

# Association of Functional Gene Polymorphisms of Interleukin-1 $\beta$ and Transforming Growth Factor- $\beta$ 1 with the Progression of Liver Fibrosis in Japanese Patients with Hepatitis C Virus-Related Chronic Liver Disease

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Interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-1 receptor antagonist (IL-1RN) are key cytokines in an inflammatory response, and transforming growth factor  $\beta$ 1 (TGF- $\beta$ 1) promotes hepatic fibrogenesis. The association of polymorphisms in the genes for these cytokines with liver fibrosis is controversial. The aim of this study was to examine the possible association of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 polymorphisms with the progression of liver fibrosis in the Japanese population using cross-sectional and longitudinal study designs. We examined 183 patients with hepatitis C virus (HCV)-related chronic liver disease (93 chronic hepatitis and 90 with cirrhosis). Some of the chronic hepatitis cases were divided into progressive fibrosis and non-progressive fibrosis. IL-1 $\beta$  -31T/C, IL-1RN variable number of tandem repeats (VNTR) and TGF- $\beta$ 1 +869 T/C polymorphisms were analyzed using a polymerase-chain reaction-based assay. In the cross-sectional study, there were no significant differences in the genotype distributions of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 between chronic hepatitis and liver cirrhosis. No significant differences were found among Child-Pugh grades in cirrhosis patients. In the longitudinal study, there were no significant differences in the genotype distributions of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 between progressive fibrosis and non-progressive fibrosis. No significant differences in the speed at which liver fibrosis develop were found among the genotypes of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1. In disagreement with other studies, the functional gene polymorphisms of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 were not associated with the progression of liver fibrosis in Japanese patients with HCV-related chronic liver disease.

**Key words:** interleukin-1 $\beta$ ; interleukin-1 receptor antagonist; liver fibrosis; polymorphism; transforming growth factor- $\beta$ 1

In hepatitis C virus (HCV)-related chronic liver disease, the progression of liver fibrosis is a common problem, and the rate of progression varies markedly between patients (Marcellin et al., 2002; McCaughan and George, 2004; Feld and Liang, 2006; Massard et al., 2006). Some patients with

Abbreviations: AST, L-aspartate aminotransferase; ECM, extracellular matrix; HCV, hepatitis C virus; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; RFLP, restriction fragment length polymorphism; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats

chronic hepatitis C rapidly develop significant fibrosis and cirrhosis, while others remain asymptomatic without progression. In general, an older age at infection, excessive alcohol consumption, male gender, overweight and immunodeficiency are known to have an association with a more rapid progression of fibrosis in chronic hepatitis C. In addition, evidence indicates that genetic factors influence the natural course of chronic hepatitis C. Since fibrosis of the liver is a highly dynamic process, a number of polymorphisms in functional genes as host genetic factors influence its progression in patients with chronic hepatitis C, contributing to inter-individual variability (Bataller et al., 2003). Among these polymorphisms, especially the proinflammatory or profibrogenetic cytokine gene polymorphisms are considered to strongly influence the progression of fibrosis (Bataller et al., 2003). Indeed, the capacity to produce cytokines in individuals largely depends on genetic polymorphisms. Several studies have examined the possible association of gene polymorphisms of proinflammatory cytokines, interleukin-1 $\beta$  (IL-1 $\beta$ ) and interleukin-1 receptor antagonist (IL-1RN), and profibrogenetic cytokine, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1), with the progression of liver fibrosis in chronic hepatitis C (Powell et al., 2000; Gewaltig et al., 2002; Bahr et al., 2003; Suzuki et al., 2003; Tanaka et al., 2003; Wang et al., 2005), but these results are controversial. In this study, we investigated cross-sectionally and longitudinally the association of functional polymorphisms in the genes for these cytokines with

the progression of liver fibrosis in Japanese patients with HCV-related chronic liver disease.

