●PEG-IFN/RBV/TVR 療法では，IL28B が TT 群(rs8099917)であれば，SVR 率は 80% 以上得られる．

●しかし，同療法でも IL28B が TG/GG 群かつ core aa70 (mutant) かつ ISDR (0-1) の場合は，治療効果が低い．

立されている．血小板減少例，ヘモグロビン低値例，肝硬変症例などには，IFN 単独・少量・長期投与により肝発癌の抑制を考えた治療も加わり，多くのC型肝炎患者に対して病態に応じた IFN 治療が確立されているので，ガイドラインを参照にして最も効率のよい安全な治療を行うことが必要である．治療の選択・実施においては肝臓専門医と密接な連携をとり実行すべきである．

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C型急性肝炎の動向と診断・治療

Epidemiology, diagnosis and treatment for acute hepatitis C in Japan

野村秀幸

Key words：C型急性肝炎，インターフェロン治療，治療開始時期，治療期間

はじめに

急激な肝細胞障害を起こし，全身倦怠感や黄疸などの症状が出現する急性肝炎において，原因が明らかなものはウイルス性急性肝炎が最も多い．我が国では，A型，B型，C型，E型のウイルス性急性肝炎がみられる．

本稿では，C型急性肝炎についてその動向，感染経路，診断，治療などについて述べる．

1. C型急性肝炎の動向と感染経路

a. 動　向

我が国におけるウイルス性急性肝炎の年次的な発生状況に関する検討は少ない．ここでは，国立病院機構共同研究の平成21年度の研究報告書をもとに述べる．1980年から2009年にかけての年次別推移では，C型急性肝炎は散発性急性肝炎の8.5％を占める．特に2000年以降の10年間でも9.5％を占めている．非A非B非C型急性肝炎を除いた，A型，B型，C型ウイルス性急性肝炎のうちC型急性肝炎は14.5％を占めていた．C型急性肝炎は症状に乏しく，劇症化しないため見落とされがちであるが，だいたい急性肝炎の約1割を占め，年次的にも流行などはなく一定している[1]（**図1**）．

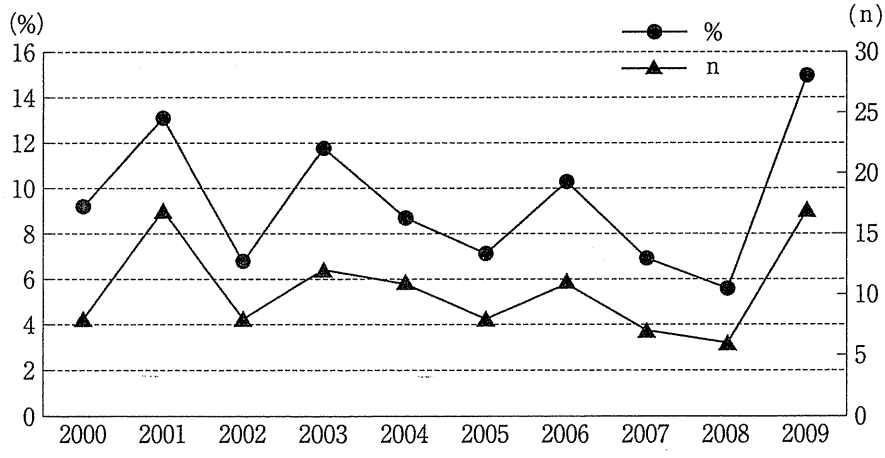

図1　C型急性肝炎の年次推移（2000-2009）
（文献[1]より引用）

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b. 感染経路

1991年までは輸血によるC型急性肝炎が多くみられたが，HCVが同定され，1992年以降第2世代HCV抗体を献血血液スクリーニングに導入して以来，輸血後C型急性肝炎は著明に減少した．その後核酸増幅検査(NAT)の導入により，2000年以降輸血後C型急性肝炎はほとんどみられなくなった．国立病院機構共同研究においても，輸血後C型急性肝炎は2000年以降3例のみの報告であり，特に2004年以降は疑いが1例みられたが，確定症例はみられない．感染経路は原因不明な場合が多いが，原因としては，針刺し事故，麻薬常用者，tattoo施行者，ピアス使用者，医療原性，性行為，である．欧米では静脈内薬物使用(覚醒剤など)がC型急性肝炎の原因の約25–54％を占めている．性的接触による感染は，4–25％である．ほかには，外科的手術などの医療原性などである．一方日本では，針刺し事故による報告が多くみられる．最近は日本でも覚醒剤使用者，tattoo施行者やピアス使用者のC型急性肝炎患者が増加し，若年化している[2–7]．

2. 診断と臨床症状，慢性化

a. C型急性肝炎の診断

C型急性肝炎の診断には，A型肝炎やB型肝炎のように急性肝炎を診断するIgM型抗体がないので，HCV抗体やHCV RNA(real time PCR法)により診断する．HCV抗体はC型肝炎のスクリーニングに広く使用されているが，C型肝炎ウイルスの初感染後数カ月間は陰性である．このためC型急性肝炎発症初期にはHCV抗体が陰性かまたは抗体価が低いことが多い．経時的観察中に抗体が陽性化したり，抗体価が上昇してくることがしばしばみられる．HCV抗体の1回のみの測定でC型急性肝炎を診断するのは不可能である．そのためHCV抗体価の経時的観察は重要である．一方，抗体価が最初から高値の場合は，C型急性肝炎の診断には感染前にHCV抗体が陰性であることが必要である．HCV抗体陰性で，C型急性肝炎の疑いがある場合は，HCV RNA定量(real time PCR法など)検査を行う．

C型急性肝炎の診断は以下に示す．まず，IgM–HA抗体陰性，HBs抗原陰性で，ALTの上昇がみられる急性肝機能障害を認め，C型肝炎ウイルスマーカーとしては，以下の3条件のいずれかを満たすことが必要である．①HCV抗体陰性で，HCV RNA陽性，②HCV抗体陰性かまたは抗体価が低く，経過観察中に抗体価の上昇を認める，③発症数カ月以前にHCV抗体陰性で，発症時陽性である[3]．IgM–HA抗体陰性，HBs抗原陰性，HCV抗体陰性の急性肝炎の場合，まずはC型急性肝炎ではないかと疑ってみることが重要であり，数カ月前に覚醒剤の使用やピアス，tattoo，針刺し事故などの問診を十分に行うことも大切である．

b. 臨床症状

発症時の臨床症状は発熱などの感冒様症状に引き続き，全身倦怠感，食欲不振，悪心などの症状をきたし，他のウイルス性急性肝炎と同様であるため，臨床症状のみでの鑑別は困難である．一般にA型急性肝炎やB型急性肝炎に比べC型急性肝炎の臨床症状は軽く，ごく軽度か無症状の場合もある．日本人では黄疸が出現しない場合もある[3]．HCV感染から発症までの潜伏期間は，5–12週間と考えられているが[8]，感染から発症までを検討した報告では約4週から9週で平均6.8週であった．著者らの針刺し事故患者からの検討では，感染から発症までの期間は約7.3週間(5–12週)であった[3]．C型急性肝炎の激症化は最近の報告ではほとんどみられない．一般的にC型急性肝炎は症状が軽く，重症化例は少ないが，慢性化しやすい．

c. C型急性肝炎の慢性化

C型急性肝炎は発症後約3カ月以内に自然治癒する症例もみられる[2]．また，発症後1–2カ月間，総ビリルビン(T.Bil)，AST，ALTの高値が続き，その後低下することが多い．HCV量は発症時高値のこともあるが，2カ月以内に低下し，その後自然排除して陰性化し自然治癒する場合もある．多くは一度ウイルス量は低下するものの陰性化することもなく，HCV RNAが持続陽性化して，慢性肝炎へとなる．3カ月以上

