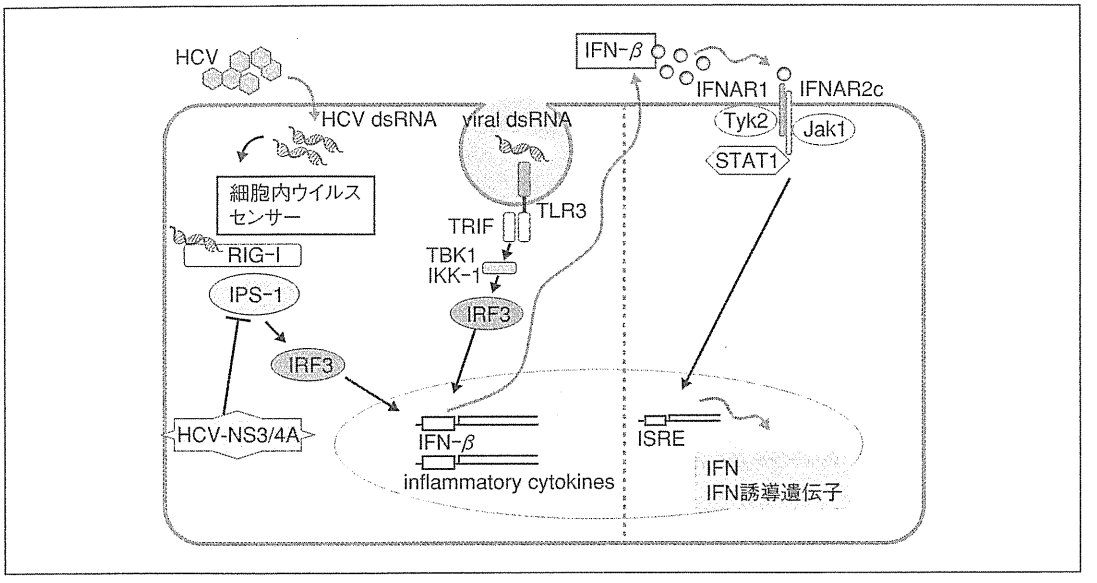

図 3　RIG-I/IPS-1 系を中心とした宿主自然免疫機構
RIG-I：retinoic acid inducible gene I，IPS：インターフェロン-β promoter stimulator

御にかかわる宿主因子である自然免疫機構と治療効果について注目した。

近年の *in vitro* の研究によると，HCV に対する生体防御には宿主自然免疫機構が重要とされる。すなわち，HCV が細胞に感染すると，まず HCV 由来の RNA が細胞内のウイルスセンサーである RIG-I(retinoic acid inducible gene I)によって探知され，そのシグナルがアダプター分子である IPS-1 (IFN-β promoter stimulator-1：別名 MAVS，Cardif，VISA) を介して核に伝達され，IFN-β が産生されるとされる。この RIG-I/IPS-1 系による自然免疫の作動が HCV 感染に際して生体側で起こる最初の防御機構であるとされ，IFN-β は I 型の IFN 受容体に結合し，Jak-STAT 系を介し大量の IFN の産生や IFN 誘導遺伝子（ISG：interferon stimulated gene）の誘導を引き起こし，宿主を抗ウイルス状態にすると考えられている（**図 3**）。

一方，HCV の NS3/4 A セリンプロテアーゼは IPS-1 を分解することが示されており，HCV は RIG-I/IPS-1 系を標的とすることで，巧みに宿主の自然免疫系から逃れているとされる（**図 3**）。したがって，RIG-I/IPS-1 系は宿主による HCV の排除，およびそれに対する HCV の抵抗性に重要な鍵を握っていることは間違いないと考えられるが，ヒトにおける臨床的意義はほとんど解明されていなかった。

そこで，われわれは治療により大量に外因性 IFN を投与してもウイルス排除が起こらない NVR の症例では，この宿主自然免疫機構になんらかの特徴があると考え，Peg-IFN-α2b＋リバビリン併用療法を施行した 1b 高ウイルス量の C 型慢性肝炎 74 例を対象として，細胞内ウイルスセンサーである RIG-I およびアダプター分子である IPS-1，さらに IFN 誘導遺伝子である ISG15 などの mRNA の治療前肝生検組織における肝内発現量を定量した[3]。その結果，RIG-I や ISG15 の肝内遺伝子発現は，治療中 HCV が減衰しない NVR 群で SVR 群に比し有意に高発現していたのに対して，IPS-1 の治療前肝内遺伝子発現は NVR 群で有意に低値で，RIG-I/IPS-1 比は NVR 群で有意に高かった（NVR：SVR＝1.3：0.4）（**図 4**）。ROC 解析では ISG15，USP18 発現および RIG-I/IPS-1 比の area under the curve は 0.9 以上で，これらの遺伝子の治療前における肝内発現を定量することは，これまで困難とされてきた Peg-IFN-α＋リバビリン併用療法の最終治療効果を治療前に予測

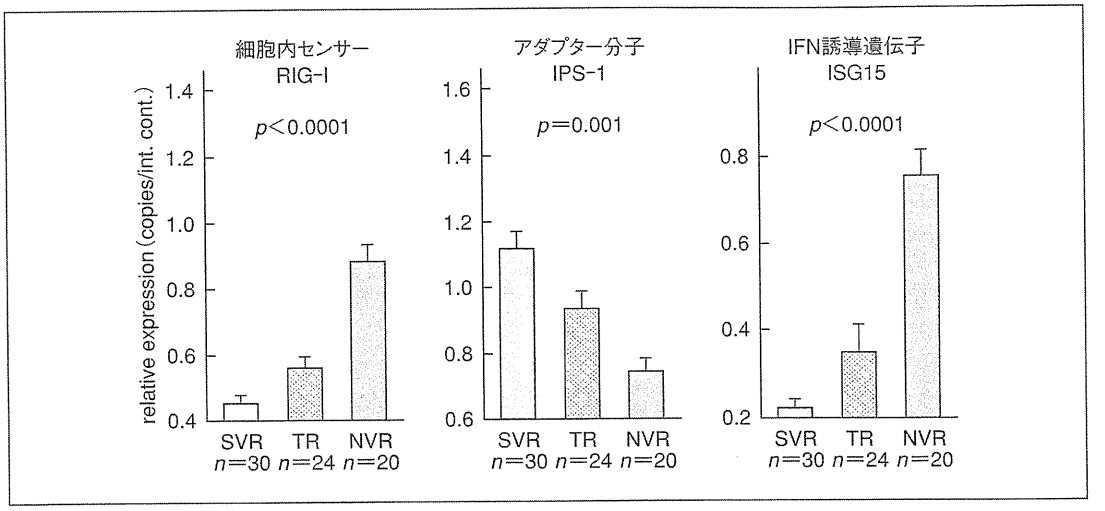

図 4　自然免疫系分子の肝内遺伝子発現とウイルス学的治療効果（文献 3 を引用，改変）

するのにきわめて有用と考えられた。

一方，治療による自然免疫系遺伝子の発現誘導の反応性を検討すると，治療前に高発現状態となっていた RIG-I や ISG15 は，Peg-IFN-α＋リバビリン投与による発現誘導が NVR 例では有意に低いことがわかった[3]。したがって，NVR 例では治療前に内因性 IFN により自然免疫系がすでに up regulation されているため，治療である外因性 IFN に対する反応性が減弱していることが示唆され，IFN に対する不応性のメカニズムを探る糸口となると考えられた。

● IL28B 近傍の一遺伝子多型（SNP）と治療効果および自然免疫系遺伝子発現との関連

さらに，最近 IL28B 近傍の SNP と Peg-IFN-α＋リバビリン併用 48 週投与における治療効果との関連が報告されている[4〜6]。これについては他稿に詳しいが，当院においても IL28B 近傍の SNP と Peg-IFN-α2b＋リバビリン併用療法中の血中 HCV 動態と治療成績を検討した。それによると IL28B の minor allele の症例では，治療中に HCV 減衰がほとんど得られない，いわゆる null responder が多いことがわかり，最終的治療効果も IL28B の major allele の症例における NVR 率が 10% であったのに対し，minor allele の症例では約 70% が NVR であった。したがって，IL28B の minor allele は，NVR ときわめて強い関連があることが示唆された。また，IL28B minor allele にかかわる臨床的背景を単変量解析で検討すると，血清 γ-GTP 高値や LDL コレステロール低値，肝脂肪化などの宿主因子と関連していることがわかった。そこで，前述の宿主自然免疫系遺伝子発現と IL28B 近傍の SNP との関連を検討すると，IL28B の minor allele の症例では，有意に RIG-I や ISG15 の治療前の肝内発現が major allele の症例に比し高く，反対に IPS-1 の発現は低い傾向を認めた。多変量解析では NVR にかかわる独立因子として，RIG-I/IPS-1 比と IL28B minor allele が抽出されたことから，RIG-I/IPS-1 系を中心とした宿主自然免疫機構は IL28B 近傍の SNP とともに NVR と関連していることが示唆された。

● おわりに

Peg-IFN-α＋リバビリン併用療法が臨床応用され広く施行されるようになり，その効果規定因子や難治要因も次第に明らかとなってきた。したがって，今後はこれらの要因を個々の症例において検討し，的確な治療効果予測を行い，より有効な対策を講じることで治療成績の向上をはかる必要がある。

文献
1) Asahina Y, Tsuchiya K, Tamaki N, et al. Effect of aging on risk for hepatocellular carcinoma in chronic hepatitis C virus infection. Hepatology 2010；52：518-27.
2) Enomoto N, Sakuma I, Asahina Y, et al. Mutations in the nonstructural protein 5A gene and response to interferon in patients with chronic hepatitis C virus 1b infection. N Engl J Med 1996；334：77-81.
3) Asahina Y, Izumi N, Hirayama I, et al. Potential relevance of cytoplasmic viral sensors and related regulators involving innate immunity in antiviral response. Gastroenterology 2008；134：1396-405.
4) Tanaka Y, Nishida N, Sugiyama M, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009；41：1105-9.
5) Ge D, Fellay J, Thompson AJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009；461：399-401.
6) Thomas DL, Thio CL, Martin MP, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009；461：798-801.

