Hepatic iron accumulation

Most of the body's excess iron is stored in the liver, and in the normal adult male, liver iron stores can range from 0.5 to 1 g [7]. The hepatic iron concentration in normal liver tissue obtained at autopsy has been reported to be 16.51 (7.82–39.93) mmol/kg dry tissue [median and (5–95 percentile interval)] [8]. These values are estimated to be equivalent to a hepatic iron content of 300–900 μg/g dry weight liver tissue. Extensive studies reported median hepatic iron concentrations of 396 (range 0–2,105) and 458 (range 114–2,190) μg/g dry weight liver tissue in patients with chronic hepatitis C [9, 10]. In addition, the reported percentages of patients with hepatic iron concentrations ≥1,000 μg/g dry weight were 14 and 19 %, respectively [9, 10]. Therefore, it should be noted that among patients with chronic hepatitis C some have high hepatic iron content, whereas others have normal hepatic iron content. In contrast, a hepatic index (μmol Fe/g liver tissue/patients age) of 1.9 or more has been reported to be typical of patients with hereditary hemochromatosis [11]. If the hepatic index of a patient aged 60 with hereditary hemochromatosis is 1.9, the hepatic iron concentration of this patient is assumed to be 6,384 μg/g liver tissue. Thus, we should understand that hepatic iron content is much less in chronic hepatitis C than in hereditary hemochromatosis and within the normal range in some of patients with chronic hepatitis C, even though it is recognized to be one of liver diseases that show hepatic iron accumulation.

There also remains uncertainty as to whether iron predominantly accumulates in hepatocytes or the reticuloendothelial system, mainly Kupffer cells, in patients with chronic hepatitis C. Some clinical studies showed that iron was mainly localized in the reticuloendothelial system [1, 12], whereas others reported its localization in hepatocytes [13]. Interestingly, Fiel et al. [14] documented that even ribavirin-associated hemolysis deposited iron preferentially in hepatocytes in patients with chronic hepatitis C. Hepatocytic iron accumulation may indicate potential DNA damage and genetic instability in association with HCV-induced oxidative stress, while iron deposition in Kupffer cells may contribute to cytokine release, leading to inflammation or fibrosis. However, further investigations are needed to clarify this issue.

Hepcidin expression

Hepcidin, which was originally isolated from human serum and urine as a peptide with antimicrobial activity [15, 16], is a hormone exclusively synthesized in the liver and a negative regulator of iron release into the systemic circulation by duodenal enterocytes and reticuloendothelial macrophages [17, 18]. Hepcidin binds to the iron exporter ferroportin, which results in internalization and degradation of ferroportin [19]. The lack of hepcidin expression in knockout mice leads to iron overload [20], and conversely, overexpression of hepcidin in transgenic mice causes severe iron deficiency [21]. Iron transferrin also regulates hepcidin synthesis through hemojuvelin and bone morphogenetic protein (BMP) 2/4 [22].

Fujita et al. [23] showed for the first time that hepatic hepcidin mRNA levels adjusted by serum ferritin values were significantly lower in patients with chronic hepatitis C than in those with chronic hepatitis B or those without hepatitis B virus (HBV) or HCV infection. Of note, the relative expression of hepcidin for iron stores was lower in chronic hepatitis C than in chronic hepatitis B or chronic liver diseases without HBV or HCV infection, even though hepcidin expression levels were strongly correlated with serum ferritin and the degree of hepatic iron deposition. These results suggested that hepcidin might play a pivotal role in iron overload in patients with chronic hepatitis C. A recent study using a validated immunoassay of the 25-amino acid bioactive hepcidin in serum also revealed that serum hepcidin levels were lower in patients with chronic hepatitis C than in controls despite a significant correlation between hepcidin and serum ferritin or the histological iron score in both groups [24]. Thus, the relatively decreased synthesis of hepcidin in chronic hepatitis C contrasts with the absolute deficit or lack in hepcidin synthesis observed in hereditary hemochromatosis and may account for the mild to moderate hepatic iron overload observed in some patients with chronic hepatitis C.

Mechanisms underlying hepatic iron accumulation

Elucidating the mechanisms of iron accumulation in chronic hepatitis C may provide new tools for the management of the condition or for the prevention of its complications, or both. Hepcidin appears to provide a critical clue for elucidating the mechanisms of iron accumulation because its decreased synthesis has been reported in chronic hepatitis C in previous studies [23–25]. Disruption of hepcidin regulation resulting from inhibited activity of the transcription factor CCAAT/enhancer-binding protein α (C/EBPα) has been postulated as a possible mechanism causing iron overload in alcoholic liver disease [26, 27]. We investigated the mechanism by which hepatic iron accumulates using transgenic mice expressing the HCV polyprotein [28]. These mice had reduced hepcidin mRNA expression, which was attributed to HCV protein-induced reactive oxygen species (ROS), with consequent upregulation of an inhibitor of the binding of C/EBPα to the hepcidin promoter. Thus, the mechanisms underlying HCV-related hepatic iron overload appear to have some similarities with alcohol-induced iron overload

in terms of disrupted hepcidin transcription through suppressed activity of C/EBPα. In agreement with our observation, an in vitro study by Miura et al. [29] using hepatoma cells showed that HCV-induced ROS inhibited the binding activity of C/EBPα to the hepcidin promoter through increased histone deacetylase activity.

Hepcidin is transcriptionally regulated in response to the iron concentration, inflammation, hypoxia, and erythropoiesis [30]. BMPs, members of the transforming growth factor beta superfamily, play a crucial role in regulating hepcidin transcription through SMAD signaling [31–33]. Hepcidin is regulated by both the circulating transferrin-bound iron and intracellular iron stores. Its exact pathway is still unknown but seems to involve the BMP pathway. As yet there is no convincing evidence that accounts for the suppressive transcription of hepcidin through the BMP/SMAD cascade in chronic hepatitis C. The significant correlations between hepcidin and serum ferritin or the histological iron score in chronic hepatitis C [23, 24] suggest that hepcidin transcription is properly regulated in response to the iron concentration in chronic hepatitis C. Thus, hepcidin transcription in chronic hepatitis C may be potentially regulated by the opposing effects of HCV-induced hepcidin-suppressive factors and the iron load-induced hepcidin-stimulation factors. As suggested by Girelli et al. [24], in the early phase of chronic hepatitis C, hepcidin may be prominently suppressed by HCV but, as iron accumulates, the negative influence of viral factors may be masked by the positive stimulation of iron.

Inflammation also regulates hepcidin transcription. Proinflammatory cytokines such as IL-6 mediate this response by inducing transcription of hepcidin mRNA via STAT3, which binds to a STAT-responsive element within the hepcidin promoter [34–36]. Our transgenic mice expressing the HCV polyprotein did not show any inflammation in the liver. A possible pitfall in this experimental model was that we could not take the inflammatory effect on hepcidin regulation into account, which is different from what is observed in patients with chronic hepatitis C. Serum levels of IL-6 have been shown to be elevated in patients with HCV-related chronic liver disease [37], which raises the possibility that IL-6 acts to stimulate hepcidin expression through the STAT3 pathway. This would be expected to counteract the decrease in hepcidin transcription caused by HCV-induced ROS. However, no significant relationship has been found between serum IL-6 and hepcidin in patients with chronic hepatitis C [24, 38], even though a paracrine effect of local IL-6 release on hepcidin transcription in the liver cannot be excluded. On the other hand, chronic inflammation with production of proinflammatory cytokines has the potential to deliver an additional burden of ROS, which would be expected to reinforce the decrease in hepcidin transcription. Most likely, during chronic inflammation states in vivo like chronic hepatitis C, the regulation of hepcidin is more complex and may depend on many variables, including the particular stage of systemic and/or hepatic inflammatory disease. This might explain the variations in hepatic iron concentrations reported among patients with HCV-related chronic liver disease. As an iron regulatory molecule other than hepcidin, upregulation of transferrin receptor 1 has been reported in patients with chronic hepatitis C [39]. The schematic outline in Fig. 1 depicts the assumed mechanisms underlying the hepatic iron accumulation in chronic hepatitis C.

Impact of hepatic iron overload on disease progression and relevance to hepatocarcinogenesis

Iron is a cofactor that influences the severity and progression of nonhemochromatic liver diseases, especially steatohepatitis and viral hepatitis [40–44]. The importance of iron as a comorbidity factor in chronic hepatitis C is emphasized by several reports of more fibrosis and a greater risk of HCC development with more hepatic iron [45–47]. Recently, it has been prospectively shown in the HALT-C (hepatitis c anti-viral long-term treatment to prevent cirrhosis) trial cohort that stainable iron in hepatocytes and portal tract cells predicts progression and outcomes (Child Pugh score >7, ascites, encephalopathy, variceal bleeding, spontaneous bacterial peritonitis, HCC, death) in advanced chronic hepatitis C [48].