HCV RNAが持続陽性を続けると，慢性化する確率が非常に高くなる．欧米では黄疸出現症例ほど自然治癒する．黄疸の出現した症例では約50％が慢性化し，黄疸がみられなかった症例では約90％が慢性化した[4]．日本人の場合，必ずしもこのとおりではなく，自然治癒率は低いのではないかと思われる[3]．日本におけるC型急性肝炎は臨床症状に乏しい症例が多く，黄疸が出現しない症例も多くみられるので，慢性化率は70–80％と考えられ，欧米に比べ高率に慢性化するものと思われる．この違いについては不明である．また，発症後のALTに二峰性以上の変動がみられる場合は，より慢性化すると考えられている．

3. 治療と予後

a. 一般的治療

C型急性肝炎は一過性に経過し，自然経過で緩解し，一般的に予後良好な疾患である．急性期の治療は入院治療が原則である．安静と栄養療法が最も大切で保存的治療が中心となり，2–3カ月で治癒する．安静臥床により肝血流の増加を促し，肝障害の治癒を促す．プロトロンビン時間の改善，トランスアミナーゼ値やビリルビン値の低下，自覚症状の改善が確認できれば，安静度を軽減する．劇症化，重症化はまれであるので，食欲が改善すれば点滴や食事の制限の必要はない．

b. インターフェロン治療

1）インターフェロン治療開始時期

C型急性肝炎は，ALT値の変動が一峰性で，発症後2–3カ月以内にHCV RNAが陰性化する症例がみられるため，発症後2–3カ月の経過観察が必要であるが，その後にHCV RNAが陰性化することは少なく，C型急性肝炎から慢性化が予測された場合は，積極的にインターフェロン治療を行うべきである[3,5–7]．著者らの経験ではC型急性肝炎患者34例についてインターフェロンβの8週間連日治療を行い，治療開始時期について検討した．4–12週目以内に治療を開始した16例は全例HCV RNAが持続陰性化し，SVR率は100％であった．13–24週目に治療を開始した7例中4例がSVRとなり，SVR率は57％であった．25週以降に治療を開始した11症例においてはSVR率は55％であった．12週までに治療を開始すればgenotypeやウイルス量に関係なく全例SVRとなった．C型急性肝炎への治療開始時期は8週間の経過観察を行い，8週目にHCV RNAが陽性であれば12週目までに治療を開始するのが最良の方法である．

表1 インターフェロン別，治療期間別のHCV RNA持続陰性化率（文献[3,5,6,7,9]より引用）

使用IFN	治療期間	SVR率
IFNα[5]	24W	98％
IFNα[3]	4W	87％
Peg-IFNα[7]	24W	94％
Peg-IFNα[9]	24W	80％
IFNα[6]	24W	75％

2）インターフェロン治療方法

C型急性肝炎へのインターフェロン治療効果は良好である（**表1**）．24週間治療を行えば約90％前後のSVR率が得られる．インターフェロン単独治療で十分である．従来型のインターフェロンα製剤やインターフェロンβ製剤を用いるときは，4週間の連続治療が望ましい[3,5]．短期治療を行うのであれば，インターフェロンα製剤やインターフェロンβ製剤を用いて，8週間の連日投与が望ましい．副作用のことを考えるとリバビリンの併用は，エビデンスも認められていないので避けるべきである．また，ペグインターフェロンを使用する場合は，できれば使用期間は24週の使用が望ましい[7,9,10]．

c. 予　後

C型急性肝炎は劇症化，重症化する例が少ないので，一般的に予後は良好であるが，慢性化する場合が多い．このためにも慢性化が疑われた場合は，積極的にインターフェロン治療を行うべきである．

おわりに

C型急性肝炎は，毎年ウイルス性急性肝炎の

約15％を占めている．今後はピアスやtattooの施行者，覚醒剤使用者などの若年化や増加に伴い，若年者のC型急性肝炎患者が増加することも考えられる．初期には臨床症状が軽いので，診断にはまずはC型急性肝炎ではと疑ってみることが重要である．治療はHCV RNAが3カ月間陽性の場合はインターフェロン治療を勧めるべきである．

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新しいプロテアーゼ阻害剤：MK7009

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索引用語：プロテアーゼ阻害剤，MK7009，C型慢性肝炎，ペグインターフェロン，リバビリン

1 はじめに

1. 日本におけるC型肝炎に対するインターフェロン療法の変遷

C型慢性肝炎に対して1992年からインターフェロン治療が開始された．難治例といわれる日本人に多いジェノタイプ1b型・高ウイルス量例に対しての24週間のインターフェロン単独療法の治療効果(著効率，SVR率：HCV RNAの持続陰性化)は，10％以下であった．2004年からペグインターフェロンとリバビリンによる48週間の併用療法が開始されSVR率は約48％と向上し，現在の標準治療となっている[1]．48週間治療では，HCV RNAが12週目以降に陰性化した症例では再燃率が高いので，レスポンスガイド治療を取り入れ72週間治療が推奨され，SVR率が約60％前後まで向上した．しかし，現在の標準療法であるペグインターフェロン・リバビリン併用療法にはいくつかの問題点がある．1)高齢者への治療効果が低い，2)治療期間が48週間から72週間と長い，3)再燃率が高い，4) SVR率が60％前後である，5)治療中止例のSVR率が低い，などである．治療期間の短縮，再燃率の低下，SVR率の向上を目指すため，現在のペグインターフェロン・リバビリン併用療法にプロテアーゼ阻害剤を追加した3者併用療法の登場が待たれる．

2. 国内におけるMK7009の開発

すでに米国においては，TelaprevirやBoceprevirといったプロテアーゼ阻害剤が発売されており，日本においても2011年末までにはTelaprevirとペグインターフェロン・リバビリンによる3剤併用療法が実施可能の予定である．それらのプロテアーゼ阻害剤に続き，次世代のDAAs (Direct Acting Antiviral Drugs)製剤として，新たなプロテアーゼ阻害剤も開発が続けられている．MK7009は，次世代のプロテアーゼ阻害として開発されており，日本では第Ⅱ相試験を経て，2011年には第Ⅲ相試験が開始されている．

Hideyuki NOMURA : New protease inhibitor: MK7009
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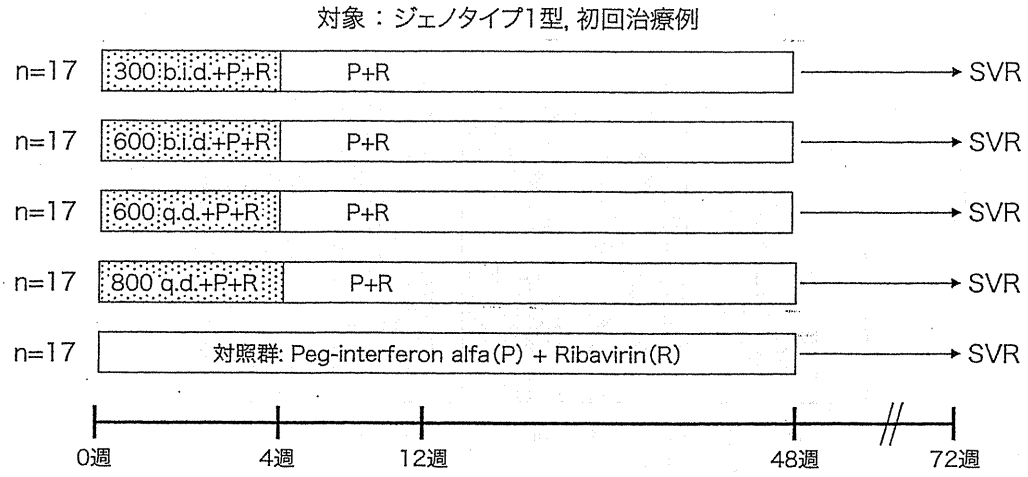

