

## 慢性肝炎の治療(C型)

treatment of chronic hepatitis C

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## 【概念】

C型肝炎ウイルス(HCV)は、感染すると自然に排除される可能性は低く、その多くが持続感染する。宿主の免疫応答は、多くの場合不完全でHCVは排除されることがなく、壊死炎症反応が持続する。この結果が慢性肝炎であり、壊死炎症の継続の結果、線維化は進展し肝硬変へと移行する。特に、肝線維化の程度と肝発癌率は密接に関連しており、肝線維化の進展に伴い発癌率が急速に増加することから、慢性肝炎の段階での診断と適切な治療がきわめて重要である。

## 治療方針

治療の第1の目標はHCVの排除である。現在、HCVを排除することができるのはインターフェロン(IFN)を用いる抗ウイルス療法のみである。かつてのIFN単独療法では全体の1/3の患者でウイルス排除が可能であるにすぎなかったが、リバビリン(RBV)を併用することで格段に治療効果が高まっている。また、IFNにポリエチレングリコール(Peg)を結合させたPeg-IFNは週1回の投与で持続的な効果を発揮し、治療効果・認容性に優れ副作用も軽減されている。Peg-IFN + RBV併用療法はきわめて強力であるが、その一方で副作用が多く、なかには重篤なものも起こりうることから慎重な適応判断が望まれる。一方、ウイルス排除が困難な場合やIFNが無効の場合は、肝炎を沈静化し肝発癌抑制を目指した治療を行うことが必要

である。

## 治療法

## ①インターフェロン療法

a) 初回治療：IFNをウイルス排除の目的で用いる場合には、予測される治療成績・副作用を考慮し、最も治療効果が得られる治療法を第1選択とし、年齢・性別・肝線維化の程度・合併症などの感染者(宿主)の条件を加味して決定する。治療成績を規定するウイルス側の因子としては、HCVのgenotype、ウイルス量が知られており、genotype 1b(セログループ1)では、さらにHCVの遺伝子変異(ISDR、コア領域70番、91番のアミノ酸置換)などが知られている。すなわちセログループ2型は1型よりIFN感受性が高く、ウイルス量は少ないと治療効果が高い。また、ISDR(インターフェロン感受性領域)内のアミノ酸変異数は多いほどIFN感受性が高く、コア領域70番・91番のアミノ酸置換があるとIFN感受性が劣る。

このような背景のもと、厚生労働省の研究班により、初回治療のガイドライン(表11-12)が示されている。ウイルス量は、現在一般的に用いられているreal time PCR法で5.0 log IU/mL以上は高ウイルス量とされ、genotype 1かつ高ウイルス量症例は最も難治であり、最も強力なPeg-IFN $\alpha$ -2b + RBVないしはPeg-IFN $\alpha$ -2b + RBV併用の48週間投与が推奨されるが、この治療法のウイルス排除率は40~50%程度にすぎない。一方、genotype 2では、この治療法を24週間行えば80~90%の高い治療効果が得られるほか、低ウイルス量症例では、RBV併用療法とIFN単独療法ではいずれも80~90%の高いSVR率で、差を認めないため、初回治療ではIFN単独療法が推奨される。また、治療効果を高めるために、治療開始12週後にHCV-RNA量が治療開始前の1/100

表 11-12 C型肝炎に対する初回治療ガイドライン

	genotype 1	genotype 2
高ウイルス量 1 Meq/mL 5.0 log IU/mL 300 fmol/L 以上	Peg-IFN $\alpha$ -2b + リバビリン (48~72週間) Peg-IFN $\alpha$ -2a + リバビリン (48~72週間)	Peg-IFN $\alpha$ -2b + リバビリン(24週間)
低ウイルス量 1 Meq/mL 5.0 log IU/mL 300 fmol/L 未満	IFN(24週間) Peg-IFN $\alpha$ -2a(24~48週間)	IFN(8~24週間) Peg-IFN $\alpha$ -2a(24~48週間)

## C型肝炎の治療(ガイドラインの補足)

- genotype 1, 高ウイルス症例へのPeg-IFN + リバビリン併用療法の投与期間延長(72週間投与)の基準：投与開始12週後にHCV-RNA量が前値の1/100以下に低下するがHCV-RNAが陽性(real time PCR法)で、36週までに陰性化した症例では、プラス24週(トータル72週間)の投与期間を延長する。
- genotype 1, 高ウイルス症例へのPeg-IFN + リバビリン併用療法で、投与開始36週後にHCV-RNAが陽性(real time PCR法)でもALT値が正常化例は、48週まで継続治療を行い、治療終了後の長期ALT値正常化維持を目指す。
- Peg-IFN + リバビリン非適応例・無反応例に対するIFN単独長期療法は、最初の2週間は通常量の連日または週3回間欠投与とし、最大8週間でHCV-RNAが陰性化しない症例は通常量の半分を長期投与する。

[平成20年度厚生労働科学研究費肝炎等克服緊急対策研究事業(肝炎分野)「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」より転載]

以下に低下するがHCV-RNAが陽性(real time PCR法)で、36週までに陰性化した例では72週間投与が可能である。

## 処方例

[体重60 kgの場合]  
Peg-IFN $\alpha$ -2b(ペグイントロン皮下注)  
80 $\mu$ g 週1回 皮下注  
リバビリン(レボトールカプセル200 mg) 3C(カプセル) 分2 朝1C・夕2C 連日

ペグイントロンは通常1.5 $\mu$ g/kgを週1回皮下投与する。投与量の目安は体重35~45 kgでは60 $\mu$ g, 46~60 kgで80 $\mu$ g, 61~75 kgで100 $\mu$ g, 76~90 kgで120 $\mu$ g, 91~120 kgでは150 $\mu$ gである。レボトールは60 kg以下では3C(朝1C・夕2C),

61 kgを超え80 kg以下では4C(朝2C・夕2C), 80 kgを超えるものでは5C(朝2C・夕3C)である。

## 処方例

Peg-IFN $\alpha$ -2a(ペガシス皮下注)  
180 $\mu$ g 週1回 皮下注

b) 再治療：初回IFN無効例の再治療にあたっては、初回治療の無効の要因を検討することが重要である。すなわち、初回治療時にHCVがいったん陰性化し再燃した例や、治療期間や薬剤投与量が不十分であった症例では再治療によりウイルス排除が可能であり、最も強力なPeg-IFN + RBV治療を行うことが基本である。RBV併用療法を施行する場合には治療効果に寄与する因子(年齢、性別、肝疾患進行度、

表 11-13 C型慢性肝炎に対する再治療ガイドライン

C型慢性肝炎に対するインターフェロン(IFN)の再治療は初回治療での無効の要因を検討し、治癒目的の治療か、進展予防(発癌予防)を目指したALT値とAFP値の正常化、あるいは安定化のための治療法を選択すべきである

1. 初回IFN無効例への再投与はIFN+リバビリン併用療法が、治療の基本である
2. リバビリン併用療法の非適応例あるいはリバビリン併用療法で無反応例では、IFNの長期投与が望ましい。なお、IFN $\alpha$ 製剤(Peg製剤を除く)は、在宅自己注射が可能
3. IFN非適応例およびIFNでALT値、AFP値の改善が得られない症例は肝庇護剤(SNMC、UDCA)、瀉血療法を単独あるいは組み合わせて治療する
4. 進展予防(発癌予防)を目指した治療のALT目標値はStage 1(F1)では、持続的に基準値の1.5倍以下にコントロールする。Stage 2~3(F2~F3)では、極力正常値ALT $\leq$ 30 IU/Lにコントロールする
5. リバビリン併用療法を行う場合には治療効果に寄与する因子である、年齢、性別、肝疾患進行度、HCVウイルスの遺伝子変異(コア領域70、91の置換、ISDR変異)、real time PCR法によるウイルス量などを参考にし、治療法を選択することが望ましい。

[平成20年度厚生労働科学研究費肝炎等克服緊急対策研究事業(肝炎分野)「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」より転載]

ISDRやコア領域のHCVの遺伝子変異などを考慮し治療法を選択することが必要である(表11-13)。この際、注意を要するのは、Peg-IFN $\alpha$ -2a + RBV療法は、Peg-IFN $\alpha$ -2b + RBV療法で無効ないしは再燃例には適応がないことである。一方、RBV併用療法の非推奨例や無反応例ではIFNを単独で2週間連日ないしは間欠投与し、8週以内にHCVが陰性化した場合は完全治癒をめざし2年間の長期投与を行うが、8週時点でHCVが陰性化しない場合には肝発癌予防を目的とし、IFN単独少量長期療法により肝機能(ALT)の正常化をはかることが必要である。注意を要するのは、Peg-IFN $\alpha$ -2a + RBV療法は、Peg-IFN $\alpha$ -2b + RBV療法で無効ないしは再燃例には適応がないことである。

c) 肝発癌抑制を目的としたIFN療法: HCVの排除が困難な場合でも、IFN投与によって、肝機能(ALT)の正常化をはかり、肝発癌抑制効果が期待できる。これは「発癌抑制を目指した血清ALT正常C型肝炎症例の抗ウイルス治療ガイドライン」(表11-14)にまとめられているが、これ

によれば血小板数が肝線維化の程度と相関することから、血小板数15万/ $\mu$ L以下の肝線維化進展例では、たとえALTが30 IU/L以下であっても、肝発癌抑制のためには抗ウイルス療法を考慮することが明記されている。また、最近ではIFN $\alpha$ 製剤 $\alpha$ -2b製剤では自己注射も可能となり、治療法の選択肢は広がっている。しかし、IFNは副作用も多いことから慎重な判断が求められる。

#### 処方例

IFN $\alpha$ (HLBI)(スミフェロンDS) 600万単位 週3回 筋注または皮下注

② 肝庇護療法: IFNの治療効果が高まったとはいえ、1/3の患者ではウイルス排除は困難である。また、高齢者・肝線維化進展例や合併症のためにIFN投与が困難な症例も存在し、IFN治療によってもALTが正常化しない例も存在する。この場合、一般に用いられるのは、ウルソデオキシコール酸(UDCA)あるいはグルチルリチン製剤による肝庇護療法である。これ

表 11-14 発癌抑制を目指した血清ALT正常C型肝炎例への抗ウイルス治療ガイドライン

ALT 値	血小板数	
	$\geq 15 \times 10^4/\mu\text{L}$	$< 15 \times 10^4/\mu\text{L}$
$\leq 30$ IU/L	2~4か月ごとに血清ALT値フォロー。ALT異常を呈した時点で完治の可能性、発癌リスクを評価し、抗ウイルス療法を考慮	線維化進展例がかなり存在する。可能なら肝生検を施行しF2A2以上の例に抗ウイルス療法を考慮。肝生検非施行例は2~4か月ごとに血清ALT値を測定し、異常を示した時点で抗ウイルス療法を考慮
31~40 IU/L	65歳以下は抗ウイルス療法の考慮	慢性肝炎治療に準じる*