8章　肝臓

I　extended criteria donors からの肝移植

谷口 雅彦，永生 高広，古川 博之

1　はじめに

1963年，Starzl[1]が世界で第1例目の脳死肝移植を施行して以来，年間6,000例を超える脳死肝移植が行われている米国を筆頭に，世界における肝移植の最大の問題は絶対的ドナー不足である．これに対し，欧米を中心として高齢者ドナー，脂肪肝などを含めたextended criteria donor（ECD）が模索されている．ここではそのECDの現状と問題点につき言及する．

2　脳死肝移植の現状

2.1　米国における移植数と待機者数の現状

1988年以降の肝移植症例数の推移を図8-I-1に示す．米国では年々の脳死肝移植数は増加し，2003年以降年間6,000例超の肝移植が行われている．2010年の年間脳死肝移植施行数は6,009例であり，これまでに延べ100,000例以上の脳死肝移植が施行されている（http://optn.transplant.hrsa.gov）．一方で，2011年7月現在，16,153名がUNOSの肝移植希望者リストに登録されているが，このうち毎年15%前後の患者が，すなわち登録者の7人に1人が臓器不足のために肝移植の機会を得ることなく亡くなっている．これが移植

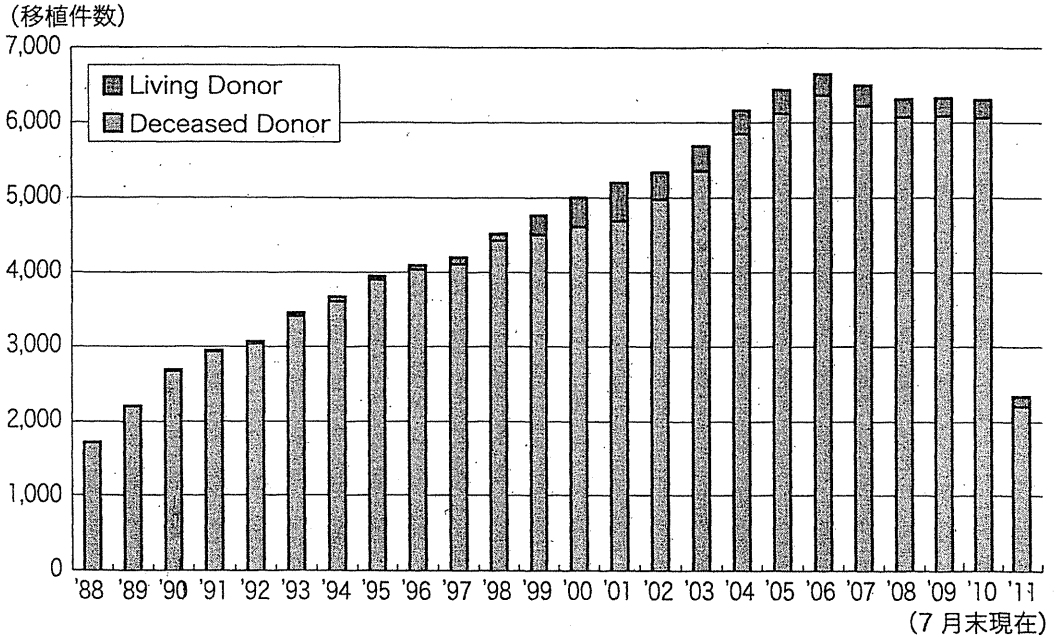

図8-I-1　米国における肝移植症例数の推移．

先進国の米国の現状であり，ここに絶対的ドナー不足が存在する．

2.2 我が国における現状

我が国では2011年6月末現在，352名が脳死肝移植を希望して日本臓器移植ネットワークに登録されている（http://www.jotnw.or.jp/）．1997年10月の脳死肝移植希望者登録開始後，2010年6月末までの希望登録者累計は1,329名であり，そのうち，実際に我が国で脳死肝移植を受けた登録者は118名（8.8%），生体肝移植を受けた登録者は214名（16.1%），海外にて脳死肝移植を受けた登録者は30名（2.3%），亡くなった登録者は485名（36.5%）であった．臓器移植法が改正され1年が経過するが，改正前と比較し実際に我が国で脳死肝移植を受けた登録者は約2%増加し，亡くなった登録者は約1%減少した．しかし，いまだ3人に1人は肝移植を受けられずに亡くなっており，絶対的ドナー不足の現状に変わりはない．

3 donor criteria

3.1 脳死肝移植における donor criteria

適切なドナー選択は，脳死肝移植の成功に不可欠である．ドナー選択に必要な検討項目として，年齢，body mass index（BMI），薬物服用，飲酒歴，肝疾患の有無，感染症の既往，悪性疾患の既往などがある．さらに死亡原因，循環動態，肝機能の推移等の検討も重要となる．理想的なドナー選択基準として，年齢50歳以下，正常な肝機能かつ肝疾患を伴わない，循環動態が安定している，重篤な腹部外傷を伴わないこと，全身の感染症がない，悪性疾患が存在しない，腎機能が正常であるなどが挙げられている[2]．

3.2 extended criteria donor（ECD）

ドナー不足に対する対策として living donor liver transplantation, split transplantation などはすでに上限に達しており，マージナルドナーからの肝移植，すなわち extended criteria donor（ECD）による肝移植に頼らざるを得ない現状がある．ECDに一定基準は存在しないが，高齢者ドナー，脂肪肝，HBV感染ドナー，HCV感染ドナー，高ナトリウム血症，ICUでの長期呼吸器管理，心停止後あるいは低血圧による一定時間の臓器虚血，高BMI，血管作動薬の使用，温・冷保存時間の延長などが挙げられる[3,4]．

3.2.1 高齢者ドナー

高齢者ドナーグラフトでの問題は，高度な動脈硬化の存在と脂肪肝をはじめとした肝実質の問題である[5,6]．これら2つは移植後早期のグラフト生着率に多大なる悪影響を及ぼす[6]．一般的にはドナー年齢が60歳を超えると移植成績に多大なる影響を及ぼすと言われている．しかし近年のドナー不足の打開策としてこの高齢者ドナーグラフトでの移植が増えている．特にスペインでは積極的に高齢者ドナーでの移植が行われている．米国における60歳以上のドナー候補は15%弱に過ぎないが，スペインのそれは実に全体の30%を超える．結果として，米国では5.5%のグラフトが移植を断念される一方で，スペインでは18%で移植が断念される[7]．しかしながら，これら高齢者ドナーの移植を考慮することが使用可能なグラフトの数を増やすことは疑いもなく，結果として待機中の死亡数を減らすことにつながる．

同様に最近の米国でのドナー年齢分布にも明らかな変化が見られる．図8-I-2に米国におけるドナーの年齢分布の変遷を示す．1989年以降，35歳までのドナー数は変化がないものの35歳以上については増加が見られ，特に2001年以降は50歳以上のドナーの全体に占める割合が増加し，全体の30%以上を占めるに至っている．

では，実際の高齢者ドナーでの移植成績はどうだろうか．Spanish Registry for Liver Transplantationによると60～69歳，80～89歳のドナーによるレシピエントの1年生存率はそれぞれ76%

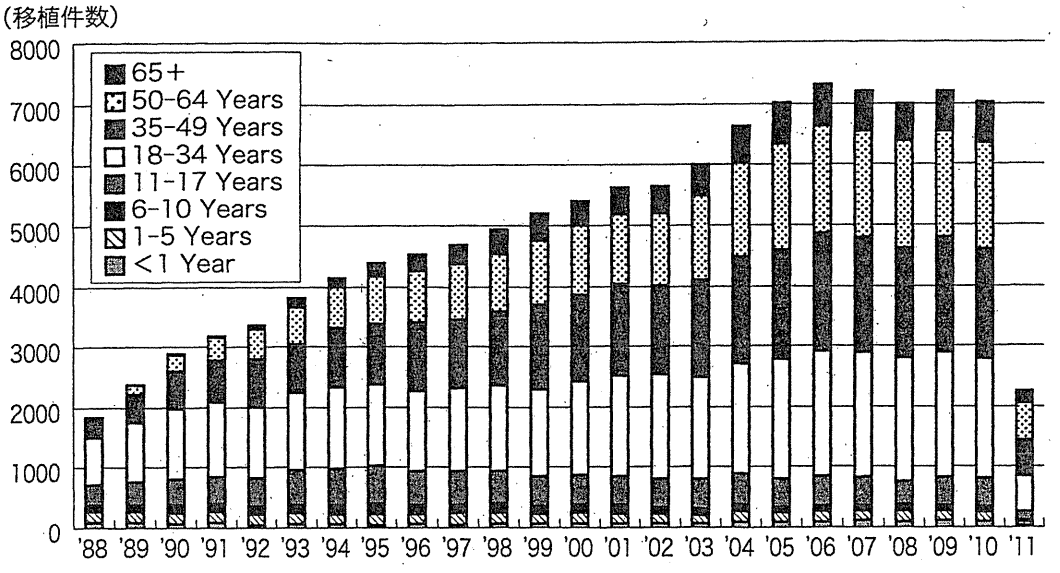

図 8-I-2 米国におけるドナーの年齢分布.

と 72%であるのに対し，15～60 歳は 80%と極端な差はない．しかしながら，5 年生存率は前者が 56%と 51%であるのに対し，後者は 66%と差は広がる．これはやはり高齢者ドナーの場合には肝癌，C 型肝炎再発，原疾患の再発が起こりやすくなるためである[8]．他方，高齢者ドナーの肝移植は現在，他の risk がなければ若年者ドナーの成績と遜色がないとも言われている[9]．これはグラフトの状態とレシピエントの risk を見極めたレシピエント選択が行われているためであり，risk の低いレシピエントに対しては高齢者ドナーによる肝移植も成績に影響を与えるものではない．このようにドナーが高齢者であるだけではもはや移植成績を下げる因子ではなくなりつつある．

3.2.2 脂肪肝

脳死ドナーにおいて脂肪肝は 13～26%に存在すると言われる[10]．肝臓への脂肪浸潤は macrovesicular と microvesicular の 2 つに分かれる．microsteatosis は可逆性であることから，primary nonfunction の発生頻度，あるいは生存率には関与しないと言われる[11,12]．一方，macrovesicular steatosis の程度は予後に関係する．通常 60%を超える severe macrovesicular steatosis は高率に primary nonfunction を発症することから，禁忌とされている[13]．他方，30～60%の moderate macrovesicular steatosis を持つドナーからの肝移植は正常肝と比較し，その primary nonfunction の頻度が高い（13%対 3%）と言われているが[14]，その他の risk factor がドナー，レシピエントにない場合には通常のドナーと比較し同等の移植後成績を得られると言われている[15]．待機死亡数が増加の一途をたどっている現状では，moderate macrovesicular steatosis を持つドナーからの移植成績を向上させる努力が必要である．

3.2.3 HBV 陽性ドナー

HBcAb 陽性ドナーの肝臓が HBsAb 陽性，あるいは HBcAb 陽性のレシピエントに移植された場合にはほとんど問題とならないが，それ以外のレシピエントに移植される場合には高率に B 型肝炎が再燃する[16]．したがって HBcAb 陽性ドナーの肝臓を移植する場合には，移植後 hepatitis-B immunoglobulin と抗ウイルス剤を用いることが必要となる[17]．

3.2.4 HCV 陽性ドナー

C 型肝硬変症例に対する HCV 陽性ドナーの肝移植は，HCV 陰性ドナーと比し，移植後生存率に有意な差を認めない[18,19]が，高齢者かつ HCV 陽性ドナーからの肝移植は高齢者かつ HCV 陰性ドナーより線維化が増悪し，グラフト不全が起こ

りやすいと言われている[20]．またドナー由来のHCVが優位なレシピエントは逆の場合より無再発生存期間が長いことが知られている[21]．HCV陽性ドナーのグラフトに線維化や炎症が存在する場合には移植の適応とならないことから，移植前の肝生検が重要である．

3.2.5 ECD graftとレシピエントのマッチング

ECD graftを用いることによって待機患者の死亡数を減少させることができると言われており[22]，2000～2007年にかけて米国で施行された肝移植は4,595例から6,228例に増加した（26.2%増）．これはMELDスコアに基づいた臓器分配システムの導入とmarginal donorの利用が誘引と考えられている．

では実際にECD graftはどのような症例に移植されるべきなのであろうか．UCLAからの1,000例を超える大規模なretrospective study[23]によると，ECDを55歳以上のドナー，5日を超えるドナーの入院，10時間を超える冷虚血時間，40分を超える温虚血時間とした場合，ECDの数（donor score = DS）が増えるほど移植後の死亡率も増えるとしている．つまりDSの数が増えるほどhigh riskになることから，移植前状態（MELDスコア）が悪いレシピエントでは移植後不幸な結果を招く恐れがあると述べている．DS 2以下の場合には，多くの症例において少ないriskで移植が可能となるが，DSが2以上の場合には緊急度が高いなどのhigh risk症例に対しては避けるべきであるとしている．同様に，スペインでの650例の検討では，primary dysfunctionに関与するECDは高齢者ドナー，30%以上の脂肪肝，冷虚血時間であり，ECD scoreが高いドナーかつMELDスコア29以上のレシピエントとの組み合わせが最もグラフト不全が起こりやすいとしている[24]．

