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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

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Background and aims: There is growing evidence that the response of hepatitis C virus (HCV) genotype 1b infected patients towards interferon (IFN) therapy is influenced by the number of mutations within the carboxy terminal region of the NS5A gene, the interferon sensitivity determining region (ISDR).

Patients and methods: In order to attain better insight into this correlation, a file comprising published data on ISDR strains from 1230 HCV genotype 1b infected patients, mainly from Japan and Europe, was constructed and analysed by logistic regression. Sustained virological response (SVR) was defined as negative HCV RNA six months after treatment.

Results: The distribution of wild-, intermediate-, and mutant-type ISDR sequences differed significantly between Japanese (n = 655) (44.1%, 37.6%, and 18.3%) and European patients (n = 525) (24.8%, 63.4%, and 11.8%; p < 0.001). There was a significant positive correlation between the number of ISDR mutations and SVR rate, irrespective of geographical region. The likelihood of SVR with each additional mutation within the ISDR was considerably more pronounced in Japanese compared with European patients (odds ratios 1.82 v 1.39; p < 0.001). Pretreatment viraemia of <6.6 log copies/ml and ISDR mutant-type infection was associated with an SVR rate of 97.1% in Japanese patients but only 52.5% in European patients. Pretreatment viraemia was a stronger predictor of SVR than ISDR mutation number in Japanese patients whereas in European patients both parameters had similar predictive power.

Conclusion: These data support the concept that mutant-type ISDR strains may represent a subtype within genotype 1b with a more favourable response towards IFN therapy.

Interferon (IFN) therapy alone or in combination with ribavirin is currently the only available treatment for chronic hepatitis C virus (HCV) infection. However, this approach is not effective in all patients. Little is known about factors that can predict a favourable or unfavourable response to IFN treatment. The only accepted predictive parameters are age, pretreatment viral load, fibrosis stage, and HCV genotype. Thus nearly all patients with HCV genotype 2 and 3 infection can be cured by modern combination therapy, including pegylated IFN plus ribavirin. In contrast, patients with HCV genotype 1b infection, the prevalent genotype in Japan and Western countries, are poor responders and a sustained virological response (SVR) was observed in less than 10% after IFN monotherapy and in approximately 50% after combination therapy.^{1–4}

Studies have been undertaken in recent years which tried to explain the resistance of genotype 1b infection to IFN therapy. Enomoto *et al* were able to demonstrate a strong correlation between the number of mutations within the carboxy terminal region of the NS5A gene spanning codons 2209–2248, the interferon sensitivity determining region (ISDR), and response to IFN therapy.⁵ Thus no patient infected with the wild-type ISDR sequence (that is, identical to the prototype Japanese HCV strain (HCV-J)) responded to IFN therapy whereas all patients infected with the “mutant-type”, defined by four or more amino acid substitutions in this region, showed an SVR.⁶ These initial findings have been confirmed by other Japanese studies but controversial data were reported from other parts of the world, particularly from Europe and the USA. This may indicate that geographical

factors account for different sensitivities of HCV genotype 1b infection towards antiviral therapy.^{7–34}

We therefore investigated, in a meta-analysis comprising published data on 1230 ISDR sequences, the relationship between the number and patterns of mutations within the ISDR and responsiveness to IFN therapy with respect to geographical area.

MATERIAL AND METHODS

Patients

A literature search with the goal of finding articles on HCV ISDR sequences was conducted using the MEDLINE database. The following criteria had to be fulfilled to be included in this analysis: consecutive patients had to belong to genotype 1b and had to be treated with IFN alone or in combination with ribavirin. Dual publications were excluded. Only those patients who became negative for serum HCV RNA six months after therapy were defined as SVR; others were labelled as non-responders (NR). Twenty two HCV infected patients from our department were also included in this study. In these patients, HCV genotyping and HCV RNA quantitation as well as sequencing of the NS5A-ISDR region were performed as described elsewhere.²⁰

Data on ISDR strains from 1230 patients were collected (table 1). They were analysed with respect to the number of mutations in the ISDR sequences and their relationship to

Abbreviations: SVR, sustained virological response; HCV, hepatitis C virus; IFN, interferon; ISDR, interferon sensitivity determining region; NR, non-response; OR, odds ratio; PKR, RNA dependent protein kinase

Table 1 Sustained response to treatment with interferon (IFN) alone or in combination with ribavirin in patients with hepatitis C virus genotype 1b infection according to the number of mutations in the interferon sensitivity determining region