\* 遺伝子型、ウイルス量、年齢などを考慮し、通常のC型肝炎治療に準じて、治療法を選択する。

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らには抗ウイルス効果はみられないもの大きな副作用がなく、一定の効果が認められる。

#### 処方例

強力ネオミノファージェンシー 40~60 mL 週2~3回 静注  
かつ/または  
ウルソデオキシコール酸(ウルソ錠 100 mg) 6錠 分3 毎食後

③ 瀉血療法: C型肝炎では肝に過剰な鉄が沈着し、鉄蓄積と肝線維化の程度が相関することや、肝臓の鉄濃度と肝機能成績が相関することから、瀉血により肝の除鉄を行うことの有用性が示されており、特に鉄制限食を併用することが重要と考えられている。IFNが無効な症例には選択肢の1つとして、2006年から保険適用されている。実際には週1回200gの瀉血をHb 11.0 g/dL未満ないしは血清フェリチン値10 ng/mL未満となるまで、最長16週間繰り返し、その後はHb 10.5~10.9 g/dL、血清フェリチン値10 ng/mLを維持するよう4週間ごとに200~400gの瀉血を1~3か月間繰り返す。

#### 【合併症・偶発症】

IFNにはさまざまな副作用がみられる。

Peg-IFNの副作用も基本的にはIFNと同様に注意深い経過観察が必要である。頻度の高い副作用としては、治療開始早期に出現するものとして発熱・頭痛などのインフルエンザ症状、食思不振・嘔気・味覚障害・心窩部痛・便秘異常などの消化器症状、皮疹・掻痒感などの皮膚症状が、治療2~3か月後に出現するものとして、脱毛や抑うつ症状などがある。また、血球成分の減少も知られ、最大の効果発揮と副作用の軽減のためには、血球検査の結果によって投与量を調整することも必要である。特にRBV併用では溶血性貧血が問題となる。一方、稀には甲状腺機能異常、眼底出血、耐糖能異常、間質性肺炎、脳出血、心筋症などの重篤な副作用も報告されている。特に65歳以上で、高血圧、糖尿病を合併しているものでは脳出血の危険性が高まるとされ注意が必要である。

#### 【患者説明のポイント】

IFN療法にはさまざまな副作用が存在することから、起こりうる副作用と対処法について十分な説明が必要である。特に、普段と異なる症状が出現した場合には主治医に連絡・受診するように説明することが重要である。またRBVには催奇形性が存在することから、投与中と投与終了6か月間は避妊の必要を十分説明しておく必要が

ある。

### 【医療スタッフへの指示】

IFNの投与中にみられる副作用に抑うつ症状がある。多くは医師の診察時に診断できるが、注射の際の表情の変化や言動により医療スタッフが気づくことがある。この際には医師に連絡するよう指導する。

## 院内感染予防対策

prevention of hospital-acquired hepatitis B and C viral infection

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### 【概念】

肝炎ウイルスによる院内感染は、①輸血や血液製剤などによる患者への感染のほか、②病室内や血液透析などの医療行為を介して患者間または患者・医療従事者間で発生することがある。これら肝炎ウイルスによる院内感染は集団発生したり、重篤な肝炎を発症することがあるので、医療機関では院内感染に対する特別な対策が必要である。医療従事者は針刺し事故など観血的処置に伴い受傷することが多く対応を要する。

### 【頻度】

輸血後肝炎の頻度は0.1%以下であり、1999年の核酸増幅検査(NAT)の導入により血液製剤の安全性はさらに高まった。しかし、感染初期のウィンドウ期間の献血による感染は、きわめて低頻度であるものの完璧な阻止は困難である。ウィンドウ期間は、HBs抗原が約59日、HBV-NATが約34日、HCV抗体が約84日、HCV-NATが約23日である。

HBV汚染血液の針事故による肝炎発生の頻度は全体で6~30%である。汚染源がHBe抗原陽性の場合には陰性に比しリスク

が高いとされ、肝炎発生率はHBe抗原陽性血液では22~31%、HBe抗原陰性では1~6%で、汚染源のウイルス量(HBV-DNA)が多いほど感染のリスクが高くなる。特に注意すべきは、全体の2~3%は重症化・劇症化することがあり、これらはHBe抗体陽性の汚染源、すなわちHBV precore 変異またはcore promoter 変異株による感染で発症することが多い。

一方、HCV汚染血液の針刺し事故による肝炎発生の頻度は約1.8%であり、HBVに比し低率であるが、発症すると無治療の場合慢性化する頻度が高い。

### 【予防策】

医療行為の際、手袋などの標準予防策をとることが基本である。血液・体液を大量に浴びる可能性が高い場合は、必要に応じてマスク、ゴーグル、フェイスシールド、ガウンなどを着用する。針刺し事故の予防の3原則は、①針のリキャップの禁止、②適切な耐貫通性の廃棄容器の管理、③安全・防御装置のついた器材の導入である。

一方、透析ユニットや手術室などの観血的処置が行われる処置室では、患者間の感染を防止するために、①医療器材や薬剤は共用としない、②ディスプレイ器材を使用する、③適切な消毒と滅菌を行う、④医療者が媒介しない対策(手洗いほか)など基本的な院内感染防止対策を徹底する。内視鏡の洗浄については、日本消化器内視鏡学会から指針が示されているので、これを参考にして適切に実施する。

### 【針刺し事故後の手順】

1) 事故直後、可及的速やかに、受傷部を流水と石けんで洗浄する。

2) 直ちに受診する。この際、汚染源が特定できる場合は、その症例のHBs抗原、HCV抗体、HIV抗体、RPR、TPLA(必要があればHTLV-1抗体)の有無を検索する。これらが未検査の場合は、患者(あるいは代理人)の了解を得たうえでこれらの

表11-15 汚染源がHBs抗原陽性の場合の対応

受傷者のHBs抗体	ワクチン接種歴	対応	経過観察
陰性 または 10 mIU/mL 未満	なし	・48時間以内にHBIG投与 および ・HBワクチン投与(直後、4週間後、20週間後)	毎月、6か月後まで HBs抗体、AST、 ALTを測定
	あり(抗体ができなかった)	・48時間以内にHBIG投与 ・ウイルス量が多い場合は1か月後にHBIGの追加投与を考慮	
10 mIU/mL 以上	あり(抗体はできたが経過とともに抗体価が低下した)	・米国疾病予防管理センターガイドラインでは処置不要としているが、HBワクチン1回投与を考慮(ブースター効果)	経過観察不要 要定期健診
	なし	・感染防御抗体があるため、薬物投与は不要	経過観察不要 要定期健診
不明 (48時間以内に判明しない)	なし	・48時間以内にHBIG投与 および ・HBワクチン投与(直後、4週間後、20週間後)	HBs抗体価判明後 対応を決定
	あり	・48時間以内にHBIG投与を考慮 および ・HBワクチン1回投与を考慮	

検査を至急実施する。針の太さや傷の深さ、注入された血液量、患者血中のウイルス量に大きく左右されるため、詳しく問診する。

3) 院内の取り決め事項に従い、感染症対策委員会などに報告する

4) 労災申請を行う。

5) BまたはC型急性肝炎を発症した場合は、治療を行うとともに、5類感染症として7日以内に最寄りの保健所に届ける。

### 【肝炎発症の予防対策】

①汚染源が不明の針刺し事故：直ちに受傷者のHBs抗原、HBs抗体、HCV抗体、HIV抗体、RPR、TPLA、AST、ALTを測定する。必要に応じてHTLV-1抗体を追加する。これらの検査は原則として毎月、6か月後まで施行する。HBs抗体が陰性(10 mIU/mL未満)でHBワクチン未接種の場合は早急にHBワクチンの接種を1

クール行うことが必要である。

### 処方例

ビームゲン 0.5mL 筋注(あるいは皮下注) 直後、4週間後、20週間後

②汚染源がHBs抗原陽性の場合：直ちに受傷者のHBs抗原、HBs抗体、AST、ALTの測定を行い、表11-15にしたがって対応する。

### 処方例

- 1) HBIG：ヘプスブリン-IH 1,000単位 + 生理食塩水 100 mL 点滴注射 受傷後48時間以内
- 2) HBワクチン：ビームゲン 0.5mL 筋注(あるいは皮下注) 直後、4週間後、20週間後

# Amino Acid Substitution in Hepatitis C Virus Core Region and Genetic Variation Near the Interleukin 28B Gene Predict Viral Response to Telaprevir with Peginterferon and Ribavirin

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Genetic variation near the IL28B gene and substitution of amino acid (aa) 70 and 91 in the core region of hepatitis C virus (HCV) genotype 1b can predict the response to pegylated interferon (PEG-IFN)/ribavirin combination therapy, but its impact on triple therapy of telaprevir/PEG IFN/ribavirin is not clear. The aims of this study were to investigate the predictive factors of sustained virological response to a 12-week or 24-week regimen of triple therapy in 72 of 81 Japanese adults infected with HCV genotype 1. Overall, sustained virological response and end-of-treatment response were achieved by 61% and 89%, respectively. Especially, the sustained virological response was achieved by 45% and 67% in the 12- and 24-week regimens, respectively. Multivariate analysis identified rs8099917 near the IL28B gene (genotype TT) and substitution at aa 70 (Arg70) as significant determinants of sustained virological response. Prediction of response to therapy based on a combination of these factors had high sensitivity, specificity, and positive and negative predictive values. The efficacy of triple therapy was high in the patients with genotype TT, who accomplished sustained virological response (84%), irrespective of substitution of core aa 70. In the patients having genotype non-TT, those of Arg70 gained high sustained virological response (50%), and sustained virological response (12%) was the worst in patients who possessed both genotype non-TT and Gln70(His70). **Conclusion:** This study identified genetic variation near the IL28B gene and aa substitution of the core region as predictors of sustained virological response to a triple therapy of telaprevir/PEG-IFN/ribavirin in Japanese patients infected with HCV genotype 1b. (HEPATOLOGY 2010;52:421-429)

Abbreviations: aa, amino acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase;  $\gamma$ GTP, gamma-glutamyl transpeptidase; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IFN, interferon; NPV, negative predictive value; PEG-IFN, pegylated interferon; PPV, positive predictive value

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Hepatitis C virus (HCV) usually causes chronic infection that can result in chronic hepatitis, liver cirrhosis, and hepatocellular carcinoma (HCC).<sup>1,2</sup> 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) in high viral loads (>100 KIU/mL) accounts for more than 70% of HCV infections, making it difficult to treat patients with chronic hepatitis C.<sup>3</sup> Such background calls for efficient treatments of Japanese patients with chronic HCV infection.

