日本財団 助成事業
The Nippon Foundation

# ２００２年度日中医学協会共同研究等助成事業報告書

－在留中国人研究者研究助成－

2003 年 3 月 1 日

財団法人 日中医学協会
理事長 殿

研究者氏名 王 岳 ㊞
所属機関名 東京大学大学院医学系消化器内科
指導責任者氏名 小俣 政男
職名 教授
所在地〒 113-8655 文京区本郷 7-3-1
電話 03-38155411 内線 33070

1．研究テーマ

ウイルス性肝炎、肝硬変、肝癌と SNP

2．本年度の研究業績

(1) 学会・研究会等における発表 (有) ・ 無 (学会名・演題)

1. 王 岳、加藤直也、椎名秀一郎、白鳥康史、小俣政男 (第 38 回日本肝臓学会総会 大阪 2002 年 6 月)
C 型肝炎における肝発癌の高危険群と IL-1b 遺転子多型

2. Yue Wang, Naoya Kato, Shuichiro Shinna, Yasushi Shiratori, Masao Omata. (53rd Annual Meeting of the AASLD, Boston, Massachusetts, November 1-5, 2002.)
Interleukin-1β gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection.

(2) 学会誌等に発表した論文 (有) ・ 無 (雑誌名・論文名)

1. Wang Y, Kato N, Hoshida Y, Yoshida H, Taniguchi H, Goto T, Moriyama M, Otsuka M, Shiina S, Shiratori Y, Ito Y, Omata M. Interleukin-1beta gene polymorphisms associated with hepatocellular carcinoma in hepatitis C virus infection. Hepatology. 2003;37(1):65-71.

日中医学協会助成事業

# C型肝炎における肝発癌の高危険群とIL-1β遺伝子多型

研究者氏名　王　岳
中国所属機関　同済大学医学部付属医院、内科医者
日本研究機関　東京大学大学院医学系消化器内科
指導責任者　教授：小俣政男
共同研究者　加藤直也、星田有人、吉田英雄、谷口博順、
五藤　忠、森山　優、大塚基之、椎名秀一朗、白鳥康史

## 要旨

Hepatitis C virus (HCV) infection is a major risk factor for developing hepatocellular carcinoma (HCC), a life-threatening sequel. However, the factors that affect disease progression to HCC have not been thoroughly elucidated.

Genetic polymorphisms in pro-inflammatory cytokines, the interleukin-1 (IL-1) family (IL-1β and IL-1ra) and tumor necrosis factor-α (TNF-α), were studied in 274 Japanese patients with chronic HCV infection and 55 healthy individuals, using standard polymerase chain reaction-based genotyping techniques. The association between these polymorphisms and disease status was evaluated, while controlling for confounding clinical variables.

The proportion of HCC patients with the IL-1β-31 T/T (55%, odds ratio to C/C was 2.63, $p$=0.009) genotype was higher than that with the T/C (44%, odds ratio to C/C was 1.64, $p$=0.149) and C/C genotypes (35%). The IL-1β-31 and -511 loci were in near-complete linkage disequilibrium, and the IL-1β-511/-31 haplotype C-T was significantly associated with the presence of HCC (odds ratio of 1.51, $p$=0.02). Polymorphisms in the TNF-α gene were not associated with disease. A multivariate analysis revealed that the IL-1β-31 T/T genotype, alpha-fetoprotein >20 μg/L, presence of cirrhosis, male sex, and age >60 years were associated with the presence of HCC, at odds ratios of 3.73 (T/T vs. C/C), 4.12, 4.03, 3.89, and 3.27, respectively.

In conclusion, IL-1β-31 genotype T/T and IL-1β-511/-31 haplotype C-T are associated with the presence of HCC in Japanese patients with chronic HCV infection.

## キーワード

cirrhosis,　inflammation, pro-inflammatory cytokine, single nucleotide polymorphism, tumor necrosis factor

## 本文

### INTRODUCTION

It has been estimated that more than 170 million people worldwide, about 3% of the world's population, are chronically infected with hepatitis C virus (HCV).[1] The majority of individuals with persistent infection develop chronic hepatitis, which can progress to cirrhosis[2,3] or hepatocellular carcinoma (HCC).[4-6] Recent epidemiological data clearly show a significant increase in the incidence of HCC, even in developed countries.[7-9] The risk for developing HCC increases with the severity of inflammation and fibrosis.[6,10,11] However, the host genetic factors that affect the clinical outcome remain unclear.

