Research Article

Table 2. Factors associated with NVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.98	0.67-1.45	0.938	1.29	0.75-2.23	0.363
Age	1.01	0.97-1.01	0.223	0.99	0.97-1.02	0.679
ALT	1.00	1.00-1.00	0.867	1.00	0.99-1.00	0.580
GGT	1.004	1.00-1.01	0.029	1.00	1.00-1.00	0.715
Platelets	0.95	0.91-0.99	0.009	0.92	0.87-0.98	0.006
Fibrosis: F3-4	2.23	1.46-3.42	0.0002	1.97	1.09-3.57	0.025
HCV-RNA: ≥600,000 IU/ml	1.83	1.05-3.19	0.035	2.49	1.17-5.29	0.018
ISDR mutation: ≤1	2.14	1.08-4.22	0.030	0.96	0.78-1.18	0.707
Core 70 (Gln/His)	3.23	2.16-4.78	<0.0001	1.41	0.83-2.42	0.207
Core 91 (Met)	1.39	0.95-2.06	0.093	1.21	0.72-2.04	0.462
IL28B: Minor allele	19.24	11.87-31.18	<0.0001	20.83	11.63-37.04	<0.0001

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Gln, glutamine; His, histidine; Met, methionine; Minor allele, heterozygote or homozygote of minor allele.

Table 3. Factors associated with SVR analyzed by univariate and multivariate logistic regression analysis.

	Univariate			Multivariate		
	Odds ratio	95%CI	*p* value	Odds ratio	95%CI	*p* value
Gender: female	0.81	0.56-1.16	0.253	0.86	0.55-1.35	0.508
Age	0.97	0.95-0.99	0.0003	0.99	0.96-1.01	0.199
ALT	1.00	1.00-1.00	0.337	1.00	1.00-1.01	0.108
GGT	1.00	1.00-1.00	0.273	1.00	1.00-1.00	0.797
Platelets	1.12	1.01-116	<0.0001	1.13	1.08-1.19	<0.0001
Fibrosis: F0-2	2.64	1.65-4.22	<0.0001	1.87	1.07-3.28	0.029
HCV-RNA: <600,000 IU/ml	2.49	1.55-3.98	0.0001	2.75	1.55-4.90	0.001
ISDR mutation: 2≤	3.78	2.14-6.68	<0.0001	2.11	1.06-4.18	0.033
Core 70 (Arg)	1.61	1.11-2.28	0.012	0.84	0.52-1.35	0.470
Core 91 (Leu)	1.28	0.88-1.85	0.185	1.26	0.81-1.96	0.300
IL28B: Major allele	6.21	3.75-10.31	<0.0001	7.41	4.05-13.57	<0.0001

ALT, alanine aminotransferase; GGT, Gamma-glutamyltransferase; ISDR, interferon sensitivity determining region; Arg, arginine; Leu, leucine; Major allele, homozygote of major allele.

Among baseline factors, IL28B was the most significant predictor of NVR and SVR. Moreover, the IL28B allele type was also correlated with early virological response: the rate of RVR and cEVR was significantly high for the IL28B major allele compared to the IL28B minor allele: 9% vs. 3% for RVR and 57% vs. 11% for cEVR (Fig. 2). On the other hand, the relapse rate was not different between the IL28B genotypes within patients who achieved RVR or cEVR (Fig. 3). We believe that optimal therapy should be based on baseline features and a response-guided approach. Our findings suggest that the IL28B genotype is a useful baseline predictor of virological response which should be used for selecting the treatment regimen: whether to treat patients with PEG-IFN and RBV or to wait for more effective future therapy including direct acting antiviral drugs. On the other hand, baseline IL28B genotype might not be suitable for determining the treatment duration in patients who started PEG-IFN/RBV therapy and whose virological response is determined because the IL28B genotype is not useful for the prediction of relapse. The duration of therapy should be personalized based on the virological response. Future studies need to explore whether the combination of baseline IL28B genotype and response-guided approach further improves the optimization of treatment duration.

The SVR rate in patients having the IL28B minor allele was 14% in the present study while it was 23% in Caucasians and 9% in African Americans in a study by McCarthy et al. [33]. On the other hand, the SVR rate in patients having the IL28B minor allele was 28% in genotypes 1/4 compared to 80% in genotypes 2/3 in a study by Rauch et al. [9]. These data imply that the impact of the IL28B polymorphism on response to therapy may be different in terms of race, geographical areas, or HCV genotypes, and that our data need to be validated in future studies including different populations and geographical areas before generalization.

Please cite this article in press as: Kurosaki M et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol (2010), doi:10.1016/j.jhep.2010.07.037

Table 4. Factors associated with IL28B genotype.

	IL28B major allele n = 345	*IL28B* minor allele n = 151	*p* value
Gender: male	166 (48%)	84 (56%)	0.143
Age (years)	57 ± 10	57 ± 10	0.585
ALT (IU/L)	79 ± 60	78 ± 62	0.842
Platelets (10^9/L)	153 ± 54	155 ± 52	0.761
GGT (IU/L)	51 ± 45	78 ± 91	0.001
Fibrosis: F3-4	76 (22%)	45 (30%)	0.063
Steatosis:			
>10%	16/88 (18%)	13/23 (57%)	0.024
>30%	6/88 (7%)	6/23 (26%)	0.017
HCV-RNA: >600,000 IU/ml	284 (82%)	125 (83%)	1.000

ALT, alanine aminotransferase; GGT, gamma-glutamyltransferase.

Four GWAS studies have shown the association between a genetic polymorphism near the IL28B gene and response to PEG-IFN plus RBV therapy. The SNPs that showed significant association with response were rs12979860 [8] and rs8099917 [6,7,9]. There is a strong linkage-disequilibrium (LD) between these two SNPs as well as several other SNPs near the IL28B gene in Japanese patients [34] but the degree of LD was weaker in Caucasians and Hispanics [8]. Thus, the combination of SNPs is not useful for predicting response in Japanese patients but may improve the predictive value in patients other than Japanese who have weaker LD between SNPs.

Other significant predictors of response independent of IL28B genotype were platelet counts, stage of fibrosis, and HCV RVA load. A previous study reported that platelet count is a predictor of response to therapy [35], and the lower platelet count was related with advanced liver fibrosis in the present study. The association between response to therapy and advanced fibrosis independent of the IL28B polymorphism is consistent with a recent study by Rauch et al. [9].

There is agreement that the viral genotype is significantly associated with the treatment outcome. Moreover, viral factors such as substitutions in the ISDR of the NS5A region [10] or in the amino acid sequence of the HCV core [4] have been studied in relation to the response to IFN treatment. The amino acid Gln or His at Core70 and Met at Core91 are repeatedly reported to be associated with resistance to therapy [4,14,15] in Japanese patients but these data wait to be validated in different populations or other geographical areas. In this study, we confirmed that patients with two or more mutations in the ISDR had a higher rate of undetectable HCV-RNA at each time point during therapy. In addition, the rate of relapse among patients who achieved cEVR was significantly lower in patients with two or more mutations in ISDR compared to those with only one or no mutations (15% vs. 31%, $p < 0.05$). Thus, the ISDR sequence may be used to predict a relapse among patients who achieved virological response during therapy, while the IL28B polymorphism may be used to predict the virological response before therapy. A higher number of mutations in the ISDR are reported to have close association with SVR in Japanese [11–13,15,36] or Asian [37,38] populations but data from Western countries have been controversial [39–42]. A meta-analysis of 1230 patients including 525 patients from Europe has shown that there was a positive correlation between the SVR and the number of mutations in the ISDR in Japanese as well as in European patients [43] but this correlation was more pronounced in Japanese patients. Thus, geographical factors may account for the different impact of ISDR on treatment response, which may be a potential limitation of our study.

To our surprise, these HCV sequences were associated with the IL28B genotype: HCV sequences with an IFN resistant phenotype were more prevalent in patients with the minor *IL28B* allele than those with the major allele. This was an unexpected finding, as we initially thought that host genetics and viral sequences were completely independent. A recent study reported that the IL28B polymorphism (rs12979860) was significantly associated with HCV genotype: the IL28B minor allele was more frequent in HCV genotype 1-infected patients compared to patients infected with HCV genotype 2 or 3 [33]. Again, patients with the IL28B minor allele (IFN resistant genotype) were infected with HCV sequences that are linked to an IFN resistant phenotype. The mechanism for this association is unclear, but may be related to an interaction between the IL28B genotype and HCV sequences in the development of chronic HCV infection as discussed by McCarthy et al., since the *IL28B* polymorphism was associated with the natural clearance of HCV [44]. Alternatively, the HCV sequence within the patient may be selected during the course of chronic infection [45,46]. These hypotheses should be explored through prospective studies of spontaneous HCV clearance or by testing the time-dependent changes in the HCV sequence during the course of chronic infection.

How these host and viral factors can be integrated to predict the response to therapy in future clinical practice is an important question. Because various host and viral factors interact in the same patient, predictive analysis should consider these factors in combination. Using the data mining analysis, we constructed a simple decision tree model for the pre-treatment prediction of SVR and NVR to PEG-IFN/RBV therapy. The classification of patients based on the genetic polymorphism of *IL28B*, mutation in the ISDR, serum levels of HCV-RNA, and platelet counts, identified subgroups of patients who have the lowest probabilities of NVR (0%) with the highest probabilities of SVR (90%) as well as those who have the highest probabilities of NVR (84%) with the lowest probability of SVR (7%). The reproducibility of the model was confirmed by the independent validation based on a second

Please cite this article in press as: Kurosaki M et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol (2010), doi:10.1016/j.jhep.2010.07.037

Research Article

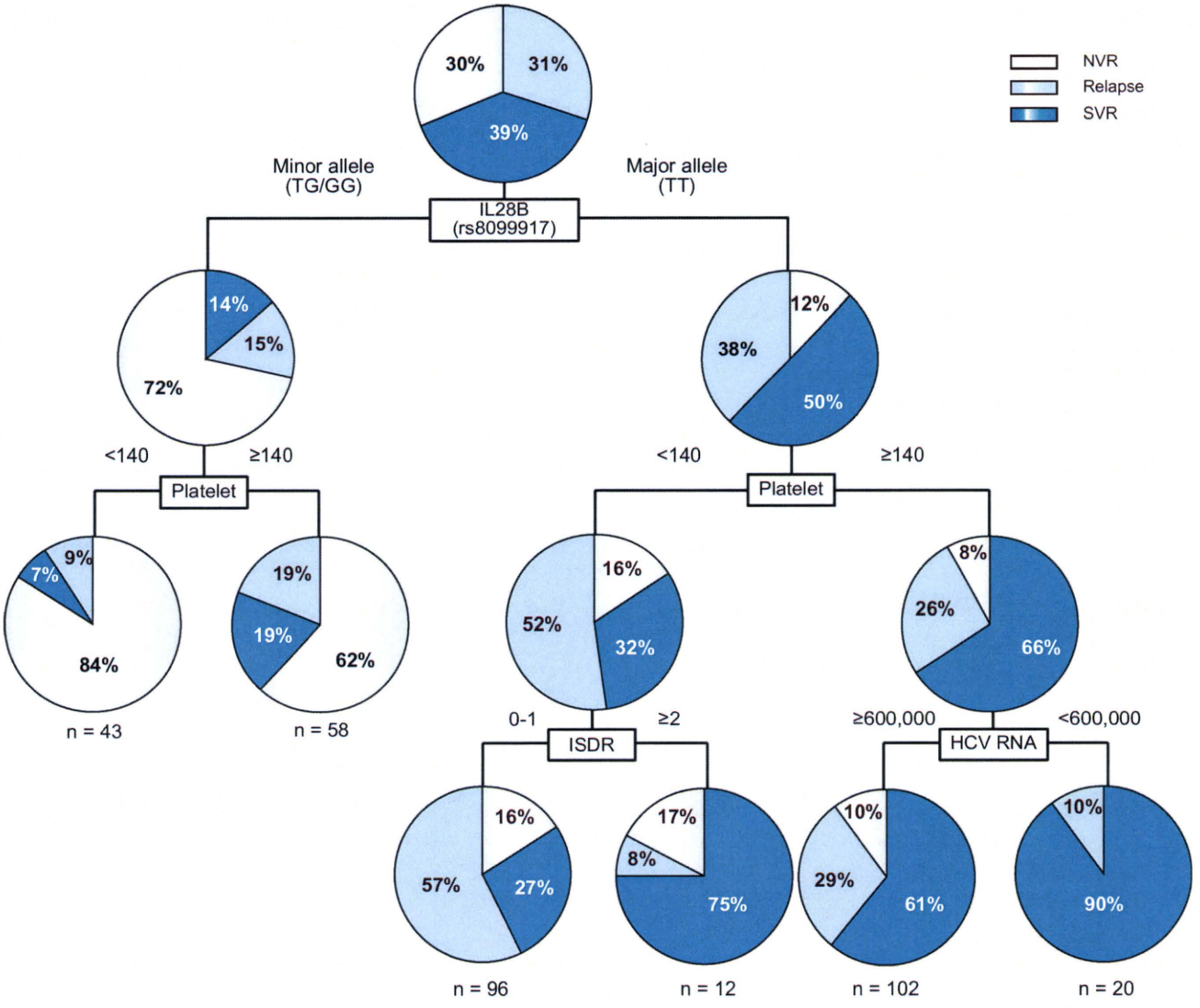

Fig. 5. Decision tree for the prediction of response to therapy. The boxes indicate the factors used for splitting. Pie charts indicate the rate of response for each group of patients after splitting. The rate of null virological response, relapse, and sustained virological response is shown. [This figure appears in colour on the web.]