Although the association of markedly increased iron accumulation in the liver with hepatocarcinogenesis in hereditary hemochromatosis has been well described [49], it remains to be elucidated whether mild to moderate increases in hepatic iron accumulation contribute to the development of HCC in patients with HCV-associated chronic liver diseases. To determine the mechanisms underlying the development of HCC in the presence of both HCV infection and mild to moderate hepatic iron accumulation, we investigated whether iron overload equivalent to that in chronic hepatitis C patients contributed to the development of HCC in transgenic mice expressing the HCV polyprotein [50]. Transgenic mice fed an excess-iron diet showed marked hepatic steatosis, including the centrilobular microvesicular type, ultrastructural alterations of the mitochondria, and decreased degradation activity of fatty acid at 6 months, as well as hepatic accumulation of lipid peroxidation products and 8-hydroxy-2′-deoxyguanosine (8-OHdG) at 12 months after the initiation of feeding. Of note, hepatic tumors including HCC developed in 5 of 11 (45 %) transgenic mice fed the excess-iron diet but not in control mice or transgenic mice fed the control diet at 12 months after the initiation of feeding. These results indicated the importance

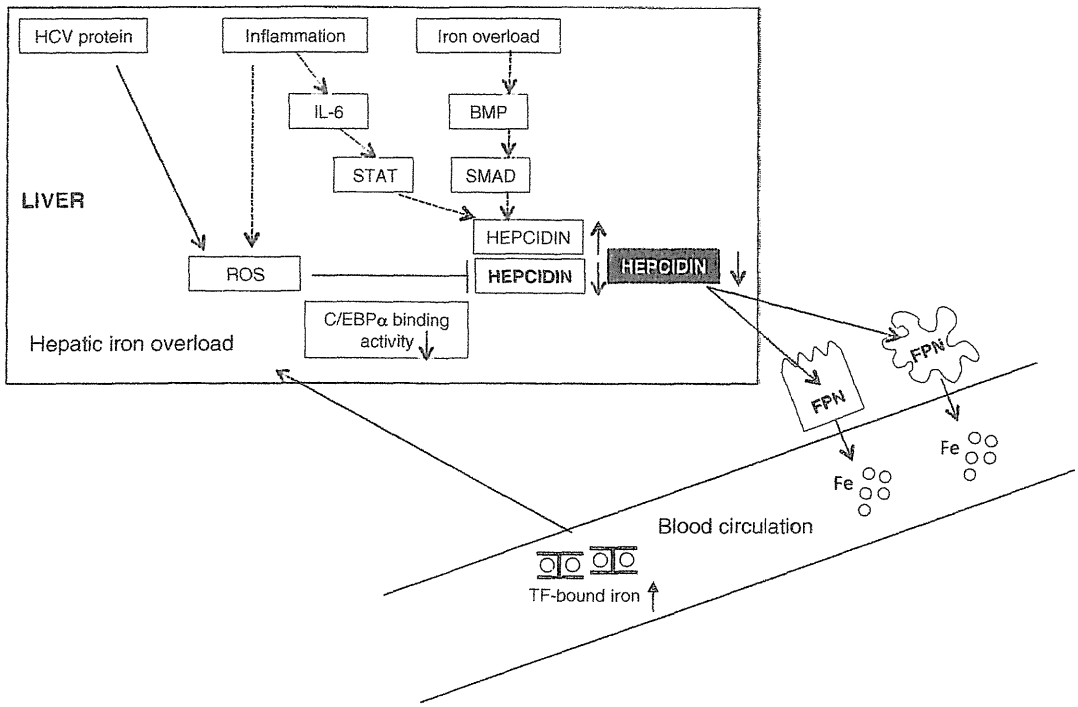

Fig. 1 Schematic diagram depicting the mechanisms underlying hepatic iron accumulation. HCV protein-induced ROS reduces hepcidin transcription through inhibition of DNA binding activity of C/EBPα. Hepcidin transcription in chronic hepatitis C may be potentially regulated by the opposing effects of HCV-induced hepcidin suppressive factors and iron load-induced hepcidin stimulation factors. Inflammation may also have the opposing effects of stimulation and suppression of hepcidin transcription through the IL-6/STAT pathway and ROS pathway, respectively. Decreased hepcidin expression enhances ferroportin (*FPN*) expression in the duodenum and macrophages, resulting in increased duodenal iron transport and macrophage iron release, which lead to hepatic iron accumulation

of oxidative stress and subsequent mitochondrial injury synergistically induced by iron loading and HCV proteins in the development of HCC. Tanaka et al. [44] showed a strong correlation of hepatic 8-OHdG levels with body and hepatic iron storage in patients with chronic hepatitis C and that oxidative DNA damage in the liver was associated with an increased risk of HCC development. Kato et al. [5] also reported that the decrease in hepatic 8-OHdG content caused by phlebotomy lowered the risk of progression to HCC, which indeed showed the critical role of the iron-overload state in the development of HCC in patients with chronic hepatitis C. Thus, there is a close relationship between oxidative DNA damage synergistically induced by hepatic iron accumulation and HCV proteins in the development of HCC in patients with HCV associated with chronic liver diseases. Whether long-term and sustained iron reduction by phlebotomy could help to prevent or delay disease progression and/or development of HCC is an important and still unresolved question, but a promising effect of phlebotomy was reported in a long-term nonrandomized prospective study [6]. As iron reduction and adherence to a low-iron diet are relatively easy and safe, combination of these treatments would seem to be beneficial to patients who cannot tolerate or have not responded to peginterfeon plus ribavirin therapy to prevent disease progression and/or the development of HCC.

Conflict of interest The authors disclose no conflicts of interest.

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石川県の肝癌撲滅計画

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肝癌撲滅には背景にある肝炎ウイルスに対する治療導入が重要である。石川県では肝炎ウイルス検診初年度より協議会を設立し，陽性者をフォローアップしてきた。インターフェロン療法導入率向上を目指してさまざまな施策を講じ，導入率は30%を越えるようになった。2010年度よりかかりつけ医と専門医の連携を強化した「石川県肝炎診療連携」を新たに開始して専門医受診勧奨，抗ウイルス療法導入を図ることにより肝癌撲滅を目指している。

はじめに

2009年度人口動態統計では肝癌による死亡者数は男性で第4位，女性では第6位であり，年間3万人を越えている肝癌の多くはウイルス性慢性肝疾患を背景に発生しており，肝癌撲滅には肝炎ウイルス感染者を早期に発見し，早期に治療することが重要である　国は2002年度より5年間で肝炎ウイルス検診を行い，肝炎ウイルス感染者の発見に努めたが，検診受診率は決して高くなく，また医療機関を受診しても適切な観察，治療導入すなわち抗ウイルス療法が行われてきたとは言い難い　本稿では肝炎ウイルス検診開始当初より石川県で取り組んできた肝炎ウイルス症例への対策について述べる

肝炎ウイルス検診

2002年肝炎ウイルス検診会誌当初より，石川県では肝炎協議会を設置し，県健康福祉部・医師会・保健所・検査センター・学術経験者が一体となって協力した検診体制を確立した　地域により専門医療機関の過不足があるため，精密検査は特に指定医療機関とはせず，かかりつけ医でも可とした。このため検診精度の向上と経過観察の重要性を考え，以下7つの項目を検診事業の柱とした。

1. 検診陽性者への行政の関与することの通知と同意
2. 精密検査の全県での統一
3. 住民，担当医用の診断手引きの作成
4. 精密検査での画像検査の義務付け
5. 全症例を対象とした事例検討会
6. 前年度陽性者に対する事後調査
7. 保健師などを対象とした研修会の開催

このなかで石川県として独自性の高いと考えているものは検診陽性者を行政が継続フォローするために必要な1，6および担当医の肝炎への理解を深めた5である　毎年検診陽性者の医療機関受診・治療状況を把握することと，担当のかかりつけ医が正しく診断，治療導入することへの意識が高まるようこれら事業を継続した

