. *Am J Gastroenterol* 1993; **88**: 240–3.
37 Ghany MG, Kleiner DE, Alter H *et al.* Progression of fibrosis in chronic hepatitis C. *Gastroenterology* 2003; **124**: 97–104.
38 Mathurin P, Moussalli J, Cardanei J-F *et al.* Slow progression rate of fibrosis in hepatitis C virus patients with persistently normal alanine transaminase activity. *Hepatology* 1998; **27**: 868–72.
39 Hui C-K, Belaye T, Montegrade K, Wright TL. A comparison in the progression of liver fibrosis in chronic hepatitis C between persistently normal and elevated transaminase. *J Hepatol* 2003; **38**: 511–17.
40 Prati D, Shiffman ML, Diago M *et al.* Viral and metabolic factors influencing alanine aminotransferase activity in patients with chronic hepatitis C. *J Hepatol* 2006; **44**: 679–85.
41 Tarao K, Ohkawa S, Tamai S, Miyakawa K. Sustained low serum GPT level below 80 INU in HCV- associated cirrhotic patients by multiagents prevent development of hepatocellular carcinoma. *Cancer* 1994; **73**: 1149–54.
42 Boccato S, Pistis R, Noventa F, Guido M, Benvegnu L, Alberti A. Fibrosis progression in initially mild chronic hepatitis C. *J Viral Hepat* 2006; **13**: 297–302.
43 Cadranel JF, Rufat P, Degos F. Practices of liver biopsy in France: results of a prospective nationwide survey. For the Group of Epidemiology of the French Association for the Study of the Liver (AFEF). *Hepatology* 2000; **32**: 477–81.
44 Castera L, Negre I, Samii K, Buffet C. Pain experienced during percutaneous liver biopsy. *Hepatology* 1999; **30**: 1529–30.
45 Afdal NH. Diagnosing fibrosis in hepatitis C: is the pendulum swinging from biopsy to blood test? *Hepatology* 2003; **37**: 972–4.
46 Imbert-Bismut F, Ratziu V, Pieroni L, Charlotte F, Benhamou Y, Poynard T. Biochemical markers of liver fibrosis in patients with hepatitis C virus infection: a prospective study. *Lancet* 2001; **357**: 1069–75.

47 Sandrin L, Fourquet B, Hasquenoph JM *et al.* Transient elastography: a new invasive method for assessment of hepatic fibrosis. *Ultrasound Med Biol* 2003; **29**: 1705–13.
48 Castera L, Vergniol J, Foucher J *et al.* Prospective comparison of transient elastography, fibrotest, APRI, and liver biopsy for the assessment of fibrosis in chronic hepatitis C. *Gastroenterology* 2005; **128**: 343–50.
49 Saito H, Tada S, Nakamoto N *et al.* Efficacy of non-invasive elastometry on staging of hepatic fibrosis. *Hepatol Res* 2004; **29**: 97–103.
50 Colletta C, Smirne C, Fabris C *et al.* Value of two noninvasive methods to detect progression of fibrosis among HCV carriers with normal aminotransferase. *Hepatology* 2005; **42**: 838–645.
51 Castera L, Foucher J, Bertet J, Couzigou P. FibroScan and FibroTest to assess liver fibrosis in HCV with normal aminotransferse. *Hepatology* 2006; **43**: 373–4.
52 Alberti A. Towards more individualized management of hepatitis V virus patients with initially or persistently normal alanineaminotransferase levels. *J Hepatol* 2005; **42**: 266–74.

