Impact of Interleukin-1β Genetic Polymorphisms on the Development of Hepatitis C Virus–Related Hepatocellular Carcinoma in Japan

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To examine the effects of polymorphisms in the gene encoding proinflammatory interleukin (IL)–1β in patients infected with hepatitis C virus (HCV) in Japan, we studied 364 patients with chronic HCV infection (146 of whom had hepatocellular carcinoma [HCC] and 218 of whom did not) and 230 healthy control subjects. IL-1B−511 and IL-1RN genotypes were ascertained, and IL-1B−511 genotype T/T was found to be a significant risk factor for the development of HCC, indicating that polymorphism in the IL-1B−511 genetic locus is one of the possible determinants of progression of hepatitis C to HCC.

Cirrhosis develops in 20%–30% of patients with chronic hepatitis C virus (HCV) infection, culminating in hepatocellular carcinoma (HCC) in some patients. However, in any individual, the factors that determine progression to fibrosis and HCC remain unknown.

Host genetic factors such as interleukin (IL)–1, IL-10, tumor necrosis factor–α, and transforming growth factor–β have been reported to influence the natural history of HCV infection [1, 2]. Of these, IL-1β is well known to be proinflammatory and to mediate several immune responses in HCV infection [1, 2]. The IL-1 gene family on chromosome 2q13–14 encodes 3 proteins: the 2 agonist forms of IL-1, IL-1β encoded by the IL-1B gene, IL-1α encoded by the IL-1A gene, and the IL-1 receptor antagonist, which competes for binding with the IL-1 receptor and is encoded by the IL-1RN gene. Of these, the IL-1B gene encoding IL-1β is highly polymorphic, and several diallelic polymorphisms have been reported. Two of these are in the promoter region at positions −511 and −31, representing C>T and T>C transitions, respectively. Several studies conducted in different ethnic populations have shown that these 2 polymorphisms are in near total linkage disequilibrium [3, 4]. The variant (and less common) alleles of these loci are associated with several proinflammatory conditions [5]. A third polymorphism, at position +3954 in exon 5, has also been reported, but the frequency of the mutant allele is low in Japanese populations [4]. Another cytokine that has an important influence on IL-1β levels is the IL-1 receptor antagonist; the gene encoding this cytokine, IL-1RN, is also known to be polymorphic [6]. The IL-1RN gene has a penta-allelic 86-bp tandem repeat polymorphism (variable number tandem repeat; VNTR) in intron 2, of which the less common allele 2 (IL-1RN*2) is associated with a wide range of chronic inflammatory and autoimmune conditions. IL-1RN*2 is associated with enhanced IL-1β production in vitro [6] and in vivo [5].

Recently, IL-1B polymorphisms were reported to be associated with progression of gastric atrophy and increased risk of gastric cancer [3, 7, 8]. However, no studies have been published on the association of these polymorphisms with chronic HCV infection. In the present study, which included ethnically homogeneous Japanese subjects, the effect of IL-1 gene cluster polymorphisms (at IL-1B−511 and IL-1RN) among subjects with chronic HCV infection and chronic hepatitis, cirrhosis, and HCC was examined.

Methods. The study involved 364 patients with chronic HCV infection (mean age ± SD, 63.4 ± 10.3 years; 63% were male), 146 with HCC and 218 without HCC (116 had liver fibrosis scores of F0–F2, and 102 had scores of F3–F4). Liver biopsy samples were obtained from patients with hepatitis or HCC at Nagoya City University Hospital, Nagoya, Japan, between February 1997 and November 1999. The degree of inflammation and fibrosis was assessed and graded according to the classification of chronic hepatitis by Desmet et al. [9]. Two hundred and thirty healthy control subjects without hepatitis virus infection (mean age ± SD, 58.9 ± 11.6 years; 51% were
male) who underwent the routine annual medical examination were also enrolled, as the control group (table 1).

For genotyping of IL-1 gene polymorphisms, DNA was extracted from peripheral blood leukocytes using the QIAamp DNA Blood Mini Kit (Qiagen). The polymorphism at position \(-11002\) of the \(IL-1B\) gene was genotyped by polymerase chain reaction (PCR)–restriction fragment–length polymorphism [7, 8]. The penta-allelic VNTR polymorphism in intron 2 of the \(IL-1RN\) gene was genotyped by PCR and amplicon sizing. The genotyping patterns were classified as C/C, C/T, or T/T for \(IL-1B\) and L/L, L*/2, or *2/*2 for \(IL-1RN\), in accordance with reports published elsewhere [7, 8]. All data were confirmed by >2 independent experiments.

Data for continuous variables are given as mean ± SD. The Mantel-Haenszel \(x^2\) test and 1-way analysis of variance, followed by the Scheffe’s multiple comparison test, were used to examine whether male-to-female ratios, mean age, and incidences of \(IL-1B\) and \(IL-1RN\) genotypes differed among control subjects with various HCV-related disorders (hepatitis with liver fibrosis score of F0–F2, hepatitis with liver fibrosis score of F3–F4, and HCC). Independent effects of \(IL-1B\) and \(IL-1RN\) genotypes were assessed by logistic regression analysis. Statistical analyses were conducted with SAS, version 8.12 (SAS Institute). \(P<.05\) was considered to be statistically significant.