group of patients. Using this model, we can rapidly develop an estimate of the response before treatment, by simply allocating patients to subgroups by following the flow-chart form, which may facilitate clinical decision making. This is in contrast to the calculating formula, which was constructed by the traditional logistic regression model. This was not widely used in clinical practice as it is abstruse and inconvenient. These results support the evidence based approach of selecting the optimum treatment strategy for individual patients, such as treating patients with a low probability of NVR with current PEG-IFN/RBV combination therapy or advising those with a high probability of NVR to wait for more effective future therapies. Patients with a high probability of relapse may be treated for a longer duration to avoid a relapse. Decisions may be based on the possibility of a response against a potential risk of adverse events and the cost of the therapy, or disease progression while waiting for future therapy.

We have previously reported the predictive model of early virological response to PEG-IFN and RBV in chronic hepatitis C [26]. The top factor selected as significant was the grade of steatosis, followed by serum level of LDL cholesterol, age, GGT, and blood sugar. The mechanism of association between these factors and treatment response was not clear at that time. To our interest, a recent study by Li et al. [47] has shown that high serum level of LDL cholesterol was linked to the IL28B major allele (CC in rs12979860). High serum level of LDL cholesterol was associated with SVR but it was no longer significant when analyzed together with the IL28B genotype in multivariate analysis. Thus, the association between treatment response and LDL cholesterol levels may reflect the underlining link of LDL cholesterol levels to IL28B genotype. Steatosis is reported to be correlated with low lipid levels [48] which suggest that IL28B genotypes may be also associated with steatosis. In fact, there were significant correlations between the IL28B genotype and the presence of steatosis in the present study (Table 4). In addition, the serum level of GGT, another predictive factor in our previous study, was significantly associated with IL28B genotype in the present study

Please cite this article in press as: Kurosaki M et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol (2010), doi:10.1016/j.jhep.2010.07.037

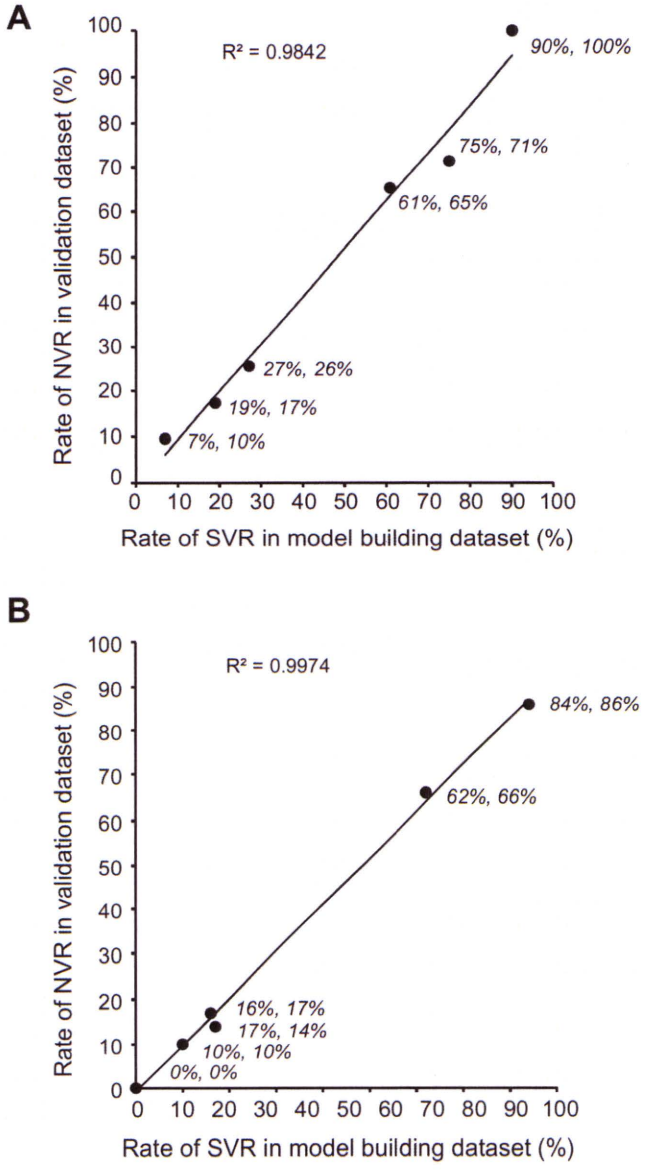

Fig. 6. Validation of the CART analysis. Each patient in the validation group was allocated to one of the six subgroups by following the flow-chart form of the decision tree. The rate of (A) sustained virological response (SVR) and (B) null virological response (NVR) in each subgroup was calculated and plotted. The *X*-axis represents the rate of SVR or NVR in the model building patients and the *Y*-axis represents those in the validation patients. The rate of SVR and NVR in each subgroup of patients is closely correlated between the model building and the validation patients (correlation coefficient: $r^2 = 0.98–0.99$).

(Table 4). The serum level of GGT was significantly associated with NVR when examined independently but was no longer significant when analyzed together with the IL28B genotype. These observations indicate that some of the factors that we have previously identified may be associated with virological response to therapy through the underlining link to the IL28B genotype.

In conclusion, the present study highlighted the impact of the *IL28B* polymorphism and mutation in the ISDR on the pre-treatment prediction of response to PEG-IFN/RBV therapy. A decision model including these host and viral factors has the potential to support selection of the optimum treatment strategy for individual patients, which may enable personalized treatment.

Conflicts of interest

The authors who have taken part in this study declare that they do not have anything to disclose regarding funding or conflict of interest with respect to this manuscript.

Financial support

This study was supported by a grant-in-aid from the Ministry of Health, Labor and Welfare, Japan, (H19-kannen-013), (H20-kannen-006).

References

[1] Ray Kim W. Global epidemiology and burden of hepatitis C. Microbes Infect 2002;4 (12):1219–1225.
[2] Fried MW, Shiffman ML, Reddy KR, Smith C, Marinos G, Goncales Jr FL, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002;347 (13):975–982.
[3] 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 (9286):958–965.
[4] Akuta N, Suzuki F, Sezaki H, Suzuki Y, Hosaka T, Someya T, et al. Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. Intervirology 2005;48 (6):372–380.
[5] Davis GL, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. Hepatology 2003;38 (3):645–652.
[6] 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;10:1105–1109.
[7] 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;10:1100–1104.
[8] 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 (7262):399–401.
[9] Rauch A, Kutalik Z, Descombes P, Cai T, Di Iulio J, Mueller T, et al. Genetic variation in IL28B is associated with chronic hepatitis C and treatment failure: a genome-wide association study. Gastroenterology 2010;138 (4):1338–1345.
[10] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, et al. Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. J Clin Invest 1995;96 (1):224–230.
[11] Enomoto N, Sakuma I, Asahina Y, Kurosaki M, Murakami T, Yamamoto C, 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 (2):77–81.
[12] Kurosaki M, Enomoto N, Murakami T, Sakuma I, Asahina Y, Yamamoto C, et al. Analysis of genotypes and amino acid residues 2209 to 2248 of the NS5A region of hepatitis C virus in relation to the response to interferon-beta therapy. Hepatology 1997;25 (3):750–753.
[13] Shirakawa H, Matsumoto A, Joshita S, Komatsu M, Tanaka N, Umemura T, et al. Pretreatment prediction of virological response to peginterferon plus ribavirin therapy in chronic hepatitis C patients using viral and host factors. Hepatology 2008;48 (6):1753–1760.
[14] Akuta N, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, et al. Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. J Hepatol 2007;46 (3):403–410.

Please cite this article in press as: Kurosaki M et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol (2010), doi:10.1016/j.jhep.2010.07.037