Organ Procurement and Transplantation Network（OPTN）のデータによると，米国の現状として2002年6月から2005年6月までに12,056例の肝移植が施行され，うち2,873例（23.8%）はECD graftの移植であり，ECD graftはMELDスコア＜15のレシピエントに最も多く移植されている（33%）[4]．Fengら[25]は米国のthe Scientific Registry of Transplant Recipientsの20,023例に及ぶ肝移植のデータ解析から，donor risk index（DRI）を算出しており，ECD graftのなかでも40歳以上のドナー，DCD donor，split/partial graftがグラフト不全と強く相関し，現状として，ECD graftはlow MELDスコアの高齢のレシピエントに移植されていると報告している．一方で，ECD graftはグラフト不全の有意なrisk factorとなるもののMELDスコアとの間に相関はなく，ECD graftを用いた移植はむしろMELDスコアの高いレシピエントに利点が多い可能性があるとも述べている．その後の同グループからの米国の28,165例に及ぶ追跡調査でも，low MELDスコアの症例の場合には，待機中の死亡率よりhigh DRI graftでの移植による死亡率の方が上まわり，一方でMELDスコア20以上の症例ではいかなるDRI graftの移植でもsurvival benefitが得られるとしている．このことからもhigh DRI graftからの移植はlow MELDスコアのレシピエントよりhigh MELDスコアのレシピエントが利点が多く，high DRI graftとlow MELDスコアのレシピエントの組み合わせはレシピエントのsurvival benefitが低いこと，また結果としてhigh MELDスコアのレシピエントが移植を受ける機会を逸することからも避けるべきであるとしている[26]．

4 おわりに

肝移植におけるECDについて言及した．上記のように，絶対的ドナー不足が世界中で問題となるなか，我が国においては脳死肝移植数自体が欧米と比較し極端に少ない．その少ない脳死肝移植において，ドナーの尊い意思に報いるためにも，臓器を決して無駄にすることなく，かつ結果として移植を成功に導くことが不可欠である．そのためにも欧米以上にこのECDによる移植に向き合

い，ECD の risk を減らすことができるよう肝移植医のさらなる努力が必要である．

参考文献

1. Starzl TE, et al. Homotransplantation of the liver in humans. Surg Gynecol Obstet 117: 659-676, 1963.
2. Mehrabi A, et al. Current concepts in transplant surgery: liver transplantation today. Langenbecks Arch Surg 393(3): 245-260, 2008.
3. Silberhumer GR, et al. Combination of extended donor criteria and changes in the Model for End-Stage Liver Disease score predict patient survival and primary dysfunction in liver transplantation: a retrospective analysis. Transplantation 83(5): 588-592, 2007.
4. Maluf DG, et al. Utilization of extended donor criteria liver allograft: Is the elevated risk of failure independent of the model for end-stage liver disease score of the recipient? Transplantation 82(12):1653-1657, 2006.
5. Grazi GL, et al. A revised consideration on the use of very aged donors for liver transplantation. Am J Transplant 1(1): 61-68, 2001.
6. Verran D, et al. Clinical experience gained from the use of 120 steatotic donor livers for orthotopic liver transplantation. Liver Transpl 9(5): 500-505, 2003.
7. Chang GJ, et al. Expanding the donor pool: can the Spanish model work in the United States? Am J Transplant 3(10): 1259-1263, 2003.
8. Cuende N, et al. Liver transplant with organs from elderly donors: Spanish experience with more than 300 liver donors over 70 years of age. Transplantation 73(8):1360, 2002.
9. Grande L, et al. Outcome of patients undergoing orthotopic liver transplantation with elderly donors (over 60 years). Transplant Proc 29(8): 3289-3290, 1997.
10. Imber CJ, et al. Hepatic steatosis and its relationship to transplantation. Liver Transpl 8(5): 415-423, 2002.
11. Urena MA, et al. An approach to the rational use of steatotic donor livers in liver transplantation. Hepatogastroenterology 46(26): 1164-1173, 1999.
12. Fishbein TM, et al. Use of livers with microvesicular fat safely expands the donor pool. Transplantation 64(2): 248-251, 1997.
13. Selzner M, Clavien PA. Fatty liver in liver transplantation and surgery. Semin Liver Dis 21(1): 105-113. 2001.
14. Strasberg SM, et al. Selecting the donor liver: risk factors for poor function after orthotopic liver transplantation. Hepatology 20(4 Pt 1): 829-838, 1994.
15. Burke A, Lucey MR. Non-alcoholic fatty liver disease, non-alcoholic steatohepatitis and orthotopic liver transplantation. Am J Transplant 4(5): 686-693, 2004.
16. Prieto M, et al. De novo hepatitis B after liver transplantation from hepatitis B core antibody-positive donors in an area with high prevalence of anti-HBc positivity in the donor population. Liver Transpl 7(1): 51-8l, 2001.
17. Nery JR, et al. Use of liver grafts from donors positive for antihepatitis B-core antibody (anti-HBc) in the era of prophylaxis with hepatitis-B immunoglobulin and lamivudine. Transplantation 75(8): 1179-1186, 2003.
18. Ghobrial RM, et al. A 10-year experience of liver transplantation for hepatitis C: analysis of factors determining outcome in over 500 patients. Ann Surg 234(3): 384-394, 2001.
19. Marroquin CE, et al. Transplantation of hepatitis C-positive livers in hepatitis C-positive patients is equivalent to transplanting hepatitis C-negative livers. Liver Transpl 7(9): 762-768, 2001.
20. Khapra AP, et al. Impact of donor age on survival and fibrosis progression in patients with hepatitis C undergoing liver transplantation using HCV+ allografts. Liver Transpl 12(10): 1496-1503, 2006.
21. Vargas HE, et al. Outcome of liver transplantation in hepatitis C virus-infected patients who received hepatitis C virus-infected grafts. Gastroenterology 117(1): 149-153, 1999.
22. Barshes NR, et al. Waitlist mortality decreases with increased use of extended criteria donor liver grafts at adult liver transplant centers. Am J Transplant 7(5): 1265-1270, 2007.
23. Cameron AM, et al. Optimal utilization of donor grafts with extended criteria: a single-center experience in over 1000 liver transplants. Ann Surg 243(6): 748-755, 2006.
24. Briceno J, et al. Prediction of graft dysfunction based on extended criteria donors in the model for end-stage liver disease score era. Transplantation 90(5): 530-539, 2010.
25. Feng S, et al. Characteristics associated with liver graft failure: the concept of a donor risk index. Am J Transplant 6(4): 783-790, 2006 .
26. Schaubel DE, et al. The survival benefit of deceased donor liver transplantation as a function of candidate disease severity and donor quality. Am J Transplant 8(2): 419-425, 2008.

Influence of *ITPA* Polymorphisms on Decreases of Hemoglobin During Treatment with Pegylated Interferon, Ribavirin, and Telaprevir

Fumitaka Suzuki,[1] Yoshiyuki Suzuki,[1] Norio Akuta,[1] Hitomi Sezaki,[1] Miharu Hirakawa,[1] Yusuke Kawamura,[1] Tetsuya Hosaka,[1] Masahiro Kobayashi,[1] Satoshi Saito,[1] Yasuji Arase,[1] Kenji Ikeda,[1] Mariko Kobayashi,[2] Kazuaki Chayama,[3] Naoyuki Kamatani,[4] Yusuke Nakamura,[5] Yuzo Miyakawa,[6] and Hiromitsu Kumada[1]

Polymorphisms of the inosine triphosphatase (*ITPA*) gene influence anemia during pegylated interferon (PEG-IFN) and ribavirin (RBV) therapy, but their effects during triple therapy with PEG-IFN, RBV, and telaprevir are not known. Triple therapy for 12 weeks, followed by PEG-IFN and RBV for 12 weeks, was given to 49 patients with RBV-sensitive (CC at rs1127354) and 12 with RBV-resistant (CA/AA) *ITPA* genotypes who had been infected with hepatitis C virus (HCV) of genotype 1. Decreases in hemoglobin levels were greater in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$), as well as at the end of treatment (−2.9 ± 1.1 vs. −2.0 ± 0.86, $P = 0.013$). Risk factors for hemoglobin <11.0 g/dL at week 4 were female gender, age >50 years, body mass index (BMI) <23, and CC at rs1127354 by multivariate analysis. RBV dose during the first 12 weeks was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$), but the total RBV dose was no different between them (49 ± 17% and 54 ± 18% of the target, $P = 0.531$). Sustained virological response (SVR) was achieved in 70% and 64% of them, respectively ($P = 0.724$). *Conclusion: ITPA* polymorphism influences hemoglobin levels during triple therapy, particularly during the first 12 weeks while telaprevir is given. With careful monitoring of anemia and prompt adjustment of RBV dose, SVR can be achieved comparably frequently between patients with CC and CA/AA genotypes. (HEPATOLOGY 2011;53:415-421)

Worldwide, 123 million people are estimated to have been infected with hepatitis C virus (HCV),[1] and ≈30% of them develop fatal liver disease such as cirrhosis and hepatocellular carcinoma.[2,3] Currently, the standard of care therapy for patients infected with HCV is pegylated interferon (PEG-IFN) and ribavirin (RBV) for 48 weeks.[4-6] However, the combined treatment can induce a sustained virological response (SVR), judged by the loss of detectable HCV RNA from serum 24 weeks after treatment completion, in at most 50% of patients infected with HCV-1, the genotype most prevalent and least responsive to IFN-based therapies.

Recently, Fellay et al.[7] reported that polymorphisms of the inosine triphosphatase (*ITPA*) gene in chromosome 20 (20p13) influence RBV-induced anemia in a genome-wide association study (GWAS). Single nucleotide polymorphism (SNP) at rs1127354 for proline-to-threonine substitution (P32T) in the second of eight

Abbreviations: BMI, body mass index; GWAS, genome-wide association study; HCV, hepatitis C virus; IFN, interferon; IL28B, *interleukin 28B;* ITPA, *inosine triphosphatase; PEG-IFN, pegylated interferon; RBV, ribavirin; SNP, single nucleotide polymorphism; SVR, sustained virological response.*

From the [1]Department of Hepatology, Toranomon Hospital, Tokyo, Japan; [2]Research Institute for Hepatology, Toranomon Hospital, Tokyo, Japan; [3]Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, Hiroshima, Japan; [4]Laboratory for Statistics, RIKEN Center for Genomic Medicine, Yokohama, Japan; [5]Laboratory of Molecular Medicine, Human Genome Center, Institute of Medical Science, University of Tokyo, Tokyo, Japan; [6]Miyakawa Memorial Research Foundation, Tokyo, Japan.

Received August 12, 2010; accepted October 1, 2010.

Supported in part by grants from the Japanese Ministry of Health, Labour and Welfare.

Address reprint requests to: Fumitaka Suzuki, Department of Hepatology, Toranomon Hospital, 1-3-1, Kajigaya, Takatsu-ku, Kawasaki City 213-8587, Japan. E-mail: fumitakas@toranomon.gr.jp; fax: +81-44-860-1623.

View this article online at wileyonlinelibrary.com.