**Results.** Table 1 shows that the ratio of the frequency of the common (C) alleles to the frequency of variant (T) alleles (C:T) at \(IL-1B\) was 0.55:0.45 in the control group. This figure is similar to previously published data from Japanese populations [4, 7]. The frequency of allele *2 of the \(IL-1RN\) locus was very low in this Japanese population, and, indeed, only 8 of 594 subjects had the \(IL-1RN\) *2/*2 genotype (1.3%) [7], a finding that is consonant with other previously published data [4, 7].

Univariate analyses of the effects of variables on groups with different HCV-related disorders showed a significant relationship between \(IL-1B\) and \(IL-1RN\) genotypes, age, and sex, in subjects with various HCV-related disorders and in control subjects, who were assessed by logistic regression analysis. Statistical analyses were conducted with SAS, version 8.12 (SAS Institute). \(P<.05\) was considered to be statistically significant.

**Table 1.** Demographic characteristics of individuals with hepatitis C and control subjects and interleukin (IL)–1\(\beta\) gene polymorphisms at \(IL-1B\) and \(IL-1RN\) in a study of the relationship between these variables and hepatocellular carcinoma (HCC).

| Characteristic or polymorphism | Control subjects (\(n = 230\)) | Without HCC | With HCC (\(n = 146\)) | \(P\) |
|--------------------------------|----------------------------------|-------------|--------------------------|------|
| No. of male subjects/no. of female subjects | 118/112 | 68/48 | 47/55 | 106/40 | <.001<sup>a</sup> |
| Age, mean ± SD, years | 58.9 ± 11.6 | 60.4 ± 13.0 | 63.7 ± 7.9<sup>c</sup> | 65.7 ± 7.7<sup>d</sup> | <.001<sup>a</sup> |
| \(IL-1B\)–511, no. (%) of subjects | | | | | |
| C/C | 62 (27.0) | 35 (30.2) | 23 (22.5) | 44 (30.1) | .003<sup>b</sup> |
| C/T | 131 (57.0) | 58 (50.0) | 56 (54.9) | 60 (41.1) | |
| T/T | 37 (16.1) | 23 (19.8) | 23 (22.5) | 42 (28.8)<sup>f</sup> | |
| C:T | 0.55:0.45 | 0.55:0.45 | 0.50:0.50 | 0.50:0.50 | |
| \(IL-1RN\), no. (%) of subjects | | | | | |
| L/L | 193 (83.9) | 103 (92.2) | 94 (89.7) | 131 (88.8) | .064<sup>d</sup> |
| L/*2 | 36 (15.7) | 10 (5.9) | 6 (8.9) | 13 (8.6) | |
| *2/*2 | 1 (0.4) | 3 (2.0) | 2 (1.4) | 2 (2.6) | |
| L:*2 | 0.92:0.08 | 0.93:0.07 | 0.95:0.05 | 0.94:0.06 | |

<sup>a</sup> Incidence among male subjects was significantly higher than that among male control subjects (\(P<.001\)), male subjects with liver fibrosis scores of F0–F2 who did not have HCC (\(P = .018\)), and male subjects with liver fibrosis scores of F3–F4 who did not have HCC (\(P<.001\)).

<sup>b</sup> Mantel-Haenszel \(x^2\) test.

<sup>c</sup> Significantly older than the control group (\(P = .002\)).

<sup>d</sup> One-way analysis of variance.

<sup>e</sup> Significantly older than the control group (\(P<.001\)) and the group of subjects with liver fibrosis scores of F0–F2 who did not have HCC (\(P = .002\)).

<sup>f</sup> Significantly higher incidence of T/T, compared with the control group (\(P = .004\)).
whereas the mean age and sex ratio in the control group were almost the same as those in the group of patients with hepatitis and liver fibrosis scores of F0–F2.

When IL-1B–511 and IL-1RN genotypes were classified as IL-1B–511 T/T or non–IL-1B–511 T/T and IL-1RN *2/*2 or non–IL-1RN *2/*2, respectively, the logistic regression analysis revealed that IL-1B–511 T/T genotype is a statistically significant risk factor for development of HCC (odds ratio, 2.19; 95% confidence interval, 1.29–3.73). Male sex and older age were also significant risks for development of HCC (table 2), whereas our independent logistic regression analysis showed no relationship between the IL-1B–511 T/T genotype and sex or age (data not shown). The IL-1RN *2/*2 genotype had no association with liver disease progression, perhaps because of the very low prevalence of the IL-1RN *2/*2 genotype in our population.