Research Article

[15] Okanoue T, Itoh Y, Hashimoto H, Yasui K, Minami M, Takehara T, et al. Predictive values of amino acid sequences of the core and NS5A regions in antiviral therapy for hepatitis C: a Japanese multi-center study. J Gastroenterol 2009;44 (9):952–963.
[16] Segal MR, Bloch DA. A comparison of estimated proportional hazards models and regression trees. Stat Med 1989;8 (5):539–550.
[17] LeBlanc M, Crowley J. A review of tree-based prognostic models. Cancer Treat Res 1995;75:113–124.
[18] Garzotto M, Beer TM, Hudson RG, Peters L, Hsieh YC, Barrera E, et al. Improved detection of prostate cancer using classification and regression tree analysis. J Clin Oncol 2005;23 (19):4322–4329.
[19] Averbook BJ, Fu P, Rao JS, Mansour EG. A long-term analysis of 1018 patients with melanoma by classic Cox regression and tree-structured survival analysis at a major referral center: implications on the future of cancer staging. Surgery 2002;132 (4):589–602.
[20] Leiter U, Buettner PG, Eigentler TK, Garbe C. Prognostic factors of thin cutaneous melanoma: an analysis of the central malignant melanoma registry of the german dermatological society. J Clin Oncol 2004;22 (18):3660–3667.
[21] Valera VA, Walter BA, Yokoyama N, Koyama Y, Iiai T, Okamoto H, et al. Prognostic groups in colorectal carcinoma patients based on tumor cell proliferation and classification and regression tree (CART) survival analysis. Ann Surg Oncol 2007;14 (1):34–40.
[22] Zlobec I, Steele R, Nigam N, Compton CC. A predictive model of rectal tumor response to preoperative radiotherapy using classification and regression tree methods. Clin Cancer Res 2005;11 (15):5440–5443.
[23] Thabane M, Simunovic M, Akhtar-Danesh N, Marshall JK. Development and validation of a risk score for post-infectious irritable bowel syndrome. Am J Gastroenterol 2009;104 (9):2267–2274.
[24] Wu BU, Johannes RS, Sun X, Tabak Y, Conwell DL, Banks PA. The early prediction of mortality in acute pancreatitis: a large population-based study. Gut 2008;57 (12):1698–1703.
[25] Fonarow GC, Adams Jr KF, Abraham WT, Yancy CW, Boscardin WJ. Risk stratification for in-hospital mortality in acutely decompensated heart failure: classification and regression tree analysis. Jama 2005;293 (5):572–580.
[26] Kurosaki M, Matsunaga K, Hirayama I, Tanaka T, Sato M, Yasui Y, et al. A predictive model of response to peginterferon ribavirin in chronic hepatitis C using classification and regression tree analysis. Hepatol Res 2010;40 (3):251–260.
[27] Nishida N, Tanabe T, Takasu M, Suyama A, Tokunaga K. Further development of multiplex single nucleotide polymorphism typing method, the DigiTag2 assay. Anal Biochem 2007;364 (1):78–85.
[28] 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 (18):1839–1850.
[29] 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 (18):1827–1838.
[30] Rossignol JF, Elfert A, El-Gohary Y, Keeffe EB. Improved virologic response in chronic hepatitis C genotype 4 treated with nitazoxanide, peginterferon, and ribavirin. Gastroenterology 2009;136 (3):856–862.
[31] Marcello T, Grakoui A, Barba-Spaeth G, Machlin ES, Kotenko SV, MacDonald MR, et al. Interferons alpha and lambda inhibit hepatitis C virus replication with distinct signal transduction and gene regulation kinetics. Gastroenterology 2006;131 (6):1887–1898.
[32] Robek MD, Boyd BS, Chisari FV. Lambda interferon inhibits hepatitis B and C virus replication. J Virol 2005;79 (6):3851–3854.
[33] McCarthy JJ, Li JH, Thompson A, Suchindran S, Lao XQ, Patel K, et al. Replicated association between an IL28B Gene Variant and a Sustained Response to Pegylated Interferon and Ribavirin. Gastroenterology 2010;138:2307–2314.
[34] Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. Λ-interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. Hepatol Res 2010;40:449–460.
[35] Backus LI, Boothroyd DB, Phillips BR, Mole LA. Predictors of response of US veterans to treatment for the hepatitis C virus. Hepatology 2007;46 (1):37–47.
[36] Mori N, Imamura M, Kawakami Y, Saneto H, Kawaoka T, Takaki S, et al. Randomized trial of high-dose interferon-alpha-2b combined with ribavirin in patients with chronic hepatitis C: correlation between amino acid substitutions in the core/NS5A region and virological response to interferon therapy. J Med Virol 2009;81 (4):640–649.
[37] Hung CH, Lee CM, Lu SN, Lee JF, Wang JH, Tung HD, et al. Mutations in the NS5A and E2-PePHD region of hepatitis C virus type 1b and correlation with the response to combination therapy with interferon and ribavirin. J Viral Hepat 2003;10 (2):87–94.
[38] Yen YH, Hung CH, Hu TH, Chen CH, Wu CM, Wang JH, et al. Mutations in the interferon sensitivity-determining region (nonstructural 5A amino acid 2209–2248) in patients with hepatitis C-1b infection and correlating response to combined therapy of pegylated interferon and ribavirin. Aliment Pharmacol Ther 2008;27 (1):72–79.
[39] Zeuzem S, Lee JH, Roth WK. Mutations in the nonstructural 5A gene of European hepatitis C virus isolates and response to interferon alfa. Hepatology 1997;25 (3):740–744.
[40] Squadrito G, Leone F, Sartori M, Nalpas B, Berthelot P, Raimondo G, et al. Mutations in the nonstructural 5A region of hepatitis C virus and response of chronic hepatitis C to interferon alfa. Gastroenterology 1997;113 (2):567–572.
[41] Sarrazin C, Berg T, Lee JH, Teuber G, Dietrich CF, Roth WK, et al. Improved correlation between multiple mutations within the NS5A region and virological response in European patients chronically infected with hepatitis C virus type 1b undergoing combination therapy. J Hepatol 1999;30 (6):1004–1013.
[42] Murphy MD, Rosen HR, Marousek GI, Chou S. Analysis of sequence configurations of the ISDR, PKR-binding domain, and V3 region as predictors of response to induction interferon-alpha and ribavirin therapy in chronic hepatitis C infection. Dig Dis Sci 2002;47 (6):1195–1205.
[43] Pascu M, Martus P, Hohne M, Wiedenmann B, Hopf U, Schreier E, et al. Sustained virological response in hepatitis C virus type 1b infected patients is predicted by the number of mutations within the NS5A-ISDR: a meta-analysis focused on geographical differences. Gut 2004;53 (9):1345–1351.
[44] Thomas DL, Thio CL, Martin MP, Qi Y, Ge D, O'Huigin C, et al. Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. Nature 2009;461 (7265):798–801.
[45] Kurosaki M, Enomoto N, Marumo F, Sato C. Evolution and selection of hepatitis C virus variants in patients with chronic hepatitis C. Virology 1994;205 (1):161–169.
[46] Enomoto N, Kurosaki M, Tanaka Y, Marumo F, Sato C. Fluctuation of hepatitis C virus quasispecies in persistent infection and interferon treatment revealed by single-strand conformation polymorphism analysis. J Gen Virol 1994;75 (Pt 6):1361–1369.
[47] Li JH, Lao XQ, Tillmann HL, Rowell J, Patel K, Thompson A, et al. Interferon-lambda genotype and low serum low-density lipoprotein cholesterol levels in patients with chronic hepatitis C infection. Hepatology 1904;51 (6):1904–1911.
[48] Serfaty L, Andreani T, Giral P, Carbonell N, Chazouilleres O, Poupon R. Hepatitis C virus induced hypobetalipoproteinemia: a possible mechanism for steatosis in chronic hepatitis C. J Hepatol 2001;34 (3):428–434.

Please cite this article in press as: Kurosaki M et al. Pre-treatment prediction of response to pegylated-interferon plus ribavirin for chronic hepatitis C using genetic polymorphism in *IL28B* and viral factors. J Hepatol (2010), doi:10.1016/j.jhep.2010.07.037

Hepatology Research

Hepatology Research 2010; **40**: 1063–1071 doi: 10.1111/j.1872-034X.2010.00741.x

Original Article

ITPA gene variant protects against anemia induced by pegylated interferon-α and ribavirin therapy for Japanese patients with chronic hepatitis C

Naoya Sakamoto,[1,2]* Yasuhito Tanaka,[3]* Mina Nakagawa,[1,2] Hiroshi Yatsuhashi,[4] Shuhei Nishiguchi,[5] Nobuyuki Enomoto,[6] Seishin Azuma,[1] Yuki Nishimura-Sakurai,[1] Sei Kakinuma,[1,2] Nao Nishida,[7] Katsushi Tokunaga,[7] Masao Honda,[8] Kiyoaki Ito,[9] Masashi Mizokami[9] and Mamoru Watanabe[1]

[1]*Department of Gastroenterology and Hepatology,* [2]*Department for Hepatitis Control, Tokyo Medical and Dental University, Tokyo,* [3]*Department of Virology and Liver Unit, Nagoya City University Graduate School of Medical Sciences, Nagoya,* [4]*Clinical Research Center, National Nagasaki Medical Center, Nagasaki,* [5]*Department of Internal Medicine, Hyogo College of Medicine,* [6]*First Department of Internal Medicine, University of Yamanashi, Yamanashi,* [7]*Department of Human Genetics, Graduate School of Medicine, The University of Tokyo, Tokyo,* [8]*Department of Gastroenterology, Kanazawa University Graduate School of Medicine, Kanazawa,* [9]*Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, Ichikawa, Japan*

Aim: Host genetic variants leading to inosine triphosphatase (ITPA) deficiency, a condition not thought to be clinically important, protect against hemolytic anemia in chronic hepatitis C patients receiving ribavirin. In this study, we evaluated the clinical significance of *ITPA* variants in Japanese hepatitis C patients who were treated with pegylated interferon plus ribavirin.

Methods: In this multicenter retrospective cross-sectional study, 474 hepatitis C patients were enrolled who were treated with pegylated interferon plus ribavirin in four geographically different hospitals in Japan. Patients were grouped according to hemoglobin decline of more than 3 g/dL at week 4. Two single nucleotide polymorphisms (SNP) within or adjacent to the *ITPA* gene (rs6051702, rs1127354) were genotyped.

Results: A functional SNP, rs1127354, within the *ITPA* exon was strongly associated with protection against anemia with only one (0.8%) in 129 patients with the *ITPA* minor variant A developing severe anemia ($P = 5.9 \times 10^{-20}$). For rs6051702, which had significant association in European-Americans, significant but weak association with severe hemoglobin reduction was found in Japanese ($P = 0.009$). In patients excluding genotype 1b and high viral load, those with the *ITPA* minor variant A achieved significantly higher sustained viral response rate than those with the major variant (CC) (96% vs 70%, respectively, $P = 0.0066$).

Conclusion: *ITPA* SNP, rs1127354, is confirmed to be a useful predictor of ribavirin-induced anemia in Japanese patients. Patients with the *ITPA* minor variant A (~27%) have an advantage in pegylated interferon plus ribavirin-based therapies, due to expected adherence of ribavirin doses, resulting in a higher viral clearance rate.

Key words: *c20orf194*, hemolytic anemia, hepatitis C virus, *ITPA* (inosine triphosphatase), pegylated interferon plus ribavirin therapy

INTRODUCTION

APPROXIMATELY 3% OF the worldwide population is infected with the hepatitis C virus (HCV), which represents 170 million people, with 3–4 million individuals newly infected each year. Chronic hepatitis C (CHC) has a variable course; although 20–25% of CHC patients maintain persistently normal serum aminotransferases and experience relatively slow histological progression, other patients present a more active biochemical course.[1–3] Overall, 30% of the CHC patients progress to cirrhosis in their lifetime,[3] and 3–8% of cirrhosis patients develop hepatocellular carcinoma (HCC) every year.[4–6] Among various factors, older age and hepatic steatosis are significant factors accelerating the rate of progression in CHC.[3,7–9]

Correspondence: Professor Masashi Mizokami, Research Center for Hepatitis and Immunology, National Center for Global Health and Medicine, 1-7-1 Konodai, Ichikawa 272-8516, Japan. Email: mmizokami@hospk.ncgm.go.jp

*These authors contributed equally to this study.

Received 28 June 2010; revision 26 August 2010; accepted 20 September 2010.

Antiviral treatment has been shown to improve liver histology and decrease incidence of HCC in CHC.[6,10] Current therapy for CHC consists of treatment with pegylated interferon (PEG IFN), which acts both as an antiviral and as an immunoregulatory cytokine, and ribavirin (RBV), an antiviral prodrug that interferes with RNA metabolism.[11,12] However, less than 50% of patients infected with HCV genotype 1 treated in this way achieve a sustained viral response (SVR) or a cure of the infection.[11,13] Older patients have showed a significantly lower SVR rate due to poor adherence resulting from adverse events and laboratory abnormalities.[14–16] In particular, hematological abnormalities and RBV-induced hemolytic anemia often necessitate dose reduction and premature withdrawal from therapy in 10–14% of patients.[11,17–20] New drugs and therapeutic approaches for CHC are actively developed and several candidates are in the early trial phase.[21,22] Given these backgrounds, effective pre-treatment screening for predictor biomarkers with the aim to evaluate possible risks over benefits from currently available treatment would allow avoiding these side-effects in patients who will not be helped by the treatment, as well as to reduce the substantial cost of the treatment.

The completion of the Human Genome Project has led to the advent of a new era of scientific research, including a revolutionary approach: the genome-wide association study (GWAS). Several recent studies have demonstrated remarkable associations between single nucleotide polymorphisms (SNP) near or within the region of the *IL28B* gene, which codes for IFN-λ3.[23–28] Another recent study indicated that genetic variants leading to inosine triphosphatase (ITPA) deficiency, a condition not thought to be clinically important, protect against hemolytic anemia in CHC patients receiving RBV.[29] The results obtained in one GWAS study need to be evaluated and confirmed in the context of different geographical and racial populations, and independent cohorts. Here, we describe clinical evaluation of two SNP within or adjacent to the *ITPA* gene (6051702 and rs1127354), that was recently highlighted by the GWAS of HCV treatment-induced anemia.[29]

METHODS

Patients

IN THIS RETROSPECTIVE cross-sectional case–control study, 474 patients with chronic HCV infection treated at Tokyo Medical and Dental University Hospital, Nagoya City University Hospital, Yamanashi University Hospital, Nagasaki Medical Center and Hyogo University of Health Science Hospital in Japan were enrolled from April 2007 to April 2009. Each patient was treated with PEG IFN-α-2b (1.5 μg/kg s.c. once a week) or PEG IFN-α-2a (180 μg/kg once a week) plus RBV (600–1000 mg daily depending on bodyweight). The treatment duration was set at a standard 48 weeks for genotype 1b high viral load (≥5 log copies/mL) patients and 24 weeks for genotype 1 low viral load (≤5 log copies/mL) and genotypes 2 and 3 patients. On-treatment dose reduction and discontinuation of PEG IFN or RBV were decided based on the recommendations of package inserts or clinical situations in individual patients to avoid possible side-effects. The rates of PEG IFN and RBV administration achieved were calculated as percentages of actual total dose administrated of a standard total dose of 24 weeks, according to bodyweight before therapy. Hepatitis B surface antigen (HBsAg) positive and/or anti-HIV positive individuals were excluded from this study. Hemoglobin (Hb) values were measured at baseline and every week until 8 weeks. We considered Hb decline at week 4 to be a clinically important time point, as previously reported.[29] The threshold of Hb reduction of more than 3 g/dL was chosen as a clinically significant Hb decline according to the previous reports.[29–31]

Informed consent was obtained from each patient who participated in the study. The study protocol conformed to the relevant ethical guidelines as reflected in a priori approval by the ethics committees of all the participating universities and hospitals.