Cytokines, as products of the host response to inflammation, play an important role in defense against viral infections. However, in HCV infection, they may play a prominent role in liver damage.[12,13] Interleukin-1β (IL-1β) and tumor necrosis factor (TNF)-α are key cytokines in the inflammatory response.[14,15] During inflammation, IL-1β mediates the immune response by inducing the expression of many other genes, either by initiating their transcription or by stabilizing their mRNA; these important genes include proinflammatory cytokine genes such as TNF-α.[16] In fact, serum levels of both cytokines are elevated in patients with chronic hepatitis C,[17,18] suggesting their

role in inflammation of the liver.

Several polymorphisms of the IL-1 gene that are thought to affect IL-1β production have been reported.[19-21] In Caucasians infected with *Helicobacter pylori*, the IL-1β-31C/IL-1β-511T haplotype and the IL-1RN (encodes interleukin 1 receptor antagonist, IL-1ra) allele 2 (IL-1RN*2) have been associated with an increased risk of gastric cancer.[20,21] IL-1RN*2 has been related to enhanced IL-1β production,[22] inflammatory bowel disease,[23,24] and autoimmune diseases.[25,26]

Six diallelic polymorphisms in the TNF-α promoter region, which are thought to affect TNF-α production, have been reported, at positions -1031, -863, -857, -376, -308, and -238.[19,27,28] A variety of infectious diseases and inflammatory disorders are associated with the TNF-α-308 allele.[19] In a British population, the TNF-α-308A allele was associated with susceptibility to type 1 autoimmune hepatitis.[29] This polymorphism in liver transplantation donors influences the inflammatory response to HCV re-infection of grafts and causes accelerated graft injury.[30] Moreover, it was recently reported that the -376 allele, which affects OCT-1 binding to the TNF-α promoter region, was associated with increased susceptibility to cerebral malaria.[31] Polymorphisms at -1031, -863, and -857 have been positively associated with susceptibility to Crohn's Disease and its location.[28]

In this study, we evaluated the association between these genetic polymorphisms and the development of HCC.

## PATIENTS AND METHODS

### Patients

Of consecutive Japanese patients with chronic HCV infection who consulted the outpatient clinic of the University of Tokyo Hospital between November 2000 and January 2001, we studied 274 patients (172 men and 102 women, 25 to 79 years old, with a median age of 61 years) whose genomic DNA was available after obtaining written informed consent for genotyping. As controls, 55 healthy Japanese volunteers (48 men and 7 women, 24 to 53 years, with a median age of 31 years) without any history of liver disease were enrolled in the study after obtaining informed consent. We also obtained approval from the institutional ethics committee.

All patients were positive for HCV antibody by the second-generation enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan), and HCV RNA was measured using the Amplicor HCV assay version 1 (Roche, Tokyo, Japan); all patients were negative for hepatitis B surface antigen (Abbott Laboratories, North Chicago, IL). HCV genotypes were determined using a genotyping assay (SRL Laboratory Co., Tokyo, Japan). Any patient with an ethanol intake of 80 g/day or above for more than 10 years was considered to have a positive history of alcohol abuse. The following clinical parameters of patients were obtained at the time of whole-blood collection: age, gender, history of blood transfusion, serum albumin level, serum total bilirubin level, serum alanine aminotransferase (ALT) level, serum alpha fetoprotein (AFP) level, prothrombin time, platelet count, and serum viral load measured by the Amplicor-HCV monitor assay (Roche). Liver biopsies were performed in 180 patients within 6 months, and diagnosis of liver cirrhosis was made based on liver histology according to the criteria of Desmet[32] and Scheuer et al.[33] In patients without biopsy specimens, diagnosis of cirrhosis was based on the presence of clinical manifestations of portal hypertension (e.g., varices, encephalopathy, or ascites), biochemical abnormalities (elevated serum bilirubin, decreased serum albumin, or prolonged prothrombin time), and obvious morphological change of the liver detected by hepatic imaging (e.g., ultrasonography, computed tomography, arteriography, or magnetic resonance imaging). Diagnosis of HCC was made by several imaging modalities and confirmed histologically by sonography-guided fine needle biopsy specimens in all 125 patients. All patients were proved not to have other cancers in an initial screening examination.