## Materials and Methods

### Subjects

One hundred and eighty-three patients with HCV-related chronic liver disease were enrolled in this study (Table 1). All the patients were Japanese and positive for HCV-RNA. The diagnosis of liver disease was confirmed by laboratory tests and/or histological examination. All patients were negative for hepatitis B surface antigen and human immunodeficiency virus antibody. The subjects of this study were 93 patients with chronic hepatitis (mean age  $63.6 \pm 10.8$  years, 53 men and 40 women) and 90 patients with liver cirrhosis (mean age  $66.4 \pm 9.4$  years, 45 men and 45 women). Among the chronic hepatitis patients, we have selected 43 who were followed up for 5 years, and divided them into the progressive fibrosis group ( $n = 26$ , 13 men and 13 women, mean age  $55 \pm 9$  years) and a non-progressive fibrosis group ( $n = 17$ , 9 men and 8 women, mean age  $63 \pm 10$  years) according to the 5-year change in the FibroIndex (Koda et al., 2007) which was determined based on the platelet count, L-aspartate aminotransferase (AST) level and  $\gamma$ -globulin level, as an index for assessing fibrotic stage at both one point and follow up:  $1.738 - 0.064 (\text{platelets} \times 10^4 / \text{mm}^3) + 0.005 (\text{AST IU/L}) + 0.463 (\gamma\text{-globulin g/dL})$ . This

**Table 1. Characteristics of patients**

		Whole patients [183]	Patients with chronic hepatitis [93]	Patients with liver cirrhosis [90]
Age	(year)	$64.9 \pm 10.2$	$63.6 \pm 10.8$	$66.4 \pm 9.4$
Gender	(male/female)	98/85	53/40	45/45
AST	(IU/mL)	$68.7 \pm 41.8$	$57.0 \pm 38.3$	$81.4 \pm 42.3$
ALT	(IU/mL)	$68.9 \pm 52.6$	$68.8 \pm 60.4$	$69.2 \pm 42.4$
Platlet	( $\times 10^4/\text{mL}$ )	$14.8 \pm 14.7$	$18.6 \pm 17.1$	$10.7 \pm 10.0$
HCV RNA	(kIU/mL)	$359 \pm 294$	$462 \pm 309$	$217 \pm 193$

ALT, L-alanine aminotransferase; AST, L-aspartate aminotransferase; HCV, hepatitis C virus.  
[ ], number of patients.

study was approved by the Committee for the Ethics of Medical Experiments on Human Subjects of the Medical Faculty of the Tottori University, and written-informed consent was obtained from each subject before blood was collected.

In addition, the rate at which fibrosis progressed was examined in some chronic hepatitis patients identified at the exact same time point of HCV-infection by careful anamnesis. The rate of progression per year was estimated as the fibrosis stage (score 0 to 4) by New Inuyama classification (Ichida et al., 1996) at the time of biopsy divided by the duration of infection with HCV or as the difference in the fibrosis stage between the first and second biopsy divided by the time interval between the 2 biopsies.

### **DNA extraction**

Genomic DNA was extracted from peripheral white blood cells using a DNA extracting kit (DNA Quick II: Dainippon Pharmaceutical, Osaka, Japan) according to the manufacturer's instructions.

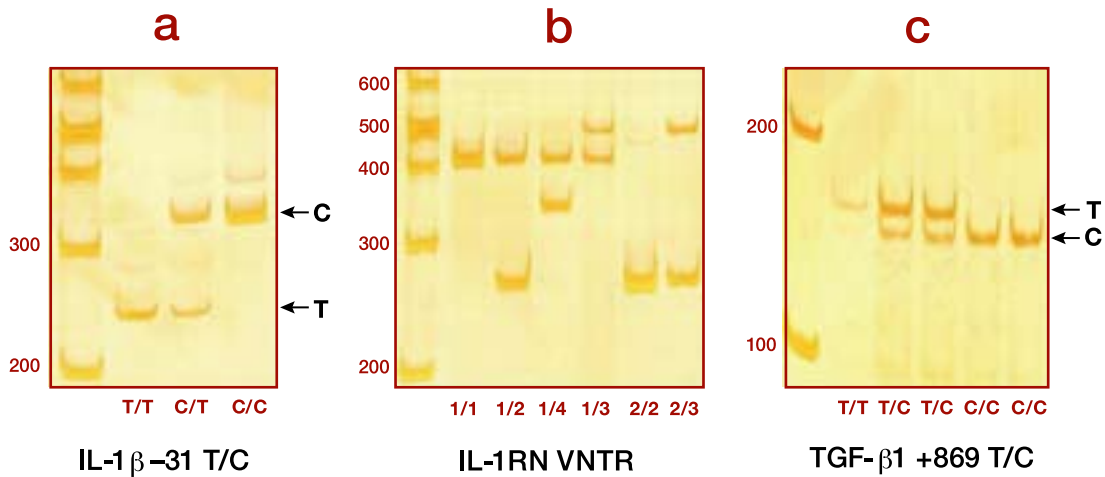
### **Analysis of the IL-1 $\beta$ -31T/C genotype**