石川県の現状

石川県では5年間の肝炎ウイルス検診受診率は36.6～41.5%と全国平均　と比べると10%ほど受診率がよかったが半数には満たない。検診陽性者の精密検査受診状況は男性67.6%，女性75.0%，年齢では若年（65歳未満）66.1%，高齢（65歳以上）74.7%であった　性年齢でわけると若年男性53.4%，若年女性71.9%，高齢男性74.0%，高齢女性74.0%と若年男性で精密検査の受診率が低いことが明らかであり，仕事等で忙しく受診機会をつくりにくい状況がうかがえる　図1に性・年齢・医療圏別での精検受診状況を示す　検診自体の受診率は能登地方および南加賀で低い傾向にあった。しかし能登地方はウイルスキャリアと判明すると医療機関をきちんと受診する傾向にある。一方，南加賀ではウイルスキャリアと判明しても医療機関への受診率が悪い，能登地方ではキャリアの発掘が重要であり，南加賀ではキャリアの発掘と受診勧奨の両面が必要なことがう

PROFILE

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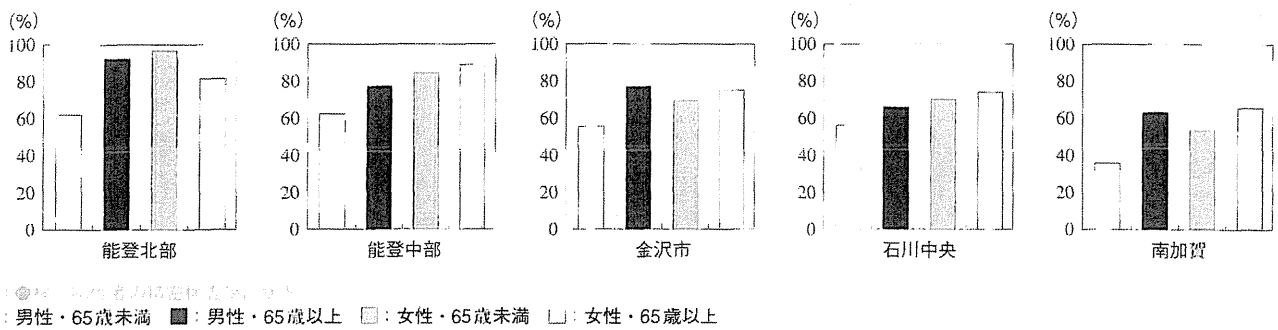
【専門領域】消化器病学，肝臓学



	検診初年度精検未受診	翌年以降医療機関受診	IFN 療法 / 受診者
能登北部	18（14.8%）	12（66.7%）	3（25.0%）
能登中部	32（17.5%）	17（53.1%）	2（11.8%）
石川中央	71（31.8%）	45（63.4%）	7（15.6%）
南加賀	88（40.6%）	52（59.1%）	10（19.2%）
金沢市	147（28.1%）	39（26.5%）	2（ 5.1%）
合計	356（28.1%）	165（46.3%）	24（14.5%）

かがえる　また医療機関受診の時間がとりにくい若年男性の受診率が悪いのは地域で共通しており，受診動機を促す啓蒙活動が必要である

前述したように石川県では保健師が面談，電話，手紙などの方法で検診陽性者の状況把握に努めている　継続して医療機関で経過観察されているのはC型肝炎では48.7～63.7%であった．一方，各市町で少なくともフォロー期間（2～7年）中に1度は医療機関を受診した症例はB型肝炎ウイルス陽性者で49～100%，C型肝炎ウイルス陽性者で80～100%であった．表1に初年度精密検査未受診者のその後の状況を示す　受診勧奨を行った結果未受診者のうち能登北部66.7%，能登中部53.1%，金沢市26.5%，石川中央63.4%，南加賀59.1%がその後に医療機関を受診し，さらに受診者のうち能登北部25.0%，能登中部11.8%，金沢市5.1%，石川中央15.6%，南加賀19.2%がインターフェロン（IFN）療法を行っていた。継続した状況把握，受診勧奨が適切な医療へと結びつくことが明らかとなった。

肝癌撲滅という目標に対してC型肝炎であればIFN療法によりウイルスが排除されることが一番である　年齢，合併症などにより全ての症例でIFN療法を行うのは困難であるが，検診症例のIFN療法の施行率が低いことが問題となっている　厚生労働省研究班の報告では当初3年間では13.8～18.2%であった[1]　石川県でも2002年131例中5例（3.8%），2003年164例中14例（8.5%）とIFN療法施行率は低かった。特に65歳以上の高齢者ではIFN施行率は2.6%と，65歳未満の9.6%に対して有意に低かった[2]　IFN導入率が高齢者を含めて低い理由を検討するために，石川県全下で内科標榜医療機関にアンケート調査を行った．設問「一度はIFN療法を患者に説明するか（複数回答可）」に肝臓専門医の約8割は条件を問わずIFN療法について説明するが，非専門医師は約5割しか条件を問わずにIFN療法を説明していなかった．また「IFN療法を行わない理由」としては高齢であることをあげる医師が多数を占めたが，「何歳までがIFN療法の適応と考えるか」という設問では専門医は70～75歳までを適応と考えているが，非専門医はおおむね70歳以下と考えており，IFN適応年齢を非専門医は低く考えがちであることも明らかとなった[2]。このような実態を踏まえ，一例ごとの事例検討会，IFN療法をテーマにした講習会などを繰り返し行い，2004年102例中24例

		初年度IFN療法施行率	
		精極受診者中	慢性肝炎中
全国	2002年	13.8%	
	2003年	13.3%	
	2004年	18.2%	
	2005年		
	2006年		
石川県	2002年	3.0%	3.8%
	2003年	5.7%	8.5%
	2004年	14.7%	23.5%
	2005年	24.5%	35.3%
	2006年	23.7%	31.0%

	キャリア（n＝13）＋慢性肝炎（n＝75） n＝88
IFN過去にあり	28（著効 6例）
現在投与中	7
投与開始	7
IFN施行数（率）	42/88（48%）
合併症不可（IP，うつなど）	4
IFN可能症例施行数（率）	42/84（50%）
IFN検討中	8

（23.5%），2005年68例中24例（35.3%），2006年71例中22例（31.0%）と後半2年間はIFN療法施行率が30%を超えていた（表2）

年々IFN施行率は上昇してきたが，さらに向上させるには専門医が関わることが重要である．石川県では精密検査を専門医が行った症例では144例中53名（36.8%）がすぐにIFN導入され，翌年以降にさらに26例でIFN療法が施行，計79例（54.9%）でIFN療法が導入されていた．一方，かかりつけ医で診られた41症例では計8例（19.5%）のIFN導入にとどまり，IFN療法施行率をあげるには専門医がその診断，治療方針決定に関わることが重要であった．2007年にでた厚生労働省の肝炎検査後診療体制のガイドラインでも「状態に変化がなくとも年一回の専門医療機関受診が望ましい」とされており，かかりつけ医から患者を年1回の専門医に受診勧奨する「石川県肝炎診療連携」を立案した．個人情報保護の問題をクリアし，行政の保持する検診データを拠点病院と専門医療機関で構成する肝炎診療連携協議会に移行するために，行政・各市町と協議の上，患者より「石川県肝炎診療連携」への参加，データ移行に関して再同意をとり，専門医療機関を受診，順次データ移管することとなった．非同意，または返答のなかった症例は引き続き行政でフォローアップをすることとした。

2,570人の肝炎ウイルス検診陽性者に同意書・調査票が送付され494人が同意，非同意が90人，専門医療機関受診し調査票が回収されたのは328人であった．HBs抗原陽性148人，HCV抗体陽性174人であった．HBs抗原陽性では無症候性キャリアと診断されたのが79例で，そのうち5例でALT31IU/L以上の異常値であったが，4例ではHBV-DNA低値の情報が付加されており，診断が妥当であることが確認された．また核酸アナログ使用率も14%とHBs抗原陽性で治療を必要とする従来の割合と合致しているデータと考えられた．HCV抗体陽性者のうち慢性肝炎またはキャリアと診断された症例の治療方針をみると専門医がIFN療法が望ましいとしたのは全体の33%であった．一方経過観察が選択された症例では，ALT値が低いか，超高齢者が多く含まれていた．今回の専門医受診を契機にIFN療法導入が7例あり，過去のIFN歴も踏まえて現在までにIFN療法が行われたのは75歳以下の検診症例で48%であった（表3）

おわりに

肝癌撲滅には背景となるウイルス性肝疾患への適切な経過観察，治療の導入が重要である．県下の肝炎ウイルス検診症例を専門医受診勧奨とデータ管理により早期に適切な治療導入に図りたい。

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2）酒井明人，他：肝炎ウイルス検診でみる高齢者C型慢性肝炎治療の現状と高齢者IFN療法の成績．消化器科 46：403，2008

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Amino Acid Substitution in HCV Core/NS5A Region and Genetic Variation Near *IL28B* Gene Affect Treatment Efficacy to Interferon plus Ribavirin Combination Therapy

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Key Words
Hepatitis C virus · Interferon · Ribavirin · Core region · NS5A region · ISDR · IRRDR · *IL28B*

Abstract
Objective: To evaluate predictive factors of treatment efficacy to interferon (IFN)/ribavirin in patients infected with HCV genotype 1b (HCV-1b). ***Methods:*** This study investigated pretreatment predictors, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in 490 Japanese adults infected with HCV-1b. ***Results:*** The proportion of patients who showed end-of-treatment response (ETR), sustained virological response (SVR), and SVR after ETR was 76, 54, and 76%, respectively. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR. Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. Furthermore, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR. By multivariate analysis, rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core aa 70/91 was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. ***Conclusion:*** aa substitution in core/NS5A region and genetic variation near *IL28B* were important predictors of treatment efficacy to IFN/ribavirin.