### Polymorphism Genotyping

Genomic DNA was extracted from 100 μl of whole blood using a SepaGene kit (Sanko Junyaku, Tokyo, Japan) according to the manufacturer's instructions. Extracted DNA was dissolved in 20 μl of Tris-HCl buffer (10 mM, pH 8.0) containing 1 mM EDTA, and stored at -30°C until use. The biallelic polymorphisms for IL-1β and TNF-α were determined by direct sequencing of an amplified gene fragment of each gene.

To determine -31 C/T polymorphisms of the IL-1β promoter, a 240-bp fragment of the IL-1β promoter was amplified by the

polymerase chain reaction (PCR) using extracted genomic DNA as a template. PCR was performed as previously described[11] using Ready-To-Go PCR beads (Amersham Pharmacia Biotech, Uppsala, Sweden) and primers L-1B-31-F (sense primer, nt -134 to -115, 5'-AGA AGC TTC CAC CAA TAC TC-3') and L-1B-31-R (antisense primer, nt +85 to +104, 5'-AGC ACC TAG TTG TAA GGA AG-3'). The thermocycling conditions were as follows: 94°C for 10 min; then 5 cycles of 94°C for 30 s, 65°C for 30 s, and 72°C for 30 s; then 30 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 30 s; then 5 cycles of 94°C for 30 s, 55°C for 30 s, and 72°C for 30 s. For single-nucleotide polymorphism (SNP) determination, direct sequencing was performed bi-directionally using 10 ng of QIAquick Spin (Qiagen, Hilden, Germany)-purified PCR product, either the sense or antisense PCR primer, and the BigDye Terminator Cycle Sequencing Ready Reaction (PE Applied Biosystems, Foster City, CA), followed by detection on an ABI 310 automated sequencer (PE Applied Biosystems).[34]

To determine -511 C/T polymorphisms of the IL-1β promoter, a 155-bp fragment of the IL-1β promoter was amplified using the same protocol used to determine IL-1β-31 polymorphisms with the primers L-1B-511-F (sense primer, nt -600 to -581, 5'-GCC TGA ACC CTG CAT ACC GT-3') and L-1B-511-R (antisense primer, nt -465 to -446, 5'-GCC AAT AGC CCT CCC TGT CT-3'). Then, SNP was determined as mentioned above.

To determine the variable number tandem repeat polymorphism in intron 2 of IL-1RN, this site was amplified using the protocol for determining IL-1β -31 and -511 polymorphisms with primers L-1B-RN-F (sense primer, nt +12472 to +12488, 5'-CCC CTC AGC AAC ACT CC-3') and L-1B-RN-R (antisense primer, nt +12897 to +12913 in allele 1 genotype, 5'-GGT CAG AAG GGC AGA GA-3'). After amplification, the amplicon was visualized on a 12.5% polyacrylamide gel with appropriate size markers. 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.

To determine -238 G/A, -308 G/A, and -376 G/A polymorphisms of the TNF-α promoter, a 691-bp fragment was amplified by PCR similarly using primers TNFA-1 (sense primer, nt -584 to -566, 5'-GCT TGT CCC TGC TAC CCG C-3') and TNFA-2 (antisense primer, nt +88 to +106, 5'-GTC AGG GGA TGT GGC GTC T-3'). The thermocycling conditions were as follows: 94°C for 10 min; then 35 cycles of 94°C for 1 min, 60°C for 1 min, and 72°C for 1 min; then 72°C for 10 min. For SNP determination, direct sequencing was performed bi-directionally using the sequencing primers TNF-forward-472/TNF seq-452 (sense primer, nt -472 to -452, 5'-TTT TCC CTC CAA CCC CGT TT-3') and TNF-reverse-138 (antisense primer, nt -157 to -138, 5'-TTC TGT CTC GGT TTC TTC TC-3').

To identify -857 G/A, -863 G/T, and -1031A/G polymorphisms of the TNF-α promoter, a 1042-bp fragment of the TNF-α promoter was amplified using the same protocol to determine the three proximal polymorphisms of TNF-α using primers TNF-2-F (sense primer, nt –1229 to –1209, 5'-GCT TGT GTG TGT GTG TCT GG-3') and TNF-2-R (antisense primer, nt –207 to –188, 5'-GGA CAC ACA AGC ATC AAG GG-3'). SNP was determined as mentioned above, using the sequencing primers TNF-2-F (sense primer, nt -1229 to –1209, 5'-GCT TGT GTG TGT GTG TCT GG-3') and TNF-2-seq (antisense primer, nt –781 to –761, 5'-TGT GGC CAT ATC TTC TTA AA-3').