The IL-1 $\beta$  -31T/C genotype was analyzed using the PCR with mutagenic primers, followed by the restriction fragment length polymorphism (RFLP) method according to Wang et al. (2003). The PCR was carried out in a final volume of 25  $\mu$ L: 2  $\mu$ L (50 ng) of genomic DNA, 10 pmol of forward primer (5'-AGAAGCTTCCACCAACTACTC-3'), 10 pmol of reverse primer (5'-AGCACCTAGTTGTAAGGAAG-3'), 200  $\mu$ M of each nucleotide, 1.5 mM MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase (Takara Shuzo, Kusatsu, Japan), and 18  $\mu$ L of sterile H<sub>2</sub>O. The conditions for amplification were as follows: initial denaturation at 95°C for 1 min, followed by 36 cycles of denaturation at 94°C for 45 s, annealing at 54°C for 50 s, and extension at 72°C for 1 min. RFLP for IL-1 $\beta$  was carried out. In brief, a mixture of 5  $\mu$ L of PCR product, 2  $\mu$ L of 10 $\times$  NE buffer (300 mM NaCl,

10 mM Tris-HCl, 0.1 mM EDTA, 1 mM DTT, 500 ng/mL bovine serum albumin and 50% glycerol, pH 7.4), 1  $\mu$ L of Alu I (10 units/ $\mu$ L) (Takara Shuzo) and 12  $\mu$ L of sterile H<sub>2</sub>O was incubated at 37°C overnight. The mixture was loaded onto a polyacrylamide gel (total concentration 10%, concentration of cross-linker 3%, 1 $\times$  TBE buffer: 89 mM Tris, 89 mM borate and 2.2 mM EDTA). Electrophoresis was performed at 200V for 70 min at 20°C using 1 $\times$  TBE buffer. Bands were visualized by silver staining (Fig. 1a).

### **Analysis of the IL-1RN VNTR genotype**

The IL-1RN variable number of tandem repeats (VNTR) genotype was analyzed by amplification followed by staining of the PCR products directly according to Wang et al. (2003). The PCR was carried out in a final volume of 25  $\mu$ L: 2  $\mu$ L (50 ng) of genomic DNA, 0.2  $\mu$ L of forward primer (5'-CCCCTCAGCAAACTCC-3'), 0.2  $\mu$ L of reverse primer (5'-GGTCAGAAGGGCAGAGA-3'), 2.0  $\mu$ L of each nucleotide, 2.5  $\mu$ L of MgCl<sub>2</sub>, 1 unit of Taq DNA polymerase (Takara Shuzo), and 18  $\mu$ L of sterile H<sub>2</sub>O. The conditions for amplification were: initial denaturation at 95°C for 10 min, followed by 5 cycles of denaturation at 94°C for 45 s, annealing at 65°C for 30 s, and extension at 72°C for 30 s, then 30 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s, and finally 5 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 30 s, and extension at 72°C for 30 s. The PCR products were loaded directly onto a polyacrylamide gel (total concentration 10%, concentration of cross-linker 3%, 1 $\times$  TBE buffer: 89 mM Tris, 89 mM borate and 2.2 mM EDTA). Electrophoresis was performed at 200 V for 120 min at 20°C using 1 $\times$  TBE buffer. Bands were visualized by silver staining. Five alleles were assigned based on amplicon size: allele 1 (4 repeats) 442 bp, allele 2 (2 repeats) 272 bp, allele 3 (3 repeats) 357 bp, allele 4 (5 repeats) 532 bp and allele 5 (6 repeats) 627 bp (Fig. 1b).



**Fig. 1.** Analysis for gene polymorphisms of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1. **a:** IL-1 $\beta$  -31 T/C genotypes. **b:** IL-1RN VNTR genotypes. **c:** TGF- $\beta$ 1 +869 T/C genotypes. IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1.