Patient evaluation

The following factors were analyzed to determine whether they were related to the efficacy of combination therapy: age, sex, previous IFN therapy, grade of inflammation and stage of fibrosis on liver biopsy, pre-treatment biochemical parameters, such as white blood cells, neutrophils, Hb, platelet count, alanine transaminase (ALT) level, serum HCV RNA level (log IU/mL). Liver biopsy specimens were evaluated blindly, to determine the grade of inflammation and stage of fibrosis, by an independent interpreter who was not aware of the clinical data. Activity of inflammation was graded on a scale of 0–3: A0, showing no activity; A1, showing mild activity; A2, showing moderate activity; and A3, showing severe activity. Fibrosis was staged on a scale of 0–4: F0, showing no fibrosis; F1, showing moderate fibrosis; F2, showing moderate fibrosis with

few septa; F3, showing severe fibrosis with numerous septa without cirrhosis; and F4, showing cirrhosis.

SNP genotyping

Human genomic DNA was extracted from whole blood of each patient. Genetic polymorphisms, rs1127354 in *ITPA*, rs6051702 in *C20orf194*, and rs8099917 around the *IL28B* gene were determined by real-time detection polymerase chain reaction with a TaqMan probe or DigiTag2 assay typing one tag SNP located within each locus.[23] Another functional SNP, rs727010 within the *ITPA* gene, was excluded due to no variants in the Asian genetic population as reported in the International HapMap Project database. Our preliminary genotyping of a 100-patient population did not find variants in that SNP.

Outcomes

The primary end-point was Hb decline and dose reduction of PEG IFN or RBV in week 4, the secondary end-point was SVR. An SVR was defined as serum HCV RNA undetectable at 24 weeks after the end of treatment. A transient viral response (TVR) meant that HCV RNA became undetectable during treatment but reappeared at the end of follow up. A null response (NR) was defined as persistently positive HCV RNA throughout the treatment. Adverse events and drug adherence were recorded.

Statistical analyses

The association between individual ITPA SNP and the incidence of significant Hb decline was tested by a basic allelic test and calculated using the χ^2-test. Multivariate logistic regression analysis with stepwise forward selection was performed with *P*-values of less than 0.05 as the criteria for model inclusion. These statistical analyses were conducted by using SPSS software package ver. 18J (Chicago, IL, USA) or Microsoft Excel Mac 2008 (Redmond, WA, USA). Discrete variables were evaluated by Fisher's exact probability test. The *P*-values were calculated by two-tailed Student's *t*-tests for continuous data and χ^2-test for categorical data, and those of less than 0.05 were considered statistically significant.

RESULTS

THE CLINICAL CHARACTERISTICS of the 474 patients are summarized in Table 1. First, we compared baseline clinical and host genetic characteristics of patient groups according to the SNP within the *ITPA* gene, rs1127354, between major homozygote (CC) and a group of heterozygote (CA) and minor homozygote (AA) (Table 2). There were no significant differences in age, sex, blood cell counts, ALT levels, serum viral loads, frequencies of core 70/91 mutations[32,33] and the numbers of NS5A interferon sensitivity determining region (ISDR) mutations[34,35] between the two groups. The SNP in the *ITPA* gene did not show significant linkage between the SNP around the *IL28B* gene, rs8099917, which is strongly associated with IFN treatment responses.[23,25] In contrast, the SNP in the *ITPA* gene showed significant linkage with the SNP in *C20orf194*, rs6051702 ($P = 7.1 \times 10^{-14}$).[29]

Next, we analyzed two SNP, rs6051702 in *C20orf194* and rs1127354 in *ITPA* loci, respectively, for their association with significant Hb decline at 4 weeks of PEG IFN plus RBV treatment using a basic allelic model that compares frequencies of alleles in cases versus controls. The SNP, rs6051702, which showed the strongest association in the European-American population,[29] was

Table 1 Baseline characteristics of participating patients

Total number	474
Age (years)	57.2 ± 10.0
Sex (male/female)	264/210
Bodyweight (kg)	61.1 ± 10.8
HCV genotypes	
1b/2a/2b/3a	416/31/26/1
1b, high viral load/others	387/87
NS5A-ISDR mutations (genotype 1b, $n = 334$, 0–1/≥2)	285/49
Core mutations (genotype 1b, $n = 379$)	
C70 (wild/mutant)	240/139
C91 (wild/mutant)	234/145
Histology at biopsy ($n = 278$)	
Grade of inflammation (A0/1/2/3)	4/97/154/23
Stage of fibrosis (F0/1/2/3/4)	8/102/74/74/20
White blood cells (/μL)†	5707 ± 1495
Neutrophils (/μL)†	2568 ± 1013
Hemoglobin (g/dL)†	14.2 ± 1.4
Platelet count ($\times 10^{-3}$/μL)†	158 ± 58
ALT (IU/L)†	89 ± 66
Serum HCV RNA (log [IU/mL])‡	6.0 ± 0.9
PEG IFN (PEG IFN-α-2a/PEG IFN-α-2b)	40/434
Hb decline at week 4 (g/dl)	2.4 ± 1.4
Severe anemia, Hb <10 g/dL at week 4	56/474 (11.8%)

†Data are expressed as mean ± standard deviation.
‡Data are shown as median (range) values.
High viral load: HCV RNA ≥ 5 log IU/mL.
ALT, alanine transaminase; Hb, hemoglobin; HCV, hepatitis C virus; IFN, interferon; ISDR, interferon sensitivity determining region; PEG, pegylated.

Table 2 Clinical and host genetic characteristics of patients according to *IPTA* gene variants

	ITPA SNP, rs1127354		
	CC ($n = 345$)	CA + AA ($n = 129$)	*P*-value
Age (years)†	57.2 ± 10.0	57.1 ± 10.1	0.87
Sex (male/female)	192/153	72/57	0.97
White blood cells (/μL)‡	5312 ± 1537	4995 ± 1388	0.92
Neutrophils (/μL)‡	1696 ± 1415	1803 ± 1516	0.49
Hemoglobin (g/dL)‡	14.2 ± 1.4	14.1 ± 1.4	0.52
Platelet count ($\times 10^{-3}$/μL)‡	157 ± 54	159 ± 70	0.81
ALT (IU/mL)‡	91 ± 70	83 ± 55	0.46
Serum HCV RNA (log copies /mL)†	6.1 ± 0.7	5.8 ± 1.1	0.093
NS5A-ISDR mutations (genotype 1b, $n = 334$, 0–1/≥2)	213/31	72/18	0.095
Core mutations (genotype 1b, $n = 389$)			
aa. 70 (wild/mutant)	179/96	61/43	0.25
aa. 91 (wild/mutant)	172/103	62/42	0.60
IL28B, rs8099917 (TT/TG/GG)	233/94/3	96/29/1	0.23
C20orf194, rs6051702 (AA/AC/CC)	254/85/6	47/72/10	7.1×10^{-14}*

*P-values were calculated by student's t-test or by χ^2 analysis.
†Data are show as median (range) values.
‡Data are expressed as mean ± standard deviation.
IL28B SNP, major allele-T and minor allele-G.
C20orf194 SNP, major allele-A and minor allele-C.
ALT, alanine transaminase; HCV, hepatitis C virus; SNP, single nucleotide polymorphisms.

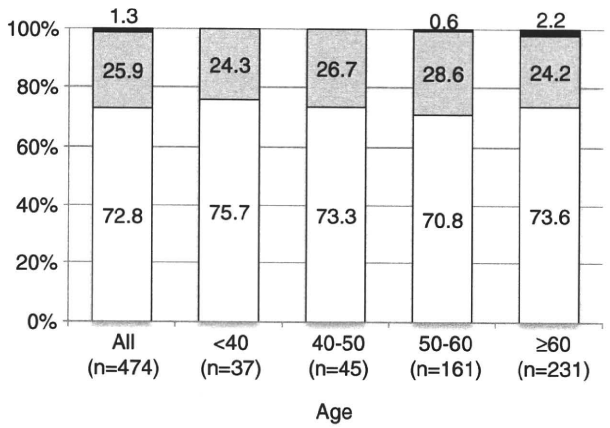

Figure 1 *ITPA* variant, rs1127354, known to be responsible for inosine triphosphatase deficiency and its age-related differences. *ITPA*, inosine triphosphatase. The numbers in parentheses denote numbers of patients.

associated with the Hb decline significantly but with smaller effect size (odds ratio [OR] = 1.40, $P = 9.0 \times 10^{-3}$, Table 3). Notably, another SNP in the *ITPA* gene, rs1127354, showed overwhelming association with the Hb decline (OR = 62.8, $P = 5.9 \times 10^{-20}$). The prevalence of *ITPA* variants is shown in Figure 1. Percentages of *ITPA*, rs1127354, major homozygote (CC), heterozygote (CA) and minor homozygote (AA) were 72.8%, 25.9% and 1.3%, respectively. There was no difference in the frequency of the *ITPA* variants throughout ages and sexes (Fig. 1).

To asses the clinical relevance of these SNP, we analyzed the proportion of patients suffering clinically significant anemia, which we defined as a decline in Hb levels of more than 3 g/dL or Hb levels of less than 10 g/dL, which is the threshold at which RBV dose reduction is recommended. As depicted in Figure 2, in *ITPA*-CC patients, Hb loss of more than 3 g/dL devel-

Table 3 Association of *C20orf194* and *IPTA* gene variants with treatment-induced Hb decline

Gene	SNP	Allele (major/minor)	MAF (%)	OR	*P*-value*
C20orf194	rs6051702	A/C	19.9	1.40	9.0×10^{-3}
ITPA	rs1127354	C/A	14.2	62.8	5.9×10^{-20}

*The SNP-phenotype associations were analyzed using a basic allelic test.
P-values were calculated by χ^2 analysis.
Hb, hemoglobin; MAF, minor allele frequency; OR, odds ratio; SNP, single nucleotide polymorphisms.

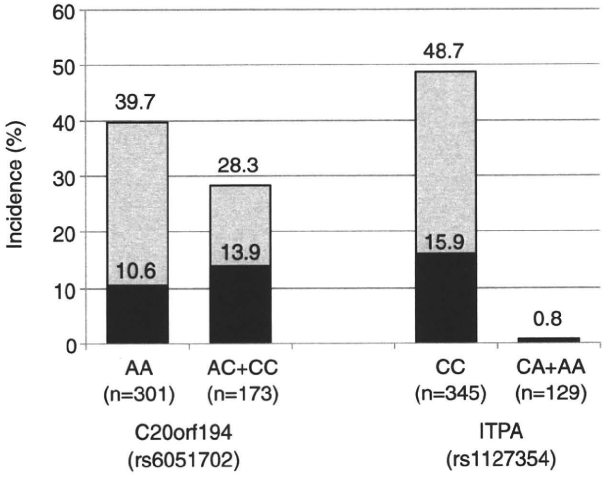

Figure 2 Effects of *c20orf194* and *ITPA* single nucleotide polymorphisms (SNP) on clinically significant anemia induced by pegylated interferon plus ribavirin treatment. Percentages of patients with hemoglobin (Hb) decline of >3 g/dL or Hb levels of >10 g/dL at week 4 of treatment are shown for each SNP in two genes, *c20orf94* (rs6051702) and *ITPA* (rs1127354).