図1　MK-7009　第Ⅱ相試験(007試験)スタディデザイン

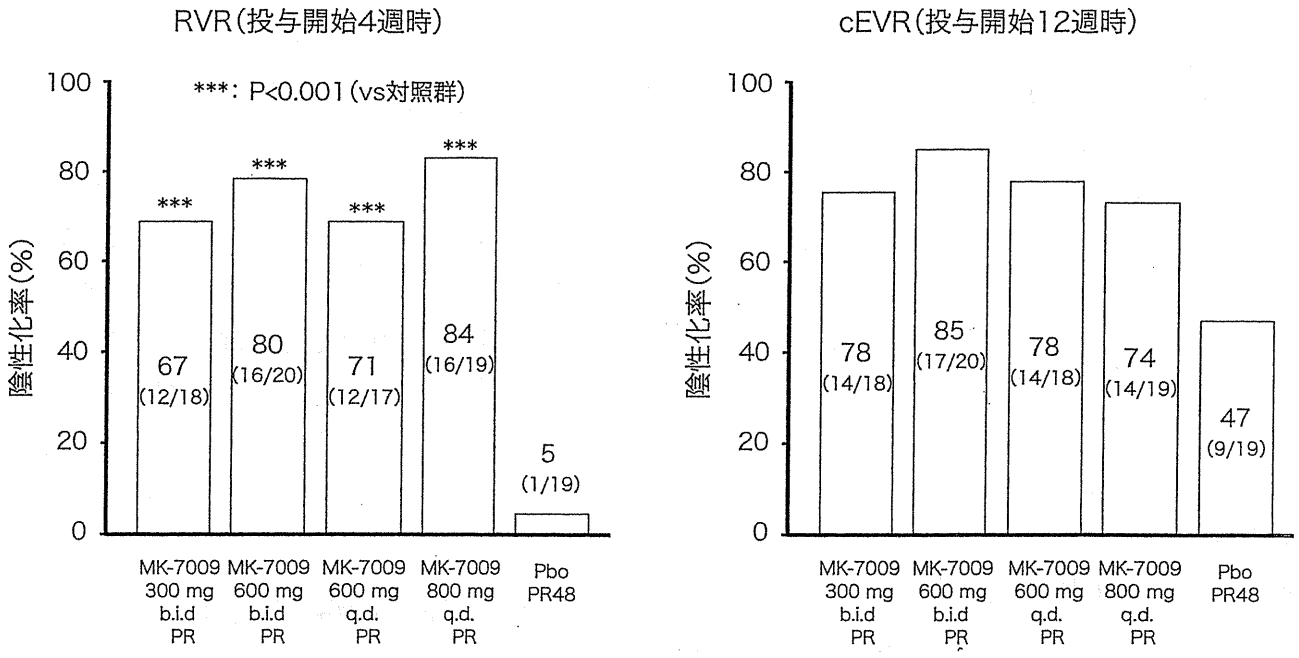

図2　MK-7009　第Ⅱ相試験(007試験)陰性化率(RVR, cEVR)

2 MK7009のプロファイル

1. 分子構造

プロテアーゼ阻害剤はその分子構造から直鎖状(linear)と大環状(macrocyclic)に分けられるが，第一世代とされるTelaprevirやBoceprevirがlinearであるのに対して，TMC435と同様にMK7009はmacrocyclicに分類される．

2. 作用部位

C型肝炎ウイルス(HCV)は，約3,000アミノ酸からなるopen reading frame (ORF)からなり，Core (C), envelope (E1/2)などで構成される構造蛋白領域と非構造領域(NS2/3/4A/4B5A/5B)がある．非構造領域のひとつであるNS3領域は，非構造蛋白質のプロセッシングに関与するだけでなく，HCVのC末端側のATPase活性およびRNAヘリカーゼ活性によって遺伝子複製に関与

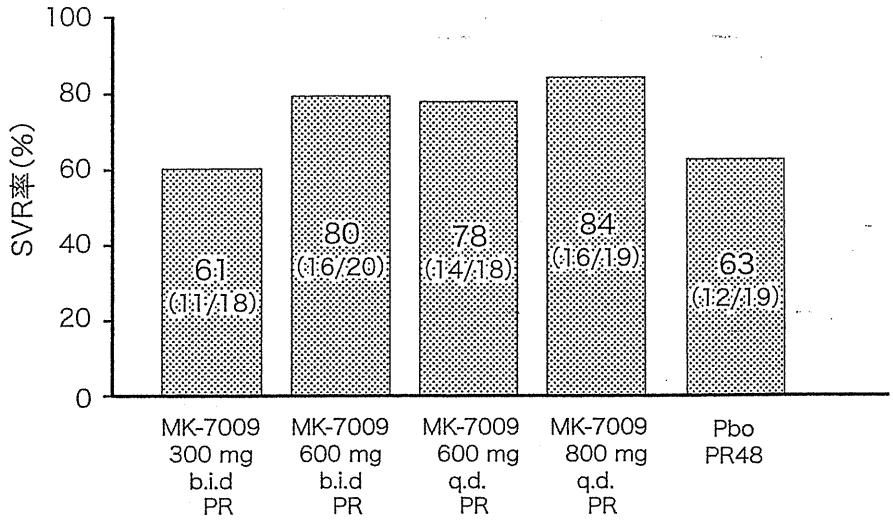

図3　MK-7009　第Ⅱ相試験(007試験) SVR率

したり，TLR (toll-like receptor)の活性化やISG (interferon stimulated gene)の発現阻害に関与することも報告される領域でもある．このNS3によりコードされるHCV由来のセリンプロテアーゼは極めて重要な働きがあり，そのセリンプロテアーゼを標的として阻害しウイルス増殖を抑制する薬剤がセリンプロテアーゼ阻害剤である．

MK7009は，NS3/4Aセリンプロテアーゼ阻害剤のひとつであり，NS3/4Aセリンプロテアーゼを選択的に阻害することによりHCV増殖抑制作用を発揮する薬剤である．

3 MK7009の海外試験（第Ⅱ相試験）成績

1. 007試験(Genotype1，初回治療例)

欧米においてMK7009は，第Ⅱa相試験が実施されており，MK7009の用量検索的な試験として少数の検討結果がAASLD2010で報告されている(図1～図3)[2]．007試験は，ジェノタイプ1型の初回治療例を対象として実施された試験であり，1群17例ずつで，MK7009を300b.i.d./600b.i.d./600q.d./800q.d.で4週間ペグインターフェロンとリバビリンに併用し3者併用療法を行い，その後44週間のペグインターフェロン+リバビリン併用療法を行う臨床試験である．その結果，SVR率はMK7009 300 mg b.i.d.群で61％ (11/18)，600 mg b.i.d.群で80％ (16/20)，600 mg q.d.群で78％ (14/18)，800 mg q.d.群で84％ (16/19)であり，プラセボ群で63％ (12/19)のSVR率に比べて，600 mg b.i.d.，600 mg q.d.群，800 mg q.d.群は高いSVR率が確認されている．

4 MK7009の特異的なメリット

1. 良好な認容性

MK7009の007試験では，有効性の向上が確認されるとともに第一世代のプロテアーゼ阻害剤で認められる特異的に増加する有害事象(皮膚障害，ヘモグロビン値減少)の報告はなく，良好な認容性が確認されている．

皮膚障害：

対照群であるプラセボ+ペグインターフェロン+リバビリン併用療法に比べて，皮膚障害の発現率に有意差はなかった．

ヘモグロビン値減少：

リバビリンに起因すると考えられるヘモグ

表1　ヒト臨床試験および *in vitro* で確認されたNS3プロテアーゼ阻害剤の耐性変異

	V36A/M	T54S/A	V55A	Q80R/K	R155K/T/Q	A156S	A156T/V	D168A/V/T/H	V170A/T
Telaprevir (linear)	■	■	*		■	■	■		*
Boceprevir (linear)	■	■	■		■	■	*		■
SCH900518 (linear)	■	■			■	■	■		■
BILN-2061** (macrocyclic)					▒		▒	▒	
ITMN191/R7227 (macrocyclic)					▒	*	*	▒	
MK-7009 (macrocyclic)					▒			▒	
TMC435350 (macrocyclic)				▒	▒			▒	
BI-201335 (macrocyclic?)					▒			▒	▒

*Mutations associated with resistance in vitro but not described in patients so far

**Mutations associated with resistance in vitro

ロビン値減少はあるものの，Telaprevirによる3剤併用療法でみられる急激なヘモグロビン値減少は認められなかった．

ビリルビン値上昇：

次世代のプロテアーゼ阻害剤に位置づけられるTMC435では，肝細胞における胆汁トランスポーターシステムへの競合阻害によるものと考えられているビリルビンの上昇が認められているが，MK7009では対照群に比べてもビリルビン値の上昇が認められる報告もなかった．