### **Analysis of the TGF- $\beta$ 1 +869 T/C genotype**

The TGF- $\beta$ 1 genotype was analyzed by PCR followed by RFLP according to Wood et al. (2000). The PCR was carried out in a final volume of 40  $\mu$ L: 3  $\mu$ L of genomic DNA, 0.32  $\mu$ L of forward primer 5'-TTCCCTCGAGGCCCTCCTA-3', 0.32  $\mu$ L of reverse primer 5'-GCCGCAGCTTGGACAGGATC-3', 2.4  $\mu$ L of each nucleotide, 4.0  $\mu$ L of 25 mM MgCl<sub>2</sub>, 0.8 units of Taq DNA polymerase (Takara Shuzo) and 29.8  $\mu$ L of sterile H<sub>2</sub>O. The conditions for amplification were: initial denaturation at 96°C for 10 min, followed by 30 cycles of denaturation at 96°C for 60 s, annealing at 62°C for 60 s, extension at 73°C for 60 s and 73°C for 5 min. The RFLP analysis for TGF- $\beta$ 1 was carried out with a mixture of 10  $\mu$ L of the PCR product, 2.0  $\mu$ L of 10 $\times$  NE buffer (50 mM NaCl, 10 mM Tris-HCl, 10 mM MgCl<sub>2</sub> and 1 mM dithiothreitol, pH 7.9), 0.2  $\mu$ L of bovine serum albumin, 0.25  $\mu$ L of MspA1-I (20 units/mL) (Takara Shuzo) and 7.55  $\mu$ L of sterile H<sub>2</sub>O. The solution was incubated at 37°C overnight. The mixtures were loaded onto a polyacrylamide gel (total concentration 15%, concentration of cross linker 3%, 1 $\times$  TBE buffer: 89 mM Tris, 89 mM borate and 2.2 mM EDTA). Electrophoresis was performed

at 200 V for 150 min at 10°C using 1 $\times$  TBE buffer as a tank buffer. Bands were visualized with silver staining (Fig. 1c).

### **Plasma IL-1 $\beta$ concentration**

The plasma concentration of IL-1 $\beta$  was measured with an ELISA kit (BioSource Europe S.A., Nivelles, Belgium).

### **Statistical analyses**

Values were expressed as the mean  $\pm$  SD. Differences in genotype distributions were tested with the  $\chi^2$  test. The differences of data between the 2 groups were assessed with a *t* test. A *P* value of < 0.05 was regarded as statistically significant.

## **Results**

### **Genotype distributions and allelic frequencies of IL-1 $\beta$ -31 T/C, IL-1RN VNTR and TGF- $\beta$ 1 +869 T/C**

Genotype distributions and allelic frequencies of IL-1 $\beta$  -31, IL-1RN and TGF- $\beta$ 1 +869 are shown in Table 2. The frequency of each genotype was

**Table 2. Genotype distributions and allelic frequencies in patients with HCV-related chronic liver disease**

		Genotype distribution					Allele frequency			
		T/T	T/C	C/C	<i>P</i> value	T	C	<i>P</i> value		
<b>IL-1<math>\beta</math> -31T/C</b>										
Chronic hepatitis	[92]	27%	49%	24%	0.539	0.51	0.49	0.488		
Liver cirrhosis	[85]	23%	46%	31%		0.46	0.54			
<b>IL-1RN VNTR</b>		1/1	1/2	1/4	2/4	1/3	<i>P</i> value	Allele 2	Others	<i>P</i> value
Chronic hepatitis	[92]	86%	11%	1%	1%	1%	0.824	0.06	0.94	0.999
Liver cirrhosis	[91]	87%	10%	2%	1%	0%		0.11	0.89	
<b>TGF-<math>\beta</math>1 +869T/C</b>		T/T	T/C	C/C	<i>P</i> value	T	C	<i>P</i> value		
Chronic hepatitis	[91]	29%	43%	28%	0.211	0.51	0.49	0.228		
Liver cirrhosis	[90]	35%	47%	18%		0.59	0.41			

For technical reasons, genotyping was not possible in all patients.

HCV, hepatitis C virus; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats.

[ ], number of patients.

in the Hardy-Weinberg equilibrium ( $\chi^2 = 1.597$ ,  $P = 0.450$  for IL-1 $\beta$ ;  $\chi^2 = 0.035$ ,  $P = 0.983$  for IL-1RN;  $\chi^2 = 0.938$ ,  $P = 0.626$  for TGF- $\beta$ 1). No significant differences in genotype distribution or allelic frequency were observed between chronic hepatitis and liver cirrhosis, although the TGF- $\beta$ 1 +869 C/C homozygote with higher transcriptional activity tended to more often be in the chronic hepatitis group. When the cases of cirrhosis were divided into grade A and grade B + C according to the Child-Pugh classification (Pugh et al., 1973), there was no significant difference in genotype frequencies between the 2 groups grade A and grade B + C groups at each gene (Table 3), although the TGF- $\beta$ 1 +869 C/C homozygote tended to be high in grade A cirrhosis but not significant ( $P = 0.067$ ).