2008年7月作成
B0023
EM0708BP

ELSEVIER

Cancer Genetics and Cytogenetics 180 (2008) 30–36

Cancer Genetics and Cytogenetics

CREB3L4, *INTS3*, and *SNAPAP* are targets for the 1q21 amplicon frequently detected in hepatocellular carcinoma

Yoshikazu Inagaki[a], Kohichiroh Yasui[a,*], Mio Endo[a], Tomoaki Nakajima[a], Keika Zen[a], Kazuhiro Tsuji[a], Masahito Minami[a], Shinji Tanaka[b], Masafumi Taniwaki[c], Yoshito Itoh[a], Shigeki Arii[b], Takeshi Okanoue[a]

[a]*Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, 465 Kajii-cho Kamigyo-ku, Kyoto 602-8566, Japan*

[b]*Department of Hepato-Biliary-Pancreatic Surgery, Tokyo Medical and Dental University, Tokyo, Japan*

[c]*Molecular Hematology and Oncology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto, Japan*

Received 10 August 2007; accepted 25 September 2007

Abstract High-density single nucleotide polymorphism (SNP) array analysis revealed novel amplification at 1q21 in cell lines derived from hepatocellular carcinomas (HCCs). Fluorescence in situ hybridization and real-time quantitative polymerase chain reaction studies verified amplification at 1q21. An increase in copy number at the region was detected in 32 of the 36 primary HCC tumors (89%). To identify the targets for amplification, we examined 19 HCC cell lines for expression levels of all 26 genes located within the 700-kb amplified region. Five genes were overexpressed in cell lines with amplification at 1q21. Among these, *CREB3L4* (cAMP responsive element binding protein 3-like 4), *INTS3* (integrator complex subunit 3), and *SNAPAP* (SNAP-associated protein) were significantly overexpressed in tumors from 18 HCC patients, compared with counterpart nontumorous tissues. The findings suggest that *CREB3L4*, *INTS3*, and *SNAPAP* are probable targets for the amplification mechanism and may therefore be involved, together or separately, in the development or progression of HCCs.

1. Introduction

Amplification of DNA in certain chromosome regions plays a crucial role in the development and progression of human malignancies, specifically when proto-oncogenic target genes within those amplicons are overexpressed. Oncogenes that are often amplified in cancers include *MYC*, *ERBB2*, and *CCND1*. The initial approach to genome-wide detection of copy number aberrations in cancers was comparative genomic hybridization (CGH) [1]. Using CGH analyses, we detected novel regions of amplification in various types of tumor. Within these amplicons, we identified a number of additional proto-oncogenes that may be upregulated by DNA amplification [2–6]. CGH has limited resolution (5–10 Mb), however, in that it detects segmental copy number changes on metaphase chromosomes [1].

The recent introduction of high-density oligonucleotide microarrays designed for typing of single nucleotide polymorphisms (SNPs) allows for high-resolution mapping of chromosomal amplifications, deletions, and loss of heterozygosity [7–11]. The GeneChip Mapping 100K array set (Affymetrix, Santa Clara, CA) contains 116,204 SNP loci, with a mean intermarker distance of 23.6 kb, and enables detailed and genome-wide identification of DNA copy number changes [12–14].

In the present study, we identified novel, high-level amplification at 1q21 using the Affymetrix GeneChip Mapping 100K array analysis against a panel of cell lines derived from hepatocellular carcinoma (HCC). Worldwide, HCC is the fifth most common malignancy in men and the eighth most common in women [15]. Previous CGH studies of HCC, including ours [3], have revealed that the most frequent copy number gains occur on 1q (58–78%) [16–18]. Gains in 1q21~q23 were identified as a genomic event associated with the early development of HCC [19]. Recurrent gains at 1q21~q23 have been observed in not only HCC but also in tumors including squamous cell carcinomas of the head and neck, lung cancer, desmoid tumors, and sarcomas [20,21]. These findings suggest that 1q21 harbors one or more proto-oncogenes whose overexpression following amplification contributes to the initiation or

* Corresponding author. Tel.: +81-75-251-5519; fax: +81-75-251-0710.

E-mail address: yasuik@koto.kpu-m.ac.jp (K. Yasui).

0165-4608/08/$ – see front matter
doi:10.1016/j.cancergencyto.2007.09.013

progression of HCC. We therefore performed molecular definition of the 1q21 amplicon. Three genes emerged as possible targets: *CREB3L4*, *INTS3*, and *SNAPAP*.

