extracellular matrix turnover (matrix metalloproteinases and their inhibitors) or cytokines that are believed to have a profibrogenic (TGFβ and related molecules) or an anti-fibrogenic (IFNγ and its receptors) activity. A total of 36 genes were selected and are shown in table 1. A selection of SNPs within each gene, totalling 384 SNPs (list in online supplementary table 1), was made using data from the first public release of HapMapII. For each gene, all HapMap SNPs in the region including the gene and the 10 kb flanking regions were initially considered. SNPs with minor allele frequency <5% or with low predicted quality for genotyping (calculated by Illumina) were filtered out, and pairwise linkage disequilibrium (LD) was estimated (from the HapMap data) between all pairs of remaining SNPs within each gene. The 384 SNP panel was then selected such that no two SNPs in the same gene had an estimated $r^2 \geq 0.8$ or were <60 bp apart.

All DNA samples were extracted from whole blood, and subjected to rigorous quality control to check for fragmentation and amplification. All SNPs were genotyped on an ultra-high throughput Illumina platform. This platform uses the GoldenGate assay followed by a bead-based technology to resolve individual SNP genotypes.[18] Discovery of SNPs within an *IFNGR2* region of ~12.3 kb from 33 689 894 to 33 702 179 bps on chromosome 21 was performed by exhaustive sequencing (figure 1). The sample consisted of 32 French Caucasian subjects (men and women with no disease history) from the Epidemiological study on the Genetics and Environment of Asthma.[19] The sample size of 32 allowed us to detect SNPs with a minor allele frequency of at least 5% with a probability of 96%. Sequencing reactions were performed with the Dye Terminator method using an ABI PRISM 3730 DNA Analyser (Applied Biosystems, Foster City, California, USA). Sequence alignment and SNP discovery were performed with Genalys software, developed by the Centre National de Génotypage (CNG).[20]

Statistical methods

Association between severe fibrosis and the panel of SNPs was first tested in sample A by a case–control analysis using the genotypic test statistic (two degrees of freedom): the cases are HCV-infected patients with severe fibrosis and the controls are infected patients without severe fibrosis. When a type I error of 0.02 was used, our initial sample A had a power of 80% for detecting a polymorphism with an additive effect, providing an odds ratio (OR) for heterozygosity of two and having a frequency >0.09. For SNPs showing association at $p<0.02$, we then tested association by a survival analysis approach using a Cox model: we considered as starting points the estimated ages at infection, and as end points either the first biopsy showing severe fibrosis (failure time) or the last biopsy showing absence of severe fibrosis in the absence of any treatment (censored time). For all these analyses, we determined the genetic model (dominant/additive/recessive) providing the best fit to the data. SNPs showing the most interesting results ($p<0.02$ in the case–control study and $p<0.05$ in the survival analysis) were then tested for replication in sample B.

We tested for heterogeneity of the association results according to different criteria, such as gender, mode of infection (blood transfusion/IVD use/others), viral genotypes (one and four vs others), age at infection (≤20 years vs >20 years). Under the hypothesis of homogeneity of association, twice the difference between the likelihood of the whole sample and the summed likelihoods of the subsamples (eg, the two subsamples of men and women) is asymptotically distributed as a χ^2 with one degree of freedom. All statistical analyses were performed using different procedures (FREQ, LOGISTIC, PHREG) implemented in SAS software version 8.2 (SAS Institute). Pairwise LD between SNPs was assessed by determining the r^2 coefficient using the Haploview software.[21] Haplotype analysis was conducted using the THESIAS software (http://www.genecanvas.org, accessed 5 May 2010).[22]

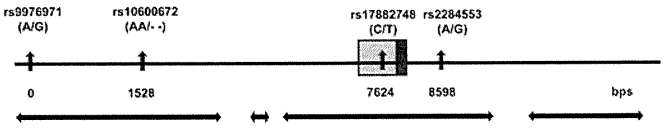

Figure 1 Schematic representation of the chromosome 21 region ranging from 33 689 800 to 33 702 200 bps, and including the 5′ region, exon 1 and part of intron 1 of *IFNGR2*. Exon 1 ranges from 33 697 072 to 33 697 792 bp with an untranslated and a translated part shown as a hatched and a solid box, respectively. Horizontal arrows indicate the regions covered by direct sequencing. Three segments could not be sequenced for technical reasons (33 694 486–33 696 000, 33 696 161–33 696 390 and 33 699 114–33 700 075 bp). The four single nucleotide polymorphisms associated with severe fibrosis are indicated by vertical arrows with distance in base pairs provided from the position of rs9976971, which is located at 33 689 967 bp.

RESULTS

Description of the two samples

Samples A and samples B consisted of a total of 267 (103 F3–4 patients with severe fibrosis and 164 F0–1 patients without severe fibrosis), and 126 (31 F3–4 and 95 F0–1 patients) patients with chronic HCV infection, respectively. The main features of the overall sample including 393 patients are shown in table 2. There was an overall excess of women (58.5% vs 41.5%, $p=8\times10^{-4}$), which might be explained, in part, by the inclusion criterion of low alcoholic consumption, but there was no significant difference ($p=0.24$) in the distribution of gender according to fibrosis status. In the 364 patients with reliable HCV acquisition data, the overall distribution of modes of infection was different ($p=0.0004$) according to the fibrosis status. The proportion of patients infected by blood transfusion was higher in patients with severe fibrosis (57.5%) than in F0–1 patients (41.3%), while the reverse was observed for IVD users (43% in F0–1 patients vs 22% in F3–4 patients). F3–4 patients were significantly older ($p=0.004$) at infection (mean 29.9 years, range 0.1–73.2 years) than F0–1 patients (25.6 years, 0.1–70.8 years), and had a longer ($p=10^{-4}$) duration of HCV infection at the time of biopsy (22.8 years, 0.7–49.6 years vs 18.9 years, 0.2–49.6 years). Finally, the distribution of viral genotypes combined as usual in three main groups according to their sensitivity to anti-viral treatment[23] was not significantly different between F0–1 and F3–4 patients.

Four variants are associated with fibrosis in the first sample

Of the 384 SNPs, 16 SNPs could not be genotyped (supplementary table 1), and we excluded an additional five SNPs because either they showed deviations ($p<0.005$) from Hardy–Weinberg equilibrium (three SNPs) or they had a minor allele frequency <0.02 (two SNPs). The 363 remaining SNPs all showed a genotyping success >96%, and were used for association analysis. Association between severe fibrosis and the panel of 363 SNPs was first tested in sample A by a classic case–control analysis where cases were the HCV-infected patients with severe fibrosis and controls were the infected patients without severe fibrosis (supplementary table 1). A total of nine

Table 2 Main features of the HCV chronically infected patients in the whole sample

	METAVIR fibrosis score		
	F0–1	F3–4	Total
Number of patients	259	134	393
Sex ratio (male/female)	0.65	0.84	0.71
Mode of infection			
Blood transfusion	98	73	171
IVD	102	28	130
Others	37	26	63
Unknown	22	7	29
Age at contamination (years)*	25.6 (12.1)†	29.9 (14.0)	27.1 (12.9)
Duration of infection (years)*	18.9 (8.2)	22.8 (9.6)	20.3 (8.9)
Viral genotypes			
1A, 1B, 4	157	96	253
2, 5	27	8	35
3	49	19	68
Unknown	26	11	37

*Data for the 364 patients with known presumed dates of acquisition.
†mean (SD).
IVD, intravenous drug.

SNPs, including two in *IFNGR2* and three in *MMP16*, provided evidence for association with a p value <0.02, and were investigated further. Of these nine SNPs, four significantly influenced (p<0.05) the rate of progression towards severe fibrosis when performing a survival analysis (table 3 and supplementary table 2). The effects of the two *IFNGR2* SNPs, rs9976971 and rs2284553, which already yielded the lowest p values in the case–control study ($p=3\times10^{-4}$ and $p=8\times10^{-4}$, respectively), were even more significant ($p=2\times10^{-5}$ and $p=8\times10^{-5}$, respectively) using the survival analysis. Conversely, the effects of the two other SNPs (one in *MMP16*, and one in *TGFBR2*) were slightly lower when accounting for time of progression. For both rs9976971 and rs2284553 (which are G/A SNPs), the risk allele was the minor allele A and the best fitting genetic model was recessive—that is, subjects who were AA homozygous were predisposed to severe fibrosis as compared with AG and GG subjects. These two SNPs were in strong LD ($r^2=0.79$), and multivariate analysis confirmed that the results observed with rs9976971 and rs2284553 reflect a single signal.

Two *IFNGR2* variants show evidence for replication in the second sample

The four SNPs providing evidence for association both in case–control (at p<0.02) and survival (at p<0.05) were tested in sample B (table 3). Only the two *IFNGR2* SNPs showed evidence for replication with the same risk allele. As in sample A, the survival analysis was more powerful than the case–control approach, leading to a significant effect in sample B for rs9976971 (p=0.011). A similar trend, although not significant (p=0.13), was observed for rs2284553. When samples A and B were combined (table 4), the overall effect of rs9976971 in the case–control design was highly significant ($p=8\times10^{-5}$), and the OR (95% CI) of presenting severe fibrosis for AA subjects as compared with AG or GG subjects was 2.95 (1.70 to 5.11). This effect was much stronger ($p=9\times10^{-7}$) in the survival analysis design, and the HR (95% CI) of progressing towards severe fibrosis for AA subjects as compared with AG or GG subjects was 2.62 (1.76 to 3.91) (figure 2).

Consistent with this result taking into account the duration of infection, we observed that the effect of rs9976971 in the classical case–control design was much stronger (OR=4.46 (2.28 to 8.72)) in the 57 F3–4 patients with rapid progression (≤20 years of infection) than in the 69 F3–4 patients with slow progression (>20 years of infection, OR=2.10 (1.04 to 4.25)). Even if we considered the fact that these results were obtained in a one-step strategy (without the use of a replication sample), and that we applied the classical and stringent Bonferroni correction for multiple testing (assuming we have tested the 363 SNPs in the whole sample), the corrected p values for rs9976971 remained significant and equal to 0.029 and 0.0003 in the case–control and the survival analysis, respectively. These results indicate that one SNP in *IFNGR2*, either rs9976971 or another variant in strong LD with it, strongly influences the rate of progression towards severe fibrosis in patients chronically infected by HCV.