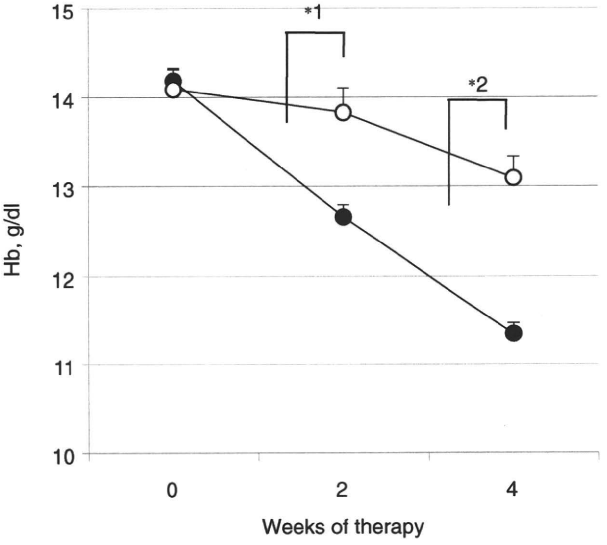

Figure 3 Time-dependent hemoglobin (Hb) decline in *ITPA* major and minor variants. Error bars indicate mean + standard error. Asterisks 1 and 2 indicate statistical significance of $P = 6.6 \times 10^{-13}$ and $P = 3.0 \times 10^{-29}$, respectively.

oped in 48.7% at week 4, and 15.9% of patients achieved Hb levels of less than 10 g/dL. In contrast, only one patient (0.8%) with *ITPA*-CA/AA developed anemia. These differences in the incidence of the treatment-induced Hb decline were consistent throughout ages. The time-dependent Hb decline in patients with *ITPA*-CC and *ITPA*-CA/AA is shown in Figure 3. In patients with *ITPA*-CC, mean Hb drop was 2.9 ± 1.3 g/dL, which was significantly higher than that of patients with *ITPA*-CA/AA (1.1 ± 0.7 g/dL). These results demonstrate that the *ITPA* minor variant A has a protective phenotype for the treatment-induced anemia. The positive predictive value of the *ITPA*-major (CC) for the development of severe anemia was 48.7%, while the negative predictive value of *ITPA*-hetero/minor (CA/AA) was 99.2%. In accordance with the incidence of anemia, there was significant difference in the incidence of RBV dose reduction. At week 4 of treatment, RBV doses were reduced in 27.9% of *ITPA*-CC patients while in only 14.4% of *ITPA*-CA/AA patients ($P = 0.012$, Fig. 4). Similarly to RBV, PEG IFN dose reduction was apparently higher in *ITPA*-CC patients though it did not reach statistical significance.

Knowing that significantly less frequent drug reduction occurred in patients with the *ITPA*-minor variant A, we next investigated if the *ITPA* gene variants affected final treatment outcomes. The treatment outcomes were available in 339 patients with genotype 1b and high viral load

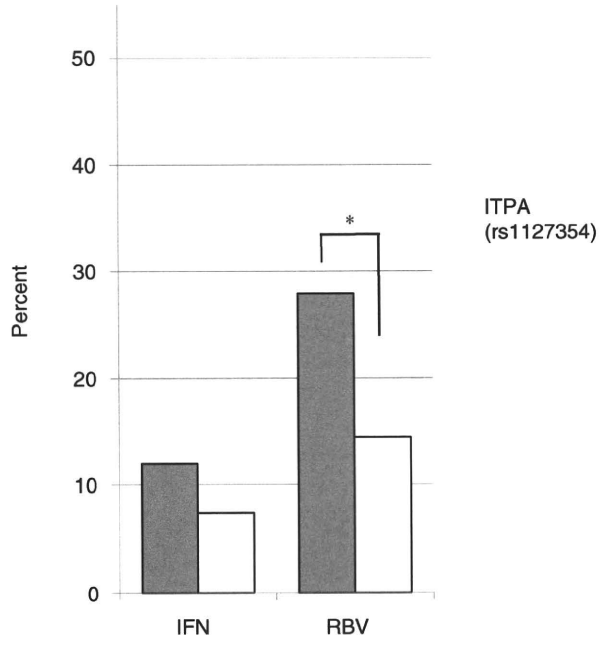

Figure 4 Percentages of patients requiring pegylated interferon (IFN) or ribavirin (RBV) dose reduction at week 4 in *ITPA* major and minor variants. *Y*-axis indicates percents of patients who required dose reduction. *$P = 0.012$.

Table 4 Sustained viral response rates of each group according to *IPTA* gene variants

IPTA SNP, rs1127354	Genotype 1b, high viral load		Others	
	CC	CA + AA	CC	CA + AA
SVR	92 (37.1%)	39 (42.9%)	41 (70%)	25 (96%)
TVR	90 (36.3%)	34 (37.3%)	15 (25%)	1 (4%)
NR	66 (26.6%)	18 (19.8%)	3 (5%)	0 (0%)
Total	248	91	59	26
P-value	0.33		**0.0066**	

High viral load; serum HCV RNA ≥ 5 logIU/mL.
"Others" include genotypes genotype 1b, serum HCV RNA <5 logIU/ml, genotypes 2a, 2b and 3a.
P-values were calculated by χ^2-test analyses of SVR versus TVR plus NR.
HCV, hepatitis C virus; SVR, sustained viral response; TVR, transient viral response; NR, null response.

(HCV RNA ≥5.0 log IU/ml) and 85 others, which included genotype 1b, low viral load and genotype 2a, 2b and 3a patients (Table 4). In patients with genotype 1b and high viral load, there was no significant difference in SVR rates between *ITPA*-CC and *ITPA*-CA/AA patients (37.1% and 42.9%, respectively). In contrast, there was a striking difference in SVR rates between *ITPA*-CC and *ITPA*-CA/AA in the other IFN-sensitive group (non-1b or low viral load); the SVR rate was 70% in *ITPA*-CC patients, while 96% of *ITPA*-CA/AA patients achieved SVR ($P = 0.0066$). These results indicate that the *ITPA* minor variant A is significantly associated with SVR in the IFN-sensitive group excluding genotype 1b and high viral load. Using those subpopulations of patients, we conducted a statistical analysis for association of several host and viral parameters with SVR. As shown in Table 5, univariate analysis identified four significant parameters including age, platelet count, stages of fibrosis and the ITPA SNP, rs1123354. Multivariate logistic regression analysis identified that only age and the ITPA SNP were significantly associated with SVR.

DISCUSSION

RECENT GWAS ON HCV infection have identified two important host genetic polymorphisms. One is the SNP in the *IL28B* gene, which is strongly associated with response to therapy of chronic genotype 1 HCV infection,[23–28] and another is the SNP in the *ITPA* gene, which precisely predicts RBV treatment-associated anemia in the European-American population.[29] In our present study, a functional SNP in the *ITPA* locus, rs1127354, is strongly associated with protection against anemia among 474 Japanese patients ($P = 5.9 \times 10^{-20}$, Table 3). Only one of 129 patients (0.8%) who carry the rs1127354 minor allele A had severe anemia (Figs 2,3). These data are consistent with the previous study in the US population[29] as well as a recent Japanese study by

Table 5 Univariate and multivariate logistic regression analyses of host and viral characteristics of patients excluding genotype 1b high virus load based on therapeutic responses ($n = 85$)

Variable	*P*-value (univariate)	*P*-value (multivariate)	OR	95% CI
Age	**0.017**	**0.047**	0.916	0.840–0.999
Sex (male vs female)	0.19	–		
Baseline Hb level	0.17	–		
Baseline platelet count	**0.019**	0.307	1.092	0.923–1.292
Stage of fibrosis (F0–2 vs 3–4)	**0.0020**	0.083	4.221	0.827–21.531
PEG IFN adherence (≥80% vs <80%)	0.67	–		
RBV adherence (≥80% vs <80%)	0.30	–		
ITPA SNP rs1127354 (CC vs CA + AA)	**0.0066**	**0.023**	12.680	1.386–116.042
IL28B SNP rs8099917 (TT vs TG + GG)	0.29	–		

CI, confidence interval; Hb, hemoglobin; IFN, interferon; ITPA, inosine triphosphatase; OR, odds ratio; PEG, pegylated; RBV, ribavirin; SNP, single nucleotide polymorphisms.

Ochi *et al.*[31] Our data were similar to these two reports; rs1127354 was the most significant SNP that was associated with RBV-induced anemia in Asian genetic populations. Additionally, we have demonstrated that the incidence of early dose reduction was significantly higher in *ITPA*-major (CC) patients as expected (Fig. 4) and, more importantly, that a significantly higher SVR rate was achieved in *ITPA*-hetero/minor (CA/AA) patients with HCV non-1b or low viral load strains (70% vs 96%, $P = 0.0066$, Table 4). Taken together, our results demonstrate that the *ITPA* minor variant A is not only a protective allele of PEG IFN and RBV treatment-associated anemia in the Japanese population, but also a significant predictor of SVR in certain HCV strains that show good response to IFN.

An SNP in *C20orf194*, rs6051702, which showed significant association with Hb reduction in European-Americans ($P = 1.1 \times 10^{-45}$),[29] was also significant in our study of a Japanese population, but with smaller effect size on Hb reduction ($P = 0.014$). The discrepancy may be due to the low levels of the linkage disequilibrium (LD) with the functional SNP in the *ITPA* gene in the Japanese/Asian population as compared with the high LD in white subjects. Indeed, it is reported that the predictive values of the *C20orf194* SNP varied between different races including African-Americans ($P = 0.19$) and Hispanics ($P = 9.5 \times 10^{-3}$).[29]

Ochi *et al.* sequenced the Japanese patient genome including ITPA and DDRGK1 loci, which are located adjacently on chromosome 20. They identified 83 SNP with major allele frequency of more than 0.05, of which four SNP including rs1127354 were significantly associated with RBV-induced anemia and which were in almost absolute LD with each other.[31] Their report indicates that the ITPA SNP, rs1127354, which we genotyped in the present study, represent a dominant variant of ITPA deficiency that protects against RBV-induced anemia in Japanese/Asian genetic populations. In our study, however, 51.3% of the ITPA-major (CC) patients did not develop significant Hb decline (Fig. 2). This finding suggests that there are other low-frequency ITPA variants or SNP in other enzymes that are involved in erythrocyte purine nucleoside metabolism.

The response to PEG IFN plus RBV treatment is affected by several viral and host factors such as age, sex,[36,37] NS5A-ISDR[38] and core region.[32,33] To maintain good adherence to drugs, especially RBV, it is important to achieve good treatment responses. Increased RBV exposure during the treatment phase was associated with an increased likelihood of SVR in the US[39] and Japanese studies.[40] Because patients with *ITPA* minor variant A are refractory to RBV-induced anemia, they are advantaged in maintaining good adherence to RBV and may be given even higher doses of RBV, resulting in a higher SVR rate. However, a study by Fellay *et al.* and a very recent replication study by Thompsom *et al.*[30] did not observe any significant association between the *ITPA* minor variants and early or late anti-HCV treatment outcomes.[29] A possible explanation for the discrepancy is that older and histologically more advanced patients were predominant in our study. Mean age in the US study was 47.5 years while 57.2 years in our present study. The percentage of advanced fibrosis (F3 or F4) was 12.0% in the US study while 34% in our study. It is well known that the incidence of drug dose reduction or discontinuation could increase according to old age as well as advanced stages, that may compromise final treatment outcomes.[14,15] Importantly, we have additionally demonstrated that in patients with other than genotype 1b HCV, ITPA minor variant A was significantly associated with better SVR rates in univariate and multivariate analyses. Because the typical PEG IFN plus RBV treatment period is shorter (24 weeks) in genotype 1 low viral load and genotype 2 patients than in genotype 1 high viral load (48 weeks), early dose reduction of RBV may be more critical to the final treatment outcome.