Introduction

Treatment of chronic hepatitis C virus (HCV) infection with interferon (IFN) combined with ribavirin carries potential serious side effects and is costly, especially when used long enough to achieve a high sustained virological response (SVR) in patients infected with HCV genotype 1b (HCV-1b) and high viral loads. For these rea-

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sons, those patients who do not achieve SVR need to be identified, so as to free them of unnecessary side effects and reduce costs, preferably before the start of the combination therapy.

Viral- and host-related factors are useful as predictors of treatment efficacy to 48-week IFN/ribavirin combination therapy. With regard to viral factors, amino acid (aa) substitutions at position 70 and/or 91 in the core region of HCV-1b are pretreatment predictors of virological response to combination therapy [1–4], and also affect clinical outcome, including hepatocarcinogenesis [5, 6]. Furthermore, the NS5A region of HCV-1b, including IFN-sensitivity-determining region (ISDR) [7, 8] and IFN/ribavirin resistance-determining region (IRRDR) [9, 10], are also useful as pretreatment predictors of virological response to combination therapy [11, 12]. With regard to host factors, genetic variations near *IL28B* gene (rs8099917, rs12979860) on chromosome 19, which encodes IFN-λ-3, are pretreatment predictors of virological response to combination therapy in individuals infected with HCV-1 [13–16], and also affect clinical outcome, including spontaneous clearance of HCV [17]. A recent report identified genetic variation near *IL28B* gene and aa substitution of the core region as predictors of SVR to triple therapy of telaprevir/pegylated (PEG)-IFN/ribavirin in Japanese patients infected with HCV-1b [18]. However, to our knowledge, there are no previous reports of IFN/ribavirin combination therapy based on multivariate analysis to investigate pretreatment predictors, including all of aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR, and genetic variation near *IL28B* gene.

The aim of the present study was to investigate predictive factors of treatment efficacy, including viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene), to 48-week IFN/ribavirin in Japanese adults infected with HCV-1b.

Patients and Methods

Study Population

A total of 1,249 HCV-1b-infected Japanese adult patients were consecutively recruited into the study protocol of combination therapy with IFN (PEG-IFNα-2b or IFNα-2b) plus ribavirin between December 2001 and January 2009 at Toranomon Hospital, Tokyo, Japan. Among these, 490 patients, who could complete a total of 48 weeks of combination therapy, were enrolled in this retrospective study, and fulfilled the following criteria: (1) negativity for hepatitis B surface antigen (HBsAg) in serum; (2) HCV-1b only confirmed by sequence analysis; (3) HCV-RNA levels of ≥5.0 log IU/ml determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan) within the preceding 2 months of enrolment; (4) no hepatocellular carcinoma; (5) body weight >40 kg; (6) lack of coinfection with human immunodeficiency virus; (7) no previous treatment with antiviral or immunosuppressive agents within the preceding 3 months of enrolment; (8) none was an alcoholic; lifetime cumulative alcohol intake was <500 kg; (9) none had other forms of liver diseases, such as hemochromatosis, Wilson disease, primary biliary cirrhosis, alcoholic liver disease, or autoimmune liver disease, and (10) none of the females was pregnant or breastfeeding.

The study protocol was in compliance with the Good Clinical Practice Guidelines and the 1975 Declaration of Helsinki, and was approved by the institutional review board. Each patient gave their informed consent before participating in this trial.

The treatment efficacy was evaluated in terms of HCV-RNA negativity at the end of treatment (end-of-treatment response (ETR)) and 24 weeks after the completion of therapy (SVR), based on the COBAS TaqMan HCV test (Roche Diagnostics). SVR in patients who achieved ETR was defined as SVR after ETR. ETR, SVR, and SVR after ETR could be evaluated in 487 (99%), 448 (91%), and 321 (66%) of 490 patients, respectively.

422 (86%) patients received PEG-IFNα-2b at a median dose of 1.4 μg/kg (range 0.7–1.9) subcutaneously each week plus oral ribavirin at a median dose of 11.1 mg/kg (range 3.7–15.1) daily for 48 weeks. The remaining 68 (14%) patients received 6 million units of IFNα-2b intramuscularly each day for 48 weeks (daily for the initial 2 weeks, followed by three times per week for 46 weeks), and oral ribavirin at a median dose of 11.3 mg/kg (range 6.8–13.4) daily for 48 weeks.

Table 1 summarizes the profiles and laboratory data of the 490 patients at the commencement of treatment. They included 310 males and 180 females aged 20–75 years (median 54).

Measurement of HCV RNA

The antiviral effects of treatment on HCV were assessed by measuring plasma HCV-RNA levels. In this study, HCV-RNA levels were evaluated at least once every month before, during, and after therapy. HCV-RNA concentrations were determined using the COBAS TaqMan HCV test (Roche Diagnostics). The linear dynamic range of the assay was 1.2–7.8 log IU/ml, and the undetectable samples were defined as negative.

Detection of aa Substitutions in Core, and NS5A Regions of HCV-1b

With the use of HCV-J (accession No. D90208) as a reference [19], the sequence of 1–191 aa in the core protein of HCV-1b was determined and then compared with the consensus sequence constructed on the previous study to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91) [1]. The sequence of 2,209–2,248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al. [7, 8] was determined, and the number of aa substitutions in ISDR was defined as wild-type (WT) (0, 1) or non-wild-type (non-WT) (≥2) in comparison with HCV-J. Furthermore, the sequence of 2,334–2,379 aa in the NS5A of HCV-1b (IRRDR) reported by El-Shamy et al. [9, 10] was determined and then compared with the consensus sequence constructed on the previous study. In the present study, aa substitutions of the core region and NS5A-ISDR/IRRDR of HCV-1b were analyzed by direct sequencing [10, 18].

Genetic Variation near IL28B Gene

Samples for genome-wide association survey were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip. Genotyping data were subjected to quality control before the data analysis. Genotyping for replication and fine mapping was performed by use of Invader assay, TaqMan assay, or direct sequencing as described previously [20, 21].

In this study, genetic variations near *IL28B* gene (rs8099917), reported as the pretreatment predictors of treatment efficacy in Japanese patients [14, 18], were investigated.

Statistical Analysis

Non-parametric tests (Mann-Whitney U test, χ^2 test and Fisher's exact probability test) were used to compare the characteristics of the groups. Correlation analysis was evaluated by the Spearman rank correlation test. Uni- and multivariate logistic regression analyses were used to determine those factors that significantly contributed to ETR, SVR, and SVR after ETR. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All p values <0.05 by the two-tailed test were considered significant. Variables that achieved statistical significance ($p < 0.05$) on univariate analysis were entered into multiple logistic regression analysis to identify significant independent predictive factors. Each variable was transformed into categorical data consisting of two simple ordinal numbers for uni- and multivariate analyses. Potential predictive factors associated with ETR, SVR, and SVR after ETR included the following variables: sex, age, history of blood transfusion, familial history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, γ-glutamyl transpeptidase (GGT), leukocyte count, hemoglobin, platelet count, level of viremia, α-fetoprotein, total cholesterol, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, triglycerides, uric acid, ribavirin dose/body weight, genetic variation near *IL28B* gene, and aa substitution in the core region, and NS5A-ISDR/IRRDR. Statistical analyses were performed using SPSS software (SPSS Inc., Chicago, Ill., USA).

Results

Response to Therapy

ETR was achieved by 372 of 487 (76%) patients, SVR by 244 of 448 (54%), and SVR after ETR by 244 of 321 (76%).

Number of aa Substitutions in NS5A-ISDR and NS5A-IRRDR

As a whole, 0, 1, and ≥2 aa substitutions in ISDR were found in 56% (227 of 406), 23% (95 of 406), and 21% (84 of 406) of patients, respectively. Thus, the percentage of patients with ≤1 aa substitution in ISDR (WT) was 79% (322 of 406). Furthermore, ≤3, 4–5, and ≥6 aa substitutions in IRRDR were found in 36% (73 of 200), 34% (67 of 200), and 30% (60 of 200) of patients, respectively (fig. 1).