Search for other polymorphisms in linkage disequilibrium with the two *IFNGR2* variants

Next, we searched for other variants in strong LD with rs9976971 along three lines. First, we looked for long range LD (from 33 380 000 to 34 000 000 bp) using the European population of the HapMap database, NCBI build 36 (http://www.hapmap.org/, accessed 5 May 2010). Substantial LD (r^2 ranging from 0.3 to 0.48) with rs9976971 was observed with three clusters of SNPs. We genotyped one tag-SNP within each cluster (rs2834208, rs13047599, and rs7279549), and no association with severe fibrosis (p>0.2) was observed with any of these tag-SNPs. No other SNPs showed r^2 >0.3 within this interval, and, in particular, all SNPs between 33 524 000 and 33 655 000 bp where the genes *IFNAR1* and *IFNAR2* encoding interferon α/β receptors are located, provided r^2 <0.11 with rs9976971. Second, we explored the SeattleSNPs variation discovery resource database as it contained results for *IFNGR2*

Table 3 Results in both samples for the four single nucleotide polymorphisms (SNPs) providing the strongest evidence for association with severe fibrosis in sample A (ie, p<0.02 in case–control study and p<0.05 in the survival analysis). The full distribution of genotypes for these four SNPs is shown in online supplementary table 2

				Sample A				Sample B			
				Case/control (n=103/164)		Survival analysis (n=267)		Case/control (n=31/95)		Survival analysis (n=97)	
Marker	Gene	Risk allele (frequency)	Model*	OR (95% CI)†	p	HR (95% CI)‡	p	OR (95% CI)	p	HR (95% CI)	p
rs2284553	IFNGR2	A (0.41§)	rec	3.10 (1.57 to 6.13)	8×10^{-4}	2.41 (1.54 to 3.79)	8×10^{-5}	1.02 (0.26 to 4.05)	-¶	3.01 (0.66 to 13.7)	0.13
rs9976971	IFNGR2	A (0.44)	rec	3.22 (1.68 to 6.18)	3×10^{-4}	2.54 (1.64 to 3.93)	2×10^{-5}	2.04 (0.68 to 6.17)	0.19	3.56 (1.26 to 10.1)	0.011
rs2664357	MMP16	C (0.27)	add	1.86 (1.24 to 2.79)	0.002	1.47 (1.12 to 1.93)	0.006	0.62 (0.32 to 1.21)	0.16	0.81 (0.40 to 1.65)	–
rs9831477	TGFBR2	T (0.58)	dom	2.93 (1.35 to 6.38)	0.005	2.27 (1.14 to 4.50)	0.016	1.58 (0.49 to 5.08)	–	0.97 (0.32 to 2.91)	–

*Genetic model (recessive (rec), additive (add), or dominant (dom)) for the risk allele.
†OR (with 95% CI) under the corresponding genetic model.
‡HR (with 95% CI) under the corresponding genetic model estimated by Cox model analysis.
§frequency estimated in sample A.
¶p > 0.2.

Table 4 Results in the whole sample for the four *IFNGR2* variants providing the strongest evidence for association

Marker (type)	Position	Genotypes	No fibrosis F0–1, n (%)	Fibrosis F3–4, n (%)	Case/control study OR (95% CI)	p	Survival analysis HR (95% CI)	p
rs9976971 (G/A SNP)	33689967* 5′ region†	GG and AG	231 (89.2)	98 (73.7)	1		1	
		AA	28 (10.8)	35 (26.3)	2.95 (1.70 to 5.11)	8×10^{-5}	2.62 (1.76 to 3.91)	9×10^{-7}
		Total	259	133				
rs10600672 (AA/– ins/del)	33691495 5′ region	–/– and AA/–	228 (88.4)	98 (73.1)	1		1	
		AA/AA	30 (11.6)	36 (26.9)	2.79 (1.63 to 4.79)	10^{-4}	2.47 (1.66 to 3.66)	4×10^{-6}
		Total	258	134				
rs17882748 (T/C SNP)	33697591 Exon 1, 5′UTR	TT and CT	216 (83.7)	92 (68.7)	1		1	
		CC	42 (16.3)	42 (31.3)	2.35 (1.43 to 3.84)	6×10^{-4}	2.05 (1.40 to 3.01)	2×10^{-4}
		Total	258	134				
rs2284553 (G/A SNP)	33698565 Intron 1	GG and AG	233 (90.3)	105 (78.4)	1		1	
		AA	25 (9.7)	29 (21.6)	2.57 (1.44 to 4.61)	0.001	2.39 (1.57 to 3.66)	3×10^{-5}
		Total	258	134				

*Position in base pairs on chromosome 21.
†position relative to *IFNGR2* gene.

sequencing in 23 European subjects for a region of ~ 38 kb (33 695 200–33 733 200) (http://pga.gs.washington.edu/data/ifngr2/, accessed 5 May 2010). Finally, as rs9976971 is located in 5′ of *IFNGR2* (33 689 967), we also sequenced a sample of 32 French Caucasian subjects for a region of 12.3 kb from 33 689 894 to 33 702 179 (figure 1 and supplementary table 3). Based on these sequencing data from both the SeattleSNPs database and our own results, we identified two additional variants in strong LD with rs9976971 (table 4). One is the T/C SNP rs17882748 (r^2=0.83 with rs9976971) located in the untranslated region of exon 1, and the other is an AA insertion/deletion denoted as rs10600672 (r^2=0.98 with rs9976971) located in the 5′ region of the gene at position 33 691 495.

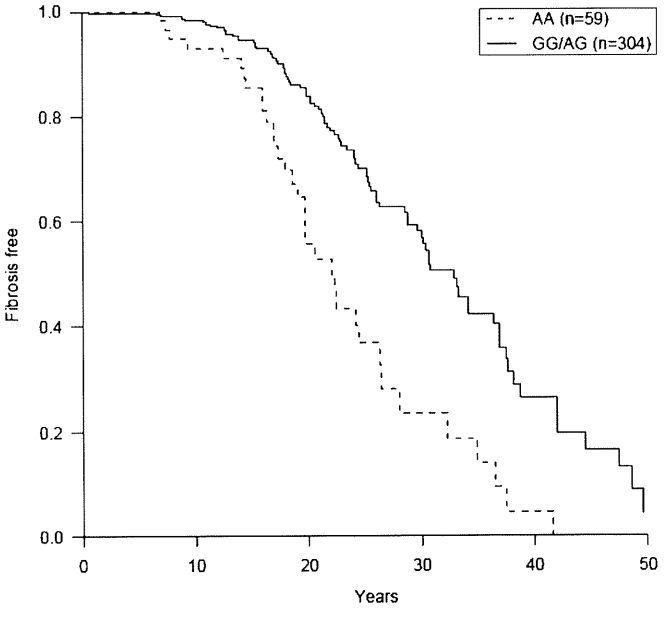

Figure 2 Effect of single nucleotide polymorphism rs9976971 on fibrosis progression. The figure shows the variation with time of the proportion of fibrosis-free patients according to genotypes at rs9976971. The time of follow-up was estimated from the presumed year of infection to the year of either the first biopsy showing severe fibrosis (F3–4 patients) or the last biopsy showing no fibrosis without any treatment (for F0–1 patients).

A cluster of four *IFNGR2* variants is strongly associated with liver fibrosis progression

Table 4 shows the results of both the case–control and the survival analysis with the four variants of interest over the combined samples A and B. Although all variants were strongly associated with development of severe fibrosis, the most significant results were observed with rs9976971 ($p=9\times10^{-7}$) and the AA ins/del ($p=4\times10^{-6}$) when using survival analysis. The AA insertion is in almost perfect LD with the A allele of rs9976971 (only three subjects had discordant genotypes) so that the HR of progressing towards severe fibrosis for AA/AA subjects as compared with AA/– or –/– subjects was 2.47 (1.66 to 3.66), and the curve of progression towards fibrosis with age according to this ins/del variant was extremely similar to that shown in figure 2 for rs9976971. We did not find any significant heterogeneity of these associations according to gender, mode of infection (blood transfusion/IVD use/others), viral genotypes (1 and 4 vs others), age at infection (≤20 years vs >20 years). We also conducted an analysis considering the different haplotypes that could be derived from these four variants using the method developed in the THESIAS program.[22] As expected by LD, two common haplotypes accounted for >90% of the estimated haplotypes (table 5). The common haplotype carrying the risk alleles at the four *IFNGR2* variants had a frequency of 0.338 and 0.447 in F0–1 and F3–4 patients, respectively. Under a recessive model (table 5), the effects of this at-risk haplotype (using a case–control or a survival analysis) were slightly lower than those estimated from rs9976971 or rs10600672 alone. As expected by the r^2 value at 0.98, the two variant haplotypes consisting of rs9976971 and rs10600672 provided results almost identical to the analysis of any of these variants alone (data not shown). However, none of the tested haplotypes consisting of three or four of these *IFNGR2* variants provided stronger evidence for association than rs9976971 or rs10600672 when considered alone.

DISCUSSION

In this study, we investigated whether the development of liver fibrosis in HCV chronically infected patients might be influenced by polymorphisms located in a panel of 36 genes involved in the fibrogenesis/fibrolysis process. To reduce the variability in assessing fibrosis stage by biopsy,[24] only F0–1 and F3–4 stages were included to define phenotypes. We found a single convincing signal of association in *IFNGR2*, which was the most

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Table 5 Results of the analysis considering haplotypes derived from the cluster of the four *IFNGR2* variants providing the strongest evidence for association

Polymorphisms				Haplotype frequencies		Case–control study		Survival analysis	
rs9976971	rs10600672	rs17882748	rs2284553	No fibrosis	Fibrosis	OR (95% CI)	p	HR (95% CI)	p
G	–	T	G	0.570	0.466				
A	AA	C	A	0.338	0.447	2.70 (1.48 to 4.92)*	0.0011	2.32 (1.52 to 3.56)*	10^{-4}
G	–	T	A	0.049	0.034				
A	AA	T	A	0.035	0.034				
A	AA	C	G	0.004	0.004				
G	AA	C	A	0.004	0.004				

*Results obtained with a recessive model for the common haplotype A/AA/C/A consisting of the risk alleles of the four *IFNGR2* variants.

significant in our primary sample and was exactly replicated (same allele at risk and same genetic model) in our second sample. The evidence for association was even higher (by one order of magnitude) when using the information obtained by the time of progression, providing strong additional support for our findings. The *IFNGR2* signal results from a cluster of four variants in strong LD. These variants are quite common with a frequency in the HapMap Caucasian population of 0.41 for risk allele A of the most associated SNP rs9976971, and 26% AA homozygosity in our sample of F3–4 patients. Sequencing data obtained either from existing databases or from our present analysis excluded the role of any other SNPs located within a region of ~43.5 kb (33 689 894–33 733 200 bps) encompassing the *IFNGR2* gene. Analysis of the HapMap database also made quite unlikely the hypothesis that this signal could be due to another SNP in long-range LD with this cluster of four *IFNGR2* variants. Refined analysis showed that no haplotypes derived from this cluster of four variants provided stronger evidence for association than any of the four SNPs when analysed alone. From a statistical point of view, the strongest evidence was obtained with rs9976971 and the AA ins/del (rs10600672), which are in almost perfect LD. Nevertheless, the roles of rs2284553 (in intron 1) and rs17882748 (in untranslated region of exon 1) could not be ruled out.