2. 少ない耐性変異の出現

新しい治療薬として，日常臨床への応用が可能となっているTelaprevirでは，治療効果や安全性に加えて耐性ウイルスの出現についても懸念されている．プロテアーゼ阻害剤には，直鎖状(linear)と大環状(macrocyclic)があり，それぞれの耐性変異の特性については違いがあることが報告されている[3]．

つまり，プロテアーゼ阻害剤を単独で投与した場合には，直鎖状プロテアーゼ阻害剤ではNS3領域に起こりうる変異の箇所も種類も多い．一方，大環状プロテアーゼ阻害剤では直鎖状のものに比べて箇所も種類も少ない(表1)．耐性ウイルスが出現した場合，その後にでてくるさらに新しい薬剤あるいは違う作用機序を有する薬剤での治療により効果があげられるのかがポイントとなるが，将来的なことを想定した場合においても耐性変異に与える影響が少ない薬剤が望まれると考えられる．

今回現在第Ⅲ相試験が進行中のMK7009について述べた．プロテアーゼ阻害剤を加えたペグインターフェロン・リバビリン3者併用

療法により，高いSVR率を到達することが可能となった．第一世代のプロテアーゼ阻害剤に比べ，副作用の少ない第二世代のプロテアーゼ阻害剤が開発され，副作用のより少ない，治療効果のより高い治療が今後望まれる．しかし，まだ未知の副作用，耐性ウイルスの出現など多くの課題が残されている．今後，現在臨床治験中の抗ウイルス剤の開発を見守って行く必要がある．

文　献

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Mutations in Two PKR-Binding Domains in Chronic Hepatitis C of Genotype 3a and Correlation With Viral Loads and Interferon Responsiveness

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Interferon (IFN) induces the double-stranded RNA-dependent protein kinase (PKR) to inhibit viral replication. Two motifs of the PKR-binding domain exist in the E2 and the NS5A regions of the hepatitis C virus (HCV). These regions are called the PKR-eukaryotic transcription factor (eIF2-alpha) phosphorylation homology domain (PePHD), and the IFN sensitivity-determining region (ISDR). Both regions are inhibited by PKR. Thus, several studies have reported the relationship between these regions and IFN responsiveness and the HCV viral load. However, the data obtained from these studies remain controversial. The aim of this study was to investigate the genomic heterogeneity of the PePHD and the ISDR in patients with genotype 3a and how this impacts HCV replication and the response to IFN therapy. Twenty-one male patients infected with HCV genotype 3a were studied. The PePHD was well conserved, and mutations were found in only one amino acid position in two patients. Patients with three or more mutations in the ISDR had lower viral loads than those with fewer than two mutations (192.2 ± 176.7 vs. 1279.4 ± 997.6 KIU/ml, $P = 0.0277$). Ten (71.4%) of 14 patients achieved a sustained virological response to IFN therapy. No specific amino acid substitutions in the PePHD and the ISDR were associated with IFN responsiveness; however, the number of mutations in the ISDR was significantly associated with the HCV viral load. The findings from this study suggest that the ISDR plays an important role in regulating viral replication in patients infected with HCV genotype 3a. ***J. Med. Virol. 83:1727–1732, 2011.*** © 2011 Wiley-Liss, Inc.

KEY WORDS: hepatitis C virus (HCV); genotype3a; double-stranded RNA-dependent protein kinase (PKR); E2-PePHD; NS5A-ISDR

INTRODUCTION

Hepatitis C virus (HCV) is a member of the Flaviviridae and causes chronic hepatitis that can develop into cirrhosis and hepatocellular carcinoma (HCC) [Seeff, 2002]. HCV is classified into six genotypes that have different clinical courses, including the incidence of HCC and interferon (IFN) responsiveness. A part of the NS5A region of HCV genotype 1b is closely associated with the response to IFN, and is named the IFN sensitivity-determining region (ISDR) [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004]. IFN acts to inhibit viral replication by inducing the double-stranded RNA-dependent protein kinase (PKR). The ISDR is at the front of the PKR-binding domain and is thought to be inhibited by PKR, according to an in vitro study [Gale et al., 1998]. Thus, ISDR heterogeneity is likely to be an important factor determining the response to IFN treatment. However, despite recent reports investigating ISDR analysis as a predictor of the response to IFN therapy [Zeuzem et al., 1997; Squadrito et al., 2002], the clinical applicability of this approach remains to be established. In addition, it is thought that the mode of HCV infection and the racial background influence the predictions of IFN outcome by the ISDR [Layden-Almer et al., 2005; Jenke et al., 2009]. Although PKR-independent effects on NS5A have been reported, some reports have not confirmed the interaction between PKR and NS5A [Podevin et al., 2001; Tan and Katze, 2001; Liu et al., 2006]. Thus, the role of the ISDR in the interaction of NS5A with PKR remains controversial.

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Another PKR-binding domain, the protein kinase-RNA activated (PKR)-eukaryotic transcription factor (elF2-alpha) phosphorylation homology domain (PePHD), exists in the E2 region of HCV. Several studies have reported a relationship between these two regions and IFN responsiveness [Saiz et al., 1998; Berg et al., 2000; Sarrazin et al., 2000; Durante et al., 2003; Hung et al., 2003; Ukai et al., 2006]. However, this is controversial for genotype 1b, and there are no reports analyzing the NS5A-ISDR and the E2-PePHD in genotype 3a in Japanese patients because of the rarity of genotype 3a in the Japanese population [Hayashi et al., 2003]. The aim of the present study was first to determine whether the genomic heterogeneity of the E2-PePHD and the NS5A-ISDR in Japanese patients with genotype 3a impacts HCV replication. The second aim was to evaluate the efficacy of IFN therapy in Japanese patients with HCV genotype 3a.

MATERIALS AND METHODS

Patients

A total of 21 hemophiliac patients infected with HCV (mean age 45.5 ± 14.6 years; range, 25–80 years) were studied. None of the patients had a history of intravenous drug use, chronic alcohol abuse, autoimmune disease, or metabolic disease. Six patients were co-infected with human immunodeficiency virus (HIV) and were well controlled by highly active antiretroviral therapy (HAART). The clinical characteristics of each patient are summarized in Table I.

Schedule of IFN Therapy

Six patients received six mega units (MU) of IFN alpha 2b (INTRON A; Schering-Plough, Osaka, Japan) injected subcutaneously daily for the first 2 weeks and then three times a week for 22 weeks, in conjunction with oral ribavirin (Rebetol; Schering-Plough) at a dose of 800 mg (for those with a weight <80 kg) daily. Seven patients received Peginterferon alfa-2b (PEG-Intron, Schering-Plough) at a dose of 1.5 μg per kilogram of body weight injected subcutaneously once a week for 48 weeks. Ribavirin (Rebetol; Schering-Plough) was given orally for 48 weeks. One patient had severe anemia, which is a contraindication for ribavirin, and received monotherapy with IFN beta (Feron; Toray, Tokyo, Japan) 600 MIU three times a week for 24 weeks. After the end of the treatment, the patients were followed for a further 24 weeks. HCV-RNA in serum samples was examined at 2, 4, 8, and 12 weeks, at the end of IFN therapy, and again 6 months after the end of the treatment. Patients who were persistently negative for serum HCV-RNA with normal serum ALT levels at 24 weeks after withdrawal of IFN treatment were considered to have a sustained virological response, and all others had non-sustained virological response. Informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki. Serum was stored at −80°C until required.