2. Materials and methods

2.1. Cell lines and tumor samples

A total of 19 HCC-derived cell lines were examined: HLE [22], HLF [22], PLC/PRF/5 [23], Li7 [24], Huh7 [25], Hep3B [26], SNU354 [27], SNU368 [27], SNU387 [27], SNU398 [27], SNU423 [27], SNU475 [27], JHH-1 [28], JHH-2 [28], JHH-4 [28], JHH-5 [28], JHH-6 [28], JHH-7 [28], and Huh-1 [29]. All cell lines were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. We obtained 36 primary HCC tumors from patients undergoing surgery at the hospital of Tokyo Medical and Dental University and Kyoto University. Genomic DNA was isolated from all cell lines and primary tumors using the Puregene DNA isolation kit (Gentra Systems, Minneapolis, MN).

Informed consent and Ethics Committee approval were obtained before initiation of the study.

2.2. SNP assay

GeneChip Mapping 100K array set (Affymetrix, Santa Clara, CA) analyses were performed according to the manufacturer's instructions. Briefly, 250 ng of genomic DNA was digested with a restriction enzyme (*Xba*I or *Hin*dIII), ligated to an adaptor, and amplified by polymerase chain reaction (PCR) [8,9,30]. Amplified products were fragmented, labeled by biotinylation, and hybridized to microarrays. Hybridization was detected by incubation with streptavidin–phycoerythrin conjugate, followed by scanning, and analysis was performed as described previously [30,31].

Copy number changes were calculated based on SNP hybridization signal intensity data from the experimental sample relative to intensity distributions derived from a reference set containing > 100 individuals using the Affymetrix Chromosome Copy Number Analysis Tool software and algorithm (CNAT version 2.0; http://www.affymetrix.com/support/developer/tools/affytools.affy) [32].

2.3. Real-time quantitative PCR

We quantified genomic DNA and mRNA using the real-time fluorescence detection method. Total RNA was obtained using Trizol reagent (Invitrogen, Carlsbad, CA). Residual genomic DNA was removed by incubating RNA samples with RNase-free DNase I (Takara Bio, Shiga, Japan) prior to reverse transcriptase PCR. Single-stranded complementary DNA (cDNA) was generated using SuperScript III reverse transcriptase (Invitrogen) according to the manufacturer's directions. Real-time quantitative PCR

Table 1
Primer sequences used for real-time quantitative polymerase chain reaction (PCR)