DOI 10.1002/hep.24058

Potential conflict of interest: Nothing to report.

exons in the *ITPA* gene, as well as that at rs7270101 in the second intron, affects the expression of ITPA.[8-11] Patients infected with HCV-1 carrying the CC genotype at rs1127354 are more prone to develop anemia than those with CA/AA genotypes during the combination therapy, and the decrease in hemoglobin is greater in patients with the AA than AC/CC genotypes at rs7270101.[7] Their observations have been extended to many patients in a large-scale trial with pegIFN-α-2a on Caucasian and African Americans,[12] as well as in the Japanese receiving PEG-IFN-α-2b and RBV who were infected with HCV-1.[13]

For improving SVR in HCV-1 patients, protease inhibitors have been added to the standard treatment with PEG-IFN and RBV, and increased SVR by ≈20%.[14-16] However, such a gain in efficacy is not without trade-offs, represented by aggravation of anemia. Early decreases in hemoglobin levels during the triple therapy reach 4 g/dL, and they exceed ≈3.0 g/dL in the standard treatment.[14,15] Because there have been no reports focusing on the influence of *ITPA* genotypes on anemia developing in patients during triple therapy, hemoglobin levels were followed in 61 Japanese patients with HCV-1 who had received it. The results were correlated with polymorphisms at rs1127354 in the *ITPA* gene because the Japanese are monoallelic at rs7270101 and have the AA genotype exclusively.[11]

Patients and Methods

Study Cohort. This retrospective cohort study was performed in 61 patients with chronic hepatitis C who met the following inclusion and exclusion criteria. Inclusion criteria were: (1) diagnosed with chronic hepatitis C; (2) HCV-1 confirmed by sequence analysis in the NS5B region; (3) HCV RNA levels ≥5.0 log IU/mL determined by the COBAS TaqMan HCV test (Roche Diagnostics K.K. Tokyo, Japan); (4) Japanese aged from 20 to 65 years at the entry; and (5) body weight between ≥40 kg and ≤120 kg at the time of registration. Exclusion criteria were: (1) decompensated liver cirrhosis; (2) hepatitis B surface antigen in serum; (3) hepatocellular carcinoma or its history; (4) autoimmune hepatitis, alcoholic liver disease, hemochromatosis, or chronic liver disease other than chronic hepatitis C; (5) chronic renal disease or creatinine clearance ≤50 mL/min at the baseline; (6) hemoglobin ≤12 g/dL, neutrophil ≤1,500/mm^3 or platelet ≤100,000/mm^3 at baseline.

Of the 61 patients, 44 (72%) had received IFN-based treatment before. Relapse occurred in 29 (47%) and the remaining 15 (25%) did not respond (null-responders). All patients gave consent for analysis of SNPs in *ITPA* and interleukin 28 (*IL28B*) genes. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and was approved by the Ethics Committee of Toranomon Hospital. Written informed consent was obtained from each patient.

Triple Treatment with PEG-IFN-α-2b, RBV, and Telaprevir. Telaprevir (MP-424; Mitsubishi Tanabe Pharma, Osaka, Japan), 750 mg, was administered 3 times a day at an 8-hour (q8) interval after each meal. Pegylated-IFN-α-2b (PEG-Intron, Schering Plough, Kenilworth, NJ) was injected subcutaneously at a median dose of 1.5 μg/kg (range: 1.32-1.71 μg/kg) once a week. RBV (Rebetol, Schering Plough) 200-600 mg was administered after breakfast and dinner. The RBV dose was adjusted by body weight: 600 mg for ≤60 kg; 800 mg for >60 kg ≈≤80 kg; and 1,000 mg for ≥80 kg. The triple therapy with PEG-IFN-α-2b, RBV, and telaprevir was continued for 12 weeks, and then switched to PEG-IFN-α-2b and RBV for an additional 12 weeks. It was withdrawn when hemoglobin levels decreased <8.5 g/dL. After the therapy was completed or discontinued, patients were followed for 24 weeks for SVR.

The RBV dose was cut by 200 mg in patients receiving 600 or 800 mg (by 400 mg in those receiving 1,000 mg) when hemoglobin decreased <12 g/dL, and by another 200 mg when it was below <10 g/dL. In addition, RBV was reduced by 200 mg in patients with hemoglobin <13 g/dL at baseline and those in whom it decreased by 1 g/dL to <13 g/dL within a week. PEG-IFN dose was reduced by one-half when the leukocyte count decreased <1,500/mm^3, neutrophil count <750/mm^3, or platelet count <80 × 10^3/mm^3; PEG-IFN was withdrawn when they decreased <1,000/mm^3, 500/mm^3, or 50 × 10^3/mm^3, respectively.

The triple therapy was withdrawn or stopped temporarily when hemoglobin decreased <8.5 g/dL. In patients in whom hemoglobin increased ≥8.5 g/dL within 2 weeks after the withdrawal, treatment was resumed with PEG-IFN and RBV 200 mg. A reduction of telaprevir (MP-424) dose was not permitted. It was discontinued when severe side effects appeared, whereas PEG-IFN and RBV were continued. Growth factors were not used for elevating hemoglobin levels.

Determination of ITPA Genotypes. *ITPA* (rs1127354) and *IL28B* (rs8099917 and rs12979860) were genotyped by the Invader assay, TaqMan assay, or direct sequencing, as described.[17,18]

Statistical Analyses. Continuous variables between groups were compared by the Mann-Whitney test (*U* test), and discontinuous variables by the chi-square test

Table 1. Baseline Characteristics of the 61 Patients Infected with HCV-1 Who Received Triple Therapy with Pegylated-Interferon, Ribavirin, and Telaprevir

		ITPA Genotypes at rs1127354	
	Total	CC	CA + AA
Demographic data			
Number	61	49	12
Sex (male/female)	34/27	28/21	6/6
Age (years)	56 (23-65)	55 (23-65)	58 (28-62)
Body weight (kg)	61.5 (41.0-92.9)	61.5 (41.0-92.9)	62.1 (44.4-81.1)
Body mass index (kg/m^2)	22.6 (17.6-32.4)	22.2 (17.6-32.4)	22.9 (17.8-26.5)
Genotypes of the *IL28B* gene			
rs8099917 (for 59 patients)			
(TT/TG + GG)	33/26	27/21	6/7
rs12979860 (for 57 patients)			
(CC/CT + TT)	30/27	36/22	4/5
Laboratory data			
Hemoglobin (g/dL)	14.4 (12.5-16.6)	14.4 (12.5-16.6)	14.2 (12.8-16.3)
Platelets (x 10^4/mm^3)	17.8 (9.1-33.8)	17.7 (9.1-33.8)	19.5 (13.1-31.6)
Albumin (g/dL)	3.9 (3.2-4.6)	3.9 (3.2-4.6)	3.9 (3.5-4.1)
Alanine aminotransferase (U/L)	39 (12-175)	41 (12-175)	28 (17-57)
Aspartate aminotransferase (U/L)	32 (15-137)	35 (15-137)	28 (20-35)
HCV RNA (log IU/mL)	6.7 (5.1-7.6)	6.8 (5.7-7.6)	6.6 (5.1-7.5)
HCV genotype 1a/1b	1/60	1/48	0/12
Previous IFN-based treatment			
Treatment naïve	17	12 (24%)	5 (42%)
Relapsed	29	23 (47%)	6 (50%)
Null response	15	14 (29%)	1 (8%)

Data are median values (range) or n.

and Fisher's exact test. Kaplan-Meier analysis and the log-rank test were applied to estimate and compare decreases of RBV dose between groups. Factors evaluated for influence on hemoglobin decrease by univariate analysis were: sex; age; body mass index (BMI); body weight; hemoglobin levels; initial PEG-IFN and RBV doses; amino acid substitutions in the HCV core protein; number of amino acid substitutions in the interferon sensitivity determining region; and *IL28B* polymorphisms (at rs8099917 and rs12979860). Factors associated with a decrease in hemoglobin levels ($P < 0.10$) were assessed by multiple logistic regression analysis, and the odds ratio (OR) with 95% confidence interval (CI) was determined. All analyses were performed using SPSS software (SPSS II v. 11.0, Chicago, IL), and a P-value < 0.05 was considered significant.

Results

Triple Therapy in Patients with HCV-1 Infection. Baseline characteristics of the 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 in the *ITPA* gene are compared in Table 1. They all were infected with HCV-1. There were no significant differences between them, except that alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels were higher in patients with CC than CA/AA genotypes ($P = 0.041$ and $P = 0.008$, respectively). Overall, *IL28B* genotypes resistant to PEG-IFN and RBV, TT/TG at rs8099917, and CC/CT at rs12979860 were rather frequent, and possessed by 44% and 47%, respectively, of the patients. This was due to inclusion of 15 nonresponders to previous IFN-based therapies, corresponding to 25% of the 61 patients studied, most of whom (14/15 [93%]) possessed IFN-resistant genotypes (TT/TG and CC/CT). Six of them had low hemoglobin levels (<13 g/dL) at baseline and were started with an RBV dose decreased by 200 mg; they included five with CC and one with CA genotypes of the *ITPA* gene.

Modification of RBV Dose During Triple Therapy. RBV dose was reduced by ≥200 mg in all 61 patients studied during triple therapy because hemoglobin had decreased <12.0 g/dL in them. During the first 12 weeks of therapy while telaprevir was given, the proportion of patients receiving the full RBV dose differed between those with CC and CA/AA genotypes (Fig. 1). RBV dose reduction was started earlier in the 49 patients with CC than the 12 with CA/AA genotypes (2.6 ± 1.3 vs. 4.8 ± 3.1 weeks after the start, respectively, $P = 0.010$). Thus, during the first 12 weeks with telaprevir the RBV dose was smaller in patients with CC than CA/AA genotypes (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the next 12

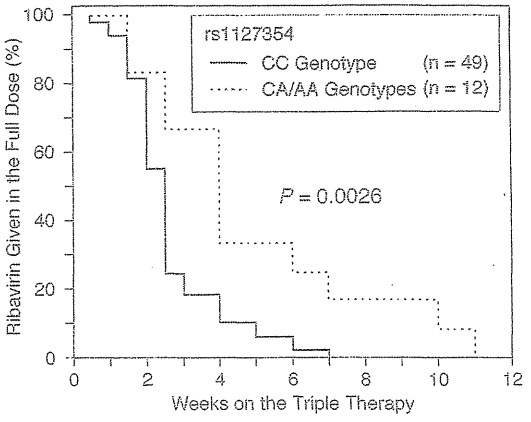

Fig. 1. Patients who received the full ribavirin dose during 12 weeks on triple therapy. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared.

weeks without telaprevir, in contrast, the RBV dose was somewhat larger in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). The total RBV dose during 24 weeks on therapy was comparable between the 49 patients with CC and the 12 with CA/AA genotypes (49 ± 17% vs. 54 ± 18%, $P = 0.531$). In patients with the CC genotype, the RBV dose was no different between those who achieved SVR and those who did not (50 ± 18% vs. 47 ± 13%, $P = 0.728$). The RBV dose did not differ either in patients with CA/AA genotypes with and without SVR (57 ± 17% vs. 48 ± 20%, $P = 0.368$).

The total dose of PEG-IFN was comparable among 49 patients with CC and 12 with CA/AA genotypes (87 ± 23% vs. 86 ± 20% of the target, $P = 0.488$). The total telaprevir dose was no different either between them (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$). Telaprevir was discontinued in 10 of the 49 (20%) patients with CC and 5 of the 12 (42%) with CA/AA genotypes ($P = 0.147$).