Author	Total	Wild-type (no mutation)	Intermediate type (1–3 mutations)	Mutant-type (≥4 mutations)	p Value*
Japan					
Komatsu 1997 ³⁵	3/10 (30.0%)	3/8 (37.5%)	0/2	0/0	NS
Kurosaki 1997 ⁷	4/22 (18.2%)	0/10	0/6	4/6 (66.7%)	0.002
Fukuda 1998 ⁸	5/31 (16.1%)	0/16	2/11 (18.2%)	3/4 (75.0%)	0.001
Arase 1999 ⁹	8/46 (17.4%)	3/25 (12.0%)	2/16 (12.5%)	3/5 (60.0%)	0.049
Nakano 1999 ¹⁰	12/52 (23.1%)	2/21 (9.5%)	5/23 (21.7%)	5/8 (62.5%)	0.005
Murashima 1999 ¹¹	24/57 (42.1%)	1/17 (5.9%)	6/16 (37.5%)	17/24 (70.8%)	0.001
Chayama 1997 ¹²	31/103 (30.1%)	9/47 (19.1%)	8/37 (21.6%)	14/19 (73.7%)	0.001
Watanabe 2001 ¹³	81/334 (24.3%)	12/145 (8.3%)	20/135 (14.8%)	49/54 (90.7%)	0.001
Total	168/655 (25.6%)	30/289 (10.4%)	43/246 (17.5%)	95/120 (79.2%)	0.001
Taiwan					
Lo 2001 ¹⁴	6/11 (54.5%)	5/9 (55.6%)	1/2 (50.0%)	0/0	NS
Europe					
McKechnie 2000 ¹⁵	2/8 (25.0%)	0/1	1/4 (25.0%)	1/3 (33.3%)	NS
Rispeter 1998 ¹⁶	4/13 (30.8%)	0/0	4/13 (30.8%)	0/0	NS
Frangoul 1998 ¹⁷	3/17 (17.6%)	0/3	3/13 (23.1%)	0/1	NS
Duvelie 1998 ¹⁸	8/19 (42.1%)	1/5 (20.0%)	5/12 (41.7%)	2/2 (100.0%)	NS
This study†	6/22 (27.3%)	2/3 (66.7%)	3/16 (18.8%)	1/3 (33.3%)	NS
Zeuzem 1997 ¹⁹	1/22 (4.5%)	0/11	1/10 (10.0%)	0/1	NS
Berg 2000 ²⁰ †	3/23 (13.0%)	0/4	2/17 (11.8%)	1/2 (50%)	NS
Stratidaki 2001 ²¹	2/28 (7.1%)	0/5	0/21	2/2 (100.0%)	0.001
Gerotto 2000 ²²	8/30 (26.7%)	1/6 (16.7%)	5/18 (27.8%)	2/6 (33.3%)	NS
Saiz 1998 ²³	8/36 (22.2%)	0/10	2/20 (10.0%)	6/6 (100.0%)	0.001
Khorsi 1997 ²⁴	17/43 (39.5%)	3/13 (23.1%)	13/28 (46.4%)	1/2 (50.0%)	NS
Squadrito 1997 ²⁵	5/48 (10.4%)	0/14	4/28 (14.3%)	1/6 (16.7%)	NS
Halfon 2000 ²⁶	8/70 (11.4%)	0/16	7/38 (18.4%)	1/16 (6.3%)	NS
Sarrazin 1999 ²⁷ †	12/72 (16.7%)	0/19	9/47 (19.1%)	3/6 (50.0%)	0.004
Puig-Basagoini 2001 ²⁸	11/74 (14.9%)	1/20 (5.0%)	4/48 (8.3%)	6/6 (100.0%)	0.001
Total	98/525 (18.0%)	8/130 (6.2%)	63/333 (18.9%)	27/62 (43.5%)	0.001
USA					
Murphy 2002 ²⁹	1/5 (20.0%)	0/1	1/4 (25.0%)	0/0	NS
Hofgärtner 1997 ³⁰	1/6 (16.7%)	0/1	1/5 (20.0%)	0/0	NS
Nousbaum 2000 ³¹	2/6 (33.3%)	0/1	2/5 (40.0%)	0/0	NS
Chung 1999 ³²	2/22 (9.1%)	0/6	2/11 (18.2%)	0/5	NS
Total	6/39 (15.4%)	0/9 (0%)	6/25 (24.0%)	0/5	NS
Total	278/1230 (22.6%)	43/437 (9.8%)	113/606 (18.6%)	122/187 (65.2%)	0.001

Values are number of patients with a sustained response/total number of patients (%).