Even with pegylated IFN (PEG-IFN) combined with ribavirin, a sustained virological response lasting over 24 weeks after the withdrawal of treatment is achieved in at most 50% of the patients infected with HCV-1b and high viral loads.<sup>4,5</sup> Recently, a new strategy was introduced in the treatment of chronic HCV infection by

means of inhibiting protease in the NS3/NS4 of the HCV polyprotein. Of these, telaprevir (VX-950) was selected as a candidate agent for treatment of chronic HCV infection.<sup>6</sup> Later, it was found that telaprevir, when combined with PEG-IFN and ribavirin, gains a robust antiviral activity.<sup>7,8</sup> Specifically, HCV RNA is suppressed below the limits of detection in the blood in almost all patients infected with HCV-1 during triple therapy of telaprevir with PEG-IFN and ribavirin.<sup>9</sup> However, treatment-resistant patients who do not achieve sustained virological response by the triple therapy have been reported.<sup>9-11</sup> The underlying mechanism of the response to the treatment is still not clear.

Amino acid (aa) substitutions at position 70 and/or 91 in the HCV core region of patients infected with HCV-1b and high viral loads are pretreatment predictors of poor virological response to PEG-IFN plus ribavirin combination therapy,<sup>12-14</sup> and also affect clinical outcome, including hepatocarcinogenesis.<sup>15,16</sup> Furthermore, a recent report showed that aa substitutions in the core region can also be used before therapy to predict very early dynamics (within 48 hours) after the start of triple therapy of telaprevir with PEG-IFN and ribavirin.<sup>17</sup> However, it is not clear at this stage whether aa substitutions in the core region can be used before therapy to predict sustained virological response to triple therapy.

Recent reports showed that genetic variations near the IL28B gene (rs8099917, rs12979860) on chromosome 19 is a host-related factor, which encodes IFN- $\lambda$ -3, are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,<sup>18-21</sup> and also affect clinical outcome, including spontaneous clearance of HCV.<sup>22</sup> However, it is not clear at this stage whether genetic variation near the IL28B gene can be used before therapy to predict sustained virological response to triple therapy.

The present study included 81 patients with HCV-1b and high viral loads who received the triple therapy of telaprevir with PEG-IFN plus ribavirin. The aims of the study were to identify the pretreatment factors that could predict sustained virological response, including viral- (aa substitutions in the HCV core and NS5A regions) and host-related factors (genetic variation near the IL28B gene).

## Patients and Methods

**Study Population.** Between May 2008 and September 2009, 81 patients infected with HCV were

recruited for this study at the Department of Hepatology in Toranomon Hospital in Metropolitan Tokyo. 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 informed consent before participating in this trial. Patients were divided into two groups: 20 (25%) patients were allocated to a 12-week regimen of triple therapy (telaprevir [MP-424], PEG-IFN, and ribavirin) (the T12PR12 group), and 61 patients (75%) were assigned to a 24-week regimen of the same triple therapy for 12 weeks followed by dual therapy of PEG-IFN and ribavirin for 12 weeks (the T12PR24 group).

All of 81 patients met the following inclusion and exclusion criteria: (1) diagnosis of chronic hepatitis C. (2) HCV-1 confirmed by sequence analysis. (3) HCV RNA levels of  $\geq 5.0$  log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics, Tokyo, Japan). (4) Japanese (Mongoloid) ethnicity. (5) Age at study entry of 20-65 years. (6) Body weight  $\geq 35$  kg and  $\leq 120$  kg at the time of registration. (7) Lack of decompensated liver cirrhosis. (8) Negativity for hepatitis B surface antigen (HBsAg) in serum. (9) Negative history of HCC. (10) No previous treatment for malignancy. (11) Negative history of autoimmune hepatitis, alcohol liver disease, hemochromatosis, and chronic liver disease other than chronic hepatitis C. (12) Negative history of depression, schizophrenia or suicide attempts, hemoglobinopathies, angina pectoris, cardiac insufficiency, myocardial infarction or severe arrhythmia, uncontrollable hypertension, chronic renal dysfunction or creatinine clearance of  $\leq 50$  mL/minute at baseline, diabetes requiring treatment or fasting glucose level of  $\geq 110$  mg/dL, autoimmune disease, cerebrovascular disorders, thyroidal dysfunction uncontrollable by medical treatment, chronic pulmonary disease, allergy to medication or anaphylaxis at baseline. (13) Hemoglobin level of  $\geq 12$  g/dL, neutrophil count  $\geq 1500/\text{mm}^3$ , and platelet count of  $\geq 100,000/\text{mm}^3$  at baseline. Pregnant or breast-feeding women or those willing to become pregnant during the study and men with a pregnant partner were excluded from the study. Furthermore, 72 of 81 patients were followed for at least 24 weeks after the completion of triple therapy. The treatment efficacy was evaluated by HCV-RNA negative at the end of treatment (end-of-treatment response) and 24 weeks after the completion of therapy (sustained virological response), based on the COBAS TaqMan HCV test (Roche Diagnostics).

Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan) was administered at 750 mg or 500 mg

**Table 1. Profile and Laboratory Data at Commencement of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1**

Demographic data	
Number of patients	81
Sex (M/F)	44 / 37
Age (years)*	55 (23-65)
History of blood transfusion	24 (29.6%)
Family history of liver disease	13 (16.0%)
Body mass index (kg/m <sup>2</sup> )*	22.5 (13.2-32.4)
Laboratory data*	
HCV genotype (1a/ 1b)	1/80
Level of viremia (log IU/mL)	6.7 (5.1-7.6)
Serum aspartate aminotransferase (IU/L)	34 (15-137)
Serum alanine aminotransferase (IU/L)	42 (12-175)
Serum albumin (g/dL)	3.9 (3.2-4.6)
Gamma-glutamyl transpeptidase (IU/L)	36 (9-229)
Leukocyte count (/mm <sup>3</sup> )	4,800 (2,800-8,100)
Hemoglobin (g/dL)	14.3 (11.7-16.8)
Platelet count (× 10 <sup>4</sup> /mm <sup>3</sup> )	17.1 (9.1-33.8)
Alpha-fetoprotein (μ g/L)	4 (2-39)
Total cholesterol (mg/dL)	180 (110-216)
Fasting plasma glucose (mg/dL)	92 (64-125)
Treatment	
PEG-IFNα-2b dose (μ g/kg)*	1.5 (1.3-2.0)
Ribavirin dose (mg/kg)*	11.7 (7.2-18.4)
Telaprevir dose (1,500 / 2,250 mg/day)	10/71
Treatment regimen (T12PR12 group / T12PR24 group)	20/61
Amino acid substitutions in the HCV genotype 1b	
Core aa 70 (arginine / glutamine [histidine] / ND)	47/33/1
Core aa 91 (leucine / methionine / ND)	43/37/1
ISDR of NS5A (wild-type / non wild-type / ND)	76/4/1
Genetic variation near IL28B gene	
rs8099917 genotype (TT / TG / GG / ND)	42/30/2/7
rs 12979860 genotype (CC / CT / TT / ND)	42/32/2/5
Past history of IFN therapy	
Treatment-naive / Relapsers to previous treatment / nonresponders to previous treatment	27/33/21

Data are number and percentages of patients, except those denoted by asterisk (\*), which represent the median (range) values. ND, not determined.

three times a day at an 8-hour (q8) interval after the meal. PEG-IFNα-2b (PEG-Intron; Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose 1.5 μg/kg (range: 1.3-2.0 μg/kg) once a week. Ribavirin (Rebetol; Schering Plough) was administered at 200-600 mg twice a day after breakfast and dinner (daily dose: 600-1000 mg).

PEG-IFN and ribavirin were discontinued or their doses reduced, as required, upon reduction of hemoglobin level, leukocyte count, neutrophil or platelet count, or the development of adverse events. Thus, the dose of PEG-IFN was reduced by 50% when the leukocyte count decreased below 1500/mm<sup>3</sup>, neutrophil count below 750/mm<sup>3</sup>, or platelet count below 80,000/mm<sup>3</sup>; PEG-IFN was discontinued when these counts decreased below 1000/mm<sup>3</sup>, 500/mm<sup>3</sup> or 50,000/mm<sup>3</sup> respectively. When hemoglobin decreased to <10 g/dL, the daily dose of ribavirin was reduced from 600 to 400 mg, from 800 to 600 mg

and 1000 mg to 600 mg, depending on the initial dose. Ribavirin was withdrawn when hemoglobin decreased to <8.5 g/dL. However, the dose of telaprevir (MP-424) remained the same, and its administration was stopped when the discontinuation was appropriate for the development of adverse events. In those patients who discontinued telaprevir, treatment with PEG-IFNα-2b and ribavirin was also terminated.

Table 1 summarizes the profiles and laboratory data of the 81 patients at the commencement of treatment. They included 44 males and 37 females, ages 23 to 65 years (median, 55 years).

**Measurement of HCV RNA.** The antiviral effects of the triple therapy on HCV were assessed by measuring plasma HCV RNA levels. In this study, HCV RNA levels during treatment 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 Amino Acid Substitutions in Core and NS5A Regions of HCV-1b.** In the present study, aa substitutions of the core region and NS5A-ISDR (IFN-sensitivity determining region) of HCV-1b were analyzed by direct sequencing. HCV RNA was extracted from serum samples at the start of treatment and reverse transcribed with random primer and MMLV reverse transcriptase (Takara Syuzo, Tokyo). Nucleic acids were amplified by polymerase chain reaction (PCR) using the following primers: (1) Nucleotide sequences of the core region: The first-round PCR was performed with CE1 (sense, 5'-GTC TGC GGA ACC GGT GAG TA-3', nucleotides: 134-153) and CE2 (antisense, 5'-GAC GTG GCG TCG TAT TGT CG-3', nucleotides: 1096-1115) primers, and the second-round PCR with CC9 (sense, 5'-ACT GCT AGC CGA GTA GTG TT-3', nucleotides: 234-253) and CE6 (antisense, 5'-GGA GCA GTC GTT CGT GAC AT-3', nucleotides: 934-953) primers. (2) Nucleotide sequences of NS5A-ISDR: The first-round PCR was performed with ISDR1 (sense, 5'-ATG CCC ATG CCA GGT TCC AG-3', nucleotides: 6662-6681) and ISDR2 (antisense, 5'-AGC TCC GCC AAG GCA GAA GA-3', nucleotides: 7350-7369) primers, and the second-round PCR with ISDR3 (sense, 5'-ACC GGA TGT GGC AGT GCT CA-3', nucleotides: 6824-6843) and ISDR4 (antisense, 5'-GTA ATC CGG GCG TGC CCA TA-3', nucleotides: 7189-7208) primers. ([1,2]; nested PCR.) All samples were initially denatured at 95°C for 2 minutes. The 35 cycles of

amplification were set as follows: denaturation for 30 seconds at 95°C, annealing of primers for 30 seconds at 55°C, and extension for 1 minute at 72°C with an additional 7 minutes for extension. Then 1  $\mu$ L of the first PCR product was transferred to the second PCR reaction. Other conditions for the second PCR were the same as the first PCR, except that the second PCR primers were used instead of the first PCR primers. The amplified PCR products were purified by the QIA quick PCR purification kit (Qiagen, Tokyo) after agarose gel electrophoresis and then used for direct sequencing. Dideoxynucleotide termination sequencing was performed with the Big Dye Deoxy Terminator Cycle Sequencing kit (PerkinElmer, Tokyo).