Decreases in Hemoglobin Levels During Triple Therapy. Figure 2 compares decreases in hemoglobin levels between 49 patients with CC and 12 with CA/AA genotypes of the *ITPA* gene. Data of six patients were omitted because the triple therapy was withdrawn 4-10 weeks after the start, including five with CC and one with CA genotype. Hemoglobin decreased more in patients with CC than CA/AA genotypes at week 2 (−1.63 ± 0.92 vs. −0.48 ± 0.75 g/dL, $P = 0.001$) and week 4 (−3.5 ± 1.1 vs. −2.2 ± 0.96, $P = 0.001$). During week 8 through 12, hemoglobin reached the nadir of approximately −4 g/dL both in patients with CC and CA/AA genotypes. Thereafter, differences in hemoglobin decrease started to widen between patients with CC and CA/AA genotypes and were significant at week 20 (−3.0 ± 1.2 vs. −2.4 ± 0.88 g/dL, $P = 0.048$) and week 24 (−2.9 ± 1.1 vs. −2.0 ± 0.85 g/dL, $P = 0.013$).

SVR was achieved by 35 (71%) of the 49 patients with CC and 8 (67%) of the 12 with CA/AA genotypes ($P = 0.736$). Hemoglobin levels did not differ between them 24 weeks after the completion of triple therapy (−0.57 ± 1.1 vs. −0.17 ± 0.87 g/dL, $P = 0.271$). Of the 32 patients with TT genotype of the *IL28B* gene at rs8099917, 30 (94%) gained SVR, more frequently than 10 of the 26 (38%) with TG/GG genotypes ($P < 0.001$). Likewise, 29 of the 30 (97%) patients with CC genotype at rs12979860 achieved SVR, more frequently than 11 of the 27 (41%) with CT/TT genotypes ($P < 0.001$).

Factors Influencing Decreases in Hemoglobin Levels. Hemoglobin decreased <11 g/dL at week 4 during the triple therapy in 27 of the 61 (44%) patients. Factors for hemoglobin <11.0 g/dL were female gender, age >50 years, body weight <60 kg, BMI <23, and baseline hemoglobin <15 g/dL, as well as the CC genotype of the *ITPA* gene, in the univariate analysis (Table 2). Of them, female gender, age >50 years, BMI <23, and the CC genotype remained significant in the multivariate analysis. Hemoglobin levels lowered <8.5 g/dL during the triple therapy in 13 of the 61 (21%) patients. Factors for hemoglobin <8.5 g/dL were female gender, age >60 years, body weight <60 kg, BMI <23, and baseline hemoglobin <14 g/dL in the univariate analysis (Table 3). Of

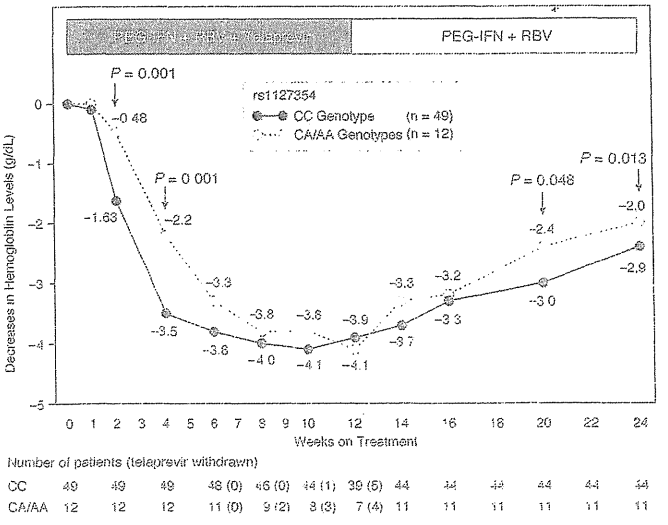

Fig. 2. Decreases in hemoglobin levels during triple therapy with telaprevir, PEG-IFN, and RBV. The 49 patients with CC and the 12 with CA/AA genotypes at rs1127354 are compared. Patients evaluated at each timepoint are indicated below, with the number of patients in whom telaprevir was withdrawn (PEG-IFN and RBV continued) in parentheses.

Table 2. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Low Hemoglobin Levels (< 11.0 g/dL) at Week 4 of Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	14.3 (4.1-50.0)	< 0.001	29.41 (3.8-250.0)	0.001
Age (> 50 years)	4.3 (1.0-17.5)	0.030	7.3 (1.1-47.6)	0.039
Body weight (< 60 kg)	11.5 (3.4-38.2)	< 0.001		
Body mass index (< 23)	8.4 (2.6-27.1)	< 0.001	17.2 (2.6-112.0)	0.003
Hemoglobin (< 15g/dL)	14.2 (3.5-57.4)	< 0.001		
ITPA gene (CC genotype)		0.062	36.8 (2.5-550.2)	0.009

Abbreviations: OR, odds ratio; CI, confidence level.

them, only age and body weight remained significant in the multivariate analysis.

Discussion

Anemia is a substantial risk in the standard of care therapy with PEG-IFN and RBV.[4-6] Triphosphorylated RBV accumulates in erythrocytes of patients who receive RBV, increasingly with RBV dose and duration, and causes oxidative damage to erythrocyte membranes toward extravascular hemolysis by the reticuloendothelial system.[19,20] Inosine triphosphate accumulates also in erythrocytes of individuals who have mutations in the *ITPA* gene, and results in benign red-cell enzymopathy.[8] The expression of *ITPA* is genetically controlled and reduced in individuals who have point mutations in the *ITPA* gene.[8-11] As another achievement of GWAS in hepatology,[21] in the wake of polymorphisms of the *IL28B* gene that influence the response to PEG-IFN and RBV,[22-24] polymorphisms in the *ITPA* gene has been reported to influence anemia caused by RBV.[7] How inosine triphosphate protects erythrocytes from hemolysis caused by RBV needs to be sorted out by *in vivo* and *in vitro* experiments. Inosine triphosphate may prohibit the accumulation of RBV in erythrocytes, or rather, it might act directly toward prohibition of hemolysis.

In the present study, 61 patients infected with HCV-1 received triple therapy with PEG-IFN, RBV, and telaprevir in the first 12 weeks followed by PEG-IFN and RBV in the second 12 weeks. Then the RBV dose and hemoglobin were compared between patients with CC and CA/AA genotypes in the *ITPA* gene. Two polymorphisms in the *ITPA* gene, in close linkage disequilibrium with an r^2 value of 0.65,[7] have been recognized in Caucasians (rs1127354 and rs7270107); the respective CA/AA and AC/CC genotypes decrease the activity of inosine triphosphatase and protect against anemia induced by RBV.[7,12] Because the Japanese are monoallelic at rs7270107 and possess the AA genotype exclusively,[11,25] only polymorphisms at rs1127354 were examined.

Of the 61 patients, 49 possessed the RBV-sensitive CC genotype and the remaining 12 had RBV-resistant CA/AA genotypes. Hemoglobin levels decreased both in patients with CC and CA/AA genotypes. They lowered ≈4 g/dL during weeks 8-12 on the triple therapy with telaprevir, and increased thereafter (Fig. 2). Between the two groups of patients, differences in hemoglobin decrease were greatest at week 4 (1.3 g/dL), as in the standard treatment with PEG-IFN and RBV.[7,12,13]

When anemia and other side effects occurred, doses of RBV, PEG-IFN, and telaprevir were modified. Of the 61 patients studied, 27 (44%) were women and most of them were in old age. Beyond 50 years of age, women are less responsive than men to the standard treatment with PEG-IFN and RBV, probably because estrogens with an antifibrotic potential decrease after menopause.[26] Stringent precautions had to be taken, therefore, by reducing the RBV dose in the patients in whom hemoglobin levels decreased <12 g/dL, rather than the conventional threshold of <10 g/dL.

Reductions of RBV dose due to anemia in patients who receive PEG-IFN and RBV are influenced by *ITPA* polymorphisms.[12] Also, in patients who had received the triple therapy the RBV dose had to be reduced more in

Table 3. Univariate and Multivariate Analyses of Host and Viral Factors Associated with Very Low Hemoglobin Levels (<8.5 g/dL) During Triple Therapy

	Univariate Analysis		Multivariate Analysis	
Parameter	OR (95% CI)	P	OR (95% CI)	P
Sex (female)	6.1 (1.5-25.1)	0.007		
Age (>60 years)	6.8 (1.8-26.0)	0.004	10.1 (1.9-53.9)	0.007
Body weight (<60 kg)	23.8 (2.9-200.0)	<0.001	33.3 (3.4-333.3)	0.003
Body mass index (<23)	14.1 (1.7-125.0)	0.001		
Hemoglobin (<14 g/dL)	4.3 (1.2-15.6)	0.023		

Abbreviations: OR, odds ratio; CI, confidence level.

patients with CC than CA/AA genotypes during the first 12 weeks while they received telaprevir (52 ± 14% vs. 65 ± 21% of the target dose, $P = 0.039$). During the second 12 weeks off telaprevir, the RBV dose was somewhat greater in patients with CC than CA/AA genotypes (47 ± 24% vs. 43 ± 20%, $P = 0.649$). Thus, the total RBV dose during 24 weeks of therapy was comparable between patients with CC and CA/AA genotypes (51 ± 15% and 57 ± 18%, $P = 0.724$). Likewise, the total dose of PEG-IFN (87 ± 23% vs. 86 ± 20% of the target, $P = 0.806$), as well as that of telaprevir (87 ± 27% vs. 71 ± 36% of the target, $P = 0.098$), was no different between patients with CC and CA/AA genotypes. SVR was achieved comparably frequently in them (71% vs. 67%, $P = 0.736$).

Decreases in hemoglobin levels during the first 12 week were similar between the current triple therapy cohort and previous patients receiving PEG-IFN and RBV.[12,13] The conservative hemoglobin levels chosen for RBV dose reduction may be a possible confounding factor on the impact of *ITPA* variants in anemia, which would have been greater should the RBV dose not be reduced in patients with RBV-sensitive CC genotypes.

ITPA polymorphisms at rs1127354 were associated with RBV-induced anemia in Japanese patients, without involvement of those at rs7270107 reported in Caucasian and African-American patients.[13] Thus, *ITPA* polymorphisms at rs1127354 would play a major role in protecting patients from RBV-induced anemia. CC/CA genotypes at rs1127354 occurs in 6% of the Caucasian population, much less often in the Oriental population, at 16%.[25,27] Although AC/CC genotypes at rs7270107 occurs in 13% of Caucasians, they do not exist in Orientals.[11,25] Obviously, different polymorphisms need to be examined in patients of distinct ethnicities when the influence on RBV-induced anemia is to be evaluated.

In confirmation of our previous report,[28] the triple therapy achieved SVR more frequently in patients with CC than CT/TT genotypes of *IL28* at rs12979860 (96% vs. 41%, $P < 0.001$). About two-thirds of studied patients accomplished SVR with the triple treatment, although one-fourth of them were nonresponders to previous IFN-based treatments; they are known to respond poorly to repeated treatments. This would lend further support to the efficacy of triple therapy being higher than treatment with pegylated IFN and RBV.

There are strong points in this study. First, *ITPA* polymorphisms influence RBV-induced anemia in the triple therapy. Second, polymorphisms at rs1127350, without involvement of those at rs7270107, protect against RBV-induced anemia. Third, the triple therapy can be applied with high efficacy by careful monitoring of hemoglobin and prompt modification of RBV dose. There are weak points in this study as well. First, it was a retrospective cohort study conducted in a small size of patients, especially those with CA/AA genotypes at rs1127350, and included null-responders to previous IFN-based therapies; the real impact of *ITPA* polymorphisms on RBV-induced anemia may have been obscured. Second, the study was conducted in Japanese patients, and the results may or may not be extended to patients of different ethnicities with distinct genetic backgrounds. Hopefully, the results presented herein will promote future studies in which the influence of the *ITPA* polymorphism on RBV-induced anemia will be pursued in larger scale and on patients of various ethnicities around the world.