NS, not significant.

* χ^2 test;

†IFN in combination with ribavirin.

the outcome of IFN therapy. We found 278 IFN sensitive strains and 952 IFN resistant strains, derived from patients with SVR and NR, respectively.

Phylogenetic analysis and secondary structure

Multiple sequence alignments were performed with the CLUSTALW program, version 6. Phylogenetic trees were constructed by means of the Phylogeny Interference Package (PHYLP, University of Washington, Seattle, Washington, USA) version 3.57c.³⁶ Evolutionary distances were estimated using the DNAdist program and phylogenetic trees were constructed with NEIGHBOR (PHYLP). Additionally, Seqboot (PHYLP) was used to create 100 replicates for calculation of bootstrap values.³⁷ The program used for conformational analysis was Protean version 5.03 (1994-002 DNASTAR, Inc).³⁸

Statistical analysis

Analysis of continuous variables was performed using the Mann-Whitney or Student's *t* test, and analysis of categorical variables with the χ^2 test. For adjustment of multiple testing of several ISDR sites, the Bonferroni correction by factor 40 was applied. Multiple logistic regression analysis was used to examine simultaneously the influence of several factors on viral response. Prognostic factors between European and Japanese studies were compared using interaction terms in logistic regression analyses. The level of significance was 0.05 (two sided) in all statistical tests. All analyses were carried

out using SPSS Inc. for Windows (release 11.0; Chicago, Illinois, USA).

RESULTS

Geographical distribution of ISDR mutations

Following Enomoto's classification, it emerged that the distribution of wild- (identical to HCV-J), intermediate- (1–3 mutations), and mutant- (≥4 mutations) type ISDR sequences was significantly different in Japan compared with Europe (fig 1A). In detail, only five of 40 ISDR positions (H 2218, D 2220, A 2224, E 2236, G 2239) showed a significant difference in mutation frequency between Japanese and European ISDR strains (fig 2).

ISDR mutations in relation to SVR

Clear positive correlations between SVR and types of ISDR sequences were demonstrated for Japanese as well as European patients (table 1). However, SVR rates in Japanese patients infected with the mutant ISDR type were higher compared with European patients (fig 1B). SVR increased with each additional number of mutations within the ISDR but this relationship was more pronounced in Japanese patients (odds ratio (OR) 1.82 v 1.39; $p < 0.001$) (fig 3).

OR values for SVR with respect to mutation frequency at single ISDR sites were generally higher in Japanese patients (table 2). In a multivariate logistic regression analysis, the mutation frequency at any one of 40 ISDR sites did not

Figure 1 Distribution of wild-, intermediate-, and mutant-type interferon sensitivity determining region (ISDR) sequences (A) and percentage of sustained virological response (SVR) in patients infected with wild-, intermediate-, and mutant-type ISDR strains in Japan and Europe (B).

improve the likelihood of an SVR compared with the total number of mutations per individual.

The frequency of individual amino acid substitutions, which occurred only in strains isolated from patients with SVR or NR, was low (<5%) and had no statistical relevance in the prediction of SVR.

Phylogenetic and hydrophobicity analysis of ISDR

Neither cluster of ISDR isolates recovered from Japanese or European patients nor cluster of IFN sensitive and IFN resistant ISDR isolates into different branches could be obtained by phylogenetic tree analysis (fig 4). In spite of the high diversity of amino acid substitutions, hydrophobic plots, calculated by the method of Kyte-Doolittle, demonstrated that these substitutions had no major influence on the hydrophobicity of the ISDR protein.

Factors affecting SVR

Forty four European patients received IFN in combination with ribavirin (table 1). Combination therapy influenced neither the overall percentage of SVR (18.3% in patients with

IFN monotherapy versus 25% in patients with combination therapy; $p = 0.31$) nor the proportion of SVR with respect to ISDR sequence type in the European patient group ($p = 0.35$, interaction term "number of mutations" with "therapy" in logistic regression analysis for SVR). Although not significant, the likelihood of SVR with each additional mutation within the ISDR was higher in patients receiving IFN in combination with ribavirin in comparison with patients receiving IFN alone (OR 1.67 (95% confidence interval (CI) 1.10–2.53) versus 1.36 (95% CI 1.21–1.53); $p = 0.33$).