With the use of HCV-J (Access. No. D90208) as a reference,<sup>23</sup> the sequence of 1-191 aa in the core protein of HCV 1b was determined and then compared with the consensus sequence constructed on 81 clinical samples to detect substitutions at aa 70 of arginine (Arg70) or glutamine/histidine (Gln70/His70) and aa 91 of leucine (Leu91) or methionine (Met91).<sup>12</sup> The sequence of 2209-2248 aa in the NS5A of HCV-1b (ISDR) reported by Enomoto et al.<sup>24</sup> was determined and the numbers of aa substitutions in ISDR were defined as wildtype (0, 1) or nonwildtype ( $\geq 2$ ).

**Genetic Variation Near the 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 the Invader assay, TaqMan assay, or direct sequencing as described.<sup>25,26</sup>

In this study, genetic variations near the IL28B gene (rs8099917, rs12979860), reported as the pretreatment predictors of treatment efficacy and clinical outcome,<sup>18-22</sup> were investigated.

**Statistical Analysis.** Nonparametric tests (chi-squared test and Fisher's exact probability test) were used to compare the characteristics of the groups. Univariate and multivariate logistic regression analyses were used to determine those factors that significantly contributed to sustained virological response. The odds ratios (OR) and 95% confidence intervals (95% CI) were also calculated. All *P* values less than 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 univariate and multivariate analyses. The

potential pretreatment factors associated with sustained virological response included the following variables: sex, age, history of blood transfusion, family history of liver disease, body mass index, aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin, gamma-glutamyl transpeptidase ( $\gamma$ GTP), leukocyte count, hemoglobin, platelet count, HCV RNA level, alfa-fetoprotein, total cholesterol, fasting blood sugar, PEG-IFN dose/body weight, ribavirin dose/body weight, telaprevir dose/day, treatment regimen of triple therapy, past history of IFN therapy, genetic variation near the IL28B gene, and aa substitution in the core region, and NS5A-ISDR. Statistical analyses were performed using SPSS (Chicago, IL). Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were also calculated to determine the reliability of predictors of the response to therapy.

## Results

**Virological Response to Therapy.** Sustained virological response was achieved by 44 of 72 (61.1%) patients. In all, 64 of 72 (88.9%) patients were considered end-of-treatment response. According to treatment regimen, sustained virological response were achieved by 45.0% (9 of 20 patients) and 67.3% (35 of 52 patients), in the T12PR12 group and the T12PR24 group, respectively. Of eight patients who could not achieve end-of-treatment response, six (75.0%) patients resulted in reevaluation of viral loads regardless of HCV-RNA temporary negative, and the other two patients (25.0%) did not achieve HCV-RNA negative during treatment.

Especially in the T12PR24 group, according to the past history of treatment, sustained virological response were achieved by 76.4% (13 of 17 patients), 86.4% (19 of 22 patients), and 23.1% (3 of 13 patients), in treatment-naive, relapsers to previous treatment, and nonresponders to previous treatment, respectively.

**Sustained Virological Response According to Amino Acid Substitutions in Core and NS5A Regions.** According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions (74.4%) showed sustained virological response than that of patients who showed Gln70/His70 (41.4%) (Fig. 1, *P* = 0.007). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 (65.0%) and Met91 (56.3%) (Fig. 1). Likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype

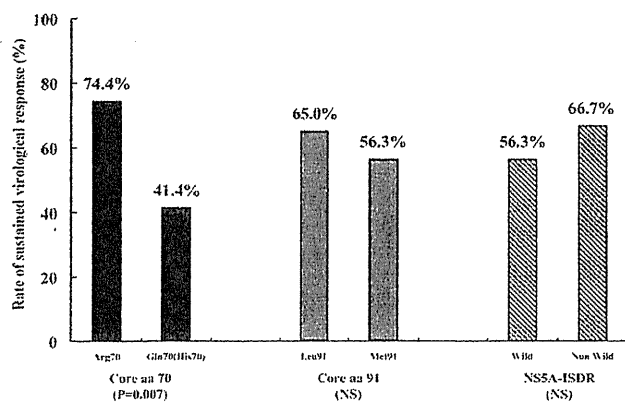


Fig. 1. According to the substitution of core aa 70, a significantly higher proportion of patients with Arg70 substitutions showed sustained virological response than that of patients who showed Gln70(His70) ( $P = 0.007$ ). In contrast, according to the substitution of core aa 91, the sustained virological response rate was not significantly different between Leu91 and Met91 likewise, according to the numbers of aa substitutions in ISDR, the sustained virological response rate was not significantly different between wildtype and nonwildtype.

(56.3%) and nonwildtype (66.7%) (Fig. 1). Thus, sustained virological response was influenced by the substitution of core aa 70.

**Sustained Virological Response According to Genetic Variation Near the IL28B Gene.** According to the genetic variation in rs8099917, sustained virological response was achieved by 83.8% (31 of 37 patients), 29.6% (8 of 27 patients), and 0% (0 of 2 patients) in patients with genotype TT, TG, and GG, respectively. Thus, a significantly higher proportion of patients with genotype TT (83.8%) showed sustained virological response than that of patients who showed genotype non-TT (27.6%) (Fig. 2,  $P < 0.001$ ) (Table 2).

According to the genetic variation in rs12979860, sustained virological response was achieved by 83.8% (31 of 37 patients), 34.5% (10 of 29 patients), and 0% (0 of 2 patients), in patients with genotype CC, CT, and TT, respectively. Thus, a significantly higher proportion of patients with genotype CC (83.8%) showed sustained virological response than that of patients who showed genotype non-CC (32.3%) (Fig. 2,  $P < 0.001$ ) (Table 2).

**Predictive Factors Associated with Sustained Virological Response.** Univariate analysis identified three parameters that correlated with sustained virological response significantly: substitution of aa 70 (Arg70; OR 4.12,  $P = 0.007$ ), genetic variation in rs8099917 (genotype TT; OR 13.6,  $P < 0.001$ ), and rs12979860 (genotype CC; OR 10.8,  $P < 0.001$ ). Two factors were identified by multivariate analysis as independent

parameters that significantly influenced sustained virological response (rs8099917 genotype TT; OR 10.6,  $P < 0.001$ ; and Arg70; OR 3.69,  $P = 0.040$ ) (Table 3).

**Assessment of Amino Acid Substitutions in Core Region and Genetic Variation Near the IL28B Gene as Predictors of Sustained Virological Response.** The ability to predict sustained virological response by substitution of core aa 70 and rs8099917 genotype near the IL28B gene was evaluated. The sustained virological response rates of patients with a combination of Arg70 or rs8099917 genotype TT were defined as PPV (prediction of sustained virological response). The nonsustained virological response rates of patients with a combination of Gln70(His70) or rs8099917 genotype non-TT were defined as NPV (prediction of nonsustained virological response).

In patients with rs8099917 genotype TT, the sensitivity, specificity, PPV, and NPV for sustained virological response were 79.5, 77.8, 83.8, and 72.4%, respectively. Thus, genotype TT has high sensitivity, specificity, and PPV for prediction of sustained virological response. In patients with Arg70 the sensitivity, specificity, PPV, and NPV were 76.9, 63.0, 75.0, and 65.4%, respectively. Thus, Arg70 has high sensitivity and PPV in predicting sustained virological response. Furthermore, when both predictors were used the sensitivity, specificity, PPV, and NPV were 61.5, 85.2, 85.7, and 60.5%, respectively. When one or more of the two predictors were used the sensitivity, specificity, PPV, and NPV were 94.9, 55.6, 75.5, and 88.2%, respectively. These results indicate that the use of the combination of the above two predictors has high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response (Table 4).

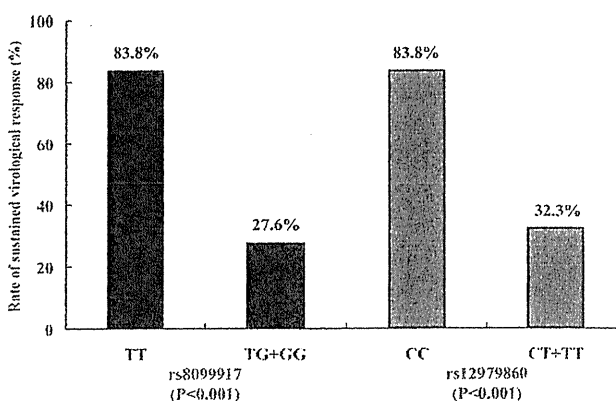


Fig. 2. According to the genetic variation in rs8099917 or rs12979860 near the IL28B gene, a significantly higher proportion of patients with genotype TT or CC showed sustained virological response than that of patients who showed genotype non-TT or non-CC, respectively ( $P < 0.001$  or  $P < 0.001$ , respectively).