Although several association studies have investigated the role of a small number of polymorphisms within the gene encoding IFNγ (*IFNG*) and progression to fibrosis in HCV infection, in particular a variant at position +874 which may influence IFNγ expression, no consistent and clearly replicated results have been reported.[10] A study also tested the role of variants within the interferon γ receptor 1 gene (*IFNGR1*), without any significant results.[25] In this context, it is interesting to note that two linkage studies conducted in Sudan[26] and Egypt[27] mapped a locus predisposing to development of severe liver fibrosis due to the parasite *Schistosoma mansoni* infection in a region including the *IFNGR1* gene. No precise variants underlying these linkage peaks have been reported yet. To our knowledge, no studies have yet investigated the influence of *IFNGR2* polymorphisms in HCV-related liver fibrosis. We cannot rule out the possibility that some *IFGNR2* variants were included in the study that tested the association with 24 823 putative functional SNPs.[12] However, it is unlikely that any of our four associated polymorphisms were included in that panel as they are not known to be functional.

IFNγ is usually considered to be an anti-fibrogenic cytokine. In an experimental model of liver fibrosis induced with carbon tetrachloride, IFNγ-deficient mice exhibited more pronounced hepatic fibrosis lesions than wild-type animals, and exogenous IFNγ administered to deficient animals reduced the level of fibrosis.[28] In human cells, IFNγ has been shown to inhibit activation, proliferation and collagen synthesis in cultures of activated hepatic stellate cells and hepatic myofibroblasts.[16 29] It is also interesting to note that IFNγ has been proposed as a treatment for idiopathic pulmonary fibrosis and was associated with reduced mortality in a meta-analysis.[30] Along the same lines, high levels of IFNγ production were associated with protection against periportal fibrosis in subjects infected with the parasites *S mansoni*[31] or *Schistosoma japonicum*.[32]

However, the role of IFNγ may be more complex, as suggested by a study performed on liver biopsy specimens from patients with chronic HCV infection, which showed that increased IFNγ expression was associated with portal inflammation and fibrosis stage.[33] This latter observation suggests that the proinflammatory effects of IFNγ may predominate over its antifibrogenic role in HCV liver fibrosis, although IFNγ expression might also be a consequence of the fibrosis process. For example, an impaired function of IFNγ receptors might lead to increased production of IFNγ, as seen in patients with complete IFNγ receptor deficiencies.[34]

In conclusion we have found that progression to severe liver fibrosis in HCV chronically infected patients of European origin is strongly associated with a cluster of four *IFNGR2* variants. Interestingly, between the two IFNγ receptors, IFNγR2 expression appears to be the deciding factor that controls the way in which target cells physiologically respond to IFNγ.[35 36] Functional studies are continuing in human liver cells to investigate the detailed biological mechanisms of this association and the potential effect of this cluster of variants on *IFNGR2* regulation. Prospective studies using large samples will also assess the predictive value of these polymorphisms with repeated validated non-invasive biomarkers.[37] IFNγ has already been found to be associated with clearance of HCV infection.[38 39] These results highlight the role of the IFNγ pathway in development of liver fibrosis that may pave the way for new treatments.

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Competing interests None.

Ethics approval This study was conducted with the approval of the institutional review board (CPP: Comité de Protection des Personnes) of Ile de France - Paris - Saint Antoine, on 5 March 2002.

Contributors All authors participated in the collection, the management, and the interpretation of clinical data, and in the writing and the final approval of the manuscript. BN, ML, CB, TP, FM, SP and LA designed the study. SP, BR, EP, SH and LA were involved in statistical analysis. RLM and FM were involved in the sequencing of IFNGR2.

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The human *AIRE* gene at chromosome 21q22 is a genetic determinant for the predisposition to rheumatoid arthritis in Japanese population

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Rheumatoid arthritis (RA) is a typical complex trait and the major cause of chronic inflammation worldwide. Although multiple genetic loci have been shown for their association with the onset of RA, they cover only a part of its genetic components and are largely ethnicity-specific. To identify novel genetic factors related to the predisposition and prognosis of RA in Japanese, we conducted a large-scale genome-wide association (GWA) study. We performed a GWA analysis by scanning the genome of 1247 RA cases and 1486 controls for 277 420 single nucleotide polymorphisms (SNPs), followed by replication analysis using two independent sample sets consisting of 1865 cases and 1623 controls, and 2303 cases and 3380 controls. We identified two SNPs, rs2075876 and rs760426, in intron of the autoimmune regulator *AIRE* gene at chromosome 21q22 that showed strong associations with the disease ($P = 3.6 \times 10^{-9}$ and $P = 4.4 \times 10^{-8}$, respectively). Rs1800250, in exon7 of *AIRE*, was in strong linkage disequilibrium ($r^2 = 0.94$) with rs2075876 and introduced an amino acid alteration (S278R) in the SAND domain of the AIRE protein. *In silico* analysis showed the decreased transcription of *AIRE* by the risk allele of rs2075876 compared with the alternative allele ($P = 6.8 \times 10^{-5}$). No correlation was observed between the rs2075876 genotype and quantitative traits reflecting the progression of RA. As AIRE is a key molecule which regulates the expression and presentation of self-antigens in thymic negative selection, its downregulation by genetic polymorphisms may result in the survival of auto-reactive T cells to trigger auto-inflammation in RA.

INTRODUCTION

Rheumatoid arthritis (RA) is a major cause of chronic arthritis worldwide and results in severe functional impairment and joint destruction. The impairment of joints and disability for social activity bring strong social and economic impact (1). Both environmental and genetic factors are considered to be associated with its onset and progression (2). Twin studies of

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the European populations showed that ~60% of RA onset could be attributed to genetic factors (3). In them, *HLA-DRB1* is the strongest genetic component of the disease beyond ethnicity, and is estimated to correspond to 30–50% of the genetic components in Europeans (4). Although extensive genetic analyses including hypothesis-independent genome-wide association (GWA) studies identified >20 genes in Europeans (5–13) and 7 genes in East Asians (14–19) as genetic risk loci for RA, they account for only a part of its genetic components. Moreover, trans-ethnic comparison demonstrated that their association with RA is mostly specific to a particular ethnicity and as little as three genes, namely, *CCR6*, *STAT4* and *TNFAIP3*, have shown their association in both populations. These results strongly suggest the existence of additional susceptibility loci to RA in East Asian populations (14–19). By these reasons, we have conducted a GWA study using large DNA collections of Japanese RA patients.

RESULTS

GWA analysis

We performed a large-scale genome scan using a Japanese DNA collection (collection 1) consisting of 1247 RA cases and 1486 general population controls with Illumina Infinium arrays (Supplementary Material, Table S1). After a standard procedure of quality control (see Materials and Methods), 241 523 single nucleotide polymorphisms (SNPs) were examined for their association with RA. Quantile-quantile plot to estimate population stratification resulted in a small inflation factor ($\lambda = 1.05$). The strongest association was detected for markers in the *HLA* locus with the strongest *P*-value of 2.4×10^{-38} for rs9296015. Another known genetic determinant, *PADI4*, also showed strong association (strongest $P = 1.8 \times 10^{-8}$ for rs2240335). Also, a modest association was found in the *CCR6* gene (strongest $P = 9.7 \times 10^{-4}$ for rs1556413) (16). However, there was no evidence of association for *STAT4* and the disease in our study ($P > 0.070$). There were no other loci that showed significant association ($P < 2.1 \times 10^{-7}$) after Bonferroni's correction for multiple testing.

We then took a strategy to select candidate genes/markers for further genotyping analysis based on their functional relevance in the immune system. For this purpose, we generated a list of SNP markers showing potential association with the disease (nominal $P < 0.001$), and investigated their chromosomal locations and corresponding genes in the order of association strength. Among the top 471 SNPs with *P*-value smaller than 0.001, we found two SNPs located in intron of the *AIRE* gene at chromosome 21q22, which is known as an autoimmune regulator. They were rs2075876 and rs760426 with *P*-value of 5.1×10^{-4} and 2.0×10^{-4}, respectively, and were ~6.7 kb apart from each other and in moderate linkage disequilibrium (LD) ($r^2 = 0.63$, Fig. 1). We performed genotyping of these two markers using an additional DNA collection (termed as collection 2) consisting of 1865 cases and 1623 controls. All the RA cases and 855 controls were newly genotyped with the Taqman method, and the genotypes of the other 768 controls were extracted from genome scan results of other population-based genetic studies. We successfully confirmed the association of rs2075876 ($P = 5.1 \times 10^{-4}$) in collection 2. The other marker, rs760426, showed a moderate association ($P = 0.011$) (Table 1).

We further examined whether or not the results of our study were reproducible in another Japanese RA GWA study of Biobank Japan Project recruiting 2303 cases and 3380 controls (termed as collection 3) (16). The statistical test again returned significant associations for these markers ($P = 3.6 \times 10^{-4}$ for rs2075876 and $P = 8.2 \times 10^{-4}$ for rs760426, Table 1). When the genotyping results of the three collections were pooled, the association *P*-value reached $P = 3.6 \times 10^{-9}$ for rs2075876 and $P = 4.4 \times 10^{-8}$ for rs760426 (Table 1).

We then investigated whether or not the association of the *AIRE* gene with RA was observed in Europeans. Our own genome scan results of German RA samples (I.M., M.L. and F.M., unpublished data) showed no associations for the SNP markers in the *AIRE* locus. Two large-scale GWA studies of European descents, namely, Wellcome Trust Case Control Consortium (9) and a meta-analysis of multiple GWA studies (12), did not identify *AIRE* as a risk locus, strongly suggesting its limited contribution to RA in East Asian populations.