References

1. Shepard CW, Finelli L, Alter MJ. Global epidemiology of hepatitis C virus infection. Lancet Infect Dis 2005;5:558-567.
2. Hoofnagle JH. Course and outcome of hepatitis C. HEPATOLOGY 2002; 36:S21-29.
3. Seeff LB. Natural history of chronic hepatitis C. HEPATOLOGY 2002;36: S35-46.
4. Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales FL Jr, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347:975-982.
5. Manns MP, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, et al. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. Lancet 2001;358:958-965.
6. McHutchison JG, Lawitz EJ, Shiffman ML, Muir AJ, Galler GW, McCone J, et al. Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. N Engl J Med 2009;361:580-593.
7. Fellay J, Thompson AJ, Ge D, Gumbs CE, Urban TJ, Shianna KV, et al. ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. Nature 2010;464:405-408.
8. Arenas M, Duley J, Sumi S, Sanderson J, Marinaki A. The ITPA c.94C>A and g.IVS2+21A>C sequence variants contribute to missplicing of the ITPA gene. Biochim Biophys Acta 2007;1772:96-102.
9. Cao H, Hegele RA. DNA polymorphisms in ITPA including basis of inosine triphosphatase deficiency. J Hum Genet 2002;47:620-622.
10. Stepchenkova EI, Tarakhovskaya ER, Spitler K, Frahm C, Menezes MR, Simone PD, et al. Functional study of the P32T ITPA variant associated with drug sensitivity in humans. J Mol Biol 2009;392: 602-613.
11. Sumi S, Marinaki AM, Arenas M, Fairbanks L, Shobowale-Bakre M, Rees DC, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency. Hum Genet 2002;111:360-367.
12. Thompson AJ, Fellay J, Patel K, Tillmann HL, Naggie S, Ge D, et al. Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. Gastroenterology 2010;139:1181-1189.
13. Ochi H, Mackawa T, Abe H, Hayashida Y, Nakano R, Kubo M, et al. ITPA polymorphism affects ribavirin-induced anemia and outcome of therapy — a genome-wide study of Japanese HCV patients. Gastroenterology 2010;139:1190-1197.
14. Hezode C, Forestier N, Dusheiko G, Ferenci P, Pol S, Goeser T, et al. Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. N Engl J Med 2009;360:1839-1850.
15. McHutchison JG, Everson GT, Gordon SC, Jacobson IM, Sulkowski M, Kauffman R, et al. Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. N Engl J Med 2009;360:1827-1838.

16. Mederacke I, Wedemeyer H, Manns MP. Boceprevir, an NS3 serine protease inhibitor of hepatitis C virus, for the treatment of HCV infection. Curr Opin Investig Drugs 2009;10:181-189.
17. Ohnishi Y, Tanaka T, Ozaki K, Yamada R, Suzuki H, Nakamura Y. A high-throughput SNP typing system for genome-wide association studies. J Hum Genet 2001;46:471-477.
18. Suzuki A, Yamada R, Chang X, Tokuhiro S, Sawada T, Suzuki M, et al. Functional haplotypes of PADI4, encoding citrullinating enzyme peptidylarginine deiminase 4, are associated with rheumatoid arthritis. Nat Genet 2003;34:395-402.
19. De Franceschi L, Fattovich G, Turrini F, Ayi K, Brugnara C, Manzato F, et al. Hemolytic anemia induced by ribavirin therapy in patients with chronic hepatitis C virus infection: role of membrane oxidative damage. HEPATOLOGY 2000;31:997-1004.
20. Russmann S, Grattagliano I, Portincasa P, Palmieri VO, Palasciano G. Ribavirin-induced anemia: mechanisms, risk factors and related targets for future research. Curr Med Chem 2006;13:3351-3357.
21. Karlsen TH, Melum E, Franke A. The utility of genome-wide association studies in hepatology. HEPATOLOGY 2010;51:1833-1842.
22. Ge D, Fellay J, Thompson AJ, Simon JS, Shianna KV, Urban TJ, et al. Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. Nature 2009;461:399-401.
23. Suppiah V, Moldovan M, Ahlenstiel G, Berg T, Weltman M, Abate ML, et al. IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. Nat Genet 2009;41:1100-1104.
24. Tanaka Y, Nishida N, Sugiyama M, Kurosaki M, Matsuura K, Sakamoto N, et al. Genome-wide association of IL28B with response to pegylated interferon-alpha and ribavirin therapy for chronic hepatitis C. Nat Genet 2009;41:1105-1109.
25. Maeda T, Sumi S, Ueta A, Ohkubo Y, Ito T, Marinaki AM, et al. Genetic basis of inosine triphosphate pyrophosphohydrolase deficiency in the Japanese population. Mol Genet Metab 2005;85:271-279.
26. Sezaki H, Suzuki F, Kawamura Y, Yatsuji H, Hosaka T, Akuta N, et al. Poor response to pegylated interferon and ribavirin in older women infected with hepatitis C virus of genotype 1b in high viral loads. Dig Dis Sci 2009;54:1317-1324.
27. Atanasova S, Shipkova M, Svinarov D, Mladenova A, Genova M, Wieland E, et al. Analysis of ITPA phenotype-genotype correlation in the Bulgarian population revealed a novel gene variant in exon 6. Ther Drug Monit 2007;29:6-10.
28. Akuta N, Suzuki F, Hirakawa M, Kawamura Y, Yatsuji H, Sezaki H, et al. 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. HEPATOLOGY 2010;52:421-429.

Gut Online First, published on November 10, 2010 as 10.1136/gut.2010.223495

HCV substitutions and IL28B polymorphisms on outcome of peg-interferon plus ribavirin combination therapy

C Nelson Hayes,[1,2] Mariko Kobayashi,[3] Norio Akuta,[3] Fumitaka Suzuki,[3] Hiromitsu Kumada,[3] Hiromi Abe,[1,2] Daiki Miki,[1,2] Michio Imamura,[1,2] Hidenori Ochi,[1,2] Naoyuki Kamatani,[4] Yusuke Nakamura,[5] Kazuaki Chayama[1,2]

[1]Laboratory for Digestive Diseases, Center for Genomic Medicine, RIKEN, Hiroshima, Japan
[2]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan
[3]Department of Hepatology, Toranomon Hospital, Tokyo, Japan
[4]Center for Genomic Medicine, Riken, Yokohama, Japan
[5]Laboratory of Molecular Medicine, Human Genome Center, The Institute of Medical Science, University of Tokyo, Tokyo, Japan

Correspondence to
Professor Kazuaki Chayama, Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan; chayama@hiroshima-u.ac.jp

Revised 21 September 2010
Accepted 26 September 2010

ABSTRACT

Background and aims A number of recent studies have shown that human polymorphisms near the *IL28B* type III interferon (IFNλ) gene influence the response to peg-interferon plus ribavirin combination therapy for infection with chronic hepatitis C virus (HCV). Viral polymorphisms, including substitutions within the HCV core and NS5A proteins, have also been shown to influence treatment outcome, but it is not known whether these factors act independently of the IL28B polymorphism or if they reflect the same or a different underlying mechanism. Multiple logistic regression was used to determine whether host and viral polymorphisms independently predict sustained virological response (SVR).

Methods Two single nucleotide polymorphisms were genotyped in the IL28B locus (rs12979860 and rs8099917) from 817 patients with chronic HCV infection, and substitutions at amino acids 70 and 91 of the HCV core protein and within the NS5A interferon sensitivity-determining region (ISDR) were analysed.

Results It was found that independent predictors of an SVR included IL28B rs12979860 CC genotype (OR=4.98; p=4.00E-08), core amino acid 70 substitutions (OR=0.53; p=0.016), age and baseline viral load. For non-virological response, the IL28B rs12979860 CT/TT genotype (OR=0.23; p=1.96E-8) and age were independent predictors. IL28B rs12979860 genotype (p=1.4E-8), core amino acid 70 substitutions (p=0.0013), ISDR substitutions (p=0.0019), baseline viral load, γ-glutamyltranspeptidase, alanine aminotransferase and platelet count were independent predictors for change in viral load by week 4 of treatment.

Conclusions IL28B polymorphisms and HCV core amino acid 70 substitutions contribute independently to an SVR to peg-interferon plus ribavirin combination therapy.

Significance of this study

What is already known about this subject?

- Clinical and viral factors influence the outcome of peg-interferon plus ribavirin combination therapy for chronic hepatitis C virus infection.
- Polymorphisms within the human IL28B locus strongly influence treatment outcome.
- Substitutions at amino acids 70 and 91 of the HCV core protein as well as within the interferon sensitivity-determining region (ISDR) also affect response to treatment.

What are the new findings?

- IL28B polymorphisms as well as substitutions at amino acid 70 both independently predict sustained virological response, suggesting that they influence treatment outcome through different mechanisms.
- IL28B polymorphisms, substitutions at core protein amino acid 70 and ISDR substitutions are each independent predictors for change in viral load after 4 weeks of treatment.

How might it impact on clinical practice in the forseeable future?

- The combination of IL28B genotyping and detection of core protein substitutions may yield more accurate pretreatment predictions of treatment efficacy.

INTRODUCTION

Hepatitis C virus (HCV) is a primary cause of chronic hepatitis and often progresses to liver cirrhosis and hepatocellular carcinoma.[1 2] Peg-interferon plus ribavirin combination therapy (PEG-RBV) is the current standard of care, but it is only effective in 50% of patients and has severe side effects often requiring discontinuation or dose modification.[3] Consequently, reliable predictors are needed to identify unsuitable candidates as early as possible.

Genome-wide association studies have reported common single nucleotide polymorphisms (SNPs) predictive of response to interferon treatment. While polymorphisms in MxA,[4 5] interferon α-receptor 1,[6] osteopontin[7] and MAPKAPK3[8] have been reported to be associated with interferon response, several linked SNPs within the IL28B locus on chromosome 19 have recently been shown to be the strongest predictors of early viral kinetics, response to treatment and spontaneous viral clearance.[9–15]

Viral polymorphisms have also been shown to be associated with treatment response. HCV genotypes 1 and 4 in particular are considered more difficult to treat than genotypes 2 and 3,[16 17] and genotype 3 is associated with steatosis.[18] Within genotype 1b, amino acid substitutions at positions 70 and 91 of the HCV core protein and accumulation of substitutions in the interferon sensitivity-determining region (ISDR) of the NS5A protein[19 20] have also been shown to be associated with treatment outcome, especially among Japanese patients.

Consequently, a number of human and viral factors are now known to affect response to treatment, but in order to identify the most important independent predictors and to identify which, if any, may be useful in guiding clinical practice, it is necessary to analyse them simultaneously in a multivariate model. In this study we therefore attempted to identify host and viral factors that independently predict treatment outcome.