TABLE I. Clinical Characteristics

	N = 21
Age (years)	45.5 ± 14.6
Sex (male/female)	21/0
AST (IU/L)	59.6 ± 26.6
ALT (IU/L)	83.6 ± 44.1
Platelet count (10^4/μl)	14.7 ± 7.2
HCV RNA level (KIU/ml)	640 (71–3,900)
HIV co-infection (yes/no)	15/6

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
AST, aspartate aminotransferase; ALT, alanine aminotransferase; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

Virological Tests

All of the patients were negative for hepatitis B surface antigen and positive for serum HCV antibody, by commercial ELISA (Dinabot, Tokyo, Japan). In all patients HCV RNA quantitative viremia load was determined by a commercial assay [Kawai et al., 2002]. Anti-HIV antibody was detected by a commercial particle agglutination assay (Fuji Rebio, Tokyo, Japan). HCV genotyping was performed by direct sequencing of the 5′-UTR and E1 regions according to a method described previously [Otagiri et al., 2002; Hayashi et al., 2003]. The genotypes were classified according to the Simmonds' nomenclature [Simmonds et al., 2005]. Direct sequencing of the E2-PePHD and the NS5A-ISDR was performed. Briefly, RNA was extracted from 140 μl of sera using the QIAamp Viral RNA kit (QIAGEN, Valencia, CA) and redissolved in 50 μl diethylpyrocarbonate-treated water. Reverse transcription using oligo and random hexamer primers was carried out on 10 ng of RNA with the iScript cDNA Synthesis Kit (BIO-RAD, Hercules, CA). Amplification of the E2-PePHD and the NS5A-ISDR was performed by nested PCR, as previously reported [Sarrazin et al., 2000; Castelain et al., 2002]. The E2-PePHD primers were: sense 5′-AGAAYSCCCCACYRCTGAGGACAT-3′ and antisense 5′-GGCAGTTTCTCYTCCTCAGCACT-3′. For NS5A-ISDR: sense 5′-GCCCAACACACAACTA-CACGCGCGC-3′ and antisense 5′-CTTTTCCTCCGAG-GAGG-3′. Amplification conditions consisted of 10 min at 94°C, followed by 40 cycles of 94°C for 10 sec, 55°C for 30 sec, and 72°C for 30 sec in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems, Foster City, CA). The second PCR was performed with the following sets of primers: for E2-PePHD, sense 5′-GTGGTA-CCATGAGCGGCGATTGGCTGCGTATCATC-3′ and antisense 5′-GCTCTAG/ATCATCAGCAGCAGACCA-CGCTCTGCTC-3′; and for NS5A-ISDR, sense 5′-TTCTTCACTGAAGTGGATGG -3′, and antisense 5′-GTTGGTGGTACATAATCCGG-3′. The PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide, and visualized under ultraviolet light. The PCR products were also subjected to cycle sequencing with an ABI PRISM Cycle Sequence Kit (Perkin Elmer, Branchburg, NJ) that uses second

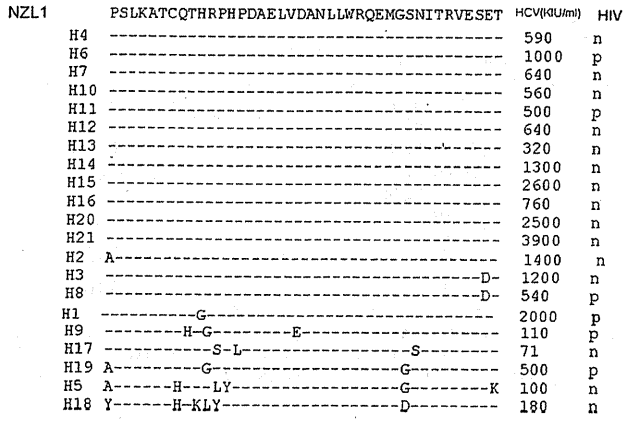

Fig. 1. Alignment of the amino acid sequence of the ISDR. HCV viral loads and co-infection with HIV are also indicated. In the sequence alignment, dashes indicate amino acids identical to the consensus sequence NZL1. ISDR, interferon sensitivity-determining region; HCV, hepatitis C virus; HIV, human immunodeficiency virus; P, positive; N, negative.

round primers as the sequencing primer, and analyzed on a ABI 310 DNA Sequencer (PE Applied Biosystems). The sequence data were aligned and analyzed with GENETYX software (version 7; Software Development, Tokyo, Japan).

Statistical Analysis

The data are expressed as the mean ± standard deviation (SD). The paired *t*-test, the chi-square test, and Fisher's exact test were used to analyze differences in variables. A value of $P < 0.05$ was considered statistically significant. The statistical software used was Statview 5.0 (SAS Institute Inc., Cary, NC).

RESULTS

Sequencing revealed that the E2 region including the PePHD was well conserved. In two cases, a mutation (S665R and H668Q) was found in the E2-PePHD. The relationship between the NS5A-ISDR substitutions, the HCV viral loads, and co-infection with HIV are shown in Figure 1. The number of mutations in the ISDR of the NZL1 strain was determined, and ISDR sequences with more than three mutations were defined as the mutant type. Five patients were classified with the mutant type and 16 patients as infected with the wild type. Six patients were co-infected with HIV. The HCV viral loads and the frequency of substitutions in the NS5A-ISDR were not significantly different between patients with HIV and those without HIV. Clinical characteristics relating to the ISDR are presented in Table II. Patients with three or more mutations in the NS5A-ISDR (mutant type) had lower viral loads than those with fewer than two mutations (wild type; 192.2 ± 176.7 vs. 1279.4 ± 997.6 KIU/ml, $P = 0.0277$). No other factors were statistically significant between the mutant and the wild types. A virological response, defined as negativity for HCV after 2, 4, 8, and 12 weeks of IFN therapy, occurred in 8, 2, 1, and 3 patients, respectively. All patients were negative for HCV-RNA at the end of the treatment. Sustained virological response was defined as no detection of HCV-RNA at 24 weeks after the end of the treatment. Ten patients remained negative for HCV-RNA, but four patients relapsed with HCV viremia after the treatment ceased. Thus, 10 (71.4%) of 14 patients achieved sustained virological response. Statistical analyses for associations between the response to IFN therapy and clinical characteristics, including substitutions in the E2-PePHD and the NS5A-ISDR, are shown in Table III. Detection of HCV RNA and an early virological response at the point of HCV negativity after 12 weeks of IFN therapy was predictive of SVR. However, other factors, including amino acid substitutions in the E2-PePHD and the NS5A-ISDR, were not associated with IFN responsiveness. There were no significant differences in HCV RNA levels between patients that were HCV negative after 12 weeks of IFN therapy with an early virological response and those without an early virological response (996.5 ± 1189.1 vs. 1666.7 ± 1040.8 KIU/ml, $P = 0.3947$). HCV RNA levels were not associated with early virological response.

DISCUSSION

HCV has been classified into six genotypes that have different geographic distributions. In Japan, genotype 1b is predominant, and genotypes 2a and 2b account for the majority of cases in Japanese patients with chronic hepatitis C. Therefore, chronic hepatitis with HCV genotypes 1b, 2a, and 2b have been well investigated

TABLE II. Clinical Characteristics According to ISDR

Factors	Wild type (n = 16)	Mutant type (n = 5)	*P*-value
Age (years)	45.2 ± 13.9	46.6 ± 18.1	0.8554
ALT (IU/L)	79.1 ± 40.0	98.0 ± 58.0	0.4157
AST (IU/L)	52.1 ± 18.2	61.8 ± 35.3	0.5034
PLT ($\times 10^4$/mm^3)	14.1 ± 8.0	16.6 ± 10.4	0.5065
HCV RNA level (KIU/ml)	1279.4 ± 997.6	192.2 ± 176.7	0.0277
HIV: P/N	4/12	2/3	0.5975
PePHD: wild/mutant	1/15	1/4	0.2857

AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; HIV, human immunodeficiency virus; P, positive; N, negative; PePDH, protein kinase-RNA activated-eukaryotic transcription factor phosphorylation homology domain; ISDR, interferes sensitivity-determining region.
Data are expressed as mean ± standard deviation.