Pretreatment viral loads were available in 459 Japanese and 315 European patients. A median HCV RNA concentration of 6.6 log copies/ml served as a cut off level to differentiate between patients with high or low viraemia. Regardless of the ISDR sequence type, pretreatment viraemia of ≥ 6.6 log copies/ml was associated with an SVR rate of 0% in Japanese but 8.8% in European patients, whereas pretreatment viraemia of < 6.6 log copies/ml was associated with an SVR rate of 86.6% in Japanese and only 21.3% in European patients (table 3). Moreover, ISDR mutant-type infection and pretreatment viraemia of < 6.6 log copies/ml were associated with an SVR rate of 97.1% in Japanese but only 52.4% in European patients (table 3).

The ISDR sequence type was a significant response predictor in patients with a pretreatment viral load of < 6.6 log copies/ml in Japan (OR 3.56 (95% CI 1.78–7.13); $p < 0.001$) and in Europe (OR 4.41 (95% CI 2.08–9.36); $p < 0.001$) (table 3). All Japanese patients with a pretreatment viral load of ≥ 6.6 log copies/ml were non-responders, and thus no dependency on ISDR sequence type was detected. In European patients with a pretreatment viral load of ≥ 6.6 log copies/ml, a non-significant dependency (OR 2.59 (95% CI 0.96–7.01); $p = 0.055$) was observed (table 3).

A negative correlation between the number of ISDR mutations and pretreatment viral load was observed in Japanese ($r = 0.58$, $p < 0.001$) as well as European patients ($r = 0.08$, $p = 0.043$) (fig 5). The difference between these correlation coefficients was significant ($p = 0.001$, interaction term in regression analysis for initial virus load).

Cumulative IFN doses prescribed within the first four and 24 weeks were significantly higher in Japanese in comparison with European patients (four weeks: 185 (48) MU (range 80–224) v 56 (29) MU (range 36–219); 24 weeks: 655 (104) MU (range 456–736) v 290 (87) MU (range 216–675)) (fig 6).

Figure 2 Frequency of mutations at each amino acid position of the interferon sensitivity determining region (ISDR) in Japanese in comparison with European patients. Differences between Japanese and European patients were tested by χ^2 test after Bonferroni correction by a factor 40 (equal to the number of positions tested, equal to the number of amino acids of ISDR, respectively). *ISDR positions significantly different between Japan and Europe.

Figure 3 Sustained virological response (SVR) rates according to number of mutations within the interferon sensitivity determining region (ISDR) in Japan and Europe. Significant correlation between number of ISDR mutations was noted in both Japanese and European patients. With each additional mutation, the SVR likelihood was higher in Japanese in comparison with European patients (odds ratio 1.82 v 1.39; $p < 0.001$). Logistic regression lines are represented as broken lines and symbols.

The influence of geographical area (Europe versus Japan) and cumulative IFN doses administered within the first four weeks with respect to SVR was analysed in a multiple logistic regression analysis (table 4). Because of the strong negative correlation with SVR in Japanese patients, thereby masking

other statistical relationships, pretreatment viraemia was not included in this analysis. Thus according to this analysis, it cannot be concluded whether, after adjustment for the number of mutations, the different cumulative IFN dose or geographical area is responsible for the different response rates between Europe and Japan: inclusion of treatment dose makes geographical area insignificant and vice versa.

Table 2 Odds ratios (OR) for a sustained virological response (SVR) with respect to mutation frequency at each of 40 interferon sensitivity determining region (ISDR) sites in Japanese and European patients