**Table 2. According to Genetic Variation Near the IL28B Gene, Background at Commencement of Triple Therapy and Treatment Efficacy**

	rs8099917 genotype			rs12979860 genotype		
	TT (n = 42)	TG+GG (n = 32)	TT vs. TG+GG P	CC (n = 42)	CT+TT (n = 34)	CC vs. CT+TT P
<b>Demographic data</b>						
Sex (M/F)	22 / 20	18 / 14	NS	22 / 20	19 / 15	NS
Age (years)*	54 (23-65)	56 (36-65)		54 (23-65)	55 (36-65)	NS
History of blood transfusion	15 (35.7%)	9 (28.3%)	NS	15 (35.7%)	9 (26.5%)	NS
Family history of liver disease	6 (14.3%)	6 (18.8%)	NS	6 (14.3%)	6 (17.6%)	NS
Body mass index (kg/m <sup>2</sup> )*	22.1 (13.2-32.4)	22.4 (18.7-26.5)	NS	22.1 (13.2-32.4)	22.3 (18.7-26.5)	NS
<b>Laboratory data*</b>						
HCV genotype (1a / 1b)	0 / 42	1 / 31	NS	0 / 42	1 / 33	NS
Level of viremia (log IU/mL)	6.9 (5.4-7.5)	6.6 (5.1-7.4)	NS	6.9 (5.4-7.5)	6.5 (5.1-7.4)	NS
Serum aspartate aminotransferase (IU/L)	38 (15-118)	31 (20-137)	0.036	38 (15-118)	31 (20-137)	0.031
Serum alanine aminotransferase (IU/L)	50 (12-175)	36 (17-136)	0.029	50 (12-175)	35 (17-136)	0.014
Serum albumin (g/dL)	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS	3.9 (3.3-4.6)	3.9 (3.2-4.6)	NS
Gamma-glutamyl transpeptidase (IU/L)	29 (9-194)	53 (9-154)	0.008	29 (9-194)	53 (9-229)	0.004
Leukocyte count (/mm <sup>3</sup> )	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS	4,800 (2,800-8,100)	4,800 (3,000-7,800)	NS
Hemoglobin (g/dL)	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS	14.3 (12.3-16.5)	14.3 (11.7-16.8)	NS
Platelet count ( $\times 10^4$ /mm <sup>3</sup> )	16.8 (9.9-33.8)	17.1 (9.1-24.8)	NS	16.8 (9.9-33.8)	17.8 (9.1-28.8)	NS
Alpha-fetoprotein ( $\mu$ g/L)	4 (2-39)	5 (2-38)	NS	4 (2-39)	5 (2-38)	NS
Total cholesterol (mg/dL)	184 (112-276)	178 (110-263)	NS	184 (112-276)	178 (110-263)	NS
Fasting plasma glucose (mg/dL)	97 (80-125)	90 (66-111)	0.038	97 (80-125)	91 (66-111)	0.030
<b>Treatment regimen</b>						
T12PR12 group / T12PR24 group	12 / 30	7 / 25	NS	12 / 30	7 / 27	NS
<b>Amino acid substitutions in the HCV genotype 1b</b>						
Core aa 70 (arginine / glutamine [histidine])	30 / 12	13 / 18	0.016	30 / 12	13 / 20	0.009
Core aa 91 (leucine / methionine)	25 / 17	13 / 18	NS	25 / 17	14 / 19	NS
ISDR of NS5A (wild-type / non wild-type)	39 / 3	30 / 1	NS	39 / 3	32 / 1	NS
<b>Past history of IFN therapy</b>						
Treatment-naive / Relapsers to previous treatment / Nonresponders to previous treatment	16 / 24 / 2	7 / 6 / 19	<0.001	16 / 24 / 2	8 / 7 / 19	<0.001
<b>Treatment efficacy**</b>						
End-of-treatment response (%)	35 (94.6%)	23 (79.3%)	NS	35 (94.6%)	25 (80.6%)	NS
Sustained virological response (%)	31 (83.8%)	8 (27.6%)	<0.001	31 (83.8%)	10 (32.3%)	<0.001

Data are number and percentages of patients, except those denoted by asterisk (\*), which represent the median (range) values.

\*\*Treatment efficacy according to rs8099917 genotype was evaluated in 66 patients, and that according to rs12979860 genotype was evaluated in 68 patients.

**Predicting Sustained Virological Response by Amino Acid Substitutions in Core Region in Combination with Genetic Variation Near the IL28B Gene.** Sustained virological response by core aa 70 in combination with rs8099917 genotype is shown in Fig. 3. In patients with rs8099917 genotype TT, sustained virological response was not different between Arg70 (85.7%) and Gln70(His70) (77.8%). In contrast, in patients with rs8099917 genotype TG and GG, a significantly higher proportion of patients with Arg70 (50.0%) showed sustained virological response than that of patients with Gln70(His70) (11.8%) ( $P = 0.038$ ).

Based on a strong power of substitution of core aa 70 and rs8099917 genotype in predicting sustained virological response (Table 3), how they increase the predictive value when they were combined was evaluated. The results are schematically depicted in Fig. 3.

Together they demonstrate three points: (1) the efficacy of triple therapy was high in patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70; (2) in patients having genotype TG and GG, those of Arg70 gained high sustained virological response (50.0%); and (3) sustained virological response (11.8%) was the worst in patients who possessed both of genotype TG and GG, and Gln70(His70).

## Discussion

Two previous studies (PROVE1 in the US, and PROVE2 in Europe) showed that the T12PR12 and T12PR24 group of telaprevir, PEG-IFN, and ribavirin could achieve sustained virological response rates of 35%-60% and 61%-69%, respectively.<sup>10,11</sup> In the

**Table 3. Multivariate Analysis of Factors Associated with Sustained Virological Response of Telaprevir, Peginterferon and Ribavirin Triple Therapy in Japanese Patients Infected with HCV Genotype 1**

Factor	Category	Odds Ratio (95% CI)	P
rs8099917 genotype	1: TG+GG	1	<0.001
	2: TT	10.6 (3.07-36.5)	
Substitution of aa 70	1: Gln70 (His70)	1	0.040
	2: Arg70	3.69 (1.06-12.8)	

Only variables that achieved statistical significance ( $P < 0.05$ ) on multivariate logistic regression analysis are shown. 95% CI: 95% confidence interval.

present Japanese study, the sustained virological response rates were 45% and 67% in the T12PR12 and T12PR24 group, respectively, as in the two previous studies. There were differences at three points between the present study and two previous studies: (1) PEG-IFN in two previous studies was used at a fixed dose of PEG-IFN $\alpha$ -2a, but that of the present study was a body weight-adjusted dose of PEG-IFN $\alpha$ -2b; (2) The body mass index of our patients (median; 23 kg/m<sup>2</sup>) was much lower than that of the participants of the previous study by McHutchison et al.<sup>10</sup> (median; >25 kg/m<sup>2</sup>); and (3) The present study was performed based on Japanese patients infected with HCV-1b, except for only one patient with HCV-1a. Especially in PROVE-1, the viral breakthrough rate was higher in HCV-1a subjects compared to HCV-1b, and one of the reasons might be due to the low genetic barrier to the emergence of the R155K variant in HCV-1a.<sup>10,27</sup> Further studies of a larger number of patients matched for background, including genotype, race, body mass index, treatment regimen, and past history of IFN therapy are required to investigate the rate of the sustained virological response by triple therapy.

IL28A, IL28B, and IL29 (IFN- $\lambda$ -2, IFN- $\lambda$ -3, and IFN- $\lambda$ -1, respectively) are novel IFNs identified recently.<sup>28,29</sup> They are similar to type I IFNs in terms

of biological activities and mechanism of action, in contrast to their differences in structure and genetics.<sup>30</sup> The antiviral effects of IFN- $\lambda$  against hepatitis B virus and HCV have been reported.<sup>31</sup> Furthermore,  $\alpha$  and  $\lambda$  IFNs act synergistically against HCV.<sup>32-34</sup> Recent reports showed that genetic variation near the IL28B gene (rs8099917, rs12979860) are pretreatment predictors of virological response to 48-week PEG-IFN plus ribavirin combination therapy in individuals infected with HCV-1,<sup>18-21</sup> and also affect clinical outcome, including spontaneous clearance of HCV.<sup>22</sup> At the 2009 meeting of the American Association for the Study of Liver Diseases, Thompson et al.<sup>35</sup> reported that genetic variation near the IL28B gene also affected the viral suppression in the first 2 to 4 weeks of PEG-IFN plus ribavirin, and this phenomenon probably explains much of the difference in treatment response rate. The present study is the first to report that genetic variation near the IL28B gene significantly also affect sustained virological response by triple therapy. These results should be interpreted with caution because races other than Japanese populations were not included. Any generalization of the results should await confirmation by studies of patients of other races to explore the relationship between genetic variation near the IL28B gene and the response to triple therapy.

The present study indicated that the use of the combination of aa substitution of the core region and genetic variation near the IL28B gene had high sensitivity, specificity, PPV, and NPV for prediction of sustained virological response. The efficacy of triple therapy was high in the patients with TT, irrespective of substitution of core aa 70. In the patients having non-TT, those of Arg70 gained high sustained virological response, and sustained virological response was the worst in patients who possessed both non-TT, and Gln70(His70). Along with a high sustained virological response, combined PEG-IFN and ribavirin are accompanied by severe side effects and entail high

**Table 4. Sensitivity, Specificity, Positive Predictive Value (PPV), and Negative Predictive Value (NPV) for Sustained Virological Response, According to Substitution of Core aa 70 and Genetic Variation Near IL28B Gene**

	% (Number)			
	Sensitivity	Specificity	PPV*	NPV**
(A) rs8099917 genotype TT	79.5 (31/39)	77.8 (21/27)	83.8 (31/37)	72.4 (21/29)
(B) Substitution at aa 70 of arginine (Arg70)	76.9 (30/39)	63.0 (17/27)	75.0 (30/40)	65.4 (17/26)
(A) and (B)	61.5 (24/39)	85.2 (23/27)	85.7 (24/28)	60.5 (23/38)
(A) and/or (B)	94.9 (37/39)	55.6 (15/27)	75.5 (37/49)	88.2 (15/17)

\*PPV; Sustained virological response rates for patients with a combination of Arg70 or rs8099917 genotype TT (prediction of sustained virological response).

\*\*NPV; nonsustained virological response rates for patients with a combination of Gln70(His70) or rs8099917 genotype non-TT (prediction of nonsustained virological response).

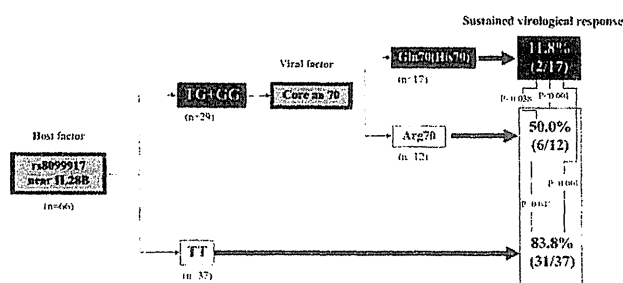


Fig. 3. Predicting sustained virological response by aa substitution in core region in combination with genetic variation near the IL28B gene. Efficacy of triple therapy was high in the patients with genotype TT who accomplished sustained virological response at 83.8%, irrespective of substitution of core aa 70. In the patients having genotype TG and GG, those of Arg70 gained a high sustained virological response (50.0%), and sustained virological response (11.8%) were the worst in patients who possessed both genotypes TG and GG, and Gln70(His70).

costs. Hence, the patients who do not achieve sustained virological response need to be identified as early as possible, in order to free them of unnecessary side effects and high costs. The present study is the first to report that the combination of aa substitution of the core region and genetic variation near the IL28B gene are very useful as pretreatment predictors of sustained virological response by triple therapy, and further studies based on a larger number of patients are necessary to investigate the present results.