Gene	Forward primer	Reverse primer	PCR product size, bp
S100A6	5′-GAAGGAGCTGAAGGAGCTGA-3′	5′-CCCTTGAGGGCTTCATTGTA-3′	177
S100A5	5′-AGAGCTGTGTCTIGGGGAGA-3′	5′-CCCTGGTCACTTGTTGTCCT-3′	166
S100A4	5′-GATGAGCAACTTGGACAGCA-3′	5′-CTICCTGGGCTGCTTATCTG-3′	127
S100A3	5′-CGAGGTGGACTTTGTGGAGT-3′	5′-GGGCCTCCTAGGTAAAATGG-3′	244
S100A2	5′-CTTCCTGGGTCTGTCTCTGC-3′	5′-TCCCCCTTACTCAGCTTGAA-3′	142
S100A16	5′-ATGTTCCTGCCAAATTCCTG-3′	5′-GAGAGGTCTCTGCTGCTGCT-3′	142
S10014	5′-CTGACCCCTTCTGAGCTACG-3′	5′-CCAGAGGGAGTTCTCAGTGC-3′	214
S100A13	5′-TCCAACTGGAACCTIGAACC-3′	5′-GATCTGGAAGTGGGTGGAGA-3′	155
S100A1	5′-GGAGACCCTCATCAACGTGT-3′	5′-CAGCCACAAGCACCACATAC-3′	215
C1orf77	5′-GGTGGTAGAGGTCGGGGTAT-3′	5′-GCATCCAGGTGTCCTITTGT-3′	167
SNAPAP	5′-AGGAACGACTGAGACGGCTA-3′	5′-GTAAATTCCCGAATCCAGCA-3′	80
ILF2	5′-CGTGGAAAGCCTAAGAGCAC-3′	5′-GAAGATTGGGTGGCACTGTT-3′	128
NPR1	5′-GCATTGAGCTGACACGAAAA-3′	5′-CCTTGACGATGTCATTGGTG-3′	219
INTS3	5′-GGTACGGGAACTGGTGAAGA-3′	5′-CTGCTCTTCAGGACCCACTC-3′	162
SLC27A3	5′-ATACCTGGGAGCGTTTTGTG-3′	5′-CCGCTGTCCTGTGTAGTTGA-3′	104
GATAD2B	5′-TICTTTGCCCTCTGTGCTTT-3′	5′-GGCATCTCGTACCTCTGAGC-3′	221
KIAA0476[a]	5′-CTATGGGCTGTGGTICCTGT-3′	5′-TGCCCATAGTGTGAGCAGAG-3′	171
CRTC2	5′-TICAGTGCAGTCCTCAGGTG-3′	5′-GCTGAACTGCTCCAGATTCC-3′	145
SLC39A1	5′-GGATIGGGGAAGACACTTGA-3′	5′-GAAATGGGCTAGGACCAACA-3′	159
CREB3L4	5′-GACCAGAAGCTGGGTCTGAG-3′	5′-TGTTACGTCCTTGTGGGTCA-3′	77
JTB	5′-ACGTATTGTCCCTGCTCACC-3′	5′-GCTGCTCACTGGGAATTAGC-3′	165
RAB13	5′-GAGCCATGGGCATIATCCTA-3′	5′-CCTTCTGCACCTTCCTCTTG-3′	162
RPS27	5′-CGCAAAGGATCTCCTTCATC-3′	5′-CGTTTGTGCATGGCTAAAGA-3′	148
NUP210L	5′-CTGTGAACAGAGGGCTGACA-3′	5′-GCTCAATGGCATGCTCTACA-3′	96
TPM3	5′-GGGTTTGAAGCTGCTGTCTC-3′	5′-CCACAAACCCAAAGCAAAGT-3′	137
C1orf89	5′-ATGCAGGACACCATGGTACA-3′	5′-TCCTGCTGGTACTGCTGATG-3′	113

[a] The approved gene symbol for the *KIAA0476* gene is now *DENND4B* (http://www.genenames.org). For simplicity, the previous symbol is retained.