Structure and organization of the human *AIRE* locus

LD structure of the chromosomal region containing rs2075876 and rs760426 was generated using Japanese HapMap results. As shown in Figure 1, rs2075876 and rs760426 are located in an LD block encompassing the 32 kb region between intron 5 of the *AIRE* gene and intron 12 of the liver phosphofructokinase *PFKL* gene. As the SNPs around the *PFKL* gene showed weaker association with RA ($P > 0.002$) than the two SNPs, we considered that the observed association with RA was most likely with the *AIRE* polymorphisms. However, both of these SNPs were located in intron and no other SNP markers in the genotyping arrays were mapped in this LD block and showed similar degree of association with RA. Hence, we searched for SNPs in dbSNP that were located in exons of *AIRE* and introduce functional alterations of the AIRE protein. There were five non-synonymous SNPs in the coding region of *AIRE* out of which rs1800520 in exon7 showed an allele frequency similar to that of rs2075876 (0.420). Rs1800520 introduced an amino acid alteration from serine to arginine at amino acid residue 278 (S278R). We genotyped rs1800520 in the DNA samples of all the cases ($n = 1865$) and a part of controls ($n = 855$) of collection 2 and found that rs1800520 was in strong LD with rs2075876 ($r^2 = 0.94$) and was also associated with RA ($P = 0.0071$).

AIRE polymorphism and expression

Although both rs2075876 and rs760426 are located in intron, they may have functional roles such as regulation of *AIRE* transcription. The correlation between these SNPs and transcription levels of *AIRE* was examined by using the expression profiles of 210 lymphoblastoid cells in Gene Expression Omnibus (GEO) database (20). As the result, the transcription of *AIRE* was decreased by the risk allele (A) of rs2075876 ($P = 6.8 \times 10^{-5}$, Fig. 2) but not by that of rs760426 ($P = 0.24$). Although we hypothesized the presence of a transcription factor-binding site around rs2075876, *in silico* study

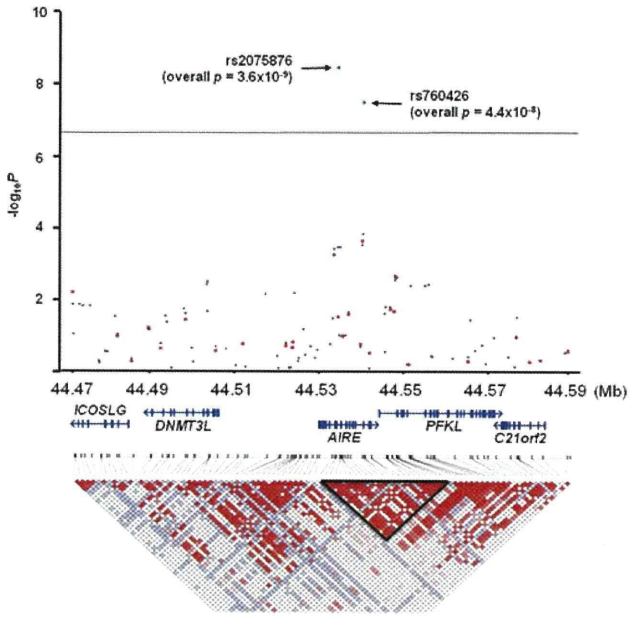

Figure 1. A schematic organization of the human *AIRE* locus at 21q22. *P*-values of the initial genome scan using collection 1 were calculated by the Trend χ^2 test and plotted in red circles. The blue circles indicate *P*-values obtained by imputation using HapMap Japanese results. Overall *P*-values of rs2075876 and rs760426 using the combined results of collections 1, 2 and 3 were also shown in green circles. A horizontal line indicates Bonferroni-adjusted $P = 2.5 \times 10^{-7}$. The structure and orientation of four genes were shown below the plots with their transcriptional orientations according to the NCBI Reference Sequence Build 36.3. LD blocks were generated according to the pairwise LD estimates of the SNPs in HapMap Japanese results.

did not predict a motif of transcription factor-binding site spanning rs2075876. Multiple nucleotide sequence alignment around rs2075876 showed a high degree of conservation among seven mammalian species (human, chimpanzee, rhesus macaque, bushbaby, horse, cow and dog). The corresponding region of rodents (mouse and rat) showed much weaker conservation (Supplementary Material, Fig. S1).

AIRE polymorphism and difference in clinical phenotypes and disease activity

RA is often subdivided into two groups based on the presence of circulating antibodies to citrullinated peptide antigen (ACPA), a specific predictive biomarker for destructive RA (21–22). In our patient collections (collection 1 and collection 2), there were 803 patients with ACPA quantification of which 176 patients were negative for ACPA. We compared the allele frequency of rs2075876 between APCA(+) and ACPA(−) groups and found no significant difference [0.39 for ACPA(+) and 0.40 for ACPA(−), $P = 0.66$]. We next tested whether rs2075876 was associated with the disease activity and prognosis. For this purpose, 212 RA patients for whom the quantitative DAS28 score was available were chosen to evaluate the correlation of RA activity and rs2075876 genotypes. Statistical analysis did not return correlations between rs2075876 genotypes and DAS28 (Supplementary Material, Fig. S2).

DISCUSSION

AIRE is a transcriptional regulator primarily expressed in medullary thymic epithelial cells (mTEC), and plays a functional role in thymocyte education and negative selection by controlling the expression of peripheral antigens in thymus (23). The expression of *AIRE* in non-thymic tissues is still controversial; some studies detected *AIRE* transcripts at a lower level in secondary lymphoid organs and in periphery while others did not (24–25), and the expression of the AIRE protein in such tissues is yet to be established. In human, dysfunction of *AIRE* caused a rare systemic multi-organ autoimmune disease known as autoimmune polyendocrinopathy–candidiasis–ectodermal dystrophy (APECED) (26). However, the patients rarely show joint destruction as observed in RA (27). In mice deficient for *aire* which develop APECED-like multi-organ autoimmune features and do not manifest with arthritis, a dramatic decrease in the expression of type II collagen was observed in mTEC and the incidence and severity of collagen-induced arthritis were augmented when compared with the wild-type (28). Such observations indicate the possible involvement of *AIRE* in immunopathology both in the human and in the mouse. However, the involvement of *AIRE* in human multigenetic autoimmune diseases still remains to be elucidated. Our study is the first successful case which clearly showed the involvement of *AIRE* in systemic autoimmunity. The function of the AIRE protein in the secondary lymphoid organs is not fully understood. Elucidation of the functions of AIRE in peripheral organs may provide hints to the involvement of *AIRE* in the predisposition or progression in RA.

In silico analysis using the GEO database showed that the risk allele of rs2075876 decreased the transcription level of *AIRE*. This may cause lower expression of various peripheral tissue antigens (PTAs), resulting in the failure of negative selection in the thymus resulting in the survival of autoreactive T cells. Although low amount of *AIRE* transcripts in B-lymphocytes was detected in most of the reported experiments, the conclusive answer for the functional impact of rs2075876 to the immune regulation needs further studies using the tissues in which *AIRE* is strongly expressed. The S278R replacement by rs1800520 is located in the SAND domain, a conserved sequence motif in nuclear proteins including Sp100 family and plays a key role in transcription regulation. However, the SAND domain of AIRE lacks the canonical KDWF motif for the interaction with DNA. Also amino acid sequence alignment of the SAND domains in different nuclear proteins revealed that S278R was located at the poorly conserved carboxyl terminal (29). Moreover, the interaction of AIRE with histone H3 through a plant homeodomain finger was suggested to be important to up-regulation of PTA genes (30). On the other hand, an assessment of mRNA stability by a computerized modeling showed lower stability of *AIRE* mRNA with the risk allele of rs1800520 than the alternative allele, suggesting the possibility of shorter half-life of the transcripts and thus lower amount of the AIRE protein. As such, we cannot conclude whether or not these SNPs have functional impact to the regulation of *AIRE* expression. The existence of unidentified SNPs that are in strong LD with them and play important functional roles is also conceivable. Extensive analyses of the *AIRE* locus by fine mapping and

Table 1. Association analysis of two SNPs in the *AIRE* gene with RA in Japanese

rs2075876		Genotype counts			Frequency A	OR (95% CI)	*P*-value
		GG	GA	AA			
Collection 1	Case	480	554	201	0.39	1.22 (1.09–1.36)	5.1×10^{-4}
	Control	639	680	167	0.34		
Collection 2	Case	706	887	243	0.37	1.18 (1.07–1.31)	9.4×10^{-4}
	Control	710	671	192	0.34		
Collection 3	Case	905	1061	330	0.37	1.15 (1.07–1.25)	3.6×10^{-4}
	Control	1462	1506	398	0.34		
Combined study	Case	2091	2502	774	0.38	1.18 (1.11–1.24)	3.6×10^{-9}
	Control	2811	2857	757	0.34		
rs760426		Genotype counts			Frequency G	OR (95% CI)	*P*-value
		AA	AG	GG			
Collection 1	Case	464	559	219	0.40	1.23 (1.10–1.38)	2.0×10^{-4}
	Control	608	709	169	0.35		
Collection 2	Case	684	897	265	0.39	1.13 (1.03–1.25)	0.011
	Control	666	741	205	0.36		
Collection 3	Case	866	1078	357	0.39	1.14 (1.06–1.23)	8.2×10^{-4}
	Control	1408	1520	450	0.36		
Combined study	Case	2014	2534	841	0.39	1.16 (1.10–1.22)	4.4×10^{-8}
	Control	2682	2970	824	0.36		

OR, odds ratio; 95% CI, 95% confidence interval.

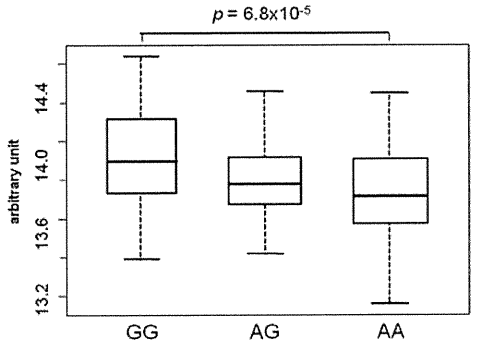

Figure 2. Comparison of the expression levels of *AIRE* among three subgroups of cell lines according to the genotype of rs2075876. 'G' and 'A' correspond, respectively, to the risk and the alternative alleles of rs2075876.

extensive sequencing in combination with examination of promoter activity will answer this question.

There was no association between *AIRE* and RA in Europeans even in the large-scale meta-analysis of GWA studies with a strong detection power (12). Although the frequency of the risk allele of rs2075876 is much lower in Caucasians (0.15 in Caucasian HapMap results and 0.097 in our own genome scan results) compared with that of the current study (0.34), this does not fully explain the lack of association in Europeans. This suggests that the association of *AIRE* with RA is, like that of *PADI4*, specific to East Asian populations including Japanese. The future validation study using other Asian population will address this issue.