TABLE III. Univariate Analysis: Factors Predictive of SVR

	SVR (n = 10)	Non-SVR (n = 4)	*P*-value
Age (years)	41.9 ± 13.3	52.5 ± 8.7	0.1705
ALT (IU/L)	82.4 ± 37.3	88.3 ± 57.1	0.8223
AST (IU/L)	52.1 ± 18.2	61.8 ± 35.3	0.5034
PLT ($\times 10^4$/mm^3)	15.1 ± 5.2	19.3 ± 10.3	0.3188
HCV RNA level (KIU/ml)	706.1 ± 735.3	2225.0 ± 1403.2	0.0190
HIV: P/N	2/8	2/2	0.5205
PePHD: wild/mutant	10/0	3/1	0.2857
ISDR: wild/mutant	7/3	3/1	0.9999
EVR: yes/no	10/0	1/3	0.0110

SVR, sustained virologic response; AST, aspartate aminotransferase; ALT, alanine aminotransferase; PLT, platelet count; HCV, hepatitis C virus; HIV, human immunodeficiency virus; P, positive; N, negative; PePDH, protein kinase-RNA activated-eukaryotic transcription factor phosphorylation homology domain; ISDR, interferon sensitivity-determining region; EVR, early virologic response.
Data are expressed as mean ± standard deviation.

in Japan. However, the other genotypes (3, 4, 5, and 6) are uncommon, and clinical data are scarce. Genotype 3a, a rare strain in Japan, is limited to patients with hemophilia because they receive clotting factors of foreign origin [Fujimura et al., 1996; Otagiri et al., 2002]. Therefore, little is known about the clinical characteristics including IFN responsiveness and the genomic heterogeneity of the E2-PePHD and the NS5A-ISDR in patients with chronic hepatitis C, genotype 3a in Japan. HCV genotype is one of the most important factors relating to the response to IFN therapy. For example, genotypes 1 and 4 show resistance to IFN therapy, whereas genotypes 2 and 3 show a good response. The present study revealed that 10 (71.4%) of 14 patients achieved sustained virological response. The high virological response rate in genotype 3a has been confirmed in the present Japanese patients as it has in Caucasian patients. HCV RNA levels and early virological response were also associated with sustained virological response, as reported previously. The role of mutations in the E2-PePHD region, which is a PKR-binding domain, among Japanese patients with chronic hepatitis C infected with genotype 3a was analyzed. The E2-PePHD was well conserved, as previously reported, and all strains but two were identical to the consensus sequence [Chayama et al., 2000; Sarrazin et al., 2000]. It was difficult to clarify the relationship between the HCV viral load and the response to IFN therapy because of the small sample number in the present study. An in vitro study has revealed the potential of the E2-PePHD [Taylor et al., 1999]; however, many more patients with this mutation would be needed to determine the function of the E2-PePHD. Mutations in the E2-PePHD are quite rare, so this region, as well as HCV genotype 1, is unsuitable for routine clinical assessment [Muñoz et al., 2008].

The usefulness of the NS5A-ISDR for the prediction of IFN therapy differs between regions, such as in Asia and Europe [Enomoto et al., 1996; Zeuzem et al., 1997; Nakano et al., 1999; Squadrito et al., 2002; Pascu et al., 2004; Muñoz et al., 2008; Yen et al., 2008; Okanoue et al., 2009; Hayashi et al., 2010]. HCV genotype 1b is distributed worldwide, yet subtypes differ in each region. It has been hypothesized previously that the geographic distribution of genotype 1b would be a factor that influences the usefulness of the ISDR. Subtype J, found only in Japan, and subtype W, distributed worldwide, have been identified. Subtype J is closely associated with the ISDR and confers a good response to IFN therapy [Nakano et al., 1999, 2001]. Thus, the prevalence of specific subtypes is one reason behind the conflicting opinions towards the use of the ISDR. This extends to racial differences [Reddy et al., 1999; Shiffman et al., 2007]. Forming a control group when comparing racial effects on chronic hepatitis C proved difficult due to the heterogeneity of HCV genotypes and subtypes. Thus, a study to evaluate racial differences is not easy to perform because of varying HCV genotype distribution. Japanese hemophiliacs receive clotting factors that are imported from foreign countries and are frequently infected with genotype 3a. Thus, the present study on genotype 3a allowed the assessment of racial differences by avoiding the distribution bias of HCV genotype. In the present study, the ISDR was not associated with the response to IFN therapy, as in Western reports [Sarrazin et al., 2000]. The prevalence of HCV genotypes and subtypes impacts the association between the ISDR and the IFN response to a far greater degree than racial differences.

There are several limitations to the present study. Firstly, the small number of patients examined prevented the identification of a correlation between the ISDR and IFN responsiveness. Secondly, the low-virological response rate and the small number of mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness, as previously reported for genotype 1 [Herion and Hoofnagle, 1997]. Furthermore, the high virological response rate and the low prevalence of patients with more than three mutations in the ISDR do not favor the use of ISDR analysis in predicting IFN responsiveness. Four patients had more than three mutations in the ISDR, which was defined as the mutant type, and only one patient did not achieve sustained virological response. This patient was co-infected with HIV. HIV co-infection is known to be a negative factor for sustained virological response [Hayashi et al., 2000; Shire et al., 2006, 2007]. Thus, patients with more than three mutations in the ISDR tend to have good IFN responsiveness, though no statistically significant differences have been noted.

This might explain why a strong correlation between the ISDR and IFN responsiveness was not observed. The number of amino acid substitutions in the ISDR inversely correlates with the HCV viral load in patients with genotype 1 [Enomoto et al., 1996; Nakano et al., 1999; Pascu et al., 2004]. Similar observations were made in our study for genotype 3a suggesting an important role for the NS5A-ISDR in regulating viral replication in these patients. However, because of the small sample size in the present study, larger cohorts are needed to confirm these results.

In addition to racial factors, host factors also contribute to IFN treatment outcomes. Many investigators have reported that gender [Alvarez, 1996; Izopet et al., 1998], age [Hayashi et al., 1998], and past history of transfusion [Kleter et al., 1998] are associated with sustained virological response in IFN-treated patients with HCV, although this is somewhat controversial. Since all of the patients in the present study were males with a past transfusion history, and they were younger than the average HCV-infected patients, we were not able to investigate a possible effect of these factors in our study population.

In conclusion, in Japanese patients with HCV genotype 3a, the E2-PePHD and the NS5A-ISDR are not associated with IFN responsiveness. The NS5A-ISDR is significantly associated with the HCV viral load suggesting an important role for NS5A-ISDR in regulating viral replication in these patients.

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CLINICAL STUDIES

Association of interleukin 28B and mutations in the core and NS5A region of hepatitis C virus with response to peg-interferon and ribavirin therapy

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Keywords
core region – genotype 1b – hepatitis C virus – interleukin 28B – NS5A

Abbreviations
aa, amino acid; ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; SVR, sustained virological response.

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Abstract

Background and aims: Mutations in the core and NS5A region of hepatitis C virus (HCV) genotype 1b have been associated with response to interferon (IFN) therapy. Genome-wide association studies have revealed that the single-nucleotide polymorphism (SNP) of interleukin 28B (IL28B) contributes to IFN response. The aim of this study was to investigate whether the SNP of IL28B (rs8099917) and amino acid substitutions in the core and NS5A region affect the response to IFN therapy. *Methods:* A total of 299 patients (157 men, 142 women; mean age, 55.9 ± 10.3 years) infected with HCV genotype 1b were studied. The fibrosis stage was diagnosed as F0 ($n=23$), F1 ($n=121$), F2 ($n=62$), F3 ($n=32$) and F4 ($n=7$) by liver biopsy. *Results:* Of the 299 patients, 138 achieved sustained virological response (SVR). On univariate analysis, predictors of SVR were age < 60 years, male gender, higher platelet count, lack of fibrosis, non-Q at core 70, mutant-type interferon sensitivity-determining region (ISDR) and IL28B genotype TT. The factors related to SVR on multivariate analysis were IL28B ($P=0.0001$), fibrosis ($P=0.0111$) and mutations in the core region70 ($P=0.0267$) and ISDR ($P=0.0408$). The best SVR was achieved in patients with non-Q70, mutant-type ISDR and T allele (74.5%), and the worst was achieved in patients with Q70, wild-type ISDR and G allele (8.1%). *Conclusions:* The SNP of IL28B and mutations in the core region and NS5A are associated with IFN responsiveness. Both host and viral factors might be useful for predicting IFN response.