**Statistical analysis**

We calculated the statistical power required to detect the effect of an SNP on the risk of harboring HCC using a multivariate logistic regression model including other known risk factors for HCC. The SNP status was assigned a continuous value of $X = 0$, 1, or 2, which represent homozygous for an allele, heterozygous, and homozygous for a different allele, respectively. The required sample size, $n$, for the multivariate logistic regression analysis was calculated using the following formula:[35]

$$n = (Z_{1-\alpha/2} + Z_{1-\beta})^2 / [P(1-P)\beta^{*2}(1-R^2)]$$

where $Z_u$ is the upper $u^{th}$ percentile of the standard normal distribution, $P$ is the proportion of patients with HCC when $X = 1$ (we used 0.44 from Table 3), $\beta^*$ is the effect size of an SNP, and $R^2$ is the multiple correlation coefficient relating $X$ with the other covariates (we used 0.009 from our data), which were the following four factors, based on previous studies: the presence of cirrhosis, male sex, high serum AFP level, and older age.[36-38] When the effect size of an SNP was assumed to be 0.69, which corresponds to an odds ratio of 2, the required sample size was 75 or 100 for statistical powers

of 80 or 90%, respectively. When the effect size of an SNP was assumed to be 1.39, which corresponds to an odds ratio of 4, the required sample size was 18 or 25 for statistical powers of 80 or 90%, respectively. Based on these calculations, our sample size was sufficient when the odds ratio of an SNP exceeds 2.

Fourteen clinical parameters (Table 1) were evaluated to determine an association with the presence of HCC using the *t*-test, the Mann-Whitney *U*-test, and the $\chi^2$ test. The association between the genotype of each locus and the presence of HCC was evaluated using the $\chi^2$ test. The Cochran-Armitage test was used to test for trend. Possible confounding effects among these variables were adjusted using a multivariate logistic regression model, and odds ratios and 95% confidence intervals were calculated. A two-tailed $p<0.05$ was considered significant. All these data analyses were performed using SPSS ver. 10.0 (SPSS Inc., Chicago, IL, 1999). Hardy-Weinberg equilibrium of alleles at individual loci was evaluated using the program HWE (ftp://linkage.rockefeller.edu/software). The haplotype frequencies for pairs of alleles were estimated using the software EH for estimating haplotype frequencies (ftp://linkage.rockefeller.edu/software). Linkage disequilibrium coefficients $D'=D/D_{min\ or\ max}$ were calculated using the program 2BY2 (ftp://linkage.rockefeller.edu/software).

## RESULTS

### Patient characteristics

As shown in Table 1, there was no significant difference in alcohol abuse, history of blood transfusion (BTF), time from BTF, HCV genotype, or viral load between the patients with and without HCC. In patients with HCC, age, the proportion that were male, the proportion of patients with cirrhosis, serum total bilirubin level, serum ALT level, and serum AFP level were higher, and serum albumin level, prothrombin time, and platelet count were lower than in patients without HCC.

### Polymorphisms in the IL-1 and TNF-α genes

The alleles at the individual IL-1β, IL-1RN, and TNF-α loci were in Hardy-Weinberg equilibrium in both patients and controls ($p>0.15$ for all loci except TNF-α-857, $p>0.30$ for the IL-1β loci, data not shown). The genotype frequencies in the patients without HCC were similar to those of healthy controls (Table 2). The proportion of the T/T genotype of IL-1β-31 in patients with HCC (36%) was higher than in patients without HCC (25%) and healthy controls (20%) (Table 2). Of the patients with IL-1β-31 T/T, C/T, and C/C genotypes, 55, 44, and 35% had HCC, respectively. Having a T allele gradually increased the proportion of patients with HCC ($p=0.02$). The IL1-β-31 genotypes T/T and C/T increased the risk of harboring HCC compared to genotype C/C with odds ratios of 2.63 (95% CI: 1.27-5.43, $p=0.009$) and 1.64 (95% CI: 0.84-3.19, $p=0.149$), respectively. The attributable risks of IL-1β-31 genotype T/T to C/T and T/T to C/C were 9 and 20%, respectively.

The frequency of the IL-1β-31 allele T in patients with HCC (61%) was higher than that in patients without HCC (51%), with an odds ratio of 1.49 (95% CI: 1.10-2.33, $p=0.02$). Significant linkage disequilibrium was observed between the IL-1β-31 and -511 loci with linkage disequilibrium coefficients of near 1.00 (complete linkage disequilibrium, data not shown). In both patients and controls, the major haplotypes of IL-1β-511/-31 were C-T and T-C. The estimated frequency of the IL-1β-511/-31 C-T haplotype in the patients with HCC (61%) was higher than in the patients without HCC (50%) with an odds ratio of 1.51 (95% CI: 1.01-2.01, $p=0.02$), which was nearly the same as that of the IL-1β-31 allele.