MATERIALS AND METHODS

Study subjects

RA collections 1–3 consisted of 1247 affected individuals and 1486 controls, 1865 cases and 1623 controls, and 2303 cases and 3380 controls, respectively (summarized in Supplementary Material, Table S1). The case subjects of collections 1 and 2 were recruited at the rheumatology departments of Kyoto University Hospital, Dohgo Spa Hospital, Sagamihara National Hospital, Tokyo University Hospital and Tokyo Women's Medical University. The control subjects for collection 1 were from Aichi Cancer Center Hospital and Research Institute and the Department of Ophthalmology and Visual Science at Kyoto University Hospital. DNA samples of healthy Japanese volunteers in collection 2 were from Pharma SNP Consortium (31) and the Center for Genomic Medicine, Graduate School of Medicine, Kyoto University. The case and control subjects in collection 3 were recruited in the Biobank Japan Project at the Institute of Medical Science, the University of Tokyo; the Department of Allergy and Rheumatology, Graduate School of Medicine, the University of Tokyo (32). All cases fulfilled the revised criteria (1987) of the American College of Rheumatology for RA. Among the RA cases, DAS28 score for RA activity in 212 RA patients was obtained at each institution. Written informed consent was obtained from all the participants at the institute of sample collection after being approved for genetic studies by the local ethical committee.

GWA analysis

Genome scan for collection 1 was performed using Infinium Technology (Illumina Inc., San Diego, CA, USA). Case subjects were genotyped with Human-Hap300 (version 1.0, 302 627 SNPs), Human CNV370-Duo (version 1.0, 332 270 SNPs) or Human610-Quad (version 1.0, 577 348 SNPs). For control subjects, they were genotyped with Human610-Quad (version 1.0, 577 348 SNPs) and HumanHap550 (version 3.0, 547 163 SNPs). For validation analysis, Taqman

technology (Life Technologies Corp., Foster City, CA, USA) was employed.

Quality control and statistical tests for case–control association

A total of 277 420 SNPs that were common among the four types of arrays described above were selected for the association study. One thousand two hundred and forty-six cases and 1486 controls with call rate being >0.90 and not showing high degree of kinship (PI_HAT < 0.10 by PLINK) were examined for the association analysis. A total of 241 523 SNPs with call rate >0.95 for both cases and controls and minor allele frequency >0.05 either in the case or in the control were used for the analysis. The case–control association was examined with the Cochran–Armitage trend for each collection as well as for the combined pooled study. Population stratification was examined and corrected with Genomic Control. SNPs that showed P-value $<10^{-3}$ were selected as candidates for further evaluation. SNPs in the *HLA*, *PADI4* and *CCR6* loci were not selected for validation studies. Haploview version 4.1 software (33) was used for LD evaluation, and MapViewer (build 36.3) (34) was used to identify the location and structure of the genes in the region.

Analysis of *AIRE* expression

A gene-expression data set in lymphoblastoid cell lines derived from 210 unrelated HapMap populations was obtained from GEO database (20). The correlation between the expression of *AIRE* and genotypes of SNPs in the region was examined using the calculation program recommended by GEO. The association P-values were obtained by the Joncheere–Terepstra method using R software or SPSS (version 18).

Bioinformatics analysis

Genome sequence alignment of 14 placental mammals was obtained from the UCSC genome browser (http://genome.ucsc.edu). Motif search was carried out by the Jaspar database (35) (http://jaspar.cgb.ki.se) using 'Jasper Core Subset' which contains 138 matrices for known *cis*-acting elements. The matrices were converted into bit scores and used to search against the genomic sequences around the SNP of interest. Identification of orthologs of the *AIRE* gene in different mammals and multiple nucleotide sequence alignment was performed using KEGG SSDB Database (www.genome.jp/kegg/ssdb).

SUPPLEMENTARY MATERIAL

Supplementary Material is available at *HMG* online.

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Conflict of Interest statement. None declared.

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APPENDIX

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Predictive Value of Early Viral Dynamics During Peginterferon and Ribavirin Combination Therapy Based on Genetic Polymorphisms Near the *IL28B* Gene in Patients Infected With HCV Genotype 1b

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A study was carried out to determine whether early viral dynamics retain prediction of the outcome of peginterferon (PEG-IFN) and ribavirin combination therapy based on different genetic polymorphisms near the *IL28B* gene, the strongest baseline predictor of response to this therapy. A total of 272 patients infected with hepatitis C virus (HCV) genotype 1b were grouped according to genetic polymorphisms near the *IL28B* gene (rs8099917). The ability of reduced HCV RNA levels at 4 and 12 weeks after starting therapy to predict a sustained virologic response was evaluated based on these genotypes. Among patients with the TT genotype for rs8099917 (associated with a favorable response), the rates of sustained virologic response were higher in patients with a $\geq$3 $\log_{10}$ reduction in serum HCV RNA levels at 4 weeks after starting therapy ($P < 0.0001$). In contrast, among patients with the TG/GG genotype (associated with an unfavorable response), there were no differences in this rate based on the reduction in HCV RNA levels at 4 weeks. Early viral dynamics at 4 weeks after starting therapy retains its predictive value for sustained virologic response in patients with the TT genotype for rs8099917, but not in patients with the TG/GG genotype. Patients who are likely to achieve sustained virologic response despite unfavorable TG/GG genotype cannot be identified based on early viral dynamics during therapy. In contrast, lack of early virologic response at 12 weeks retains a strong predictive value for the failure of sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy. ***J. Med. Virol. 84:61–70, 2012.*** © 2011 Wiley Periodicals, Inc.

KEY WORDS: chronic hepatitis C; early viral dynamics; genetic polymorphisms near the *IL28B* gene; peginterferon; response-guided therapy; ribavirin

INTRODUCTION

The current standard antiviral therapy for patients with chronic hepatitis C is combination therapy with peginterferon (PEG-IFN) and ribavirin [Ghany et al., 2009]. Although this treatment regimen has increased markedly the number of patients with a sustained virologic response, i.e., the eradication of hepatitis C virus (HCV), only 50% of patients infected with HCV genotype 1 achieved a sustained virologic response approximately.

Many investigators have examined factors that predict the treatment outcome of PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1. In addition to the baseline factors, the response of HCV during combination therapy, i.e., the changes in serum HCV RNA levels after starting therapy, has been shown to be an important predictor of the treatment outcome [Zeuzem et al., 2001; Buti

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et al., 2002; Berg et al., 2003], with the emphasis on "response-guided therapy" [Lee and Ferenci, 2008; Marcellin and Rizzetto, 2008]. Recent reports have emphasized the importance of evaluating the viral dynamics at 4 weeks after starting therapy to predict a sustained virologic response. A rapid virologic response, in which serum HCV RNA is undetectable at 4 weeks after starting therapy, has been the strongest predictive factor of a sustained virologic response reportedly [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, the predictive value of reduced serum HCV RNA levels at 4 weeks after starting therapy has been clarified further, and a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy has high predictive value that a patient will achieve a sustained virologic response as a final outcome, even in the absence of a rapid virologic response [Toyoda et al., 2011].

In contrast, the lack of an early virologic response, defined as either undetectable serum HCV RNA or HCV RNA levels decreased by >2.0 $\log_{10}$ from the pretreatment level at 12 weeks after starting therapy, has been the most important predictor for the failure of a sustained virologic response in patients infected with HCV genotype 1 reportedly [Fried et al., 2002; Davis et al., 2003]. Therefore, treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment, according to the recommendation in the AASLD guidelines [Ghany et al., 2009].

More recently, several studies reported that genetic polymorphisms near the *IL28B* gene (rs8099917, rs12979860) on chromosome 19 affect the virologic response to PEG-IFN and ribavirin combination therapy in patients infected with HCV genotype 1 [Ge et al., 2009; Suppiah et al., 2009; Tanaka et al., 2009; McCarthy et al., 2010; Rauch et al., 2010]. Furthermore, genetic polymorphisms near the *IL28B* gene are the strongest baseline predictive factor of the final outcome of combination therapy. An additional report showed the effects of genetic polymorphisms near the *IL28B* gene on HCV viral dynamics during PEG-IFN and ribavirin combination therapy [Thompson et al., 2010].

Although early HCV viral dynamics during therapy was shown originally to have a high predictive value for a sustained virologic response in HCV genotype 1-infected patients before genetic polymorphisms near the *IL28B* gene were linked to a therapeutic response, it is not clear whether early viral dynamics retain their predictive value in light of this additional information. The purpose of the present study was to investigate whether response-guided therapy based on viral dynamics at 4 or 12 weeks after initiating therapy retains its ability to predict the final outcome of PEG-IFN and ribavirin combination therapy after accounting for genetic polymorphisms near the *IL28B* gene.

MATERIALS AND METHODS

Patients and Treatment

Between January 2007 and June 2008, a total of 402 patients with chronic hepatitis C received antiviral combination therapy with PEG-IFN and ribavirin for HCV infection at the Ogaki Municipal Hospital or the Nagoya University Hospital. Among these patients, 272 were infected with HCV genotype 1b and had pretreatment HCV RNA levels >5.0 $\log_{10}$ IU/ml based on a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System; Roche Molecular Systems, Pleasanton, CA; Lower limit of quantification, 1.7 $\log_{10}$ IU/ml: Lower limit of detection, 1.0 $\log_{10}$ IU/ml) [Colucci et al., 2007; Pittaluga et al., 2008]. This study did not include any patients infected with HCV genotype 1a because this genotype is not found in the general Japanese population.

All patients were given PEG-IFN alpha-2b (Pegintron, Schering-Plough, Tokyo, Japan) weekly and ribavirin (Rebetol, Schering-Plough, Kenilworth, NJ) daily. The PEG-IFN and ribavirin doses were adjusted based on the patient's body weight. Patients weighing ≤ 45 kg were given 60 μg of PEG-IFN alpha-2b once a week, those weighing >45 and ≤ 60 kg were given 80 μg, those weighing >60 and ≤ 75 kg were given 100 μg, those weighing >75 and ≤ 90 kg were given 120 μg, and those weighing >90 kg were given 150 μg. Patients weighing ≤ 60 kg were administered 600 mg of ribavirin per day, those weighing >60 and ≤ 80 kg were given 800 mg per day, and those weighing >80 kg were administered 1000 mg per day. The PEG-IFN and ribavirin doses were modified based on the manufacturer's recommendations. All patients were scheduled to undergo 48 weeks of treatment. The treatment duration was extended up to 72 weeks in some patients. In addition, treatment was discontinued before 48 weeks in some patients who had a low likelihood of achieving an eradication of HCV due to the presence of serum HCV RNA at 24 weeks after starting therapy.