### **Difference in genotype frequency of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 between progressive and non-progressive fibrosis**

The chronic hepatitis patients were divided into those with progressive fibrosis and those with non-progressive fibrosis according to the 5-year change of FibroIndex (Table 4). No significant differences in genotype frequency were observed between the 2 groups. In addition, the 5-year

**Table 3. Genotype distributions and the Child-Pugh classification in patients with liver cirrhosis**

Genotype	Child-Pugh		<i>P</i> value
	A	B + C	
<b>IL-1<math>\beta</math> -31 T/C</b>			
T homozygote	33 (79%)	24 (71%)	0.424
C carrier	9 (21%)	10 (29%)	
<b>IL-1RN VNTR</b>			
Allele 2	5 (11%)	4 (11%)	0.999
Others	40 (89%)	32 (89%)	
<b>TGF-<math>\beta</math>1 +869T/C</b>			
T carrier	33 (77%)	34 (92%)	0.067
C homozygote	10 (23%)	3 (8%)	

IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; LC, liver cirrhosis; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1.

changes of FibroIndex did not differ between the genotypes in the progressive or non-progressive group.

### **Speed at which liver fibrosis developed according to genotypes for IL-1 $\beta$ , IL1-RN and TGF- $\beta$ 1**

Among the chronic hepatitis patients in whom the rate at which liver fibrosis developed was estimated by careful anamnesis, comparisons

**Table 4. Genotype frequency in the progressive and non-progressive fibrosis groups**

Genotype	Non-progressive group		Progressive group		P value
	FibroIndex ( $\Delta$ )	Genotype frequency	FibroIndex ( $\Delta$ )	Genotype frequency	
IL-1 $\beta$ T homozygote	-0.311 $\pm$ 0.266	6 (24%)	0.433 $\pm$ 0.504	3 (17%)	0.832
-31T/C C carrier	-0.235 $\pm$ 0.146	16 (76%)	0.392 $\pm$ 0.424	15 (83%)	
IL-1RN Allele 2	-0.153 $\pm$ 0.159	3 (11%)	0.344 $\pm$ 0.279	2 (11%)	0.999
VNTR Others	-0.284 $\pm$ 0.197	24 (89%)	0.407 $\pm$ 0.444	16 (89%)	
TGF- $\beta$ 1 T carrier	-0.234 $\pm$ 0.192	18 (67%)	0.427 $\pm$ 0.502	12 (71%)	0.999
+869T/C C homozygote	-0.284 $\pm$ 0.197	9 (33%)	0.407 $\pm$ 0.444	5 (29%)	

$\Delta$ , increment variable; IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats.

**Table 5. The speed at which liver fibrosis developed estimated by careful anamnesis among genotypes**

Genotype	Liver fibrosis speed (stage/infection duration) (mean $\pm$ SEM)		P value
	IL-1 $\beta$ T homozygote [ 6]	0.117 $\pm$ 0.038	
-31T/C C carrier [16]	0.167 $\pm$ 0.050		
IL-1RN Allele 2 [ 1]	0.108 $\pm$ 0.000	0.772	
VNTR Others [23]	0.159 $\pm$ 0.036		
TGF- $\beta$ 1 T carrier [21]	0.162 $\pm$ 0.040	0.534	
+869 T/C C homozygote [ 3]	0.094 $\pm$ 0.007		

IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats.  
[ ], number of patients.

**Table 6. The speed at which liver fibrosis developed estimated by follow-up liver biopsies among genotypes**

Genotype	Liver fibrosis speed (stage/infection duration) (mean $\pm$ SEM)		P value
	IL-1 $\beta$ T homozygote [ 4]	0.046 $\pm$ 0.030	
-31T/C C carrier [ 8]	0.091 $\pm$ 0.126		
IL-1RN Allele 2 [ 2]	-0.240 $\pm$ 0.023	0.858	
VNTR Others [11]	0.027 $\pm$ 0.113		
TGF- $\beta$ 1 T carrier [ 6]	0.121 $\pm$ 0.169	0.613	
+869 T/C C homozygote [ 6]	0.032 $\pm$ 0.028		

IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats.  
[ ], number of patients.

were made according to genotype (Table 5). No significant differences in clinical findings such as age, gender, infection duration, and laboratory data were observed (Table 1). Although the C/C homozygote of TGF- $\beta$ 1 tends to develop fibrosis at a slower speed than the T carrier, there were no significant differences in terms of each gene.