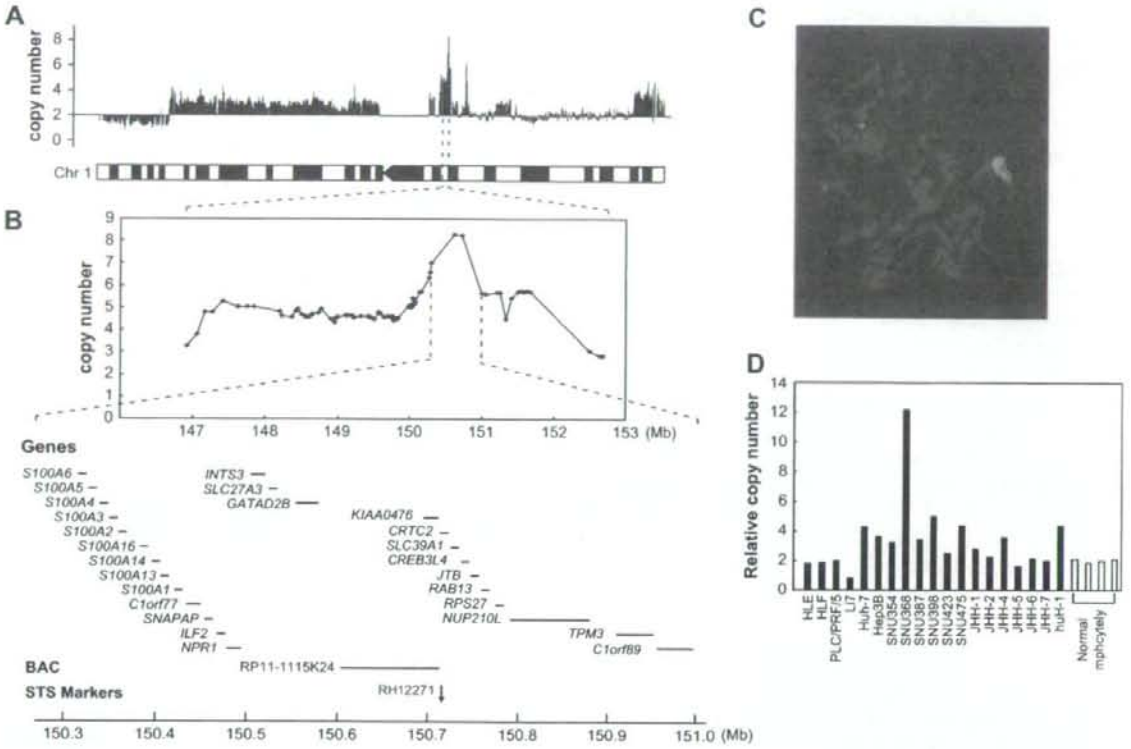

Fig. 1. Map of the amplicon at 1q21 in the hepatocellular carcinoma (HCC) cell line SNU368. (A) Copy number determined by SNP 100 K arrays (upper) and chromosome 1 cytoband map (lower) are shown. Copy number values were estimated using the Affymetrix CNAT chromosome copy number analysis tool. Green lines show copy number gains; red lines correspond to losses of genomic material. (B) Map of 1q21 amplicon. The graph represents the copy number determined by SNP arrays. The positions of the 26 genes within the amplicon, the bacterial artificial chromosome (BAC; RP11-1115K24) used as a probe for fluorescence in situ hybridization (FISH), and the STS marker (RH12271) used for real-time quantitative polymerase chain reaction (PCR) are shown, according to the UCSC genome database (http://genome.ucsc.edu/). (C) Representative FISH images using BAC RP11-1115K24 on metaphase chromosomes from SNU368 cells. The image shows strong homogeneously staining region signals on a marker chromosome. (D) Copy numbers at the STS marker locus RH12271 in 19 HCC cell lines and four normal peripheral blood lymphocytes, as measured by real-time quantitative PCR with reference to LINE-1 controls. Values are normalized such that the average copy number in genomic DNA derived from four normal lymphocytes has a value of 2.

experiments were performed with the LightCycler system using FastStart DNA Master Plus SYBR Green I (Roche Diagnostics, Penzberg, Germany) according to the manufacturer's protocol. Primers used for PCR (listed in Table 1) were designed using Primer3 version 0.4 (http://frodo.wi.mit.edu/cgi-bin/primer3/primer3_www.cgi) on the basis of sequence data obtained from the U.S. National Center for Biotechnology Information Entrez Gene database (http://www.ncbi.nlm.nih.gov/). GAPDH [33] and long interspersed nuclear element 1 (LINE-1) [9] were used as endogenous controls for mRNA and genomic DNA levels, respectively.

2.4. Fluorescence in situ hybridization

Fluorescence in situ hybridization (FISH) experiments were performed using bacterial artificial chromosome (BAC; RP11-1115K24) as a probe, as described previously [2]. Briefly, the probe was labeled with biotin-16-dUTP

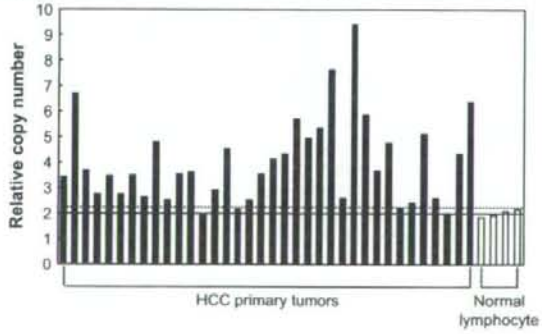

Fig. 2. Copy number gain at 1q21 in primary HCC tumors. Copy number at the STS marker locus RH12271 in 36 primary HCC tumors and four normal peripheral blood lymphocytes were determined by real-time quantitative PCR with reference to LINE-1 controls. Values are normalized such that the average copy number in genomic DNA derived from four normal lymphocytes has a value of 2 (solid horizontal line). The mean + 2 × SD of normal lymphocytes was used to determine the cutoff value for copy number gain (dotted horizontal line).