Other limitations of the present study were that aa substitutions in areas other than the core region and NS5A-ISDR of the HCV genome, such as the interferon/ribavirin resistance determining region (IRRDR),<sup>36</sup> were not examined. Furthermore, HCV mutants with aa conversions for resistance to telaprevir during triple therapy, such as the 156S mutation,<sup>37</sup> were also not investigated. In this regard, telaprevir-resistant HCV mutants were reported to be susceptible to IFN in both *in vivo* and *in vitro* studies.<sup>38,39</sup> Thus, viral factors before and during triple therapy should be investigated in future studies and identification of these factors should facilitate the development of more effective therapeutic regimens.

In conclusion, triple therapy with telaprevir, PEG-IFN, and ribavirin in Japanese patients infected with HCV-1 and high viral load achieved high sustained virological response rates. Furthermore, the aa substitution pattern of the core region and genetic variation near the IL28B gene seem to affect treatment efficacy. Further large-scale prospective studies are necessary to investigate whether the present results relate to the efficacy of triple 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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# Influence of Amino-Acid Polymorphism in the Core Protein on Progression of Liver Disease in Patients Infected With Hepatitis C Virus Genotype 1b

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The substitution of amino acid (aa) 70 of arginine for glutamine and/or that of aa91 of leucine for methionine in the core protein in patients infected with hepatitis C virus (HCV) genotype 1b is associated with a poor response to pegylated interferon and ribavirin. Factors influencing these substitutions were sought in 1,097 patients infected with HCV-1b who had not received antiviral treatment. HCV variants with Arg70 and Leu91 (wild-type) decreased, while those with Gln70 and/or Met91 (mutant types) increased with age ( $P < 0.001$ ). Of the 1,097 patients, 464 (42.3%) were infected with the Gln70 variant and the remaining 633 patients with the Arg70 variant. The proportion of patients with the Gln70 variant increased with the severity of liver disease ( $P < 0.001$ ), elevated  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP) levels ( $P < 0.001$ ) and a decrease in platelet count ( $P = 0.008$ ). In univariate analysis patients with hepatocellular carcinoma, elevated aspartate aminotransferase (AST  $\geq 58$  IU/L) and  $\gamma$ -GTP ( $\geq 61$  IU/L), and decreased albumin levels ( $< 3.9$  g/dl) were more frequent in the patients with the Gln70 variant than the Arg70 variant ( $P = 0.003$ ,  $0.005$ ,  $< 0.001$ , and  $0.031$ , respectively). In multivariate analysis HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and  $\gamma$ -GTP  $\geq 61$  IU/L (1.647 [1.268–2.139]) increased the risk for the Gln70 variant. In conclusion, the substitution of amino aa70 of Arg for Gln in patients infected with HCV-1b increases with age, and it is associated with severe liver disease accompanied by elevated AST and  $\gamma$ -GTP levels, as well as the development of hepatocellular carcinoma. *J. Med. Virol.* 82:41–48, 2010. © 2009 Wiley-Liss, Inc.

**KEY WORDS:** cirrhosis; core protein; hepatitis C; hepatocellular carcinoma; interferon; ribavirin

## INTRODUCTION

Worldwide, an estimated 170 million people are infected with hepatitis C virus (HCV) persistently [Cohen, 1999]. Decompensated cirrhosis and hepatocellular carcinoma (HCC) can develop in about 30% of patients infected with HCV [Alberti et al., 1999; Seeff, 2002]. HCV has six major genotypes and dozens of subgenotypes, and they have distinct geographic distributions and are associated with the progression of liver disease [Simmonds, 1995]. Host and virological factors can influence the severity of liver disease and the response to antiviral treatment. HCV infection in the childhood and women runs a milder course than that in adulthood and men, and the intake of alcohol accelerates the progression of liver disease [Poynard et al., 1997; Kenny-Walsh, 1999; Vogt et al., 1999; Wiese et al., 2000]. Genotypes 1 and 4 aggravate liver disease and decrease the response to antiviral treatment, in comparison with genotypes 2, 3, and 6 [Tsubota et al., 1994; Hui et al., 2003; Hadziyannis et al., 2004; Legrand-Abbravanel et al., 2005; Yuen and Lai, 2006]. High levels of HCV RNA in the serum can induce severe liver disease and decrease treatment response [Tsubota et al., 1994].

In Japan, genotype 1b in a high viral load ( $> 100$  KIU/ml) accounts for  $> 70\%$  of HCV infection, and decreases the treatment response in patients with chronic hepatitis C [Kumada et al., 2006]. Even with pegylated interferon (PEG-IFN) combined with ribavirin, the sustained virological response for longer than 24 weeks after the withdrawal of treatment is achieved merely in

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50% of the patients with HCV-1b in high levels [Manns et al., 2001; Fried et al., 2002]. It is necessary to predict the response to PEG-IFN/ribavirin before the start of antiviral therapy, to avoid severe side-effects in the patients who will barely gain sustained virological response.

The core protein of HCV is coded for by the C gene, and consists of 191 amino acids (aa) [Rosenberg, 2001]. Although the core protein is conserved better than the other structural and non-structural proteins of HCV, polymorphisms of core protein are known, and they influence the response to antiviral treatment. In patients infected with HCV-1b, for example, the substitution of arginine at position 70 (Arg70) for glutamine (Gln70) and that of leucine at position 91 (Leu91) for methionine (Met70) decrease sustained virological response in the patients with chronic hepatitis C who are treated with PEG-IFN/ribavirin and increase the development of HCC [Akuta et al., 2007a,b,d, 2008].

In the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo, the amino-acid sequence of the core-protein was determined in 1,079 patients infected with HCV-1b who had not received antiviral treatment. The substitution of Arg70 for Gln70 and that of Leu91 or Met 91 were correlated with the age at presentation, liver function tests and the severity of liver disease. Based on the results obtained, Gln70 would contribute to the progression of chronic hepatitis C.

## MATERIALS AND METHODS

### Patients

During 1966–2008, 1,097 patients infected with HCV-1b visited the Department of Hepatology at the Toranomon Hospital in Metropolitan Tokyo. They were: (1) negative for hepatitis B surface antigen by radio-immunoassay (Dainabot, Tokyo, Japan) or antibody to human immunodeficiency virus type-1; (2) positive for anti-HCV by a third-generation enzyme immunoassay (Chiron Corp., Emeryville, CA) and HCV RNA by the polymerase chain reaction (PCR) (Cobas Amplicor HCV Monitor ver.2.0, Roche Diagnostics, Tokyo, Japan); (3) infected with HCV genotype 1b but not with other genotypes; (4) without previous antiviral treatment; (5) without other forms of hepatitis, including hemochromatosis, Wilson's disease, primary biliary cirrhosis, alcoholic liver disease and autoimmune liver disease; and (6) had serum samples stored at  $-80^{\circ}\text{C}$ . Of the 1,097 patients, 778 (70.9%) had chronic hepatitis, 221 (20.1%) cirrhosis, and 98 (8.9%) HCC. Amino acids in the core protein at positions 70 and 91 were determined, and were correlated with liver disease and biochemical and virological markers. Informed consent was obtained from each patient in this study, and the protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki as reflected by approval by Ethic Committee of the institution.

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## Histopathological Diagnoses of Liver Disease

Liver biopsy was performed under laparoscopy by a modified Vim Silverman needle (Tohoku University style, Kakinuma Factory, Tokyo). The sample was fixed in 10% formalin, and was stained with hematoxylin and eosin, Masson's trichrome, silver impregnation, and periodic acid-Schiff. It contained at least six portal areas. The pathological diagnosis was made by one of the authors (H.K.) who was blinded to the clinical data. Chronic hepatitis was diagnosed based on the scoring system of Desmet et al. [1994]. Cirrhosis was diagnosed by imaging on ultrasonography (US), computed tomography (CT), or magnetic resonance imaging (MRI). HCC was diagnosed by US and/or CT. Angiography was performed when HCC was strongly suspected by US, CT, MRI, or liver biopsy. An increasing trend of tumor markers was taken into consideration for the diagnosis of HCC.

## Determination of Amino-Acid Substitutions in the Core Protein

Amino acid (aa) at position 70 of Arg or Gln and aa91 of Leu or Met were determined by PCR with primers specific for each of them [Okamoto et al., 2007]. It is highly reproducible, and has a sensitivity of 94.4% in the determination of aa70 or aa91 in samples with HCV RNA titers  $>10$  KIU/ml. The concordance of the results of this method with those of direct sequencing reached 97.1%. Amino acids at positions 70 and 91 were confirmed by direct sequencing of most samples [Akuta et al., 2005].

## Statistical Analysis

Changes of Arg70/Leu91 (wild-type) and Gln70 and/or Met91 (mutant types) with age were analyzed by the Cochran–Armitage trend test (SAS version 9.1.3; SAS Institute, Inc., Cary, NC). Frequencies were compared between groups by the Kruskal–Wallis test and Fisher's exact test. Univariate and multivariate logistic regression analyses were used for the evaluation of factors independently associated with the substitution of aa70. They included the following ten variables: age, sex, liver disease, platelet count, hemoglobin, albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT),  $\gamma$ -glutamyl transpeptidase ( $\gamma$ -GTP), and substitution of aa at position 91 in the core protein. Each variable was transformed into categorical data consisting of two simple ordinal numbers for univariate and multivariate analyses. Variables that achieved statistical significance on univariate analysis were tested by the multivariate Cox proportional hazard model to identify independent factors. Statistical comparisons were performed using SPSS ver.11.0 (SPSS, Inc., Chicago, IL). A *P*-value  $<0.05$  by the two-tailed test was considered significant.

## RESULTS

## Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With HCV-1b

Table I lists the baseline characteristics of the 1,097 patients who were infected with HCV-1b and had not received antiviral treatment. They had the median age of 60 years and included 590 (53.8%) men. The median transaminase levels were elevated, and alpha-fetoprotein was within the normal limit (<10 µg/L). The majority of the patients (70.9%) had chronic hepatitis, while HCC had developed in 8.9% of the patients. Amino acids at positions 70 and 91 in the core protein were both the wild-type (Arg70 and Leu91) in 37.6% of them, and both mutant types (Gln70 and Met91) in 16.4%. The Gln70 variant was detected in 464 of the 1,097 (42.3%) patients.