It has been estimated that 170 million worldwide are infected with hepatitis C virus (HCV), which causes chronic hepatitis that can develop into potentially fatal cirrhosis and hepatocellular carcinoma (1). Therefore, HCV infection is a major global health problem. Pegylated-interferon (IFN)-α and ribavirin combination therapy is standard treatment for patients with chronic hepatitis C, but it eradicates HCV for only 50% of patients with genotype1 (2, 3). The difference in response was investigated, and several factors were identified, including age, liver fibrosis, HCV genotype, HCV RNA levels and race (4–7). Viral factors were frequently the focus for investigation of IFN responsiveness, and amino acid (aa) substitutions in the core and NS5A regions were reported as markers that could be used to predict the response to IFN therapy (8–14). However, these relationships were controversial (15, 16), and investigations were limited to viral factors alone to clarify IFN responsiveness. However, host genetic factors, as well as genetic heterogeneity in the HCV genome, contribute to IFN treatment outcomes. Therefore, several genome-wide association studies were performed to understand the host factors that were associated with IFN responsiveness; these revealed that interleukin 28B (IL28B) polymorphisms are strongly associated with response to IFN therapy (17–20). The single-nucleotide polymorphisms (SNPs) of IL28B, rs12979860 and rs8099917 genotypes are significantly associated with the outcome of IFN therapy. Although Caucasians and Hispanics have weak linkage-disequilibrium between these two SNPs, Japanese patients have strong linkage-disequilibrium, with no discrepancy between rs12979860 and rs8099917. Thus, rs8099917, which is strongly associated in Japanese reports, was selected for the present study (21). SNP of IL28B and mutations in the core and NS5A regions had different effects on IFN responsiveness, and their combined use might improve the ability to predict the response to IFN. However, the relationships between IL28B and viral factors such as mutations in the core and NS5A regions are little known. The aim of this study

was to investigate whether the SNP of IL28B and aa substitutions in the core and NS5A regions in patients with HCV genotype 1b affect the response to pegylated-IFN-α 2b and ribavirin combination therapy.

Methods

A total of 432 patients with chronic hepatitis C genotype1b and high viral load who were treated at Nagoya University Hospital, Fujita Health University Hospital and Ogaki Municipal Hospital were enrolled; 299 patients who completed IFN treatment for 48 weeks and had complete clinical data were selected for this study. Patients whose HCV RNA levels were < 100 KIU/ml were excluded. The patients' clinical characteristics are summarized in Table 1. The core region (aa 30–110) and interferon sensitivity-determining region (ISDR) (aa 2209–2248) were examined by direct sequencing. Identification of the SNP of IL28B (rs8099917) was performed by a real-time polymerase chain reaction (PCR) system. Liver biopsy was performed in 245 patients, and fibrosis stage was diagnosed according to the METAVIR criteria (22). Patients received subcutaneous injections of pegylated-IFN-α 2b (1.5 μg/kg) once each week plus oral ribavirin (600 mg for < 60 kg, 800 mg for 60–80 kg, 1000 mg for > 80 kg) daily for 48 weeks. Serum was stored at − 80 °C for virological examination at pretreatment. Patients who were persistently negative for serum HCV RNA at 24 weeks after withdrawal of IFN treatment were considered to have a sustained virological response (SVR). The other patients were considered to have non-SVR. This study was approved by each hospital's ethics committee. Written informed consent was obtained from each patient, and the study protocol conformed to the ethical guidelines of the Declaration of Helsinki.

Virological analysis

The HCV-RNA quantitative viraemia load was determined by PCR. HCV was genotyped by direct sequencing of the 5′-untranslated region and/or E1 regions as described previously (23, 24). Genotypes were classified according to the nomenclature proposed by Simmonds *et al.* (25). Direct sequencing of the HCV core and NS5A-ISDR region was performed as reported previously (9, 14). In brief, RNA was extracted from 140 μl of serum with a commercial kit (QIAamp Viral RNA Kit, Qiagen, Valencia, CA, USA) and dissolved in 50 μl of diethylpyrocarbonate-treated water. RNA (10 ng) was used for reverse transcription with oligo and random hexamer primers using a commercial kit (iScript cDNA Synthesis Kit, Bio-Rad, Hercules, CA, USA). The HCV core region and NS5A-ISDR were amplified by nested PCR. In brief, each 50 μl PCR reaction contained 100 nM of each primer, 1 ng of template cDNA, 5 μl of GeneAmp 10 × PCR buffer, 2 μl of dNTPs and 1.25 U of AmpliTaq Gold (Applied Biosystems, Foster City, CA, USA). Primers for the core region were sense, 5′-GGGAGGTCTCGTA GACCGTGCACCATG-3′ and antisense, 5′-GAGMGG KATRTACCCCATGAGRTCGGC-3′, and primers for the NS5A-ISDR were sense 5′-TGGATGGAGTGCGGTTGCA CAGGTA-3′ and antisense 5′-TCTTTCTCCGTGGAGG TGGTATTG-3′. Amplification conditions consisted of 10 min at 94 °C, followed by 40 cycles of 94 °C for 10 s, 55 °C for 30 s and 72 °C for 30 s in a thermal cycler (GeneAmp PCR System 9700, Applied Biosystems). The second PCR was performed in the same reaction buffer with the first-round PCR product as template and with the following sets of primers: for the core region, sense primer 5′-AGACCGTGCACCATGAGCAC-3′, and antisense 5′-TACGCCGGGGGTCAKTRGGGCCCCA-3′; and for the NS5A-ISDR, sense 5′-CAGGTACGCTCCGGCGTGCA-3′ and antisense 5′-GGGGCCTTGGTAGGTGGCAA-3′. PCR products were separated by electrophoresis on 2% agarose gels, stained with ethidium bromide and visualized under ultraviolet light. PCR products were then purified and sequenced with the second-round PCR primers with a dye terminator sequencing kit (BigDye Terminator v1.1 Cycle Sequencing Kit, Applied Biosystems) and an ABI 310 DNA Sequencer (Applied Biosystems). A mutation mixture was defined as viral mutants that constituted 50% or more of the total viral population.

Table 1. Baseline characteristics of the patients

Clinical characteristics	*N* = 299
Age (years)	55.9 ± 10.3
Sex: male/female	157/142
AST (IU/L)	58.7 ± 48.9
ALT (IU/L)	69.8 ± 66.9
Platelet count (10^4/μL)	16.6 ± 5.3
HCV RNA level (KIU/ml)	1760
The fibrosis stage	(100–7200)
F0, F1, F2, F3, F4	23,121, 62, 32, 7
Body weight (kg)	57.9 ± 12.7

Data are expressed as mean ± standard deviation.
HCV RNA level was shown by median (range).
ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus.

Genomic analysis

Detection of the SNP of IL28B (rs8099917) was carried out by a real-time PCR system. In brief, genomic DNA was extracted from 150 μl of whole blood using a commercial kit (QIAamp DNA Blood mini Kit, Qiagen) and was dissolved in 50 μl of diethylpyrocarbonate-treated water. DNA (10 ng) was used for PCR with primers and probes from a commercial kit (Taqman SNP Genotyping Assays, Applied Biosystems). The SNP of IL28B (rs8099917) was amplified, and the results were analysed by real-time PCR in a thermal cycler (7300 Real-time PCR System, Applied Biosystems).

Statistical analysis

Data are expressed as means ± standard deviation. The paired *t*-test, the χ^2-test and Fisher's exact test were used

to analyse differences in variables. A P value < 0.05 was considered significant. Multiple logistic regression models were used to identify factors predictive of SVR. The statistical software used was SPSS software (SPSS Inc., Chicago, IL, USA).

Results

Virological response

Of 299 patients, 35 (11.7%) showed a rapid virological response (RVR), with HCV negativity at 4 weeks, and 172 (57.5%) showed an early virological response (EVR), with HCV negativity at 12 weeks. Overall, 234 patients became HCV negative at the end of treatment (78.3%). However, 138 patients continued to be HCV negative after withdrawal of IFN treatment, and 138 of 299 (46.2%) patients were defined as achieving SVR. Of 35 patients with RVR, 33 (94.3%) achieved SVR. Of 172 patients with EVR, 126 (73.3%) achieved SVR. Of 127 patients without EVR, 115 became non-SVR (90.6%). Thus, RVR and EVR were associated with SVR ($P < 0.001$).