ISDR	Japan OR (95% CI)	Europe OR (95% CI)
P 2209	27.46 (13.6–55.5)	3.25 (1.6–6.8)
S 2210	6.0 (0.5–67.3)	0.81 (0.7–0.8)
L 2211	25.21 (3.1–203.2)	0.18 (0.1–0.2)
K 2212	7.29 (3.7–14.5)	1.70 (0.5–5.5)
A 2213	7.71 (1.5–40.1)	*
T 2214	16.8 (8.8–32.0)	5.12 (2.4–11.0)
C 2215	15.25 (4.3–53.8)	6.43 (1.1–39.1)
T 2216	16.87 (8.0–35.8)	1.56 (0.7–3.5)
T 2217	8.78 (4.8–16.1)	3.04 (1.4–6.5)
H 2218	2.50 (1.7–3.6)	1.99 (1.2–3.3)
H 2219	6.46 (3.3–12.7)	1.64 (0.6–4.7)
D 2220	5.20 (3.1–8.8)	0.80 (0.7–0.8)
S 2221	17.98 (6.1–53.2)	3.09 (1.9–9.7)
P 2222	0.24 (0.2–0.3)	0.81 (0.7–0.8)
D 2223	4.03 (1.5–11.0)	2.86 (0.8–10.3)
A 2224	5.1 (3.3–8.0)	9.73 (4.9–19.7)
D 2225	2.01 (0.3–12.2)	4.31 (1.1–17.6)
L 2226	10.93 (2.2–53.2)	0.81 (0.7–0.8)
I 2227	13.74 (6.8–27.6)	3.56 (1.9–6.8)
E 2228	7.93 (2.5–25.7)	8.84 (2.2–36.0)
A 2229	15.45 (1.8–133.3)	0.19 (0.1–0.2)
N 2230	6.13 (1.1–33.8)	*
L 2231	12.28 (1.4–110.8)	0.80 (0.7–0.8)
L 2232	3.84 (1.0–14.5)	0.81 (0.7–0.8)
W 2233	9.12 (0.9–88.7)	17.39 (2.0–157.4)
R 2234	7.29 (4.1–12.8)	1.17 (0.4–3.2)
Q 2235	10.52 (3.4–32.8)	3.44 (0.9–13.1)
E 2236	9.15 (0.9–88.6)	1.64 (0.6–4.7)
M 2237	2.75 (1.0–7.3)	0.80 (0.7–0.8)
G 2238	6.06 (0.5–67.3)	4.24 (0.6–30.5)
G 2239	0.74 (0.7–0.8)	7.29 (2.7–19.3)
N 2240	18.38 (8.4–40.4)	2.71 (1.1–6.7)
I 2241	1.50 (0.1–16.7)	2.55 (0.6–10.9)
T 2242	0.75 (0.7–0.8)	0.80 (0.7–0.8)
R 2243	*	0.80 (0.7–0.8)
V 2244	*	8.68 (1.6–48.1)
E 2245	0.75 (0.7–0.8)	17.39 (1.9–157.4)
S 2246	0.75 (0.7–0.8)	*
E 2247	0.25 (0.2–0.3)	10.43 (2.7–41.1)
N 2248	11.29 (4.1–31.1)	2.86 (0.8–10.3)

*No mutation.

DISCUSSION

A database comprising information on ISDR strains from 1230 genotype 1b patients, mainly from Japan and Europe, was constructed and analysed by logistic regression analysis with the aim of studying the relationship between number of mutations within the ISDR and response to IFN treatment.

A clear positive association was observed between the SVR rate and number of mutations within the ISDR, regardless of geographical region, but this correlation was more pronounced in Japan compared with Europe. SVR rates were nearly twice as high (79%) in Japanese patients infected with the mutant-type compared with the European group (43.5%). Furthermore, with each additional ISDR mutation there was a higher odds ratio for SVR in Japanese compared with European patients.

In two earlier studies it was suggested that these differences in SVR rates could be partially attributed to under representation of the mutant-type in European population.^{33–34} However, in this meta-analysis conducted in a much larger population, the mutant-type was approximately equally represented in both geographical areas. Therefore, the stronger correlation between ISDR mutation and SVR rate observed in Japanese patients cannot be explained by a different geographical distribution of ISDR mutations.

Treatment regimen (that is, IFN dose or IFN plus ribavirin combination therapy) is another factor which may account for the observed differences in SVR rates between Japan and Europe. Thus Japanese patients received significantly higher cumulative IFN doses within the first 4–24 weeks than European patients. In European patients who received IFN plus ribavirin, there was a non-significant trend for a higher SVR likelihood with each additional ISDR mutation in comparison with those who received IFN alone. However, the question of whether the different treatment regimens or geographical factors are mainly responsible for differences in SVR rates observed between Japanese and European patients cannot be answered.

Pretreatment hepatitis C viraemia was a strong predictor of SVR. In the presence of pretreatment HCV RNA levels

Figure 4 Phylogenetic tree of 158 interferon sensitivity determining region (ISDR) sequences with four or more mutations recovered from 178 Japanese and European patients with a non-response or sustained virological response to treatment. The scale bars represent 10%.