Table 1. Patient profile and laboratory data at commencement of the 48-week combination therapy of IFN + ribavirin in 490 patients infected with HCV-1b

Demographic data	
Number of patients	490
Male/female	310/180
Age, years	54 (20–75)
History of blood transfusion	169 (34%)
Family history of liver disease	96 (20%)
Body mass index, kg/m^2	22.6 (15.7–34.7)
Laboratory data	
Level of viremia, log IU/ml	6.4 (2.2–7.7)
Serum AST, IU/l	50 (16–296)
Serum ALT, IU/l	67 (12–836)
Serum albumin, g/dl	3.9 (3.1–4.7)
GGT, IU/l	44 (10–592)
Leukocyte count, n/mm^3	4,700 (1,200–10,900)
Hemoglobin, g/dl	14.4 (10.6–18.1)
Platelet count, × 10^4/mm^3	16.7 (6.4–37.5)
α-Fetoprotein, μg/l	5 (1–459)
Total cholesterol, mg/dl	170 (96–284)
High-density lipoprotein cholesterol, mg/dl	46 (13–95)
Low-density lipoprotein cholesterol, mg/dl	100 (32–190)
Triglycerides, mg/dl	90 (33–416)
Uric acid, mg/dl	5.5 (2.3–9.4)
Treatment	
PEG-IFNα-2b/IFNα-2b	422/68
Ribavirin dose, mg/kg	11.2 (3.7–15.1)
aa substitutions in the HCV-1b	
Core aa 70, arginine/glutamine (histidine)	266/151
Core aa 91, leucine/methionine	246/169
ISDR of NS5A, 0/1/≥2	227/95/84
IRRDR of NS5A, ≤3/4–5/≥6	73/67/60
Genetic variation near IL28B *gene*	
rs8099917 genotype, TT/TG/GG	150/65/4

Data represent number of patients with percentages in parentheses, or median (range) values.

The correlation between ISDR and IRRDR was analyzed. There was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR ($r = 0.308$, $p < 0.001$) (fig. 2).

aa Substitutions in the Core Region and NS5A-ISDR/IRRDR

Concerning the substitution of core aa 70, the number of aa substitutions in ISDR of 256 patients with Arg70 (median 0) was not significantly different from that of 146 patients with Gln70 (His70) (median 0) (fig. 3a). Fur-

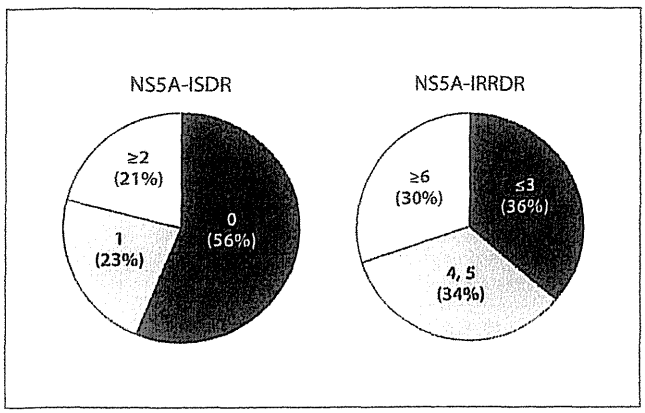

Fig. 1. The number of aa substitutions in NS5A-ISDR and NS5A-IRRDR. The percentage of patients with ≤1 aa substitution in ISDR (WT) was 79%.

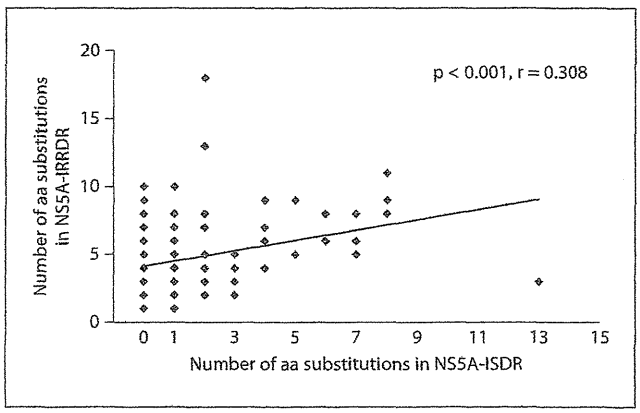

Fig. 2. Correlation between NS5A-ISDR and NS5A-IRRDR. There was a significant positive correlation between the number of aa substitutions in ISDR and that in IRRDR ($r = 0.308$, $p < 0.001$).

thermore, the number of aa substitutions in IRRDR of 123 patients with Arg70 (median 5) was also not significantly different from that of 77 patients with Gln70 (His70) (median 4) (fig. 3b).

Concerning the substitution of core aa 91, the number of aa substitutions in ISDR of 240 patients with Leu91 (median 1) was significantly higher than that of 161 patients with Met91 (median 0) ($p < 0.001$) (fig. 3c). Furthermore, the number of aa substitutions in IRRDR of 111 patients with Leu91 (median 5) was significantly higher than that of 89 patients with Met91 (median 3) ($p < 0.001$) (fig. 3d).

Viremia Level and aa Substitutions in Core Region/ISDR/IRRDR

Concerning the number of substitutions in ISDR, viremia levels of 321 patients with WT (median 6.5) were significantly higher than those of 84 patients with non-WT (median 5.7) ($p < 0.001$) (fig 4a).

Concerning the number of substitutions in IRRDR, viremia levels of 140 patients with ≤5 substitutions (median 6.4) were significantly higher than those of 60 patients with ≥6 (median 6.1) ($p = 0.027$) (fig. 4b).

Concerning the substitution of core aa 70, viremia levels of 265 patients with Arg70 (median 6.4) were not significantly different from those of 151 patients with Gln70 (His70) (median 6.3) (fig. 4c).

Concerning the substitution of core aa 91, viremia levels of 169 patients with Met91 (median 6.5) were significantly higher than those of 245 patients with Leu91 (median 6.2) ($p = 0.028$) (fig. 4d).

Thus, levels of viremia were influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

Treatment Response according to the Number of aa Substitutions in IRRDR

Concerning the number of aa substitutions in IRRDR, a significantly higher proportion of patients with ≥4 aa substitutions (58%) showed SVR compared to patients with ≤3 (42%) ($p = 0.039$). In contrast, the SVR rate was not significantly different between patients with ≤4 (49%) and those with ≥5 (57%) aa substitutions. Likewise, the SVR rate was not significantly different between patients with ≤5 (51%) and those with ≥6 (55%) aa substitutions (fig. 5a).

The ETR rate was not significantly different between patients with ≤3 (74%) and those with ≥4 (82%) aa substitutions, nor between patients with ≤4 (76%) and those with ≥5 (83%). Likewise, the ETR rate was not significantly different between those with ≤5 (79%) and those with ≥6 (80%) aa substitutions (fig. 5b).

The SVR rate after ETR was not significantly different between patients with ≤3 (61%) and those with ≥4 (74%) aa substitutions, nor between patients with ≤4 (67%) and those with ≥5 (72%). Likewise, they were not significantly different between patients with ≤5 (67%) and those with ≥6 (75%) aa substitutions (fig. 5c).

Thus, it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. However, the ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