#### **Speed of liver fibrosis estimated by follow up liver biopsies among genotypes of IL-1 $\beta$ , IL1-RN and TGF- $\beta$ 1**

Among chronic hepatitis patients in whom the rate at which liver fibrosis developed was estimat-

ed with the follow-up liver biopsies, the speed of the disease's progression was examined according to genotypes (Table 6). There were no statistically significant differences between the genotypes, although the TGF- $\beta$ 1 C/C homozygote tended to have a slower progression than the T carrier.

#### **Plasma IL-1 $\beta$ concentration according to genotype of IL-1 $\beta$ and IL-1RN**

We examined the relationship of plasma IL-1 $\beta$  concentration with the genotypes of IL-1 $\beta$  and IL-1RN, and found no significant differences in the IL-1 $\beta$  concentrations between IL-1 $\beta$  -31C carrier

and T homozygote, and between IL-1RN allele 2 and the others (Table 7).

## Discussion

In the present cross-sectional and longitudinal studies, we found no close association of functional gene polymorphisms of IL-1 $\beta$  -31T/C, IL-1RN VNTR and TGF- $\beta$ 1 +869T/C with the progression of liver fibrosis in Japanese patients with HCV-related chronic liver disease, although the TGF- $\beta$ 1 C/C homozygote tended to have a slower progression of liver fibrosis.

IL-1 $\beta$  is a key cytokine in the inflammatory response and its biological activities promotes various diseases including chronic hepatitis, in which an increase in IL-1 $\beta$  stimulates the progression of liver fibrosis. In fact, serum levels and hepatic mRNA levels of IL-1 $\beta$  are reported to be elevated in patients with chronic hepatitis C (Tilg et al., 1992; Gramantieri et al., 1999). The IL-1 gene family at 2q13-14 encodes 3 proteins. In this gene, some polymorphisms have been reported: one is in the promoter region at position -511 and -31, representing C  $\rightarrow$  T and T  $\rightarrow$  C transitions, which are in near total linkage disequilibrium (El-Omar et al., 2000). The IL-1 $\beta$  -31 C allele (-511 T) has higher transcriptional activity than the IL-1 $\beta$  -31 T allele (-511 C) (Bataller et al., 2003) suggesting that -31C allele carriers may overproduce the IL-1 $\beta$  protein, resulting in a more aggressive necroinflammation and in a rapid progression of liver fibrosis.

Takamatsu et al. (2000) have reported that the carriers of the -511 allele 2 (-31C) were significantly more common in Japanese patients with alcoholic cirrhosis than in those with noncirrhotic alcoholic liver disease. By contrast, Bahr et al. (2003) have reported that IL-1 $\beta$  -511T/C genotype distribution was similar between HCV-induced chronic hepatitis and cirrhosis in the German population. Tanaka et al. (2003) have also reported that IL-1 $\beta$  -511C/T genotype distributions did not differ between F0-F2 and F3-F4 in HCV-related

**Table 7. Plasma IL-1 $\beta$  concentration according to genotype**

Genotype		Plasma IL-1 $\beta$ (ng/mL) (mean $\pm$ SD)	<i>P</i> value
IL-1 $\beta$ -31T/C	T homo	12.4 $\pm$ 14.2	0.757
	C carrier	13.5 $\pm$ 13.7	
IL-1RN VNTR	Allele 2	12.6 $\pm$ 4.4	0.753
	Others	14.0 $\pm$ 14.1	

IL-1 $\beta$ , interleukin-1 $\beta$ ; IL-1RN, interleukin-1 receptor antagonist; TGF- $\beta$ 1, transforming growth factor- $\beta$ 1; VNTR, variable number of tandem repeats.

chronic liver disease in the Japanese population. In our study, we found no significant difference of IL-1 $\beta$  -31T/C genotype frequency between HCV-related chronic hepatitis and cirrhosis in Japanese. Taken together, the IL-1 $\beta$  -31T/C genotype does not influence the rate of progression of liver fibrosis in patients with HCV-related chronic liver disease. In fact, we found no significant difference in the plasma IL-1 $\beta$  concentration in patients with HCV-related chronic liver disease according to IL-1 $\beta$  -31T/C genotypes or IL-1RN VNTR genotypes.