A sustained virologic response was defined as undetectable serum HCV RNA at 24 weeks after ending the therapy. A patient was considered to have relapsed when serum HCV RNA was detectable between the end of treatment and 24 weeks after completing treatment, although serum HCV RNA was undetectable during and at the end of therapy. Patients were considered to have non-response if serum HCV RNA was detectable at 24 weeks after initiating therapy (i.e., null response or partial response according to the American guidelines [Ghany et al., 2009]). Patients were considered to have a rapid virologic response if they had undetectable serum HCV RNA at 4 weeks after starting therapy. An early virologic response was defined as the disappearance or decrease in serum HCV RNA levels by at least 2 $\log_{10}$ at 12 weeks after starting therapy. Patients were considered to have a complete early virologic response if serum HCV RNA was undetectable at 12 weeks after starting therapy and a partial early virologic response if the serum

HCV RNA levels had decreased by at least 2 $\log_{10}$ at 12 weeks after initiating therapy. Patients were considered not to have an early virologic response if their HCV RNA levels did not decrease by more than 2 $\log_{10}$ at 12 weeks compared to the pretreatment levels. Patients were considered to have a slow virologic response if the serum HCV RNA became undetectable between 12 and 24 weeks.

The study protocol was in compliance with the Helsinki Declaration and was approved by the ethics committee of the Ogaki Municipal Hospital and the Nagoya University School of Medicine. Prior to initiating the study, each patient provided written informed consent to use the laboratory data, analyze genetic polymorphisms near the *IL28B* gene, and test stored serum samples.

Assessments of Serum HCV RNA Levels and Genetic Polymorphisms Near the *IL28B* Gene

After a patient provided informed consent, serum samples were obtained at the patient's regular hospital visits, just prior to initiating treatment, every 4 weeks during the treatment period, and during the 24-week follow-up period after treatment. Serum samples were stored at −80°C until further use. The HCV RNA levels were measured using a quantitative real-time PCR-based method for HCV (HCV COBAS AmpliPrep/COBAS TaqMan System).

Genotyping of rs 8099917 polymorphisms near the *IL28B* gene was performed using the TaqMan SNP assay (Applied Biosystems, Foster City, California) according to the manufacturer's guidelines. A predesigned and functionally tested probe was used for rs8099917 (C__11710096_10, Applied Biosystems).

Statistical analyses. Quantitative values are reported as the mean ± SD. In between-group differences were analyzed by the chi-square test. Univariate and multivariate analyses using a logistic regression model were performed to identify factors that predict a sustained virologic response, including age, sex, body weight, serum alanine aminotransferase activity, serum aspartate aminotransferase activity, serum gamma-glutamyl transpeptidase levels, serum alkaline phosphatase values, serum albumin levels, total serum bilirubin values, white blood cell counts, hemoglobin, platelet counts, hepatitis activity grade (A0 and A1 vs. A2 and A3), liver fibrosis grade (F0 and F1 vs. F2 and F3), pretreatment HCV RNA levels ($\geq$6.5 $\log_{10}$ vs. <6.5 $\log_{10}$), reduction in peginterferon dose and ribavirin dose, reduction in HCV RNA levels at 4 weeks after starting therapy ($\geq$3 $\log_{10}$ vs. <3 $\log_{10}$), and the type of an early virologic response. All *P*-values are two-tailed, and $P < 0.05$ was considered significant statistically.

RESULTS

The characteristics of the patients examined in this study are shown in Table I. Liver histology was evaluated according to the METAVIR score [The French METAVIR Cooperative Study Group, 1994]. Although some patients had a reduction in their PEG-IFN and ribavirin doses during therapy, respectively, all patients except for those who discontinued the therapy had more than 80% adhesion to both the PEG-IFN and ribavirin regimens. No patients discontinued the therapy because of adverse effects. The treatment duration was extended up to 72 weeks in 51 of 71 patients (71.8%) who exhibited a slow virologic response. As a final outcome, 118 patients (43.4%) achieved a sustained virologic response, 84 patients (30.9%) relapsed, and the remaining 70 patients (25.7%) had no response.

TABLE I. Characteristics of all Study Patients (n = 272)

Age (years)	56.0 ± 10.9
Sex (female/male)	139 (51.1)/133 (48.9)
Body weight (kg)	57.8 ± 10.5
Alanine aminotransferase (IU/L)	64.6 ± 56.4
Aspartate aminotransferase (IU/L)	53.9 ± 42.7
Gamma-glutamyl transpeptidase (IU)	48.5 ± 43.9
Alkaline phosphatase (IU/L)	267.9 ± 101.3
Albumin (g/dl)	4.04 ± 0.37
Total bilirubin (mg/dl)	0.79 ± 0.30
White blood cell count (/μl)	4892 ± 1333
Hemoglobin (g/dl)	14.0 ± 1.3
Platelet count ($\times 10^3$/μl)	163 ± 51
Liver histology-activity (A0/A1/A2/A3)*	3 (1.2)/136 (55.3)/ 92 (37.4)/15 (6.1)
Liver histology-fibrosis (F0/F1/F2/F3)*	27 (11.0)/114 (46.3)/ 70 (28.5)/35 (14.2)
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.35 ± 0.79
Reduction in the peginterferon dose	81 (29.8)
Reduction in the ribavirin dose	130 (47.8)
Final outcomes (sustained virologic response /relapse/ no response)	118 (43.4)/ 84 (30.9)/70 (25.7)

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

Reduction in Serum HCV RNA Levels at 4 Weeks after Starting Therapy and Treatment Outcome According to Genetic Polymorphisms Near the *IL28B* Gene

An analysis of genetic polymorphisms at rs8099917 near the *IL28B* gene indicated that 207 patients (76.1%) had a TT genotype, 3 patients had a GG genotype (1.1%), and the remaining 62 patients were TG heterozygote (22.8%). Table II shows the comparison of the background characteristics between patients with the favorable TT genotype and those with the unfavorable TG/GG genotype. As reported previously [Abe et al., 2010], gamma-glutamyl transpeptidase level was higher significantly in patients with the TG/GG genotype. As a final outcome, the rate of a sustained virologic response was higher significantly in patients with the TT genotype. Among 207 patients with the TT genotype, serum HCV RNA became undetectable in 19 patients (9.2%) at 4 weeks after starting therapy (a rapid virologic response). In the remaining 188 patients, the decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.12

TABLE II. Characteristics of Study Patients According to the Genetic Polymorphisms Near the *IL28B* Gene

	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)	*P*-value
Age (years)	56.5 ± 10.4	54.4 ± 12.4	0.4112
Sex (female/male)	107 (51.7)/100 (48.3)	32 (49.2)/33 (50.8)	0.8384
Body weight (kg)	57.8 ± 10.9	57.8 ± 9.4	0.8361
Alanine aminotransferase (IU/L)	65.1 ± 53.3	62.8 ± 65.6	0.2548
Aspartate aminotransferase (IU/L)	53.6 ± 34.8	54.7 ± 62.0	0.3339
Gamma-glutamyl transpeptidase (IU)	44.2 ± 37.1	62.3 ± 59.0	0.0003
Alkaline phosphatase (IU/L)	263.1 ± 90.3	282.8 ± 129.9	0.3875
Albumin (g/dl)	4.04 ± 0.36	4.05 ± 0.43	0.8020
Total bilirubin (mg/dl)	0.79 ± 0.30	0.76 ± 0.32	0.3010
White blood cell count (/μl)	4826 ± 1333	5100 ± 1320	0.1608
Hemoglobin (g/dl)	13.9 ± 1.3	14.1 ± 1.4	0.3339
Platelet count ($\times 10^3$/μl)	161 ± 49	169 ± 57	0.3871
Liver histology-activity (A0/A1/A2/A3)*	2 (1.1)/98 (52.4)/ 74 (39.6)/13 (6.9)	1 (1.7)/38 (64.4)/ 18 (30.5)/2 (3.4)	0.3241
Liver histology-fibrosis (F0/F1/F2/F3)*	21 (11.2)/83 (44.4)/ 57 (30.5)/26 (13.9)	6 (10.2)/31 (52.5)/ 13 (22.0)/9 (15.3)	0.6401
Pretreatment HCV RNA concentration ($\log_{10}$ IU/ml)	6.37 ± 0.85	6.29 ± 0.55	0.0582
Reduction in the peginterferon dose	61 (29.5)	20 (30.8)	0.9644
Reduction in the ribavirin dose	101 (48.8)	29 (44.6)	0.5565
Final outcomes (sustained virologic response /relapse/ no response)	106 (51.2)/ 69 (33.3)/32 (15.5)	12 (18.4)/15 (23.1)/ 38 (58.5)	<0.0001

HCV, hepatitis C virus.
Percentages are shown in parentheses.
*Liver biopsy was not performed in 26 patients.