MATERIALS AND METHODS

Patients

Data from 817 patients who were treated with PEG-RBV combination therapy for chronic hepatitis C genotype 1b infection between 2002 and 2008 were collected from Toranomon Hospital (Tokyo) and hospitals that belong to the Hiroshima Liver Study Group (http://home.hiroshima-u.ac.jp/naika1/hepatology/english/study.html) in Hiroshima, Japan. Study subjects tested positive for HCV RNA over a span of >6 months, were negative for hepatitis B and HIV, and showed no evidence of other liver diseases. Patients received weekly injections of peg-interferon-α2b at 1.5 g/kg body weight for 48 weeks and ribavirin was administered orally. The amount of ribavirin was adjusted based on body weight (600 mg for <60 kg, 800 mg for 60–80 kg, 1000 mg for >80 kg). Patients with low baseline viral load (<5 log IU/ml) were excluded, as were patients who received <0.89 g/kg of peg-interferon or <8.3 mg/kg of ribavirin. Treatment success was evaluated based on a sustained virological response (SVR), defined as undetectable HCV RNA levels 24 weeks after cessation of treatment. Some patients showed a transient response (TR or relapser), in which HCV RNA dropped to undetectable levels during treatment but then later rebounded. In those with a non-viral response (NVR), HCV RNA levels failed to decline by 2 log10 IU/ml by week 12 of treatment and never dropped below detectable levels. Histopathological diagnosis was made according to the criteria of Desmet *et al.*[21] All subjects gave written informed consent to participate in the study according to the process approved by the ethical committee of each hospital and conforming to the ethical guidelines of the 1975 Declaration of Helsinki.

HCV RNA levels

HCV RNA levels were monitored throughout the course of treatment at 1 or 2 month intervals for a total of at least six time points via reverse transcription–PCR (RT–PCR) using the original Amplicor method, the high range method or the TaqMan RT–PCR test. The measurement ranges of these assays were 0.5–850 kIU/ml, 5–5000 kIU/ml and 1.2–7.8 log IU, respectively. Samples exceeding the measurement range were diluted with phosphate-buffered saline (PBS) and reanalysed. All values were reported as log IU/ml.

ISDR and core amino acid substitutions

Amino acid substitutions in the HCV core and ISDRs were determined by direct sequencing of PCR products following extraction and reverse transcription of serum HCV RNA. Core amino acid substitutions at positions 70 and 91 (core70 and core91) were determined according to Akuta *et al*,[22 23] and the number of ISDR substitutions was established as in Enomoto *et al.*[19 21 24] Of the 817 patients in the study, substitutions for both ISDR and core70 could be determined for 379 patients.

SNP genotyping

We genotyped each patient for two IL28B SNPs previously reported to be associated with treatment outcome, rs12979860 and rs8099917.[9–11] Samples were genotyped using the Illumina HumanHap610-Quad Genotyping BeadChip or the Invader assay, as described previously.[25 26] The two SNPs are in strong linkage disequilibrium, with a correlation coefficient of 0.99. SNP genotypes for both rs12979860 and rs8099917 were determined for 815 patients (99.7%).

Statistical analysis

All analyses were performed using the R statistical package (http://www.r-project.org). Non-parametric tests (χ^2 and Mann–Whitney U tests) were used to detect significant associations. All statistical analyses were two sided, and $p<0.05$ was considered significant. Simple and multiple logistic regression analyses were used to examine the association between viral substitutions and clinical factors using $p<0.05$ as the criterion for inclusion in the initial multivariate model. Multivariate logistic regression analysis was performed using forward/backward stepwise selection based on Akaike Information Criterion (AIC) score and validated using the rms package in R. ORs and 95% CIs were calculated for each factor.

RESULTS

Patient characteristics

Patient profiles are shown in table 1. Forty-five per cent of patients achieved an SVR, 22% were transient responders and 33% failed to respond to treatment (NVR). Males were significantly more likely to achieve an SVR than females (50% and 38%, respectively; p=0.0011), and younger patients were more likely to achieve an SVR than older patients (59.2% and 40.9% above and below median age 58, respectively; p=1.57E-6). Patients who achieved an SVR also had lower γ-glutamyl-transpeptidase (γGTP) levels (36 IU/l vs 45 IU/l; p= 0.008) and higher platelet counts (17.1 vs 15.3×10^{10}/L; p=3.649E-05) than those who did not.

IL28B SNP genotypes

The genotypes of two IL28B SNPs were measured for each patient. Because of linkage disequilibrium, SNP results are nearly interchangeable. However, six patients showed an intermediate haplotype consisting of the favourable genotype for rs8099917 (TT) but an unfavourable genotype for rs12979860 (CT), whereas only one of the six patients achieved an SVR, suggesting that rs12979860 is a better predictor of SVR in this data set.

The frequency of the risk allele (T) for rs12979860 was 0.15 among all patients and 0.08 in SVR patients, 0.14 in TR patients and 0.27 in NVR patients. Patients homozygous for the rs12979860 favourable allele (CC) were significantly more likely to achieve an SVR compared with those with TC or TT genotypes (53% vs 24%, OR=3.55, p=3.95E-13). Conversely, patients with the risk allele (TC or TT) were significantly more likely to show an NVR (55% vs 25%; OR=0.265; p=4.4E-16). Patients with the rs12979860 CC genotype had a marginally lower baseline viral load (6.6 vs 6.4 log IU/ml; p=0.093), but showed significantly greater reduction in viral load by week 4 of treatment (−3.2 vs −0.8 log IU/ml; p<2.2E-16). The rs12979860 CC genotype was also associated with wild type core70 (78% vs 54%; p=1.6E-6) and non-wild type ISDR (67% vs 83%; p=0.007).

The frequency of the rs8099917 risk allele (G) was 0.15 among all patients, 0.08 in SVR patients, 0.13 in TR patients and 0.26 in NVR patients. Patients with the rs8099917 TT genotype were significantly more likely to achieve an SVR than patients with GT or GG genotypes (53% vs 24%, OR=3.43, p=2.18E-12), and GT/GG patients were significantly more likely to show an NVR

Table 1 Patient profiles by response to treatment

	All (813)	SVR (366)	TR (176)	NVR (271)
Sex (M/F)	459/354	231/135	84/92	144/127
Age	58 (51–65)	56 (47–63)	60.5 (56–65.25)	59 (52.5–66)
Body weight (kg)	59 (52–67)	60 (52–68.25)	58 (51–66)	60 (52–66.4)
BMI (kg/m^2)	22.61 (20.81–24.65)	22.44 (20.46–24.58)	22.85 (20.85–24.89)	22.76 (21.12–24.63)
Hypertension (yes/no)	141/672	61/305	29/147	51/220
Diabetes (yes/no)	97/716	31/335	25/151	41/230
Fibrosis (0–2/3–4)	138/421	52/227	34/81	52/113
Activity (0–1/2–3)	274/272	136/138	53/56	85/78
ISDR (0, 1/≥2)	78/298	43/128	15/71	20/99
Amino acid 70 (wild-type/mutant)	256/139	137/45	54/35	65/59
Amino acid 91 (wild-type/mutant)	221/178	112/72	51/40	58/66
WBC (/L)	4.71×10^9 (3.9×10^9–5.7×10^9)	4.9×10^9 (4.0×10^9–6.0×10^9)	4.6×10^9 (3.8×10^9–5.4×10^9)	4.6×10^9 (3.7×10^9–5.5×10^9)
Haemoglobin (g/dl)	14.1 (13.2–15)	14.2 (13.3–15.22)	13.9 (13.1–14.8)	14.1 (13.05–14.9)
Platelets ($\times10^4$L)	16.1×10^6 (12.5×10^6–19.9×10^6)	17.1×10^6 (13.7×10^6–20.7×10^6)	15.5×10^6 (11.3×10^6–18.8×10^6)	15.1×10^6 (12×10^6–19.2×10^6)
AST (IU/l)	45 (34–65.5)	43 (32.25–64)	43.5 (33.25–66)	48 (37–66.5)
ALT (IU/l)	55 (37–87)	57 (37–92)	50 (33–78)	53 (39–82.5)
γGTP (IU/l)	40 (25–72)	36 (23–65.75)	36 (23–69)	52 (32–86)a
Albumin (g/dl)	3.9 (3.7–4.1)	3.9 (3.7–4.1)	3.8 (3.7–4)	3.8 (3.7–4.1)
Total cholesterol (mg/dl)	171 (150–192)	169 (149.2–192)	175 (158–191)	170 (148.5–192.5)
Viral load (log IU/ml)	6.5 (6.1–6.9)	6.4 (5.9–6.825)	6.6 (6.3–7)	6.6 (6.2–7)
PEG-IFN-α2b (μg)	80 (80–100)	80 (80–100)	80 (75–100)	80 (60–100)
PEG-IFN-α2b/kg (μg/kg)	1.19 (1.19–1.48)	1.36 (1.19–1.48)	1.19 (1.19–1.48)	1.19 (1.02–1.48)
Ribavirin (mg)	600 (600–800)	600 (600–800)	600 (600–800)	600 (400–800)
Ribavirin/kg (mg/kg)	8.9 (8.9–11.87)	10.29 (8.9–11.87)	8.9 (8.9–11.87)	8.9 (7.8–11.86)
rs12979860 (CC/CT/TT)	582/203/27	311/51/4	128/43/4	143/109/19
rs8099917 (TT/TG/GG)	588/199/25	311/51/3	132/40/4	145/108/18

For categorical data, the number of patients in each category is shown. For continuous data, the median and range are displayed.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; F, female; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; M, male; NVR, non-virological response; PEG-IFN, pegylated interferon; SVR, sustained virological response; TR, transient response; WBC, white blood cells.

(56% vs 25%; OR=0.26; p=3.33E-16). Patients with the rs8099917 TT genotype had marginally higher baseline viral load (6.6 vs 6.4 log IU/ml; p=0.077) but showed a significantly greater drop in viral load by week 4 of treatment (−3.1 vs −0.8 log IU/ml; p<2.2E-16). The rs8099917 TT genotype was also associated with wild-type core70 (79% vs 56%; p= 3.1E-6) and non-wild-type ISDR (68% vs 83%; p=0.015).

Viral substitutions

Patients who achieved an SVR had significantly lower initial HCV RNA levels than those who did not (6.4 vs 6.6 log IU/ml; p=2.1E-6). The 140 patients (17%) with a substitution at position 70 of the HCV core protein (core70) were significantly less likely to achieve an SVR than patients with wild type core70 (33% vs 53%; p=0.00019) and were significantly more likely to show an NVR (42% vs 25%; p=0.0013). The 179 (22%) of patients with a substitution at position 91 (core91) were marginally less likely to achieve an SVR (41% vs 50%; p=0.08) but were significantly more likely to show an NVR (37% vs 27%; p=0.039). The 78 (10%) of patients who had two or more substitutions in the ISDR of NS5A were only marginally less likely to achieve an SVR than those with wild-type ISDR (43% vs 55%; p=0.066) and were not more likely to show an NVR (33% vs 26%; p=0.24).

Predictive factors for an SVR

Significant univariate predictors for an SVR included patient clinical factors (age, sex, diabetes, platelet count, white blood cell count, haemoglobin level, γGTP level); SNP genotype (rs12979860 and rs8099917); and viral factors (baseline viral load and core70, core91 and ISDR substitutions) (table 2). Following multivariate analysis, only age, rs12979860 genotype, core70 substitution and baseline viral load were significant independent predictors (figure 1A). The joint effects of rs12979860 and core70 on response to treatments are illustrated in figure 2.

Predictive factors for an NVR

Significant univariate predictors for an NVR included age, rs12979860 and rs8099917 genotypes, core70 and core91 substitutions, diabetes, aspartate aminotransferase (AST), baseline viral load, platelet count, white blood cell count and γGTP levels (table 3). Following multivariate analysis only age and rs12979860 genotype remained as independent predictors (figure 1B).

Predictive factors for change in viral load by week 4 of treatment

Factors influencing virological response were assessed by examining change in viral load between the start of treatment and week 4. Using linear regression, sex, rs12979860, rs8099917, core70, core91, ISDR, baseline viral load, alanine aminotransferase (ALT), platelet count, white blood cell count, haemoglobin level and γGTP were found to be significant univariate predictors of change in viral load by week 4 (table 4). Independent factors included rs12979860, core70, ISDR, ALT, platelet count and γGTP. We also found a significant positive linear relationship between the total number of ISDR substitutions and change in viral load between week 0 and week 4 (slope=0.2; p=0.0047).