## The Prevalence of Amino-Acid Substitutions Stratified by Age and Sex

The 1,097 patients infected with HCV-1b were classified into three age groups, and the prevalence of Arg70/Leu91 (wild-type) and that of Gln70 and/or Met91 (mutant types) were compared (Fig. 1). Arg70/Leu91 decreased with age by trend analysis, from 63.6% in the patients aged ≤30 years to 36.6% in those ≥41 years ( $P < 0.001$  by the Cochran–Armitage trend test). Table II lists the prevalence of the Gln70 variant in men and women stratified by the age. There were no sex differences in the prevalence of the Gln70 variant.

## The Prevalence of the Gln70 Variant in Patients With Different Liver Diseases

Figure 2 compares the prevalence of the Gln70 variant among patients infected with HCV-1b who presented with different liver diseases at the baseline. The prevalence of the Gln70 variant increased with the progression of liver disease from chronic hepatitis

TABLE I. Clinical and Virological Characteristics of the 1,097 Patients Who Were Infected With Hepatitis C Virus of Genotype 1b

Age (years)	60 (19–83)
Men	590 (53.8%)
Follow-up period (years)	8 (3–28)
Hemoglobin (g/dl)	14.0 (4.5–26.8)
Platelets ( $\times 10^3/\text{mm}^3$ )	15.4 (2.0–34.1)
Aspartate aminotransferase (IU/L)	58 (8–617)
Alanine aminotransferase (IU/L)	69 (6–776)
Alpha-fetoprotein (µg/L)	6 (2–65,700)
Liver disease	
Chronic hepatitis	778 (70.9%)
Cirrhosis	221 (20.1%)
Hepatocellular carcinoma	98 (8.9%)
Amino acids in the core protein	
Arg70/Leu91 (double wild-type)	412 (37.6%)
Gln70/Leu91 (mutant type)	284 (25.9%)
Arg70/Met91 (mutant type)	221 (20.1%)
Gln70/Met91 (double mutant type)	180 (16.4%)

Values are the median with range in parentheses or the number with percentage in parentheses.

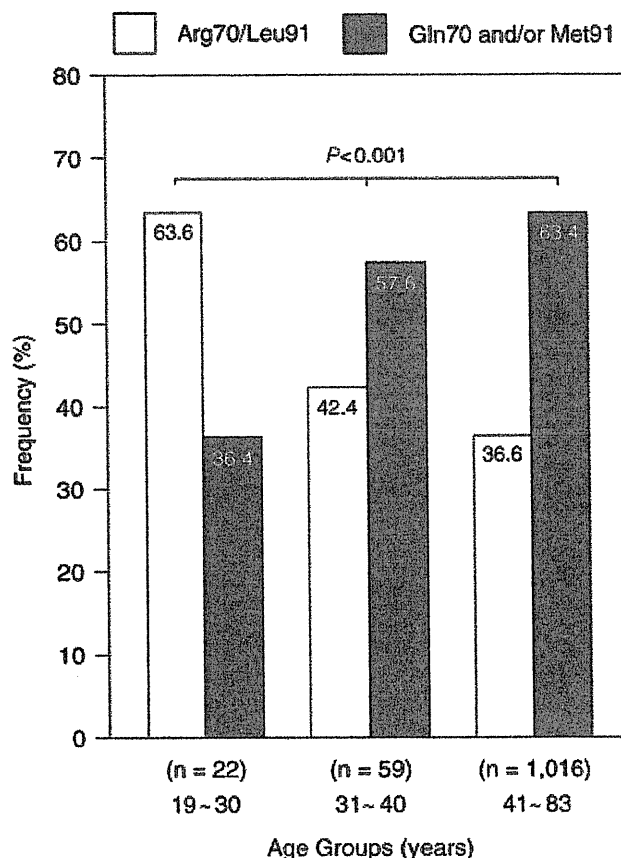


Fig. 1. The age-specific prevalence of Gln70 in treatment-naive patients infected with HCV-1b.

(32.6%) to cirrhosis (43.0%) and HCC (53.1%) ( $P < 0.001$  by the Kruskal–Wallis test). In patients with cirrhosis, the 126 patients with the Arg70 variant were aged with the mean of 62 years (range: 32–78 years) in comparison with the 95 patients with the Gln70 variant who were aged 59 years (25–80). In patients with HCC, the 47 patients with the Arg70 variant were aged with the mean of 66 years (range: 37–81 years) in comparison with the 51 patients with the Gln70 variant who were aged 66 years (46–78).

TABLE II. Frequency of Gln70 in the Core Protein in Patients Infected With HCV-1b Stratified by Age and Sex

Age (years)	Men	Women	Differences
19–30	23.5% (4/17)	20% (1/5)	1.0
31–40	34.1% (14/41)	38.9% (7/18)	0.773
41–50	37.2% (45/121)	40% (14/35)	0.763
51–60	39.1% (72/184)	40.1% (63/157)	0.912
61–70	36.0% (62/172)	30.1% (74/246)	0.205
70–83	45.5% (25/55)	43.5% (20/46)	0.842
Total	37.6% (222/590)	35.3% (179/507)	0.451

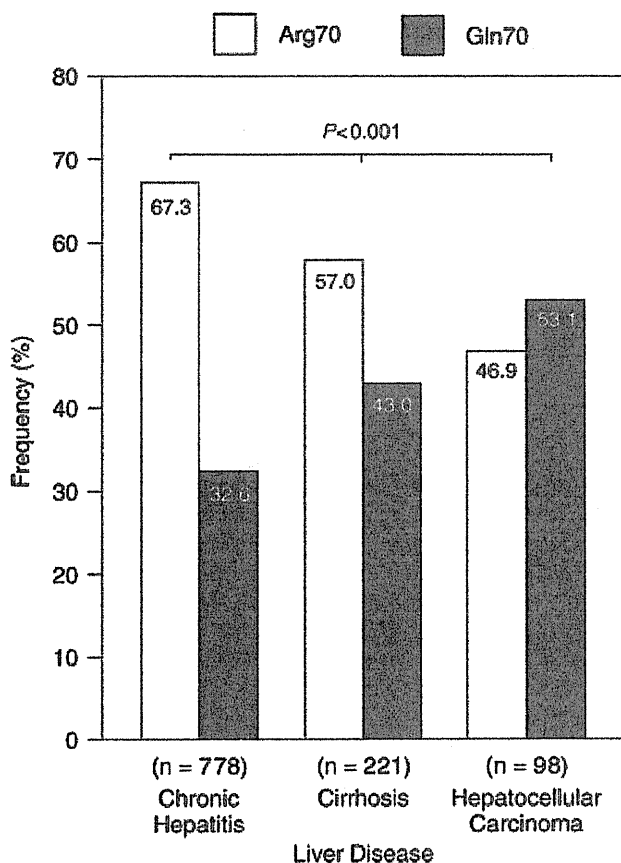


Fig. 2. The prevalence of the Gln70 variant among patients with chronic hepatitis, cirrhosis and hepatocellular carcinoma.

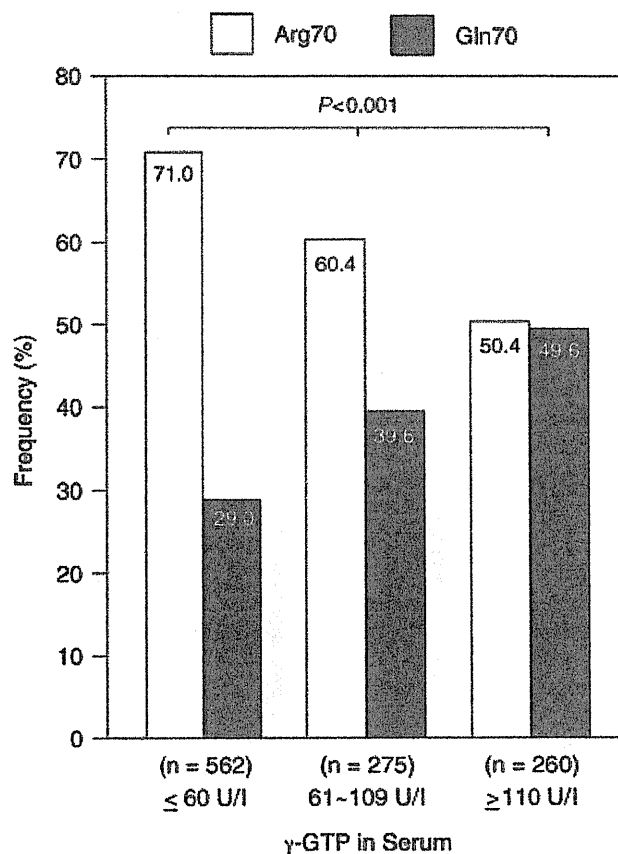


Fig. 3. The prevalence of the Gln70 variant among patients with different γ-GTP levels.

#### The Influence of γ-GTP Levels on the Prevalence of the Gln70 Variant

The prevalence of Gln70 was compared among patients with different γ-GTP levels at the baseline (Fig. 3). The prevalence of the Gln70 variant increased in parallel with the γ-GTP levels from 29.0% to 49.6% ( $P < 0.001$  by the Kruskal–Wallis test).

#### The Influence of Platelet Count on the Prevalence of the Gln70 Variant

The prevalence of the Gln70 variant was compared among three groups of patients with various platelet counts at the baseline (Fig. 4). The prevalence of the Gln70 variant increased as the platelet count decreased ( $P = 0.008$  by the Kruskal–Wallis test).

#### Factors Associated With the Gln70 Variant in Patients Infected with HCV-1b

Since the Gln70 variant, in comparison with the Arg70 variant, aggravated liver disease in patients infected with HCV-1b (Figs. 2–4), ten factors were evaluated for the association with the Gln70 variant by the univariate analysis (Table III); the cut-off value was

set at the median of studied patients. Among them, HCC, elevated levels of AST ( $\geq 58$  IU/L) and γ-GTP ( $> 61$  U/L), as well as decreased albumin concentration ( $< 3.9$  g/dl), were associated with the Gln70 variant ( $P = 0.003, 0.005, < 0.001,$  and  $0.031,$  respectively). A similar analysis was performed for the substitution of Leu91 for Met91 (Table IV). Except for the association with the substitution of Arg70 for Gln70, the Met91 variant had no influence on any variable examined.

Two factors associated independently with the Gln70 variant were identified by the multivariate analysis (Table V). The risk for the Gln70 variant was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917],  $P = 0.011$ ) and γ-GTP  $\geq 61$  IU/L (1.647 [1.268–2.139],  $P < 0.001$ ).