Discussion. This study is the first demonstration of significant association between IL-1β gene polymorphisms with HCV-related HCC in a Japanese population. The importance of these genetic polymorphisms has gained credence in recent years. El-Omar et al. [3] reported that proinflammatory genotypes in the IL-1 gene cluster (IL-1B–511 T/T; 31 C/C and IL-1RN *2/*2) were associated with increased risk of gastric cancer and its presumptive precursors, gastric atrophy and hypochlorhydria, in white populations from Poland and Scotland. Recently, the IL-1B–511 T/T genotype was reported to be associated with hypochlorhydria and atrophic gastritis in a Japanese population [7]. Interestingly, in a more recent report, gastric mucosal levels of IL-1β in carriers of the IL-1B–511 T/T genotype or IL-1RN*2 allele were shown to be higher than those of noncarriers among Helicobacter pylori–infected patients, and a synergistic effect between 2 loci was seen [5], which suggests that these polymorphisms are indeed functional in the context of an infective agent. IL-1β is a proinflammatory cytokine but also a tumor growth factor that increases production of prostaglandin E2 and hepatocyte growth factor [10]. IL-1β also up-regulates mitogen-inducible cyclooxygenase (COX)–2 [11]. Combined expression of inducible nitric oxide and COX–2 may have an important effect on the prognosis of patients with HCV-related HCC by modulating angiogenesis [12]. Thus, the IL-1B–511 T/T genotype, which is related to increased IL-1β production, might be a risk factor for the development of HCV-related HCC.

It is well established that the prevalence of HCC in the United States is lower than those reported in Japan, Spain, and Italy [13, 14]. This indicates the existence of different genetic and/or environmental factors in different ethnic groups, although a longer duration of HCV exposure is one of the most critical factors in HCC prevalence rates [15]. Interestingly, ethnic origin is a key determinant of the frequency of genetic markers in a population. The prevalence of specific polymorphisms within certain populations no doubt reflects the influence of past selective pressures and will vary from one geographic region to another. Although the role of the IL-1RN VNTR polymorphism has yet to be conclusively proven in Japan, because of the low frequency of the variant allele, there seems to be a primary facile case that the IL-1B–511 polymorphism is important in both white and Japanese populations. It is noteworthy that the proinflammatory T7 genotype is significantly more common in Japan than in white populations (see the Cancer Genome Anatomy Project SNP500Cancer Database, http://snp500cancer.nci.nih.gov/snp.cfm?snp_id=16944&ethnic=true). Approximately 16%–20% of subjects included in a study in Japan [4, 7] and 33% of African American subjects and 44% of Hispanic subjects in a study carried out in the United States (Cancer Genome Anatomy Project SNP500Cancer Database) had this genotype, compared with 12% of subjects in a study in Scotland, 11%–14% of subjects in a study in Poland, and only 10% of white subjects in a study in the United States [3, 8] (Project SNP500 Cancer database). The greater prevalence of this genotype among Japanese and Hispanic individuals might partially explain the

Table 2. Relationship of subject group with age, sex, and IL-1B–511 and IL-1RN genotypes among control subjects, subjects with hepatitis C and liver fibrosis scores of F0–F4, and subjects with hepatocellular carcinoma (HCC).

| Variable, patient group | Odds ratio (95% CI) | P  |
|-------------------------|---------------------|----|
| Age                     |                     |    |
| Control                 | 1.00 (reference)    | NA |
| F0–F2                   | 1.01 (0.99–1.04)    | .164|
| F3–F4                   | 1.04 (1.02–1.07)    | <.001|
| HCC                     | 1.08 (1.05–1.10)    | <.001|
| Male sexa               | 1.00 (reference)    | NA |
| F0–2                    | 1.38 (0.87–2.18)    | .172|
| F3–4                    | 0.90 (0.56–1.45)    | .665|
| HCC                     | 3.06 (1.91–4.90)    | <.001|
| IL-1B–511 T/T genotypeb | 1.00 (reference)    | NA |
| Control                 | 1.27 (0.71–2.27)    | .427|
| F3–4                    | 1.50 (0.83–2.71)    | .182|
| HCC                     | 2.19 (1.29–3.73)    | .004|
| IL-1RN *2/*2 genotypec  | 1.00 (reference)    | NA |
| Control                 | 1.41 (0.71–2.80)    | .38 |
| F3–4                    | 2.22 (0.98–5.01)    | .06 |
| HCC                     | 1.50 (0.76–2.95)    | .22 |

NOTE. NA, not applicable.

a Reference: female sex.

b Reference: non-T/T genotype.
c Reference: non-*2/*2 genotype.
higher incidence of HCC in Japan and Spain, compared with Western white populations.

Finally, our results might be interpreted within the context of our study’s limitations: measurement of IL-1β levels in liver tissues was not possible, and therefore the correlation between the various stages of liver disease and IL-1β gene polymorphism is open to further investigation. Nevertheless, our results indicate that genotypic testing for IL-1β gene polymorphisms could have prognostic utility for predicting the development of HCC among HCV-infected patients. The additional costs of establishing the genotype of the virus infecting these patients through direct genetic testing and of more intensive care for patients with the IL-1β−511 T/T genotype may be offset by the reduced costs associated with earlier diagnosis and more effective treatment of HCC. The clinical utility remains to be verified by further studies.

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