In patients with the favourable rs12979860 CC genotype, core70 wild type was a significant predictor of viral decline (p=0.007; figures 3A,B), but in patients with the CT or TT genotypes, viral decline did not vary with respect to core70 substitutions (p=0.18; figures 3C,D). Conversely, ISDR was not

Table 2 Predictors for a sustained virological response

	Simple			Multiple			
Variable	n	OR	p Value	n	OR	95% CI	p Value
Age	813	0.58	1.22E-08***	362	0.432	0.31 to 0.60	6.61E-07***
Sex (male vs female)	813	1.28	0.0006***	362	1.2	0.95 to 1.54	0.133
BMI (kg/m^2)	800	0.87	0.1286				
rs12979860 (CC vs TC/TT)	812	3.65	2.67E-14***	362	4.98	2.81 to 8.82	4.00E-08***
rs8099917 (TT vs GT/GG)	812	3.53	1.77E-13***				
Hypertension	813	0.92	0.6452				
Diabetes	813	0.53	0.005907**				
Core amino acid 70 (wild type vs mutant)	395	0.42	5.82E-05***	362	0.527	0.31 to 0.89	0.01575*
Core amino acid 91 (wild type vs mutant)	399	0.66	0.0419*				
ISDR	376	1.12	0.1627				
Viral load (log IU/ml)	695	0.68	2.09E-06***	362	0.77	0.62 to 0.96	0.02249*
Fibrosis (F0–1 vs F2–4)	559	0.74	0.0817				
Activity (A0–1 vs A2–4)	546	0.96	0.7975				
Total cholesterol (mg/dl)	663	0.86	0.2151				
AST (IU/l)	687	1.03	0.1069				
ALT (IU/l)	692	1.26	0.0920				
Platelets (×10^4/L)	694	1.49	3.57E-05***	362	1.39	0.97 to 1.99	0.073
WBC (/L)	693	1.31	0.0014**				
Haemoglobin (g/dl)	693	1.28	0.0043**				
γGTP (IU/l)	646	0.96	0.0052**				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.

a significant predictor of viral decline in patients with the rs12979860 CC genotype (p=0.078; figures 4A,B), but patients with the CT or TT genotypes and two or more substitutions in the ISDR showed significantly greater viral decline by week 4 than patients with zero or one ISDR substitution (p=0.007; figures 4C,D).

DISCUSSION

In this study we showed that host factors (younger age, male sex, favourable IL28B SNP genotypes) as well as viral factors (baseline viral load, wild-type core70 and two or more substitutions in the ISDR) contribute to the successful outcome of PEG-RBV combination therapy. Although some of these factors independently predict an SVR or NVR in multivariate analysis, collectively they reflect a complex genotype-by-environment interaction involving common polymorphisms in both the virus and the human host.

Genetic variation within the human IL28 locus has been reported as the strongest pretreatment predictor of an SVR,[15] and the results of this study support this finding. Several tightly linked SNPs in the non-coding region of *IL28A* and *IL28B* have been shown to be associated with spontaneous viral clearance, rapid and early virological response and/or SVR following treatment with interferon and ribavirin for HCV genotype 1b.[9–15] *IL28A*, *IL28B* and *IL29* code for type III (λ) interferons, which are similar to type I interferons but use a different receptor and show high tissue specificity.[27 28] It has not been determined which, if any, of the reported SNPs directly affects function, but the functional SNP probably affects gene expression. IRF3- and IRF7-binding sites near the transcription start

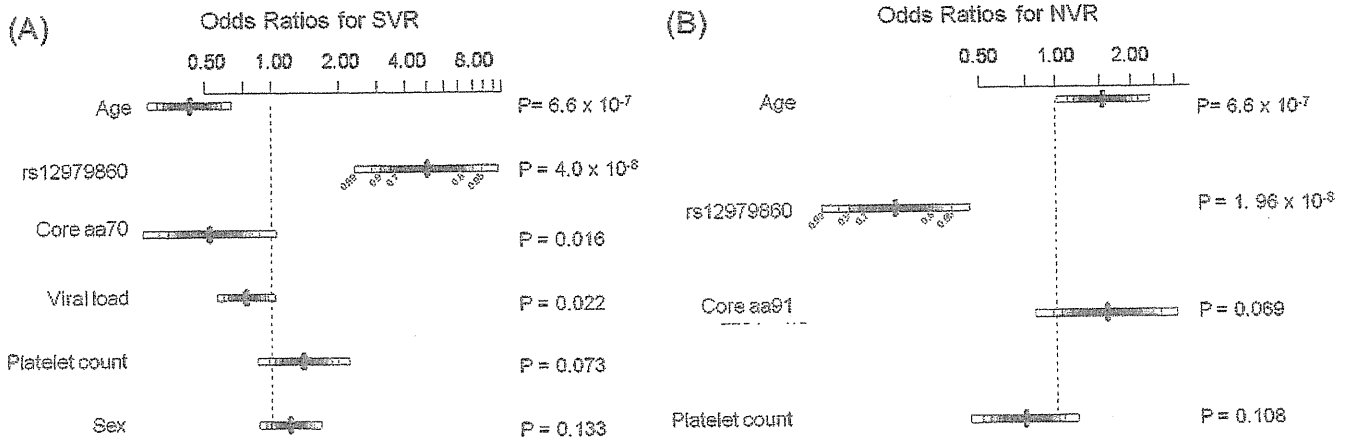

Figure 1 ORs for predictive factors response to treatment. ORs and 95% CIs are shown for predictive factors for (A) sustained virological response (SVR) and (B) non-virological response (NVR) based on multiple logistic regression with stepwise selection.

Hayes CN, Kobayashi M, Akuta N, *et al*. *Gut* (2010). doi:10.1136/gut.2010.223495

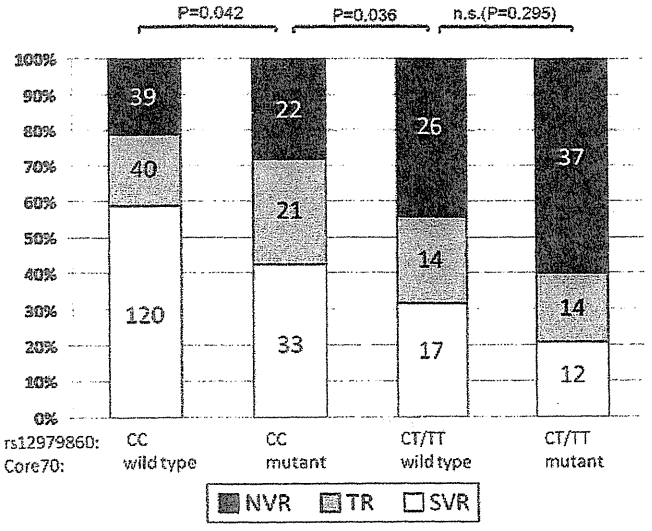

Figure 2 Cumulative effects of rs12979860 genotype and core protein amino acid 70 substitutions. The relative effects of rs12979860 genotype (favourable CC vs non-favourable CT/CC) and core amino acid 70 substitutions (favourable wild type vs unfavourable substitutions) on response to treatment are shown. NVR, non-virological response; TR, transient response/relapser; SVR, sustained virological response.

site of *IL28B* are essential for gene expression, but distal clusters of nuclear factor-κB (NF-κB)-binding sites are necessary for maximal expression,[29 30] suggesting that upstream polymorphisms may potentially disrupt transcription factor-binding sites within a distal promoter or enhancer. Unintuitively, interferon-stimulated genes are downregulated in patients with the favourable rs8099917 TT genotype,[31] implying that responders have a lower baseline expression of immune response genes.[32] This might serve to prevent desensitisation and promote maximal induction of interferon-stimulated genes, but detailed gene regulation studies are needed to resolve the role of IL28B polymorphisms in antiviral defence.

In addition to effects of human genetic polymorphisms, a number of studies have reported significant association between HCV core70/core91 substitutions and treatment outcome.[20 33 34] We found significant independent associations between core70 substitutions and an SVR, as well as change in viral load by week 4, but the association was not significant for an NVR under multivariate analysis despite being highly significant in univariate analysis. Although the role of core70 substitutions is unclear, the core protein interacts with a number of viral and host proteins and disrupts the interferon signalling pathway.[35–37] The proportion of core70 substitutions in the host viral population has been reported to increase during treatment with PEG-RBV therapy, which may indicate positive selection at this position in response to treatment.[38] Substitutions at these positions appear to affect the antiviral response during the early stages of treatment, as wild-type core70 and core91 are associated with a rapid decrease in HCV RNA levels during the first 4 weeks of treatment.[39 40] Because a rapid virological response is also a strong predictor of SVR and NVR, core70 and core91 substitutions may affect treatment outcome either directly or indirectly.[40 41]

Unlike HCV core70 substitutions, we found only a marginal association between ISDR substitutions and SVR, and no association with NVR. However, ISDR substitution was a significant independent predictor of change in viral load by week 4. The presence of two or more mutations in this 40 amino acid stretch of the NS5A protein is associated with an SVR.[24 42] Other studies have found no significant association between ISDR and SVR but have found a higher overall mutation rate in the NS5A protein among SVR patients,[43 44] and one study suggests that the association with ISDR varies by strain and is more pronounced in Japan than in Europe.[45] It is not clear whether mutations in ISDR directly affect function or whether they reflect the genetic distance from an interferon-resistant

Table 3 Predictors for a non-virological response

	Simple			Multiple			
Variable	n	OR	p Value	n	OR	95% CI	p Value
Age	813	1.30	0.01306*	370	1.55	1.12 to 2.15	0.008367**
Sex (male vs female)	813	0.90	0.178				
BMI (kg/m^2)	800	1.07	0.3899				
rs12979860 (CC vs TC/TT)	812	0.26	2.73E-17***	370	0.231	0.14 to 0.39	1.96E-08***
rs8099917 (TT vs GT/GG)	812	0.26	1.51E-17***				
Hypertension	813	1.16	0.4323				
Diabetes	813	1.55	0.04685*				
Core amino acid 70 (wild type vs mutant)	395	2.17	0.000496***				
Core amino acid 91 (wild type vs mutant)	399	1.66	0.02029*	370	1.58	0.96 to 2.60	0.06943
ISDR	376	0.92	0.06197				
Viral load (log IU/ml)	695	1.32	0.01716*				
Fibrosis (F0–1 vs F2–4)	559	1.24	0.2608				
Activity (A0–1 vs A2–4)	546	1.12	0.5499				
Total cholesterol (mg/dl)	663	0.98	0.5824				
AST (IU/l)	687	1.02	0.03148*				
ALT (IU/l)	692	0.91	0.8772				
Platelets (×10^4/L)	694	0.76	0.008222**	370	0.739	0.51 to 1.07	0.1077
WBC (/L)	693	0.83	0.04617*				
Haemoglobin (g/dl)	693	0.84	0.1201				
γGTP (IU/l)	646	1.15	1.23E-05***				

Results of simple and multiple regression are shown. Factors with a p value <0.05 were included in the multivariate model. Variables were selected using stepwise selection. Asterisks indicate level of statistical significance: *<0.05; **<0.01; ***<0.001.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; γGTP, γ-glutamyltranspeptidase; ISDR, interferon sensitivity-determining region; WBC, white blood cells.