≥ 6.6 log copies/ml, no SVR was achieved in Japanese patients in contrast with European patients who showed an SVR of 8.8% under these conditions. On the other hand, low pretreatment HCV RNA levels in association with a mutant-type infection resulted in SVR rates of up to 97% in Japanese patients while European patients had rates of only 52.4%.

There was further evidence, derived from the multivariate analysis, that in Japanese patients the pretreatment viraemia parameter is a much stronger predictor of SVR than number of ISDR mutations, and this has also been described by Chayama and colleagues.¹² In contrast, in European patients both factors (that is, number of ISDR mutations and HCV RNA levels) were of similar importance. Furthermore, a negative correlation between number of mutations within the ISDR region and pretreatment viral load was observed in both Japanese and European patients. European or North American patients infected with strains with multiple ISDR mutations have been shown to respond with a more rapid first and second phase viral decline in the course of IFN therapy.^{20–38} Taken together, these data support the existence of geographical differences in HCV genotype 1b infection which can be attributed to a genetic viral factor (that is, the

existence of a Japanese specific group of HCV 1b isolates with different biological properties) or to an as yet unidentified host or racial factor.^{10–39–40} However, data concerning pretreatment HCV RNA concentrations were based on different methods of quantification, and therefore the analysis must be interpreted with caution.

There was a high diversity of individual ISDR mutations among the different strains regardless of whether they were isolated from responders or non-responders. As shown by multivariate logistic regression analysis, the mutation frequency at any ISDR site had no effect on SVR rates compared with the total number of mutations per individual. Phylogenetic analysis revealed no clusters that could be correlated with geographical area or treatment response. In spite of the high diversity, no important changes in the hydrophobicity of the ISDR protein were observed. Thus it seems that IFN sensitive phenotype relates rather to the number of substitutions and not to specific substitutions pattern of the ISDR.

The biological role of ISDR is not clear. Studies have revealed that the NS5A protein binds and represses the IFN induced double stranded RNA dependent protein kinase

Table 3 Relationship between sustained virological response (SVR) and pretreatment viral level, geographical area, and interferon sensitivity determining region (ISDR) sequence type.

Pretreatment viraemia	ISDR type	Japan		Europe	
		NR	SVR	NR	SVR
≥ 6.6 log copies/ml*	Wild	171 (100%)	—	35 (100%)	—
	Intermediate	144 (100%)	—	71 (88.8%)	9 (11.3%)
	Mutant	10 (100%)	—	19 (86.4%)	3 (13.6%)
	Total	325 (100%)	—	125 (91.2%)	12 (8.8%)
<6.6 log copies/ml*	Wild	10 (32.3%)	21 (67.7%)	39 (95.1%)	2 (4.9%)
	Intermediate	6 (17.6%)	28 (82.4%)	91 (78.4%)	25 (21.6%)
	Mutant	2 (2.9%)	67 (97.1%)	10 (47.6%)	11 (52.4%)
	Total	18 (13.4%)	116 (86.6%)	140 (78.7%)	38 (21.3%)

For each of the 12 subgroups defined by the combinations of pretreatment viraemia, continent, and ISDR sequence type, the number (%) of patients is given. Patients with high pretreatment viral levels and SVR were only observed in European studies. In contrast, SVR rates were higher in Japanese patients with low pretreatment viral levels. SVR was dependent on the ISDR sequence type in both areas

NR, non-responders.

*6.6 log copies/ml = median viraemia.

Figure 5 Correlation between pretreatment viral load and number of interferon sensitivity determining region (ISDR) mutations in Japanese and European patients. Logistic regression lines are represented by continuous and broken lines, and symbols. This difference in correlations was significant ($p=0.001$, interaction term between continent and ISDR mutations in a linear regression analysis for initial virus load).

(PKR). The PKR interacting domain of NS5A, spanning amino acid positions 2209–2274, comprises the ISDR, and the NS5A-PKR interaction is disrupted by the ISDR mutation which corresponds to IFN sensitive HCV, resulting in repression of PKR function and phosphorylation of eIF-2 α .⁴¹ NS5A expression blocked the antiviral effects of IFN in human cells in a quantitative manner and was genotype dependent, NS5A of genotype 1b being more potent in blocking the antiviral effects of IFN than NS5A from genotype 1a.^{42–43} Thus the IFN sensitive ISDR phenotype could influence post translational processing of NS5A and thereby in conjunction with other viral or host factors acting to direct the replication of the HCV genome.⁴⁴

Mutations in other regions of the NS5A protein (that is, PKR binding domain, V3 region, carboxy-terminal) could also be correlated with SVR.^{18–19–22–31} Sarrazin *et al* identified mutations within the NS5A region, spanning amino acid positions 2350–2370, to be relevant for treatment response.⁴⁵ However, the fact that some European patients showing either no mutation within the ISDR or high pretreatment viral load have been able to eliminate the virus indicates the presence of other unidentified host or viral factors that influence the response to antiviral treatment.