IL-1 RN, an anti-inflammatory molecule which competes for binding with the IL-1 receptor, is encoded by the IL-1RN gene (Santtila et al., 1998). This gene has a penta-allelic 86-bp tandem repeat polymorphisms VNTR in intron 2. IL-1RN allele 2 has been shown to be associated with enhanced IL-1 $\beta$  production in vitro (Santtila et al., 1998) and in vivo (Hwang et al., 2002). Bahr et al. (2003) have reported that IL-1RN allele 2 is significantly more prevalent in HCV-related cirrhosis as compared with HCV-related chronic hepatitis. In the Japanese population, Tanaka et al. (2003) have reported that IL1RN VNTR genotype distributions did not differ between F0-F2 and F3-F4 in HCV-related chronic liver disease. In our study, we also found no significant difference of IL-1RN VNTR genotype between HCV-related chronic hepatitis and cirrhosis in Japanese. As the reason for the differ-

ence between Caucasian and Japanese, the allelic frequency itself has been attributed to be different between the both populations. In fact, allele 2/2 of IL-1RN was observed in 10 to 30% of the Caucasian control population (Bahr et al., 2003), but only 0 to 2% of the Japanese control population (Tanaka et al., 2003; Wang et al., 2003).

TGF- $\beta$ 1 is a pluripotent cytokine that promotes hepatic fibrogenesis by stimulating the synthesis of extracellular matrix (ECM), inhibiting the degradation of ECM, and activation of hepatic stellate cells. Indeed, the plasma TGF- $\beta$ 1 concentration is increased in patients with chronic hepatitis, and correlated with the degree of liver fibrosis (Murawaki et al., 1998; Thushima et al., 1999). In the human TGF- $\beta$ 1 gene, which is located at chromosome 19q13, eight polymorphisms are presently known including -800G/A, -509C/T, +869T/C and +915G/C (Gewaltig et al., 2002). The +869T homozygote, the +915G homozygote and the -509T carrier are reported to have higher levels of TGF- $\beta$ 1 (Grainger et al., 1999).

There are several studies about the association of TGF- $\beta$ 1 gene polymorphisms with the progression of liver fibrosis in patients with HCV-related chronic liver disease, but the results are controversial. Powell et al. (2000) have shown that the TGF- $\beta$ 1 +915G/G homozygote with high transcriptional activity was more often in stage III/IV in Australian HCV-related chronic liver disease patients, whereas TGF- $\beta$ 1 -509C/T and +869T/C did not differ among the stages of fibrosis. In contrast with Powell's data, Wang et al. (2003) have shown that the TGF- $\beta$ 1 +869 T carrier and +915 G/C heterozygote are more often in stage III/IV in German HCV patients. Suzuki et al. (2003) have shown no association of TGF- $\beta$ 1 +869T/C polymorphism with the progression of liver fibrosis in Japanese HCV patients. Since the TGF- $\beta$ 1 +915 G/C polymorphism was not observed in Japanese (Suzuki et al., 2003), we analyzed only the TGF- $\beta$ 1 +869T/C genotype in this study. In agreement with Suzuki's results, we found no significant association of TGF- $\beta$ 1 +869T/C with the progression of liver fibrosis in HCV-related chronic liver disease.

Gewaltig et al. (2002) examined the association of TGF- $\beta$ 1 polymorphisms with the rate at which fibrosis developed in 26 patients who underwent repeatable liver biopsy, and showed that the presence of proline at codon 10 and/or 25 was associated with a faster progression of fibrosis: +869 C carriers and +915G/C heterozygote developed fibrosis faster. By contrast, our longitudinal study, in which disease progression was estimated based on the duration of HCV infection or the change of stage in follow-up biopsies, has shown that the TGF- $\beta$ 1 +869 C/C homozygote had a slower rate of progression than T carriers, but the difference was not significant. We can not presently explain this difference, but race, the evaluation system, and patient background may all have had an effect. Large-scale, well-designed studies are required to clarify this issue.

In conclusion, we did not find a close association of IL-1 $\beta$ , IL-1RN and TGF- $\beta$ 1 genotype distributions with the progression of liver fibrosis in HCV-related chronic liver disease in cross-sectional and longitudinal studies.

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