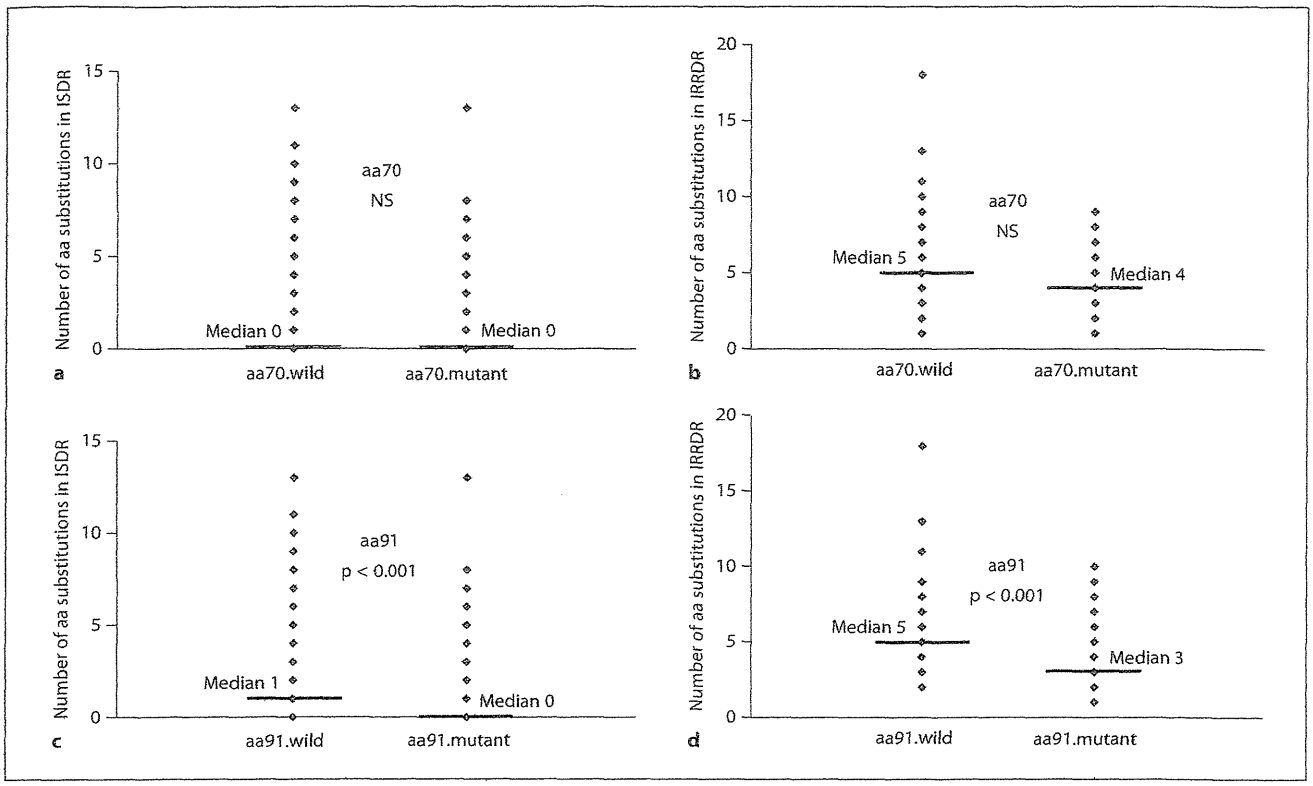

Fig. 3. aa substitutions in the core region and NS5A-ISDR/IRRDR. **a**, **b** Concerning the substitution of core aa 70, the number of aa substitutions in ISDR/IRRDR of patients with Arg70 was not significantly different from that of patients with Gln70 (His70). **c**, **d** Concerning the substitution of core aa 91, the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91 ($p < 0.001$).

Predictors of SVR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 15 parameters that correlate with SVR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.006$), AST (<58 IU/l; $p = 0.039$), leukocyte count (≥4,500/mm^3; $p = 0.043$), hemoglobin (≥14.0 g/dl; $p = 0.001$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), GGT (<50 IU/l; $p = 0.028$), uric acid (≥5.5 mg/dl; $p = 0.005$), level of viremia (<6.0 log IU/ml; $p < 0.001$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), the number of aa substitutions in ISDR (non-WT; $p < 0.001$) and IRRDR (≥4; $p = 0.039$). Figure 6 shows the SVR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 3 parameters that independently influenced SVR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), gender (male sex; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.027$) (table 2).

Predictors of ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 14 parameters that correlated with ETR: gender (male sex; $p = 0.001$), age (<55 years; $p = 0.004$), AST (<39 IU/l; $p = 0.027$), hemoglobin (≥14.0 g/dl; $p = 0.035$), platelet count (≥15.0 × 10^4/mm^3; $p < 0.001$), albumin (≥3.9 g/dl; $p = 0.014$), GGT (<50 IU/l; $p < 0.001$), uric acid (≥5.5 mg/dl; $p = 0.003$), level of viremia (<6.0 log IU/ml; $p = 0.001$), low-density lipoprotein cholesterol (≥85 mg/dl; $p = 0.004$), α-fetoprotein (<10 μg/l; $p < 0.001$), genetic variation in rs8099917 (genotype TT; $p < 0.001$), substitution of aa 70 (Arg70; $p < 0.001$), and the number of aa substitutions in ISDR (non-WT; $p = 0.021$). Figure 7 shows the ETR rate according to aa

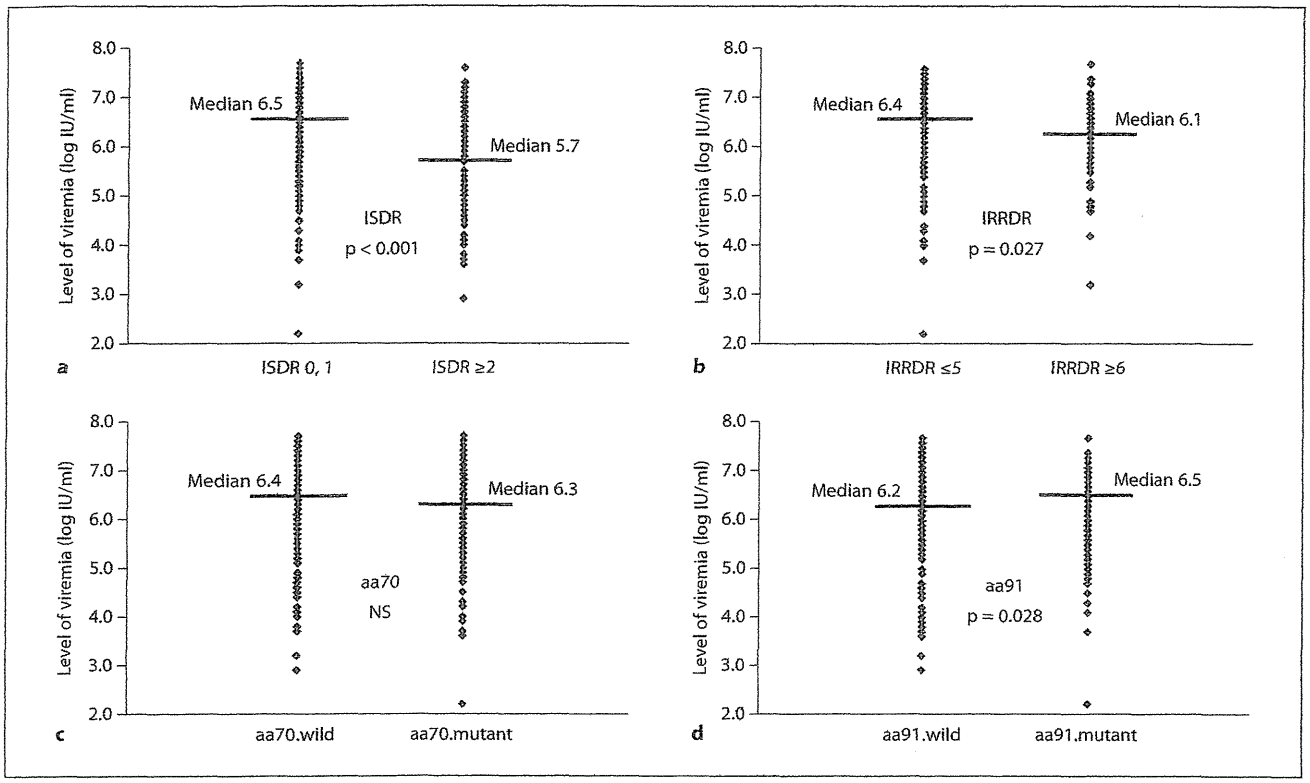

Fig. 4. Viremia level and aa substitutions in core region/ISDR/IRRDR. **a** Concerning the number of substitutions in ISDR, viremia levels of patients with WT were significantly higher than those of patients with non-WT ($p < 0.001$). **b** Concerning the number of substitutions in IRRDR, viremia levels of patients with ≤5 aa substitutions were significantly higher levels than those of patients with ≥6 ($p = 0.027$). **c** Concerning the substitution of core aa 70, viremia levels of patients with Arg70 were not significantly different from those of patients with Gln70 (His70). **d** Concerning the substitution of core aa 91, viremia levels of patients with Met91 were significantly higher than those of patients with Leu91 ($p = 0.028$). Thus, levels of viremia might be influenced by aa substitutions in core aa 91 and ISDR/IRRDR.