Figure 6 Cumulative interferon (IFN) dosages prescribed within the first four and 24 weeks in Japanese and European patients. The circle symbol represents the mean value; bottom and top edges of the box represent 25th and 75th percentile coefficient 1; whiskers indicate range 5–95 coefficient 1.5; and asterisks (*) represent 1% and 99% percentiles (minimum and maximum).

This meta-analysis suggests the existence of a subgroup of European patients which can be defined as non-responders. Thus no European patient infected with the ISDR wild-type and with a high pretreatment viraemia responded to IFN therapy. In contrast, European patients infected with the mutant ISDR type and expressing low pretreatment viral load were highly responsive to standard dose IFN monotherapy, hereby achieving SVR rates of 52.4% (table 3). This treatment response is similar to that of pegylated IFN plus ribavirin for HCV type 1 infection.^{46–47} In Japanese patients the situation is different. Regardless of ISDR type, a Japanese patient with a high pretreatment viraemia would not respond to treatment. Japanese patients infected with the ISDR mutant-type having a low pretreatment viraemia can achieve SVR rates of up to 100% when treated with high dose IFN. This is in accordance with the Markov decision analysis model proposed by Moriguchi *et al* which shows that IFN monotherapy has little effect when given to HCV genotype 1 infected patient who are older than 50 years, have hepatitis C viraemia exceeding 1.0 mEq/ml, and have no amino acid mutations in the ISDR.⁴⁸

In conclusion, there is clear evidence from this meta-analysis for an association between number of ISDR mutations and SVR rate. It is however worthwhile further investigating whether ISDR mutations also influence response in those patients who have received combination therapy with pegylated IFN. In addition, these data imply that mutant ISDR strains represent a subtype with different biological properties within genotype 1b.

Table 4 Multiple logistic regression analysis for viral response

	Model 1 ($p<0.001$, $\chi^2=237.8$, df = 1)	Model 2 ($p<0.001$, $\chi^2=246.0$, df = 2)	Model 3 ($p<0.001$, $\chi^2=246.8$, df = 2)	Model 4 ($p<0.001$, $\chi^2=247.1$, df = 3)
No of mutations within the ISDR	1.63 (1.51–1.75)	1.62 (1.51–1.75)	1.62 (1.51–1.75)	1.62 (1.51–1.75)
Geographical area (Japan v Europe)	—	1.61 (1.15–2.26)	—	1.19 (0.60–2.36)
IFN cumulative dose in the first 4 weeks	—	—	1.65 (1.18–2.30)	1.41 (0.72–2.78)

The effect of the number of mutations was not modified after inclusion of geographical area, interferon (IFN) cumulative dose, or both (models 1–4). Geographical area and IFN cumulative dose were almost equally important for the prognosis of sustained virological response (models 2 and 3). However, neither inclusion of IFN dose with number of mutations and geographical area nor inclusion of geographical area with number of mutations and IFN dose improved the model (model 4 compared with models 2 and 3, respectively).

For each model, the overall test for all covariates is given (degrees of freedom (df) are identical to numbers of covariates). Odds ratios (with 95% confidence intervals) are displayed for each covariate and separately for each model.

Model equations: for p =probability of sustained viral response, $\text{logit}=\text{log}[p/(1-p)]$, X_1 =number of ISDR mutations (quantitative variable), X_2 coding the geographical area (0=Europe, 1=Japan), and X_3 coding the cumulative dose (0=less or equal to 100 ..., 1=more than 100 ...).

The model equations are: $\text{logit}=\beta_0+\beta_1X_1$ (model 1), $\text{logit}=\beta_0+\beta_1X_1+\beta_2X_2$ (model 2), $\text{logit}=\beta_0+\beta_1X_1+\beta_3X_3$ (model 3), and $\text{logit}=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3$ (model 4).

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