Genetic heterogeneity in NS5A-interferon sensitivity-determining region and response to interferon therapy

The sequence of the HCVJ strain was defined as the consensus sequence, and the approach of counting the number of mutations to the chosen consensus sequence in ISDR was used to analyse the ISDR system as in previous reports (12–14). Seventy-one patients with more than two mutations in the ISDR were defined as mutant type, and the other 228 patients were wild type. SVR was achieved in 41.2% (95/228) of the patients with wild-type ISDR and in 60.6% (43/71) of the patients with mutant-type ISDR ($P=0.0063$). ISDR was associated with SVR.

Amino acid substitutions in core regions of the hepatitis C virus genome and response to interferon therapy

Eighty-five patients with glutamine in core region 70 were defined as Q-type, and the other 214 patients were non-Q-type, as in previous reports (14). Overall, 118 of 214 patients with non-Q in the core region achieved SVR (55.1%). The SVR rate of patients with Q in core region 70 was 23.5% (20/85). Q70 in core region 70 was significantly associated with poor response to IFN therapy ($P < 0.0001$). The distribution of mutations in the HCV core region at aa 91 was leucine (L), 210 and methionine (M), 89. There were no significant differences between mutations in the HCV core region at aa 91 and SVR.

The prevalence of the single-nucleotide polymorphism of Interleukin28B (rs8099917) T (major allele) and G (minor allele) and response to interferon therapy

The frequencies of the IL28B genotypes were major homozygotes (TT), 219; heterozygotes (TG), 76; and minor homozygotes (GG), 4. The rates of SVR in the patients with TT, TG and GG were 57.9% (127/219), 14.5% (11/76) and 0% (0/4) respectively. The G allele of the IL28B genotype was significantly associated with poor response to IFN therapy ($P < 0.0001$).

Table 2. Association between interleukin 28B genotypes and amino acid substitutions in hepatitis C virus core region and interferon sensitivity-determining region

	ISDR	
	Mutant	Wild
TG/GG	12	68
TT	59	160
P value = 0.0324		
	HCV core region 70	
	Non-Q	Q
TG/GG	35	45
TT	179	40
P value < 0.0001		
	HCV core region 91	
	L	M
TG/GG	46	34
TT	164	55
P value = 0.0044		

The number is patients' number.
HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; L, leucine; Q, glutamine; M, methionine.

The relationships between substitutions of aa in the HCV core region, NS5A-ISDR and the SNP of IL28B are shown in Table 2. NS5A-ISDR and both mutations in the HCV core regions were associated with IL28B genotypes. ISDR wild-type and Q70, which were resistant strains to IFN therapy, were more frequently found in patients with resistant TG/GG allele than in those with sensitive TT allele.

Factors associated with sustained virological response

The results of univariate analysis for factors predictive of SVR are shown in Table 3. Patients with SVR were younger than those without SVR. Males were more frequent among SVR patients than non-SVR patients. SVR patients had higher platelet counts than non-SVR patients. SVR was achieved in 23.1% (9/39) of patients with advanced fibrosis and 50.5% (104/206) of patients without advanced fibrosis ($P=0.0016$). SVR was achieved in 41.7% (95/228) of patients with wild-type ISDR and 60.6% (43/71) of patients with mutant-type ($P=0.0063$). SVR occurred more frequently in patients without Q70 (55.1%; 118/214) than in those with Q70 (23.5%; 20/85; $P=0.0001$). Achievement of SVR occurred more frequently in patients with TT allele (58%; 127/219) than in those with TG and GG alleles (13.8%; 11/80;

Table 3. Univariate analysis: factors predictive of sustained virological response

Factors	SVR ($n=138$)	Non-SVR ($n=161$)	*P* value
Age (years)	53.8 ± 11.5	57.9 ± 8.7	0.0005
Gender: male/female	82/56	75/86	0.0280
ALT (IU/L)	71.3 ± 76.7	68.4 ± 57.6	0.7110
AST (IU/L)	54.6 ± 46.7	62.1 ± 50.6	0.1983
PLT ($\times 10^4/mm^3$)	17.7 ± 5.5	15.6 ± 4.9	0.0008
Fibrosis: F0, 1, 2/3, 4	104/9	102/30	0.0016
HCV RNA level (KIU/ml)	2001.5 ± 1441.2	2168.3 ± 1432.4	0.3705
Core 70: non-Q/Q	118/20	96/65	0.0001
Core 91: L/M	104/106	34/55	0.0771
ISDR: wild/mutant	95/43	133/28	0.0063
IL28B: TT/TG+GG	127/11	92/69	0.0001

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; L, leucine; M, methionine; PLT, platelet count; Q, glutamine.

Table 4. Factors associated with sustained virological response by multivariate analysis

Factor	Category	Risk ratio	95% CI	*P* value
IL28B genotype	TT	0.106	0.043–0.259	0.0001
Fibrosis	F3, F4	3.550	1.335–9.440	0.0111
Core70	Q	2.496	1.111–5.604	0.0267
ISDR	Wild	2.206	1.034–4.710	0.0408

Only factor that achieved statistical significance ($P < 0.05$) on multivariate logistic regression analysis are shown.
IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region.

$P=0.0001$). Age, sex, platelet count, liver fibrosis, core 70, ISDR and IL28B were associated with SVR. The same 11 factors used in univariate analysis were used in multivariate analysis. The factors related to SVR on multivariate analysis were IL28B genotype, liver fibrosis, core 70 and ISDR, as shown in Table 4. The other factors were not significant.

The virological response according to interleukin28B genotypes and amino acid substitutions in the 70 core region and interferon sensitivity-determining region

The SVR rates according to IL28B genotypes and aa substitutions in the 70 core region and ISDR are shown in Table 5. The best SVR rate was achieved in patients with non-Q70, mutant-type ISDR and T allele, and the worst response was achieved in patients with Q70, wild-type ISDR and G allele.

Discussion

Viral factors associated with SVR have been the most frequently studied, and several regions, including 5′UTR, core, E2, NS5A and NS5B, have been suggested to play important roles in IFN responsiveness (8–16, 26–31). The aa substitutions in the HCV core and NS5A region would be two major viral factors that have strong associations with IFN response. The ISDR located in the

Table 5. The sustained virological response rate according to interleukin 28B and amino acid substitutions in 70 core region and interferon sensitivity-determining region

Core70/ISDR	IL28B; TT 58% (127/219)	IL28B; GT/GG 13.8% (11/80)
Q/wild 20% (13/65)	35.7% (10/28)	8.1% (3/37)
Q/mutant 35% (7/20)	50% (6/12)	12.5% (1/8)
non-Q/wild 50.3% (82/163)	57.6% (76/132)	19.4% (6/31)
non-Q/mutant 70.6% (36/51)	74.5% (35/47)	25% (1/4)

P-value = 0.0001 by Cochran–Armitage test.
IL28B, interleukin 28B; ISDR, interferon sensitivity-determining region; Q, glutamine.

NS5A region was originally reported in 1996 by Enomoto *et al.* (8) and confirmed by several Asian studies (9, 12–14), but controversial results were reported by Western studies (15, 16). Meta-analysis showed the relationships between ISDR and SVR and suggested that unidentified factors have an effect on IFN responsiveness (32). The ISDR interacts with protein kinase R (PKR) and inactivates replication of HCV *in vitro* (33). Therefore, ISDR heterogeneity plays an important role that may affect response to IFN. However, some reports have not confirmed the interaction between PKR and NS5A (34, 35), and they suggested the PKR-independent effects of NS5A (36, 37). Thus, the effects of aa substitutions of the ISDR are unclear, and investigators searched for other viral factors. The aa substitutions at 70 and 91 in the HCV core region were reported as factors that could predict IFN responsiveness (10). Thus, several studies have reported that combining aa substitutions in the HCV core region and NS5A region could improve the predictive value of SVR in patients with genotype 1b (12–14). These results were useful to develop individualized treatment strategies for chronic hepatitis C patients.