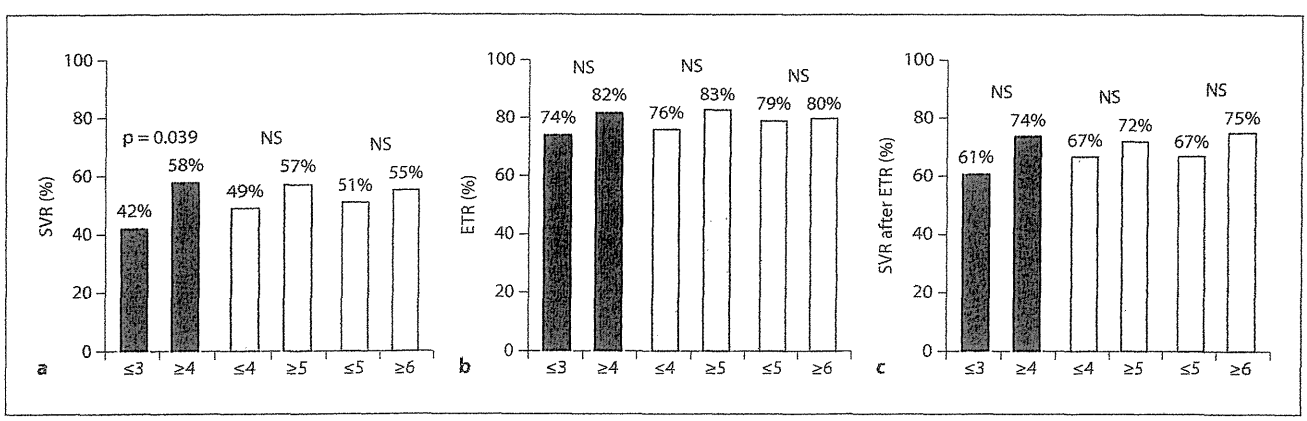

Fig. 5. Treatment response according to the number of aa substitutions in NS5A-IRRDR. **a** A significantly higher proportion of patients with ≥4 (58%) aa substitutions showed SVR compared to patients with ≤3 (42%) ($p = 0.039$), and it was useful as predictor of SVR to categorize into two groups of ≤4 and ≥5 aa substitutions by univariate analysis. **b**, **c** ETR and SVR after ETR rates were not significantly different according to the number of aa substitutions in IRRDR.

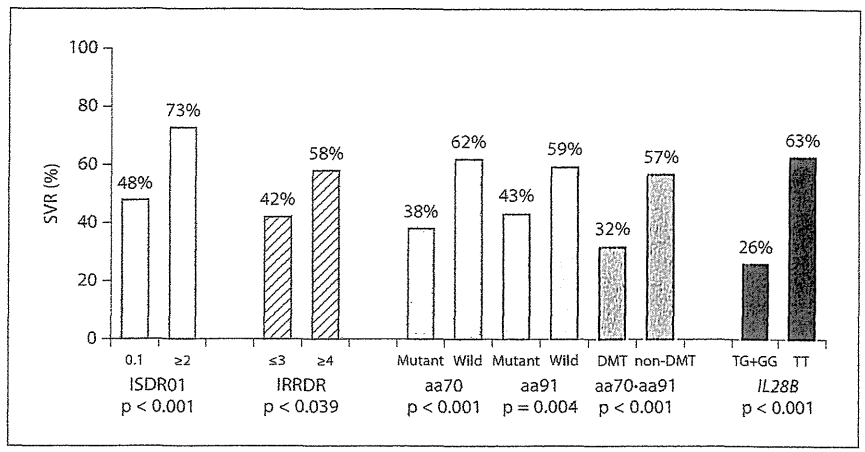

Fig. 6. SVR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

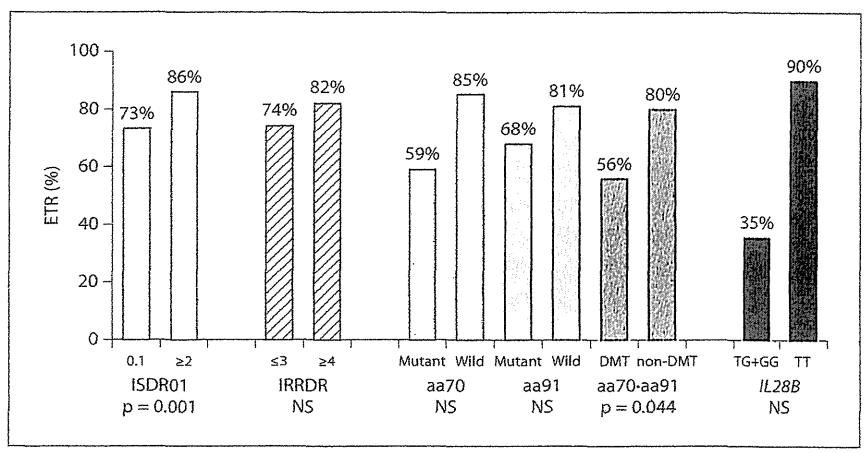

Fig. 7. ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Table 2. Factors associated with SVR to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 16.7 (4.54–61.3)	<0.001
Gender	1: Female 2: Male	1 10.5 (3.47–32.3)	<0.001
ISDR of NS5A	1: WT 2: Non-WT	1 5.68 (1.22–26.3)	0.027

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 3. Factors associated with ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
rs8099917 genotype	1: TG+GG 2: TT	1 18.2 (6.29–52.6)	<0.001
Level of viremia log IU/ml	1: ≥6.0 2: <6.0	1 9.20 (2.59–32.6)	0.001
Core aa 70	1: Gln70 (His70) 2: Arg70	1 4.68 (1.65–13.3)	0.004
Serum albumin g/dl	1: <3.9 2: ≥3.9	1 3.08 (1.11–8.47)	0.030

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

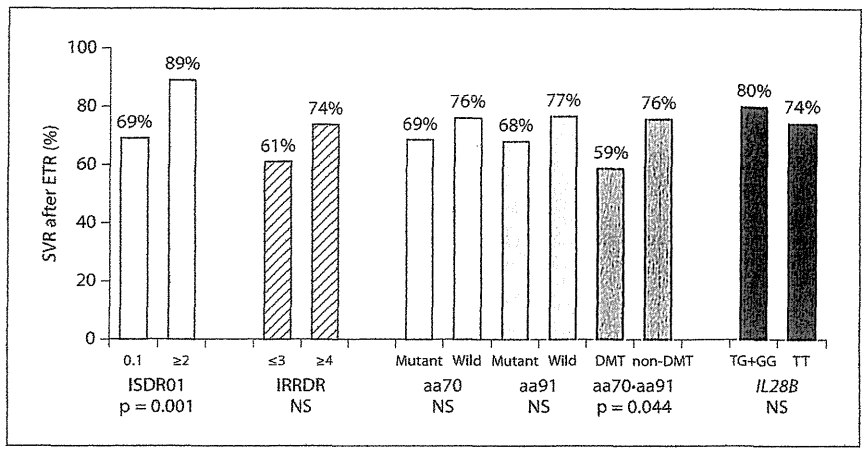

Fig. 8. SVR after ETR rate according to aa substitution in core/NS5A region and genetic variation near *IL28B* by univariate analysis.

substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 4 parameters that independently influenced ETR: genetic variation in rs8099917 (genotype TT; $p < 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.001$), substitution of aa 70 (Arg70; $p = 0.004$), and albumin (≥3.9 g/dl; $p = 0.030$) (table 3).

Predictors of SVR after ETR as Determined by Uni- and Multivariate Analyses

Univariate analysis identified 11 parameters that influenced SVR after ETR: gender (male sex; $p < 0.001$), age (<55 years; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.025$), leukocyte count (≥4,500/mm^3; $p = 0.033$), hemoglobin (≥14.0 g/dl; $p = 0.025$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.001$), level of viremia (<6.0 log IU/ml; $p = 0.020$), total cholesterol (<170 mg/dl; $p = 0.017$), α-fetoprotein (<10 μg/l; $p = 0.004$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.044$), and the number of aa substitutions in ISDR (non-WT; $p = 0.001$). Figure 8 shows the SVR after ETR rate according to aa substitution in the core/NS5A region and genetic variation near *IL28B* by univariate analysis.

Multivariate analysis that included the above variables identified 6 parameters that independently influenced the SVR after ETR: gender (male sex; $p < 0.001$), ribavirin dose (≥11.0 mg/kg; $p = 0.002$), the number of aa substitutions in ISDR (non-WT; $p = 0.012$), substitution of aa 70 and 91 (Arg70 and/or Leu91; $p = 0.023$), platelet count (≥15.0 × 10^4/mm^3; $p = 0.033$), and α-fetoprotein (<10 μg/l; $p = 0.042$) (table 4).