$\log_{10}$ to 5.71 $\log_{10}$ (mean, 3.12 $\log_{10}$). The reduction in serum HCV RNA levels was $\geq$3 $\log_{10}$ in 98 patients (47.3%), <3 $\log_{10}$ and $\geq$2 $\log_{10}$ in 52 patients (25.1%), <2 $\log_{10}$ and $\geq$1 $\log_{10}$ in 23 patients (11.1%), and <1 $\log_{10}$ in 15 patients (7.3%). Figure 1A shows the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TT genotype. The rates were higher significantly in patients who achieved a rapid virologic response or had a $\geq$3 $\log_{10}$ decrease in

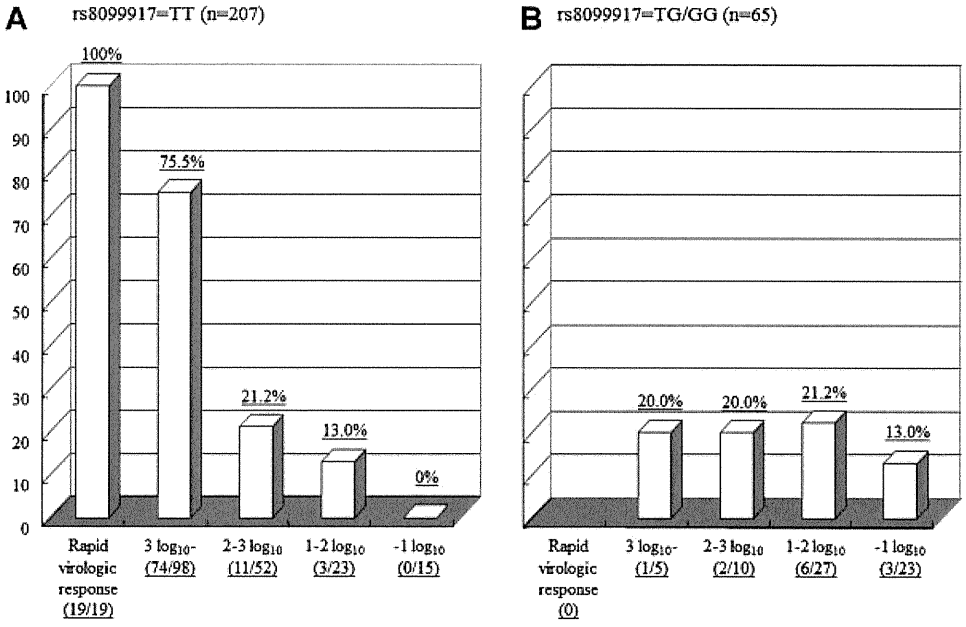

Fig. 1. The rate of sustained virologic responses (%) based on the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

serum HCV RNA levels at 4 weeks compared to those with a <3 $\log_{10}$ decrease in serum HCV RNA levels ($P < 0.0001$). When a 3 $\log_{10}$ decrease in serum HCV RNA levels was defined as the cut-off point, 56.5% of patients were considered to have a ≥3 $\log_{10}$ decrease in serum HCV RNA levels. The sensitivity, specificity, positive predictive value, and negative predictive value for a sustained virologic response were 86.8, 75.2, 78.6, and 84.4%, respectively.

Among the 65 patients who had the TG/GG genotype, no patient achieved a rapid virologic response at 4 weeks after initiating therapy. The decrease in serum HCV RNA levels at 4 weeks after starting therapy ranged from 0.11 $\log_{10}$ to 4.75 $\log_{10}$ (mean, 1.66 $\log_{10}$). The reduction in serum HCV RNA levels at 4 weeks after starting the therapy were smaller in patients with the TG/GG genotype than those with the TT genotype (1.66 ± 1.02 $\log_{10}$ in patients with the TG/GG genotype vs. 3.12 ± 1.37 $\log_{10}$ in patients with TT genotype excluding RVR, $P < 0.0001$). The reduction in serum HCV RNA levels was ≥3 $\log_{10}$ in five patients (7.7%), <3 $\log_{10}$ and ≥2 $\log_{10}$ in 10 patients (15.4%), <2 $\log_{10}$ and ≥1 $\log_{10}$ in 27 patients (41.5%), and <1 $\log_{10}$ in 23 patients (35.4%). Figure 1B shows the rates of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy in patients with the TG/GG genotype. There were no differences in the rate of a sustained virologic response based on the reduction in HCV RNA levels at 4 weeks after starting therapy; the rate of a sustained virologic response remained at 20% approximately regardless of the reduction in HCV RNA levels in 42 patients with a ≥1 $\log_{10}$ reduction in serum HCV RNA levels.

Association Between an Early Virologic Response at 12 Weeks and Treatment Outcome Based on Genetic Polymorphisms Near the *IL28B* Gene

Figure 2 shows the rate of patients with the TT genotype or TG/GG genotype for rs8099917 who achieved a complete early virologic response, a partial early virologic response, and those who did not achieve early virologic response at 12 weeks after starting therapy based on the reduction in serum HCV RNA level at 4 weeks after initiating therapy. Nearly 75% of patients with the TT genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy achieved a complete early virologic response. In contrast, 80% of patients with the TG/GG genotype whose HCV RNA levels were reduced by ≥3 $\log_{10}$ at 4 weeks after starting the therapy showed a partial early virologic response. The majority of patients with the TT or TG/GG genotypes achieved a partial early virologic response when their reduction in HCV RNA levels was <3 $\log_{10}$ and ≥2 $\log_{10}$ or <2 $\log_{10}$ and ≥1 $\log_{10}$.

Figure 3 shows the rates of a sustained virologic response according to the type of early virologic response in patients with the TT genotype (Fig. 3A) and TG/GG genotype (Fig. 3B). Among patients with the TT genotype, the rate of sustained virologic response was significantly higher in patients with a complete early virologic response than in those with a partial early virologic response ($P < 0.0001$). In contrast, there was no difference in the rate of a sustained virologic response between patients with a complete early virologic response and those with a partial early virologic response ($P = 0.8917$) among patients with

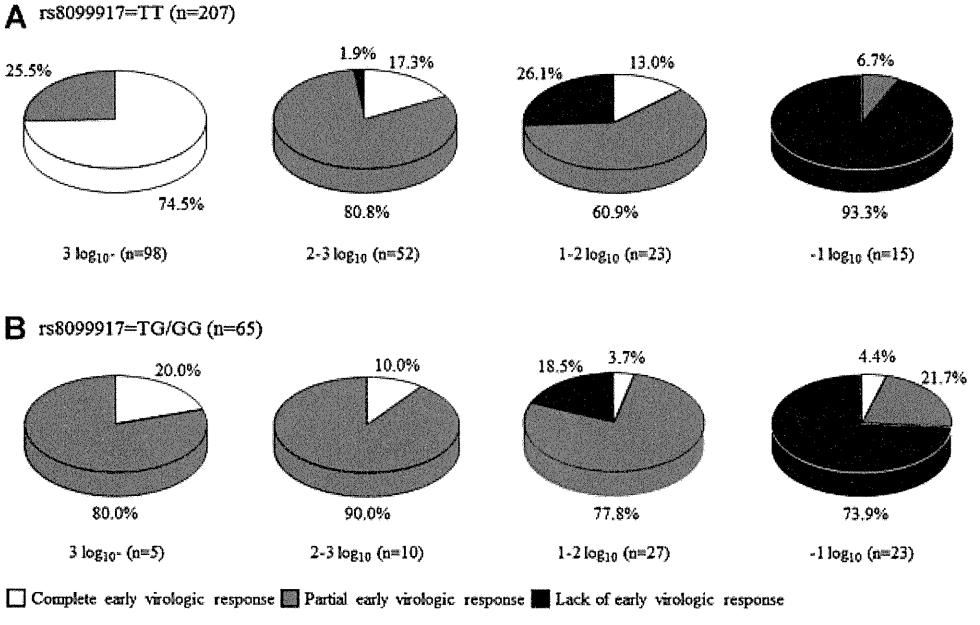

Fig. 2. The association between the virologic responses at 12 weeks after starting therapy and the reduction in serum HCV RNA levels at 4 weeks after starting therapy. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

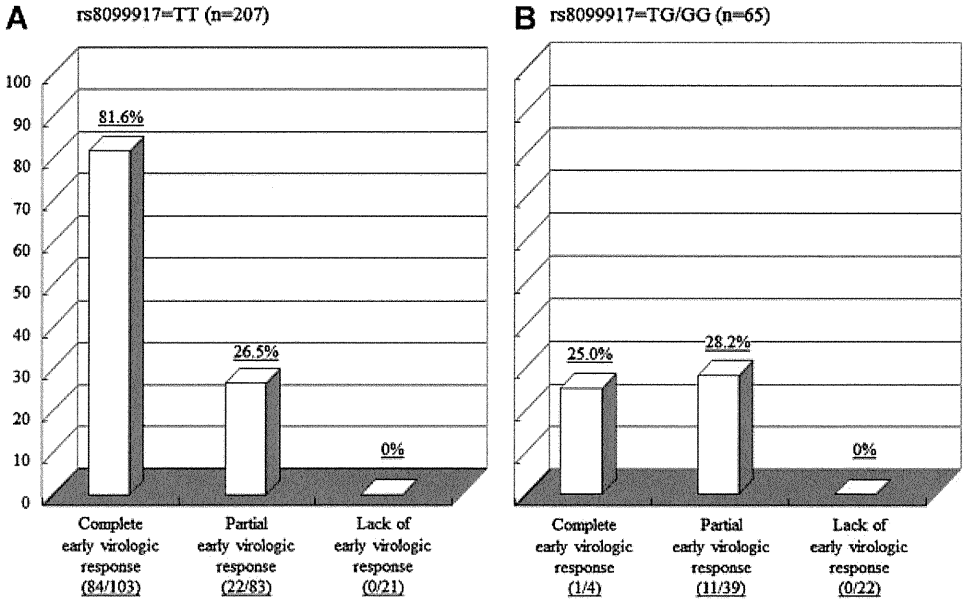

Fig. 3. The rate of sustained virologic responses based on the type of early virologic response. **A**: Patients with the TT genotype for rs8099917, (**B**) patients with the TG/GG genotype for rs8099917.

the TG/GG genotype. None of the patients with the TT genotype or TG/GG genotype who yielded a lack of an early virologic response reached a sustained virologic response.

Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

Univariate and multivariate analyses were conducted for factors associated with a sustained virologic response based on different genetic polymorphisms near the *IL28B* gene. In patients with the TT genotype, the factors that were associated with a sustained virologic response included serum alkaline phosphatase levels, serum albumin, platelet counts, hepatitis activity grade, liver fibrosis grade, reduction in HCV RNA levels at 4 weeks after starting therapy, and a complete early virologic response based on a univariate analysis (Table IIIA). In a multivariate analysis, the serum albumin levels, reduction in HCV RNA levels 4 weeks after starting therapy, and a complete early virologic response were independent factors that were significantly associated with a sustained virologic response (Table IIIB). A reduction in HCV RNA levels 4 weeks after starting therapy was the strongest factor that affected a sustained virologic response. In patients with the TG/GG genotype, the factors that were associated with a sustained virologic response included patient age, platelet counts, and pretreatment HCV RNA levels based on a univariate analysis (Table IIIA). A reduction in the HCV RNA levels at 4 weeks after starting therapy was not associated with a sustained virologic response. In a multivariate analysis, patient age and pretreatment HCV RNA levels were independent factors that were significantly associated with a sustained virologic response (Table IIIC).