#### DISCUSSION

The response to PEG-IFN and ribavirin is influenced by genotypes and viral load, and is poorest in patients with HCV-1b in high HCV RNA levels [Manns et al., 2001; Fried et al., 2002; Hadziyannis et al., 2004]. The prediction of sustained virological response would circumvent side-effects and costs in non-responders. Amino-acid substitutions in the core protein are useful



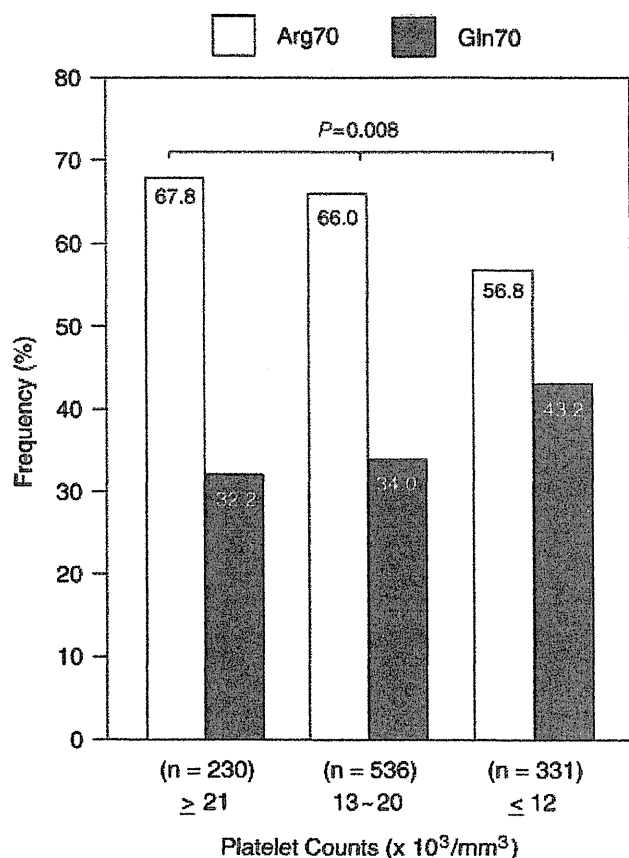


Fig. 4. The prevalence of the Gln70 among three groups of patients with different platelet counts.

for predicting the non-response in patients infected with HCV-1b. The substitution of Arg70 for Gln70 in the prototype sequence of HCV-1b [Kato et al., 1990] and/or that of Leu91 for Met91 can predict the non-response to

IFN-based treatment [Akuta et al., 2005, 2006, 2007c,d]. It has been beyond the scope of previous studies, however, whether or not these amino-acid substitutions influence the progression of hepatitis C in the patients who have not received antiviral treatment. The availability of pre-treatment sera from many patients with chronic hepatitis C permitted the evaluation of the influence of aa substitutions in the core protein on the progression of liver disease without therapeutic intervention.

First, the prevalence of the Gln70 variant increased with the age of patients until they had reached 50 years (Fig. 1). It is not certain if HCV-1b with Arg70 underwent a point mutation for Gln70 (G-to-A at nucleotide 209), or these amino-acid residues were present in HCV-1b strains prevalent at the time of infection. Follow-up of patients for aa substitutions will resolve this issue. Another possibility for this difference would be a selection bias. If the patients with the Arg90 variant fare better than those with the Gln70 variant, they would not develop liver disease severe enough to visit hospital.

Secondly, the patients infected with HCV-1b with Gln70 increased in parallel with  $\gamma$ -GTP levels and the severity of liver disease from chronic hepatitis to cirrhosis and HCC, as well as with a decrease in platelet count (Figs. 2-4). Since the Met91 variant did not make such difference, the aggravation of liver disease would have been due to the Gln70 variant, but not to the Met91 variant. Increases in the  $\gamma$ -GTP level may have been related to the development of HCC;  $\gamma$ -GTP has been proposed as a sensitive marker of cirrhosis and HCC [Penn and Worthington, 1983]. Decreased platelet counts have been associated with HCC [Ikeda et al., 2001; Lu et al., 2006; Kumada et al., 2009]. Although the proportion of the Gln70 variant increases with the severity of liver disease (Fig. 2), the median age of patients with cirrhosis or HCC did not differ between the patients with the Arg70 variant and Gln70 variant who

TABLE III. Factors Associated With the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

Factor	Category	Gln70	P-value
Sex	1: Male	38.6% (228/590)	0.663
	2: Female	37.3% (189/507)	
Age (years)	1: <60	40.6% (219/540)	0.093
	2: $\geq 60$	35.5% (198/557)	
AST (IU/L)	1: <58	33.9% (184/543)	0.005
	2: $\geq 58$	42.2% (234/554)	
ALT (IU/L)	1: <75	36.9% (213/578)	0.376
	2: $\geq 75$	39.3% (204/519)	
Albumin (g/dl)	1: <3.9	42.5% (194/457)	0.031
	2: $\geq 3.9$	35.8% (229/640)	
$\gamma$ -GTP (IU/L)	1: <61	29.0% (163/562)	<0.001
	2: $\geq 61$	44.4% (238/535)	
Hemoglobin (g/dl)	1: <14	35.1% (176/501)	0.083
	2: $\geq 14$	40.4% (241/596)	
Platelet count ( $\times 10^3/\text{mm}^3$ )	1: <150	39.9% (207/519)	0.253
	2: $\geq 150$	36.3% (210/578)	
Hepatocellular carcinoma	1: No	36.6% (366/999)	0.003
	2: Yes	53.1% (52/98)	
Substitutions of core aa91	1: Leucine	35.6% (227/638)	0.051
	2: Methionine	41.4% (190/459)	

TABLE IV. Factors Associated With the Substitution of aa91 of Leucine for Methionine in the Core Protein in 1,097 Patients Infected With HCV Genotype1b by Univariate Analysis

Factor	Category	Met91	P-value
Sex	1: Male	40.8% (241/590)	0.500
	2: Female	43.0% (218/507)	
Age (years)	1: <60	43.5% (235/540)	0.271
	2: ≥60	40.2% (220/517)	
AST (IU/L)	1: <58	43.6% (234/537)	0.196
	2: ≥58	39.7% (217/547)	
ALT (IU/L)	1: <75	42.4% (238/561)	0.618
	2: ≥75	40.8% (205/502)	
Albumin (g/dl)	1: <3.9	42.0% (177/421)	0.797
	2: ≥3.9	41.2% (249/604)	
γ-GTP (IU/L)	1: <61	40.4% (237/586)	0.327
	2: ≥61	43.4% (222/511)	
Hemoglobin (g/dl)	1: <14	40.8% (193/473)	0.658
	2: ≥14	42.3% (240/567)	
Platelet count (×10 <sup>3</sup> /mm <sup>3</sup> )	1: <150	40.5% (202/499)	0.454
	2: ≥150	42.9% (240/559)	
Hepatocellular carcinoma	1: No	42.3% (423/999)	0.334
	2: Yes	36.7% (36/ 98)	
Substitutions of core aa71	1: Arginine	49.0% (269/680)	0.051
	2: Glutamine	45.6% (190/417)	

had cirrhosis (62 years vs. 59 years) of HCC (66 years vs. 66 years). This would indicate a possibility that the Gln70 variant would be a factor for the aggravation of liver disease that might be independent of age.

The distinct capacity of Gln70 and Met91 for decreasing the response to combined treatment in patients infected with HCV-1b was proposed in a recent study [Okanoue et al., 2008]. The Gln70 variant decreased sustained virological response, while the Met91 variant did not, although the Met91 variant reduced the rate of rapid virological response within 4 weeks after the start of therapy. The role of the Gln70 variant greater than that of the Met91 variant in the progression of liver disease has been confirmed in this study (Tables III and IV). In the multivariate analysis, the risk for Gln70 was increased by HCC (odds ratio 1.829 [95% confidence interval 1.147–2.917]) and γ-GTP ≥61 U/L (1.647 [1.268–2.139]). The Gln70 variant would aggravate liver disease toward the development of HCC in patients infected with HCV-1b who have not received antiviral treatment.

It would be a matter of conjecture how the Gln70 variant influences the severity of liver disease. Previous suggestions for a reduced response of patients with the Gln70 variant were confined to interaction of the core protein with IFN receptors and IFN-signaling pathways [Alexander, 2002; Blindenbacher et al., 2003; Bode et al., 2003]; these studies were restricted to patients receiving

IFN-based treatments [Akuta et al., 2007a,b,d, 2008]. The ability of the Gln70 variant for accelerating the progression of liver disease, in the absence of exogenous IFN, has changed this issue into a wider perspective. There still remains a possibility, however, that the Gln70 variant would interact with the endogenous IFN induced by HCV infection, and aggravate liver disease.

Another possibility may be the cytotoxic T-cell (CTL) response, as has been demonstrated for the pathogenesis of chronic hepatitis B [Chisari and Ferrari, 1995]. Since both hepatitis B virus (HBV) and HCV do not have a cytopathic capacity, hepatitis B and C would be mediated by immune responses directed at viral proteins. Amino-acid sequences bearing a CTL epitope restricted by the MHC class-I are demonstrated in the HBV core protein [Bertoletti et al., 1993; Bertoletti and Gehring, 2006], and are implicated in liver disease in the patients with the HLA-2 phenotype [Penna et al., 1991; Bertoletti et al., 1994]. It is tempting to speculate that the substitution of Arg70 for Gln70 might generate a CTL epitope and stimulate cytotoxic lymphocytes toward inflammation of the liver [Kita et al., 1993; Jackson et al., 1999].

In conclusion, amino-acid substitutions in the core protein influence the progression of liver disease, and the Gln70 variant aggravates hepatic inflammation and increases the risk for HCC in the patients who have not received antiviral treatment. The ability of the Gln70

TABLE V. Factors Associated with the Substitution of aa70 of Arginine for Glutamine in the Core Protein in 1,097 Patients Infected with HCV Genotype1b by Multivariate Analysis

Factor	Category	Odds ratio (95%CI)	P-value
Hepatocellular carcinoma	1: No	1	0.011
	2: Yes	1.829 (1.147–2.917)	
γ-GTP (IU/L)	1: <61	1	<0.001
	2: ≥61	1.647 (1.268–2.139)	

variant to aggravate liver disease, in the absence of exogenous IFN, would lend further support on its capacity of predicting sustained virological response before the start of therapy. It is possible that mechanisms other than the resistance to IFN, such as cytotoxic T-cell responses, might be involved in an increased pathogenetic potential of HCV-1b with Gln70.

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