Ribavirin is a synthetic guanosine analog, and has actions *in vitro* against a wide range of RNA and DNA viruses.[41] Possible antiviral mechanisms of ribavirin include immune modulation by switching the T-cell phenotype from type 2 to type 1,[42] anti-proliferative effect by inhibition of cellular GTP synthesis,[41] and direct inhibition of virus replication.[43] Although monotherapy with RBV clinically showed minimal effect on the viral load and almost no effect on the viral clearance,[44–47] combinatory use of RBV with IFN elicits strong synergistic effects against HCV *in vitro*[48] and *in vivo*.[49,50]

Ribavirin is directly toxic to erythrocytes and is associated with hemolysis, which is usually reversible and dose-related.[49,50] RBV is incorporated into erythrocytes where it undergoes phosphorylation to its pharmacologically active forms through adenosine kinase. The RBV-phosphate conjugates are unable to cross the erythrocyte cell membrane and are thus accumulated intracellularly and cleared slowly from red cells with a half-life of approximately 40 days.[51] Inosine triphosphatase (ITP) deficiency or low activity variants, in turn, lead to an accumulation of ITP in red blood cells and may compete with RBV triphosphate, and may protect from RBV-induced hemolysis.[52,53]

There are several STAT-C agents (specifically targeted antiviral therapies for hepatitis C) being tested for

clinical efficacy against hepatitis C.[21,22] Most experts believe that when new drugs are approved to treat hepatitis C they will be used in combination with PEG IFN and RBV. Moreover, recent clinical trials including NS3 protease inhibitors have shown that PEG IFN plus RBV would be necessary to achieve optimal treatment responses.[18,19,54] Our present results may give a valuable pharmacogenetic diagnostic tool for the tailoring of RBV dosage to minimize drug-induced adverse events and for further optimization of the clinical anti-HCV chemotherapeutics.

ACKNOWLEDGMENTS

THIS STUDY WAS supported by grants from the Ministry of Education, Culture, Sports, Science and Technology Japan, the Japan Society for the Promotion of Science, Ministry of Health, Labor and Welfare Japan, Japan Health Sciences Foundation, and National Institute of Biomedical Innovation.

REFERENCES

1 Wiese M, Grungreiff K, Guthoff W, Lafrenz M, Oesen U, Porst H. Outcome in a hepatitis C (genotype 1b) single source outbreak in Germany – a 25-year multicenter study. *J Hepatol* 2005; **43**: 590–8.
2 Santantonio T, Sinisi E, Guastadisegni A *et al.* Natural course of acute hepatitis C: a long-term prospective study. *Dig Liver Dis* 2003; **35**: 104–13.
3 Massard J, Ratziu V, Thabut D *et al.* Natural history and predictors of disease severity in chronic hepatitis C. *J Hepatol* 2006; **44**: S19–24.
4 Benvegnu L, Gios M, Boccato S, Alberti A. Natural history of compensated viral cirrhosis: a prospective study on the incidence and hierarchy of major complications. *Gut* 2004; **53**: 744–9.
5 Sangiovanni A, Prati GM, Fasani P *et al.* The natural history of compensated cirrhosis due to hepatitis C virus: a 17-year cohort study of 214 patients. *Hepatology* 2006; **43**: 1303–10.
6 Yoshida H, Tateishi R, Arakawa Y *et al.* Benefit of interferon therapy in hepatocellular carcinoma prevention for individual patients with chronic hepatitis C. *Gut* 2004; **53**: 425–30.
7 Marcellin P, Asselah T, Boyer N. Fibrosis and disease progression in hepatitis C. *Hepatology* 2002; **36**: S47–56.
8 Perumalswami P, Kleiner DE, Lutchman G *et al.* Steatosis and progression of fibrosis in untreated patients with chronic hepatitis C infection. *Hepatology* 2006; **43**: 780–7.
9 Poynard T, Ratziu V, Charlotte F, Goodman Z, McHutchison J, Albrecht J. Rates and risk factors of liver fibrosis progression in patients with chronic hepatitis c. *J Hepatol* 2001; **34**: 730–9.
10 George SL, Bacon BR, Brunt EM, Mihindukulasuriya KL, Hoffmann J, Di Bisceglie AM. Clinical, virologic, histologic, and biochemical outcomes after successful HCV therapy: a 5-year follow-up of 150 patients. *Hepatology* 2009; **49**: 729–38.
11 Fried MW, Shiffman ML, Reddy KR *et al.* Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. *N Engl J Med* 2002; **347**: 975–82.
12 Manns MP, McHutchison JG, Gordon SC *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**: 958–65.
13 Hadziyannis SJ, Sette H, Jr, Morgan TR *et al.* Peginterferon-alpha2a and ribavirin combination therapy in chronic hepatitis C: a randomized study of treatment duration and ribavirin dose. *Ann Intern Med* 2004; **140**: 346–55.
14 Hiramatsu N, Oze T, Tsuda N *et al.* Should aged patients with chronic hepatitis C be treated with interferon and ribavirin combination therapy? *Hepatol Res* 2006; **35**: 185–9.
15 Iwasaki Y, Ikeda H, Araki Y *et al.* Limitation of combination therapy of interferon and ribavirin for older patients with chronic hepatitis C. *Hepatology* 2006; **43**: 54–63.
16 Sezaki H, Suzuki F, Akuta N *et al.* An open pilot study exploring the efficacy of fluvastatin, pegylated interferon and ribavirin in patients with hepatitis C virus genotype 1b in high viral loads. *Intervirology* 2009; **52**: 43–8.
17 Bruno R, Sacchi P, Maiocchi L, Patruno S, Filice G. Hepatotoxicity and antiretroviral therapy with protease inhibitors: a review. *Dig Liver Dis* 2006; **38**: 363–73.
18 Hezode C, Forestier N, Dusheiko G *et al.* Telaprevir and peginterferon with or without ribavirin for chronic HCV infection. *N Engl J Med* 2009; **360**: 1839–50.
19 McHutchison JG, Everson GT, Gordon SC *et al.* Telaprevir with peginterferon and ribavirin for chronic HCV genotype 1 infection. *N Engl J Med* 2009; **360**: 1827–38.
20 Suzuki F, Akuta N, Suzuki Y *et al.* Rapid loss of hepatitis C virus genotype 1b from serum in patients receiving a triple treatment with telaprevir (MP-424), pegylated interferon and ribavirin for 12 weeks. *Hepatol Res* 2009; **39**: 1056–63.
21 Sakamoto N, Watanabe M. New therapeutic approaches to hepatitis C virus. *J Gastroenterol* 2009; **44**: 643–9.
22 Sakamoto N, Wu GY. Prospects for future therapy of hepatitis C virus infection. *Future Virol* 2009; **4**: 453–62.
23 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.
24 Ge D, Fellay J, Thompson AJ *et al.* Genetic variation in IL28B predicts hepatitis C treatment-induced viral clearance. *Nature* 2009; **461**: 399–401.
25 Suppiah V, Moldovan M, Ahlenstiel G *et al.* IL28B is associated with response to chronic hepatitis C interferon-alpha and ribavirin therapy. *Nat Genet* 2009; **41**: 1100–4.

26 Thomas DL, Thio CL, Martin MP *et al.* Genetic variation in IL28B and spontaneous clearance of hepatitis C virus. *Nature* 2009; **461**: 798–801.

27 Tanaka Y, Nishida N, Sugiyama M, Tokunaga K, Mizokami M. lambda-Interferons and the single nucleotide polymorphisms: a milestone to tailor-made therapy for chronic hepatitis C. *Hepatol Res* 2010; **40**: 449–60.

28 Ahlenstiel G, Booth DR, George J. IL28B in hepatitis C virus infection – translating pharmacogenomics into clinical practice. *J Gastroenterol* 2010; **45**: 903–10. EPub ahead of print.

29 Fellay J, Thompson AJ, Ge D *et al.* ITPA gene variants protect against anaemia in patients treated for chronic hepatitis C. *Nature* 2010; **464**: 405–8.

30 Thompson AJ, Fellay J, Patel K *et al.* Variants in the ITPA gene protect against ribavirin-induced hemolytic anemia and decrease the need for ribavirin dose reduction. *Gastroenterology* 2010; **139**: 1181–9. EPub ahead of print.

31 Ochi H, Maekawa T, Abe H *et al.* ITPA polymorphism affects ribavirin-induced anemia and outcome of therapy – a genome-wide study of Japanese HCV patients. *Gastroenterology* 2010; 139: 1190–7. EPub ahead of print.

32 Akuta N, Suzuki F, Sezaki H *et al.* Association of amino acid substitution pattern in core protein of hepatitis C virus genotype 1b high viral load and non-virological response to interferon-ribavirin combination therapy. *Intervirology* 2005; **48**: 372–80.

33 Akuta N, Suzuki F, Kawamura Y *et al.* Predictive factors of early and sustained responses to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b: amino acid substitutions in the core region and low-density lipoprotein cholesterol levels. *J Hepatol* 2007; **46**: 403–10.

34 Enomoto N, Sakuma I, Asahina Y *et al.* Comparison of full-length sequences of interferon-sensitive and resistant hepatitis C virus 1b. Sensitivity to interferon is conferred by amino acid substitutions in the NS5A region. *J Clin Invest* 1995; **96**: 224–30.

35 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.

36 Honda T, Katano Y, Urano F *et al.* Efficacy of ribavirin plus interferon-alpha in patients aged >or=60 years with chronic hepatitis C. *J Gastroenterol Hepatol* 2007; **22**: 989–95.

37 Hung CH, Chen CH, Lee CM *et al.* Association of amino acid variations in the NS5A and E2-PePHD region of hepatitis C virus 1b with hepatocellular carcinoma. *J Viral Hepat* 2008; **15**: 58–65.

38 Nakagawa M, Sakamoto N, Ueyama M *et al.* Mutations in the interferon sensitivity determining region and virological response to combination therapy with pegylated-interferon alpha 2b plus ribavirin in patients with chronic hepatitis C-1b infection. *J Gastroenterol* 2010; **45**: 656–65.

39 McHutchison JG, Lawitz EJ, Shiffman ML *et al.* Peginterferon alfa-2b or alfa-2a with ribavirin for treatment of hepatitis C infection. *N Engl J Med* 2009; **361**: 580–93.

40 Hiramatsu N, Oze T, Yakushijin T *et al.* Ribavirin dose reduction raises relapse rate dose-dependently in genotype 1 patients with hepatitis C responding to pegylated interferon alpha-2b plus ribavirin. *J Viral Hepat* 2009; **16**: 586–94.

41 Patterson JL, Fernandez-Larsson R. Molecular mechanisms of action of ribavirin. *Rev Infect Dis* 1990; **12**: 1132–46.

42 Hultgren C, Milich DR, Weiland O, Sallberg M. The antiviral compound ribavirin modulates the T helper (Th) 1/Th2 subset balance in hepatitis B and C virus-specific immune responses. *J Gen Virol* 1998; **79**: 2381–91.

43 Crotty S, Maag D, Arnold JJ *et al.* The broad-spectrum antiviral ribonucleoside ribavirin is an RNA virus mutagen. *Nat Med* 2000; **6**: 1375–9.

44 Reichard O, Andersson J, Schvarcz R, Weiland O. Ribavirin treatment for chronic hepatitis C. *Lancet* 1991; **337**: 1058–61.

45 Di Bisceglie AM, Shindo M, Fong TL *et al.* A pilot study of ribavirin therapy for chronic hepatitis C. *Hepatology* 1992; **16**: 649–54.

46 Dusheiko G, Main J, Thomas H *et al.* Ribavirin treatment for patients with chronic hepatitis C: results of a placebo-controlled study. *J Hepatol* 1996; **25**: 591–8.

47 Bodenheimer HC, Jr, Lindsay KL, Davis GL, Lewis JH, Thung SN, Seeff LB. Tolerance and efficacy of oral ribavirin treatment of chronic hepatitis C: a multicenter trial. *Hepatology* 1997; **26**: 473–7.

48 Tanabe Y, Sakamoto N, Enomoto N *et al.* Synergistic inhibition of intracellular hepatitis C virus replication by combination of ribavirin and interferon- alpha. *J Infect Dis* 2004; **189**: 1129–39.

49 Davis GL, Esteban-Mur R, Rustgi V *et al.* Interferon alfa-2b alone or in combination with ribavirin for the treatment of relapse of chronic hepatitis C. International Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1493–9.