Characteristics of Patients who Achieved a Sustained Virologic Response to the Combination Therapy Despite the Unfavorable TG/GG Genotype Near the *IL28B* Gene

Table IV shows the characteristics of 12 patients who achieved a sustained virologic response despite having the unfavorable TG/GG genotype for rs8099917 near the *IL28B* gene. All but one patient was under 60 years old and had liver fibrosis not more than grade 2 (one patient did not undergo a liver biopsy). Except for one patient, the reduction in the serum HCV RNA levels at 4 weeks after starting therapy was less than 3 $\log_{10}$ and all but one patient showed a partial early virologic response at 12 weeks after starting the therapy. In all 11 patients with a partial early virologic response, the serum HCV RNA was undetectable up to 24 weeks after starting the therapy. All but one patient extended the treatment duration from 48 to 72 weeks (two patients discontinued therapy at 60 weeks during the extended treatment period). When the characteristics of patients who achieved a sustained virologic response were compared between those with the unfavorable TG/GG genotype and those with the favorable TT genotype, patients with the TG/GG genotype were younger (41.8 ± 14.4 years vs. 55.1 ± 10.4 years, $P = 0.0023$) and had lower pretreatment HCV RNA levels (5.91 ± 0.44 $\log_{10}$ IU/ml vs. 6.21 ± 1.05 $\log_{10}$ IU/ml, $P = 0.0199$).

TABLE III. Univariate and Multivariate Analyses for Factors Associated With a Sustained Virologic Response to Peginterferon and Ribavirin Combination Therapy in Patients With the TT and the TG/GG Genotype for the rs8099917

(A) Univariate analyses	*P*-value	
	Patients with TT genotype of rs8099917 (n = 207)	Patients with TG/GG genotype of rs8099917 (n = 65)
Age (years)	0.0505	0.0007
Sex (female/male)	0.1830	0.2296
Body weight (kg)	0.6891	0.2456
Alanine aminotransferase (IU/L)	0.7988	0.4032
Aspartate aminotransferase (IU/L)	0.5021	0.1705
Gamma-glutamyl transpeptidase (IU)	0.6340	0.6648
Alkaline phosphatase (IU/L)	0.0315	0.0599
Albumin (g/dl)	0.0002	0.6594
Total bilirubin (mg/dl)	0.2929	0.7130
White blood cell count (/μl)	0.2508	0.5549
Hemoglobin (g/dl)	0.0847	0.2289
Platelet count ($\times 10^3$/μl)	0.0454	0.0411
Liver histology-activity (A0–1/A2–3)	0.0445	0.1117
Liver histology-fibrosis (F0–1/F2–3)	0.0002	0.2283
Pretreatment HCV RNA concentration (≥ 6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.5279	0.0379
Reduction in the peginterferon dose	0.4316	0.5563
Reduction in the ribavirin dose	0.1823	0.4272
Reduction in HCV RNA levels at 4 weeks after starting the therapy (≥ 3 $\log_{10}$ vs. < 3 $\log_{10}$)	<0.0001	0.9265
Early virologic response (complete vs. partial)	<0.0001	0.9777
Early virologic response (partial vs. non)	0.8632	0.0686

(B) Multivariate analyses: Patients with TT genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Alkaline phosphatase (IU/L)	0.2617	
Albumin (g/dl)	0.0365	28.287 (1.4107–755.41)
Platelet count ($\times 10^3$/μl)	0.2599	
Liver histology-activity (A0–1/A2–3)	0.6678	
Liver histology-fibrosis (F0–1/F2–3)	0.2307	
Reduction in HCV RNA levels at 4 weeks after starting the therapy (≥ 3 $\log_{10}$ vs. <3 $\log_{10}$)	<0.0001	16.029 (6.8593–40.406)
Early virologic response (complete vs. partial)	0.0224	0.3685 (0.1557–0.8749)

(C) Multivariate analyses: Patients with TG/GG genotype of rs8099917	*P*-value	Odds ratio (95% confidence interval)
Age (years)	0.0022	0.0034 (0.0000–0.0840)
Platelet count ($\times 10^3$/μl)	0.3344	
Pretreatment HCV RNA concentration (≥ 6.5 $\log_{10}$ vs. <6.5 $\log_{10}$)	0.0304	0.0548 (0.0020–0.4950)

HCV, hepatitis C virus.

DISCUSSION

Several previous studies reported that patients who achieved a rapid virologic response, in which serum HCV RNA become undetectable at 4 weeks after starting therapy, had a high likelihood of achieving a sustained virologic response [Martinez-Bauer et al., 2006; Poordad et al., 2008; de Segadas-Soares et al., 2009; Martinot-Peignoux et al., 2009]. In addition, several recent studies reported the predictive value of the degree of reduction in serum HCV RNA levels at 4 weeks after starting therapy [Yu et al., 2007; Huang et al., 2010; Toyoda et al., 2011]. Therefore, the viral dynamics of HCV at 4 as well as 12 weeks after starting therapy is important for response-guided therapy.

Genetic polymorphisms near the *IL28B* gene have emerged as the strongest predictive factor of a sustained virologic response in patients infected with HCV genotype 1 [Hayes et al., 2011; Kurosaki et al., 2011]. In addition, Thompson et al. [2010 reported that genetic polymorphisms near the *IL28B* gene were associated strongly with early viral dynamics during PEG-IFN and ribavirin combination therapy. These findings raised an important issue of whether response-guided therapy, based on the reduction in serum HCV RNA levels at 4 or 12 weeks after starting

TABLE IV. Patients who Achieved a Sustained Virologic Response Despite the TG/GG Genotype for the rs8099917

	Age (years)	Sex	Liver histology	Pretreatment HCV RNA level (log_{10} IU/ml)	HCV RNA reduction at 4 weeks	Response at 12 weeks	HCV RNA became undetectable (weeks)	Treatment duration (weeks)
1.	31	Female	A1/F1	6.13	2.19	partial EVR	20	48
2.	55	Male	A1/F1	5.80	1.77	partial EVR	16	72
3.	57	Female	A1/F1	5.58	3.01	partial EVR	16	72
4.	57	Female	A1/F1	6.21	1.81	partial EVR	20	72
5.	62	Male	N.D.	6.23	1.13	partial EVR	24	72
6.	21	Male	A1/F2	6.04	1.83	partial EVR	24	72
7.	42	Male	A1/F1	6.27	0.57	partial EVR	24	72
8.	29	Female	A1/F2	5.83	1.83	partial EVR	20	60
9.	52	Male	A1/F0	5.91	2.12	complete EVR	12	48
10.	40	Male	A2/F1	5.84	1.34	partial EVR	20	72
11.	27	Male	N.D.	5.63	0.42	partial EVR	24	72
12.	28	Male	A1/F0	6.59	0.76	partial EVR	20	60

N.D., not done; HCV, hepatitis C virus; EVR, early virologic response.

therapy, retains a predictive value when considering genetic polymorphisms near the *IL28B* gene.

In the present study, the predictive value of the decrease in serum HCV RNA levels was evaluated at 4 and 12 weeks after starting therapy in Japanese patients infected with HCV genotype 1b based on genetic polymorphisms near the *IL28B* gene. Consistent with previous reports, patients with the TG/GG genotype for rs8099917 had a smaller reduction in serum HCV RNA levels at 4 weeks after starting treatment ($P < 0.0001$), which indicates an unfavorable response to the combination therapy. Patients with the TT genotype for rs8099917, which is associated with a favorable response to the combination therapy, exhibited a significant difference in the rate of a sustained virologic response based on the reduction in serum HCV RNA levels at 4 weeks after initiating the therapy. Patients with a rapid virologic response or with a ≥ 3 log_{10} reduction in HCV RNA levels had a higher likelihood of achieving a sustained virologic response.

In contrast, these factors did not have any predictive value in patients with the TG/GG genotype. Only 18.5% of patients achieved a sustained virologic response (12 of 65 patients), and it was difficult to identify these patients based on the reduction in HCV RNA levels at 4 weeks or the type of an early virologic response at 12 weeks after starting therapy. Patients who achieved a sustained virologic response, despite the TG/GG genotype for rs8099917, were identified among those with a <2 log_{10} and ≥ 1 log_{10} or even <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy. Interestingly and paradoxically, the possibility of a sustained virologic response can be expected in patients with a <1 log_{10} reduction in HCV RNA levels at 4 weeks after starting therapy only when they have the unfavorable TG/GG genotype.

In the evaluation at 12 weeks after starting therapy, patients with the TT genotype who achieved a complete early virologic response had a higher rate of a sustained virologic response significantly than patients who achieved a partial early virologic response, whereas this difference was not found in patients with the TG/GG genotype. No patients who failed to achieve an early virologic response achieved a sustained virologic response regardless of the genetic polymorphisms near the *IL28B* gene. Thus, the lack of an early virologic response retained a strong predictive value for the failure of achieving a sustained virologic response. This result supports the recommendation in the AASLD guidelines, in which treatment may be discontinued in patients without an early virologic response at 12 weeks of treatment.

The characteristics of patients who achieved a sustained virologic response despite the unfavorable TG/GG genotype were younger in age and lower pretreatment HCV RNA levels. Most patients with the TG/GG genotype who achieved a sustained virologic response showed a partial early virologic response and extended the treatment duration. It was difficult to identify these patients according to viral dynamics at 4 or 12 weeks after starting therapy.

There are several limitations in this study. Some patients with a slow virologic response did not have their treatment period extended from 48 to 72 weeks. This is because the effectiveness of a 72-week combination therapy regimen in patients with HCV genotype 1 with a slow virologic response [Berg et al., 2006; Pearlman et al., 2007] had not been established in Japan in the earlier part of this study. This fact might have influenced the treatment outcome especially in patients with the unfavorable TG/GG genotype. Another limitation is a smaller sample size of patients with the TG/GG genotype in comparison to that of patients with the TT genotype. This sample size could have caused the lack of statistical significance in the rate of a sustained virologic response according to the reduction in HCV RNA levels at 4 weeks after starting therapy or according to the type of an early virologic response in patients with the TG/GG genotype. In addition, the data were based on Japanese patients infected with HCV genotype 1b. Therefore, these results should be confirmed in other ethnicities and patients infected with HCV genotype 1a.

In conclusion, among patients infected with HCV genotype 1b with the TT genotype for rs8099917, a rapid virologic response or a ≥ 3 $\log_{10}$ reduction in HCV RNA levels at 4 weeks after starting therapy, or a complete early virologic response indicate strongly that these patients will achieve a sustained virologic response as a final outcome for PEG-IFN and ribavirin combination therapy. Early viral dynamics retain the predictive value in this patient subpopulation. A reduction in HCV RNA levels at 4 weeks after starting therapy or the type of an early virologic response does not predict the likelihood that patients with the TG/GG genotype will achieve a sustained virologic response. In contrast, the lack of an early virologic response retains a strong predictive value for the failure to achieve a sustained virologic response regardless of *IL28B* polymorphisms, which remains useful as a factor to stop therapy.

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