Comparison of Factors Associated with Treatment Efficacy Identified by Multivariate Analysis

Table 5 shows the variables that achieved statistical significance on multivariate logistic regression for each evaluation of treatment efficacy. Rs8099917 genotype was an important predictor of ETR and SVR. With regard to viral factors, core region was an important predictor of ETR, and SVR after ETR. ISDR was an important predictor of SVR, and SVR after ETR. Level of viremia was an important predictor of ETR. Thus, genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia) were important predictors of treatment efficacy. Furthermore, gender, α-fetoprotein, albumin, and platelet count were also identified as other important predictors of treatment efficacy, in addition to genetic variation near *IL28B* and viral factors.

Discussion

Using multivariate analysis, the present study identified viral- (aa substitutions in core aa 70/91 and NS5A-ISDR/IRRDR) and host-related factors (genetic variation near *IL28B* gene) that influenced treatment efficacy to 48-week IFN/ribavirin combination therapy, which is in agreement with recent findings [22, 23]. Identification of these viral and host factors before the start of IFN/ribavirin combination therapy should help to select better therapeutic regimens, including triple therapy of telaprevir/PEG-IFN/ribavirin [24–26], for those patients who are less likely to achieve SVR.

According to the number of substitutions in ISDR, a previous report showed that levels of viremia were sig-

Table 4. Factors associated with SVR in patients who achieved ETR response to 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	Category	OR (95% CI)	p
Gender	1: Female 2: Male	1 4.27 (2.15–8.55)	<0.001
Ribavirin dose, mg/kg	1: <11.0 2: ≥11.0	1 2.95 (1.48–5.86)	0.002
ISDR of NS5A	1: WT 2: Non-WT	1 4.00 (1.35–11.8)	0.012
Core aa 70 and 91	1: Gln70 (His70) and Met91 2: Arg70 and/or Leu91	1 2.96 (1.16–7.52)	0.023
Platelet count $\times 10^4/mm^3$	1: <15.0 2: ≥15.0	1 2.19 (1.07–4.50)	0.033
α-Fetoprotein μg/l	1: ≥10 2: <10	1 2.66 (1.04–6.80)	0.042

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.

Table 5. Comparison of factors associated with efficacy of 48-week IFN + ribavirin combination therapy in patients infected with HCV-1b, identified by multivariate analysis

Factor	ETR response (at 48 weeks)	SVR after ETR response	SVR
IL28B	rs8099917 p < 0.001, 18.2 (6.29–52.6)[a]		rs8099917 p < 0.001, 16.7 (4.54–61.3)[a]
Virus	Core aa 70 p = 0.004, 4.68 (1.65–13.3)[a]	Core aa 70 and 91 p = 0.023, 2.96 (1.16–7.52)[a]	
	Level of viremia p = 0.001, 9.20 (2.59–32.6)[a]	ISDR p = 0.012, 4.00 (1.35–11.8)[a]	ISDR p = 0.027, 5.68 (1.22–26.3)[a]
Others	Albumin p = 0.030, 3.08 (1.11–8.47)[a]	α-Fetoprotein p = 0.042, 2.66 (1.04–6.80)[a]	
		Platelet count p = 0.033, 2.19 (1.07–4.50)[a]	
		Gender p < 0.001, 4.27 (2.15–8.55)[a]	Gender p < 0.001, 10.5 (3.47–32.3)[a]
		Ribavirin dose p = 0.002, 2.95 (1.48–5.86)[a]	

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.
[a] OR (95% CI).

nificantly lower in patients with non-WT of ISDR than in those with WT [8]. The present study indicated that substitution of IRRDR and core aa 91, in addition to substitution of ISDR, also significantly influenced levels of viremia. Furthermore, there was a significant positive correlation between the number of aa substitutions in ISDR and those in IRRDR, and the number of aa substitutions in ISDR/IRRDR of patients with Leu91 was significantly higher than that of patients with Met91. To our knowledge, this is the first report of the relationship between viremia levels and aa substitutions in core region/ISDR/IRRDR. This result might be interpreted to mean

that core aa 91/ISDR/IRRDR might be associated with viremia levels involved in resistance to combination therapy. Further studies that examine the functional impact of aa substitutions to combination therapy should be conducted to confirm the above finding.

The present results showed that α-fetoprotein, albumin, platelet count, and gender were predictors of virological response to IFN/ribavirin combination therapy. Previous data indicated that absence of advanced liver fibrosis was a positive predictor of SVR to IFN monotherapy and IFN/ribavirin combination therapy [2, 3, 13, 27–29], and that advanced liver fibrosis was usually associated with higher levels of α-fetoprotein, and lower levels of albumin and platelet count [1, 3, 30–32]. Furthermore, gender is also a predictor of treatment response to IFN/ribavirin combination therapy [2, 3, 14]. In the present study based on a large number of patients, histopathological changes in the liver and gender were identified as independent predictors of virological response, in addition to genetic variation near *IL28B* and viral factors (core region, ISDR, and level of viremia).

In a previous study, multivariate analysis identified core region, gender, and stage of liver fibrosis as parameters that independently influenced the SVR of patients who achieved early virological response, but ISDR was not entered into uni- and multivariate analysis [3]. To our knowledge, the present study based on multivariate analysis is the first report to identify ISDR as pretreatment predictor of SVR after ETR to combination therapy. Interestingly, ISDR was not a predictor of ETR, but was a significant predictor of SVR to combination therapy. Thus, the underlying mechanisms of failure to develop SVR in those patients who achieve HCV-RNA negativity remain unclear. Further studies that examine the impact of aa substitutions of ISDR to combination therapy should be conducted to confirm the above finding.

One limitation of the present study was that aa substitutions in areas other than the core region and NS5A-ISDR/IRRDR of the HCV genome were not examined. Other limitations were differences in host factors including race [24, 33, 34] and differences in viral factors, such as the distribution of HCV-1a or -1b, and geographic diversities of HCV-1b [35]. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of 48-week IFN/ribavirin combination therapy, and further understanding of the complex interaction between virus- and host- related factors should facilitate the development of more effective therapeutic regimens.

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Complicated Relationships of Amino Acid Substitution in Hepatitis C Virus Core Region and *IL28B* Genotype Influencing Hepatocarcinogenesis

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The impact of amino acid (aa) 70 substitution in the core region on hepatocarcinogenesis and survival for liver-related death in patients of hepatitis C virus (HCV) genotype 1b (HCV-1b), who had not received antiviral therapy, is unknown. The relationships among aa 70 substitution, *IL28B* genotype, and hepatocarcinogenesis are also not clear. A total of 1,181 consecutive HCV-infected patients, who had not received antiviral therapy, were included in a follow-up study to determine predictive factors of hepatocarcinogenesis and survival for liver-related death. The cumulative hepatocarcinogenesis rates in HCV-1b of Gln70(His70) (glutamine (histidine) at aa 70) were significantly higher than those in HCV-1b of Arg70 (arginine at aa 70) and HCV-2a/2b. The cumulative survival rates for liver-related death in HCV-1b of Gln70(His70) were significantly lower than those in HCV-1b of Arg70 and HCV-2a/2b. Multivariate analysis identified gender (male), age (≥60 years), albumin (<3.9 g/dL), platelet count (<15.0 × 10^4/mm^3), aspartate aminotransferase (≥67 IU/L), and HCV subgroup (HCV-1b of Gln70(His70)) as determinants of both hepatocarcinogenesis and survival rates for liver-related death. In HCV-1b patients, the cumulative change rates from Arg70 to Gln70(His70) by direct sequencing were significantly higher than those from Gln70(His70) to Arg70. In patients of Arg70 at the initial visit, the cumulative change rates from Arg70 to Gln70(His70) in *IL28B* rs8099917 non-TT genotype were significantly higher than those in the TT genotype. *Conclusion:* Substitution of aa 70 in the core region of HCV-1b is an important predictor of hepatocarcinogenesis and survival for liver-related death in HCV patients who had not received antiviral therapy. The *IL28B* genotype might partly affect changes over time of dominant amino acid in core aa 70 of HCV-1b. (HEPATOLOGY 2012;56:2134-2141)

Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).[1,2] At present, treatments based on interferon (IFN), in combination with ribavirin, are the mainstay for combating HCV infection. In Japan, HCV genotype 1b (HCV-1b) and high viral loads account for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.[3]

Despite numerous lines of epidemiologic evidence connecting HCV infection and the development of HCC, it remains controversial whether HCV itself plays a direct role or an indirect role in the pathogenesis of HCC.[4] It has become evident that HCV core region has oncogenic potential through the use of transgenic mice, but the clinical impact of the core region on hepatocarcinogenesis is still unclear.[5] Previous reports indicated that amino acid (aa) substitutions at position 70 in the HCV core region of patients infected with HCV-1b are pretreatment predictors of poor virological response to pegylated IFN (PEG-IFN)/ribavirin combination therapy and triple therapy

Abbreviations: aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PEG/IFN, pegylated interferon.

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