50 McHutchison JG, Gordon SC, Schiff ER *et al.* Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1485–92.

51 Kowdley KV. Hematologic side effects of interferon and ribavirin therapy. *J Clin Gastroenterol* 2005; **39**: S3–8.

52 Shipkova M, Lorenz K, Oellerich M, Wieland E, von Ahsen N. Measurement of erythrocyte inosine triphosphate pyrophosphohydrolase (ITPA) activity by HPLC and correlation of ITPA genotype-phenotype in a Caucasian population. *Clin Chem* 2006; **52**: 240–7.

53 Fraser JH, Meyers H, Henderson JF, Brox LW, McCoy EE. Individual variation in inosine triphosphate accumulation in human erythrocytes. *Clin Biochem* 1975; **8**: 353–64.

54 McHutchison JG, Manns MP, Muir AJ *et al.* Telaprevir for previously treated chronic HCV infection. *N Engl J Med* 2010; **362**: 1292–303.

IL-6と肝炎・肝発癌

植山 真由美* 中川 美奈*
坂本 直哉* 渡辺 守*

索引用語：IL-6，肝炎，肝細胞癌，男女差

1 はじめに

多機能性サイトカインとして知られるインターロイキン6(IL-6)は，局所炎症反応や組織障害に反応してマクロファージ・リンパ球・線維芽細胞・血管内皮細胞など多岐にわたる細胞より産生され，局所炎症性細胞に対する作用をはじめとして脳神経・内分泌系，免疫系，造血系細胞に対して幅広く作用する[1]．肝臓においてはクッパー細胞より産生され，CRP・ハプトグロビンなどの急性期蛋白を誘導する生体防御機構に関与する[2]．さらに血管内皮細胞増殖因子(vascular endothelial growth factor; VEGF)の産生を誘導することにより血管新生を促進し肝組織の再生・修復に寄与している(図1)．

肝疾患においてIL-6の重要性を示唆する報告はこれまでなされており，健常人に比べて慢性肝炎，肝硬変，肝細胞癌と病態が進行するにつれて血清IL-6値が高くなるという報告や[3,4]，肝癌マウスのIL-6発現に雌雄で相違があり，肝発癌の性差にIL-6が関与している可能性が示唆されている[5]．本稿ではIL-6と肝炎・肝癌との関連について，肝癌発症率の性差にも着目して概説する．

2 肝臓におけるIL-6発現

肝臓におけるIL-6の発現にはToll様受容体(Toll-like receptor; TLR)が関与している．TLRは細胞表面またはエンドゾームに局在する膜貫通型タンパク質で，TLRを発現している細胞が病原体の侵入を認識するとアダプター分子であるMyd88(Myeloid differentiation factor; 骨髄分化因子)を経由してシグナル伝達経路が活性化し炎症性サイトカインやⅠ型インターフェロン産生が誘導される[6]．肝細胞にウイルス感染もしくは細胞障害性サイトカインが作用して肝細胞が壊死やアポトーシスをきたすと肝壊死物質がクッパー細胞表面上に発現したTLRに結合し，Myd88依存性に転写因子であるNF-κB(nuclear factor-kappa B)を核に移行させIL-6の転写制御が行われる[7]．B細胞リンパ腫細胞株であるRaji細胞にHCVを感染させると細胞表面

Mayumi UEYAMA et al : Interleukin-6 and hepatitis, hepatocellular carcinoma
*東京医科歯科大学大学院医歯学総合研究科消化器病態学［〒113-8510 東京都文京区湯島 1-5-45］

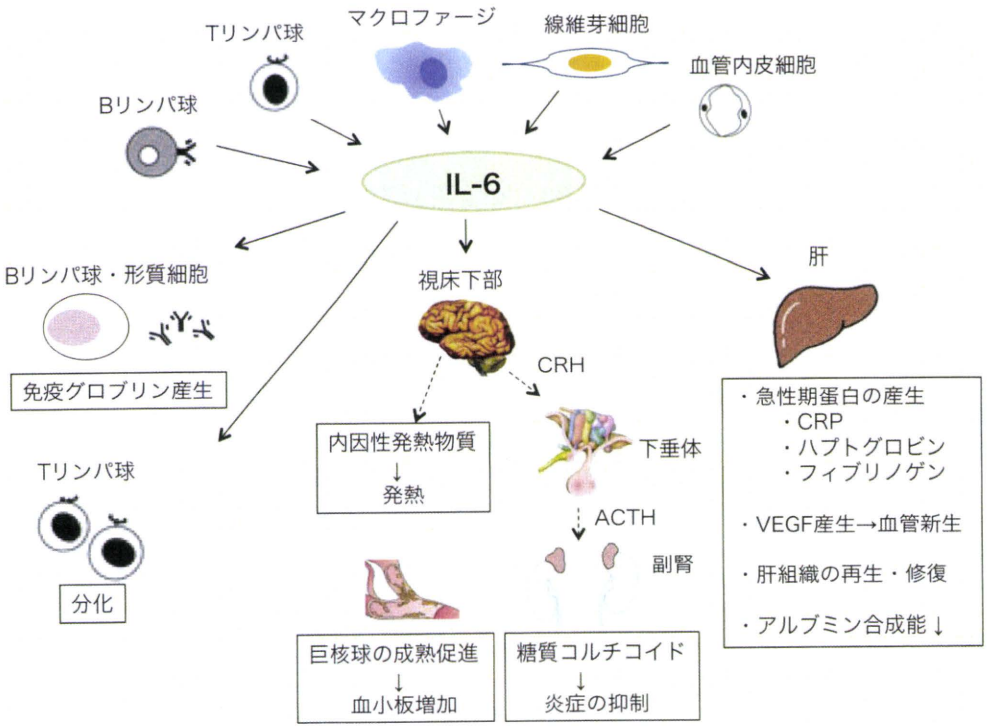

図1　IL-6の発現と全身の作用機序（文献1より引用，一部改訂）

IL-6はリンパ球・単球/マクロファージ・線維芽細胞・血管内皮細胞などから産生される．抗体産生やリンパ球分化への誘導，視床下部からのCRH放出促進などの生体防御反応をはじめ，肝臓では急性期炎症蛋白合成などに関与している．
CRH：副腎皮質刺激ホルモン放出ホルモン　ACTH：副腎皮質刺激ホルモン

にTLR4の発現が誘導され上清中IL-6濃度が上昇することが示されており[8]，IL-6が肝細胞表面上の受容体に結合すると，JAK-STAT経路を介してc-Myc，VEGF，Bcl-xLといった細胞増殖，血管新生促進，抗アポトーシス作用のある遺伝子の発現を調節し[9]，肝炎の遷延ならびに肝細胞癌への進展に関与すると考えられている（図2）．

3　IL-6と肝炎

血清中IL-6は健常人と比してウイルス性肝炎[10]・アルコール性肝炎[11]・NASH[12]などの慢性肝炎や急性肝炎・劇症肝炎で高値となる[13]ことが知られ，肝炎の回復と相関してIL-6値も正常化することから肝障害の程度を評価する指標として有用であると考えられている[13]．C型慢性肝炎に対するインターフェロン（interferon; IFN）治療経過とIL-6の推移については，投与前血清IL-6値がHAI scoreならびにHCV-RNA量と相関しSVR症例では治療後IL-6も低下すること[14]やIFN投与後の体温と血清IL-6値が相関すること[15]，Peg-interferon（Peg-IFN）α2a/Ribavirin（RBV）併用療法開始後2週目で高値となるが治療終了時には治療前レベルと同等になる[16]という報告がある．

われわれの検証ではPeg-IFN/RBV併用療法を導入したC型慢性肝炎患者173名の治療前血清IL-6値を測定すると，non SVR症例ではSVR症例に比較しIL-6が高い症例の占める

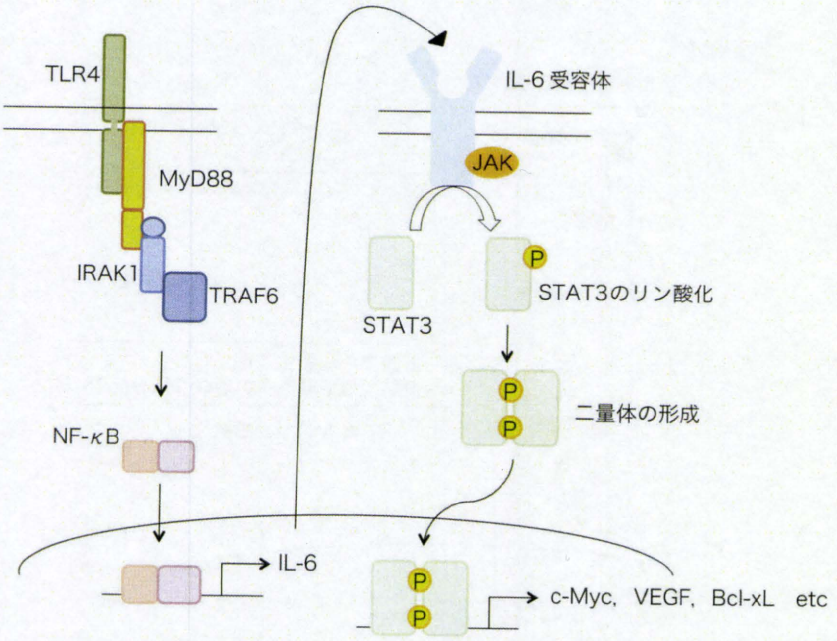

図2　Myd88依存性サイトカイン産生ならびにIL-6のシグナル伝達

TLRにリガンドが結合するとアダプターであるMyd88がTLRと結合し，IRAK1を活性化する．IRAK1はTRAF6と相互作用して転写因子のNF-κBを核に移行させ炎症性サイトカインの発現を誘導する．IL-6は細胞膜上に発現した受容体に結合するとJAK-STAT経路が活性化され，STAT3が二量体を形成後核内に移行し多くの遺伝子転写を制御する．

IRAK1（IL-1 receptor associated kinase），TRAF（TNF receptor associated factor 6），STAT3（Signal Transducers and Activator of Transcription-3）

割合が有意に高かった(カットオフ値: 5)．このことからIL-6がIFN治療抵抗性に関与している可能性が示唆された．また，男性，高齢，高ウイルス量，線維化の進展，ISDR変異少，core変異有と治療抵抗性を予測する患者背景の際に高値となる傾向を認めたことから，今後血清IL-6が治療効果予測因子となりうる可能性について引き続き検討中である．

IL-6が肝障害の進行や治療難治の程度と相関するという知見とは相反する報告もあり，肝損傷・肝切除後の再生ならびに肝機能維持において重要な因子であるとの報告もいくつかなされている[17-19]．肝切除後の肝細胞再生は1日以内に始まることが知られているが，TNF-αやIL-6が休止状態にあるG0 phaseにある肝細胞をG1周期へと移行させることが再生の契機となるという[17]．また，IL-6ノックアウトマウスを用いた研究では，IL-6-/-マウスはIL-6+/+マウスと比べて肝細胞のアポトーシスを高率にきたすものの，IL-6投与により肝障害の程度がIL-6+/+マウスと同等にまで回復することが示された[18]．また肝の一部を切除したIL-6-/-マウスでは黄疸・食欲不振を高率に認め死亡率が40％であったのに対し，IL-6+/+マウスならびにIL-6-/-マウスにIL-6を投与したマウス群の死亡率はそれぞれ10％，13.8％と有意に死亡率の低下を認めた[19]．以上のようにIL-6が肝再生や生存率に重要な役割を担っていることがノックアウト

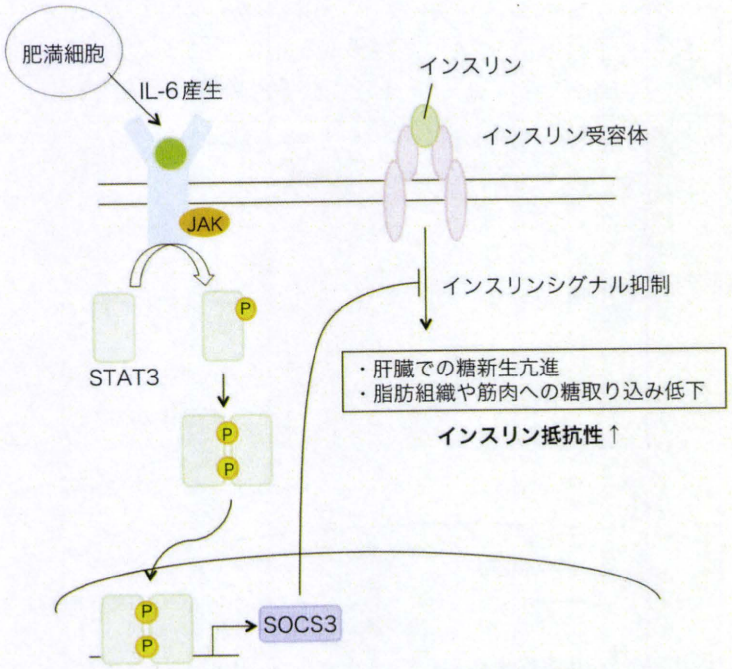

図3　IL-6シグナルとインスリン抵抗性の関係

過剰な脂肪蓄積により肥大化した脂肪細胞からIL-6が分泌される．IL-6が受容体に結合するとSTAT3が活性化されSOCS3の発現が増強する．これが肝臓，筋肉，脂肪組織でのインスリン作用を阻害しインスリン抵抗性を惹起する．

マウスの実験から得られているが，多機能性サイトカインとしてさまざまな働きをもつIL-6の多面性については今後解明すべき点が多いと思われる．

4　IL-6と脂質代謝・インスリン抵抗性

IL-6は血球系細胞などから分泌され，生体防御反応や免疫機能を制御するサイトカインである以外に脂肪組織から分泌される生理活性蛋白(アディポカイン)としての役割も担っており，非炎症時の血中IL-6の約30％が脂肪組織由来であるといわれている[20]．アディポカインは中枢神経系における食欲の制御や血圧調節，末梢組織での糖脂質代謝調節などの多様な作用を併せ持つが，血清中IL-6は肥満者[20]や2型糖尿病患者[21]で高値となることが報告されており，インスリン抵抗性の惹起因子と考えられている．その機序としてSOCS3(suppressor of cytokine signaling-3)が注目されている．過剰な脂肪蓄積により肥大化した脂肪細胞からIL-6が分泌されIL-6が受容体に結合すると，STAT3(Signal Transducers and Activator of Transcription-3)が活性化され，SOCS3の発現が増強，SOCS-3が肝臓，筋肉，脂肪組織でのインスリンシグナル伝達を阻害することでインスリン抵抗性が惹起されると報告されている[22](図3)．また，こうして惹起されたインスリン抵抗性が肝硬変や肝発癌を促進することも報告されていることから[23]，IL-6は糖尿病と肝炎・肝発癌の病態解明の接点としても，今後重要な役割を担う可能性が示唆される．

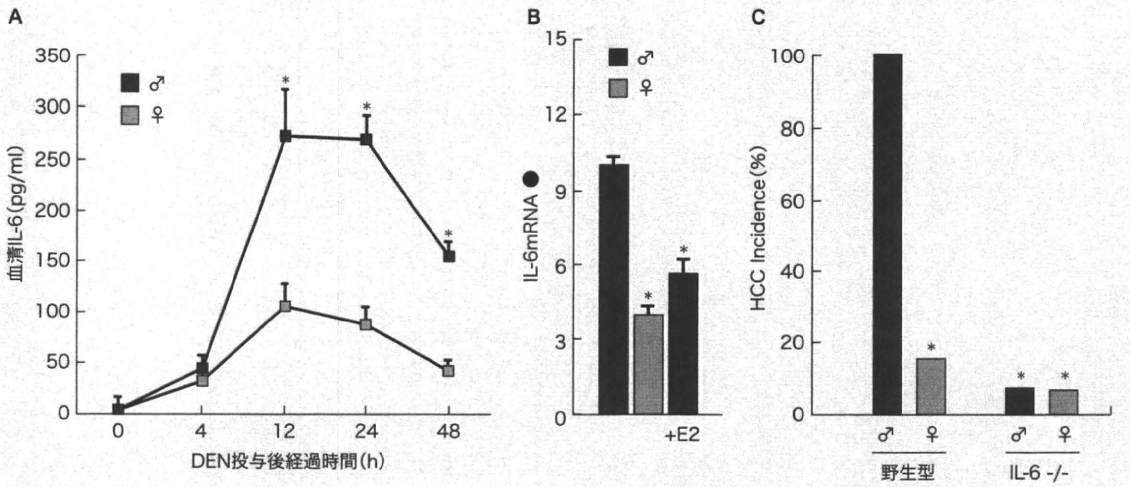

図4　DEN投与後の血清IL-6，肝臓IL-6mRNA，発癌率の性別による比較（文献5より引用，一部改変）
DEN投与後の血清IL-6値は雄では雌の約3倍(A)，肝発現するIL-6mRNAも雄は雌の2倍以上となり，雄にエストラジオールを投与すると雌の発現レベルまで低下した(B)．DEN投与にて雄マウスでは全例肝発癌を認めたのに対し，雌マウスの発癌率は約1割にまで減少し，IL-6 -/-雄マウスでの発癌率は野生型雌レベル以下に低下した(C)．
(E2：エストラジオール　*p<0.005)

5　IL-6と肝発癌

全悪性腫瘍の約1～2割は感染や炎症が発生母地となり発症するが，代表的なものとして胃癌(ピロリ菌感染)，大腸癌(炎症性腸疾患)，膵癌(慢性膵炎)などがある．肝細胞癌においてもB型肝炎ウイルス(HBV)やC型肝炎ウイルス(HCV)といった肝炎ウイルスの関与が大きいが[9]，ウイルス性肝炎以外にアルコール性肝炎，非アルコール性脂肪性肝炎(non-alcoholic steatohepatitis; NASH)などからも発生し，これら脂肪性肝炎の病態にはインスリン抵抗性の関与が大きいと考えられている[24]．

肝細胞癌は国籍を問わず男性の有病率が女性よりも高いことが報告されており(男：女＝2：1～4：1)，好発年齢は女性と比べて男性の方が約5年早いことが知られている[25]．男女差が生じる理由としてはウイルス感染率，アルコール摂取量，喫煙率，肥満の差異に由来するとされるが，遺伝子や性ホルモンの関与も報告されている．IL-6と肝炎・肝細胞癌に関してもこれまで種々の報告があり，血清中のIL-6は腫瘍径と相関することや[4]，AFPとともに測定することで診断率が高くなることから肝細胞癌の有効な腫瘍マーカーとして今後有用となり得ると考えられている[26]．

性ホルモンと肝発癌の性差についてMyD88依存性IL-6産生がDEN(diethylnitrosamine)誘発HCC発生に関与していることがNauglerら[5]により明らかにされた．同筆者らはクッパー細胞によるIL-6産生がエストロゲンの介入により阻害されるため女性では肝発癌リスクが低下することを明らかにした．DEN投与後の血清IL-6値は，雄では雌の約3倍となり(図4-A)，肝臓に発現するIL-6mRNAも雄では雌の2倍以上であるのにエストロゲンを投与した雄では雌の発現レベルまで低下した(図4-B)．またDEN投与後に雄

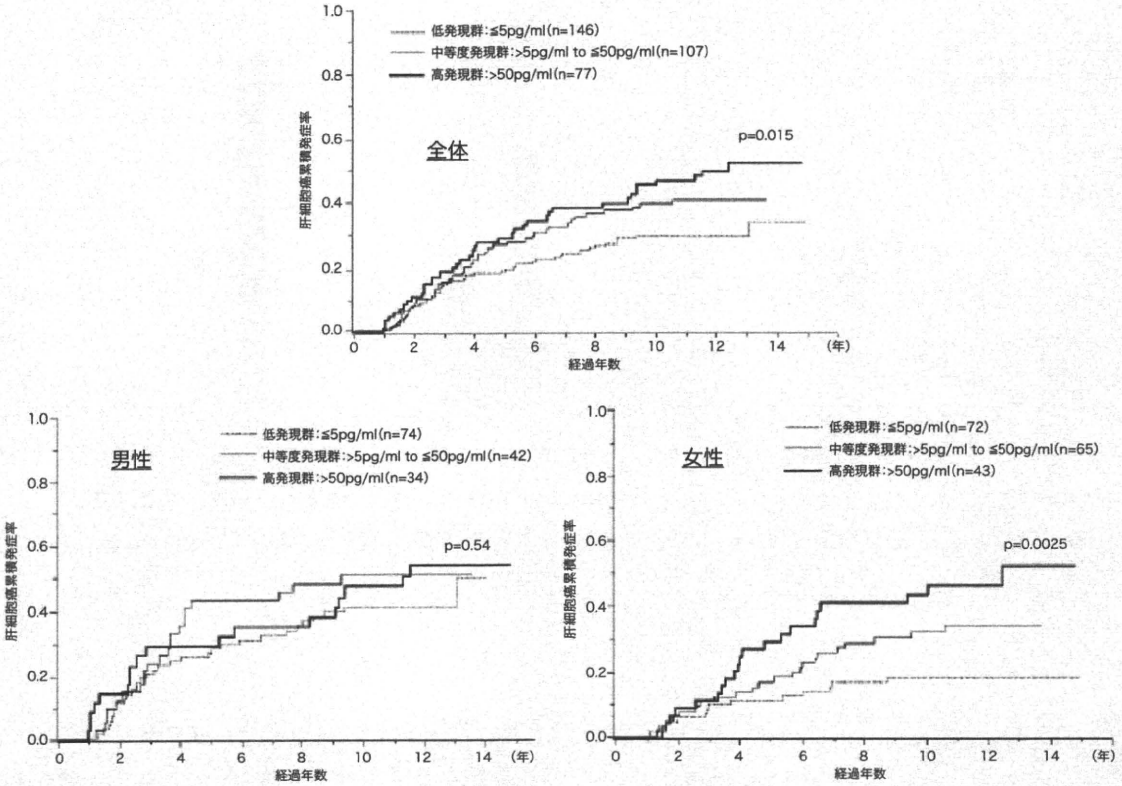

図5　血清IL-6値による肝細胞癌累積発症率の性別による比較（文献28より引用，一部改変）

血清IL-6値により低発現群(<5 pg/ml)，中等度発現群(5～50 pg/ml)，高発現群(50 pg/ml<)に分けて比較したところ，全体としてIL-6が高値になるにつれ発癌率が上昇し，男性よりも女性において有意な相関を認めた．

マウスでは全例肝発癌を認めたのに対し，雌マウスの発癌率は約1割にまで減少し，雄マウスでもIL-6をノックアウトすると発癌率は野生型雌レベル以下に低下したことがわかった(図4-C)．さらにMyd88をノックアウトした雄マウスにおいてもDEN投与後の肝発現IL-6mRNAならびに腫瘍数・腫瘍径は野生型マウスと比すると低下していることを示した．以上よりIL-6はクッパー細胞でTLRのMyd88依存性に発現制御がなされているが，エストロゲンはMyd88より上流でこの経路を阻害し，IL-6発現ならびにDEN誘発肝発癌を抑制していることが明らかとなった．

また，癌抑制遺伝子によるIL-6発現の調節ならびに肝発癌・生存率の関与を検討した報告がある．JiらはS100P(S100 calcium binding protein P)などの発現を制御する癌抑制遺伝子microRNA26a(miR26a)に着目し[27]，Microarrayにてmicro RNA26aの発現が低下するとNF-κBならびにIL-6が関与する遺伝子ネットワークが活性化することを見いだした．腫瘍内miR26a発現量は同一患者の正常組織より低下しているのに対しIL-6mRNAは対照的に腫瘍内において正常組織よりも発現量が高いことを示した．また腫瘍内miR26a発現量が低いと生存期間が短いことからmiR26a発現低下がIL-6発現増加をもたらし発癌・進展に関与すると示唆した．性別に関しては正常組織のmiR26a発現量は女性が男性よりも高いことから男性と比して発