For other loci, including IL-1RN and six polymorphic sites of the TNF-α promoter gene, there was no difference between the patients with HCC and those without HCC (Table 2). For IL-1RN and the six loci of the TNF-α promoter gene, we found no association with the presence of HCC.

### Factors associated with presence of HCC in HCV-infected patients

The IL-1β-31 genotype ($p=0.04$), age >60 years ($p<0.001$), male ($p=0.002$), the presence of cirrhosis ($p<0.001$), serum albumin level <3.9 g/dL ($p<0.001$), total bilirubin >0.7 mg/dL ($p=0.04$), ALT >80 U/L ($p=0.043$), AFP >20 μg/L ($p<0.001$), prothrombin time <70% ($p<0.001$), and platelet count $<12.5\times10^4$ /μL ($p<0.001$) were significantly associated with the presence of HCC by the $\chi^2$ test. To evaluate the effect of the IL-1β-31 polymorphism on the presence of HCC, a stepwise multivariate logistic regression analysis was performed using these fourteen variables. Five variables (IL-1β-31, serum alpha fetoprotein level, presence of cirrhosis, male, and age) were included in the

final model with odds ratios of 3.73 (T/T vs. C/C), 4.12 (>20 μg/L vs. ≤20 μg/L), 4.03 (presence vs. absence), 3.89 (male vs. female), and 3.27 (age >60 vs. ≤60 years) (Table 3), respectively.

## DISCUSSION

In this study, we identified a presumed genetic marker for susceptibility to hepatocarcinogenesis in patients with chronic HCV infection. Our results showed an effect of the IL-1β-31 polymorphism on the association with HCC, after controlling for other confounding clinical parameters. In our result, the genotype frequencies in patients with cirrhosis, but without HCC, were similar to those in patients without cirrhosis and HCC (the frequencies of the IL-1β-31 T/T genotype in these two groups were 24 and 25%, respectively), suggesting that this polymorphism is not as strongly associated with the presence of cirrhosis.

Our results showed that the proportion of patients with HCC gradually increased from IL-1β-31 genotype C/C to C/T to T/T. In addition, IL-1β-31 allele T was significantly associated with the presence of HCC. Based on these results, we can assume a codominant model, that is, one T allele increases the risk of having HCC, and two T alleles further increase the risk.

We showed that IL-1β-31 and -511 were in near-complete linkage disequilibrium, as observed previously,[20,21,39] indicating that one or both of these loci may functionally affect HCC development in patients with chronic HCV infection. The IL-1β-31 polymorphism, which involves a TATA box, is reported to affect lipopolysaccharide-induced IL-1β production by modifying DNA-protein interactions.[20,21] It is an attractive hypothesis that the IL-1β-31 T allele enhances IL-1β production in the liver, and induces hepatocyte damage that may finally lead to HCC development. However, El-Omar et al. reported that the T allele was inversely correlated with the risk of gastric cancer.[20,21] IL-1β-511 might also be associated with hepatocarcinogenesis, although it does not alter DNA-protein binding activity.[20] Thus, the implications of IL-1β genetic polymorphism in HCC development require further functional analysis. IL-1β is also reported to attenuate interferon-induced antiviral activity,[40] and is assumed to be closely associated with the pathogenesis of chronic hepatitis C. Conversely, we must consider the possibility that IL-1β-31 and -511 are simply in a state of linkage disequilibrium with another unknown functional locus.

A homozygous IL-1RN allele 2 (IL-1RN*2) is observed in 10 to 30% of the Caucasian population, and the 2/2 genotype is associated with hypochlorhydria and gastric cancer.[20,21] By contrast, we found that the IL-1RN*2 allele was less common in Japanese individuals (heterozygotes; 5 to 9%, homozygote; 0 to 2%).

It has been reported that in Japanese patients with alcoholic liver disease, IL-1β–511T/T (i.e., –31C/C) is found significantly more frequently in patients with cirrhosis (15.6%) than in those without cirrhosis (9.2%).[39] However, we found no association between the IL-1β genotype and cirrhosis using logistic regression analysis. This may be explained by the difference in the pathogenesis of C-viral and alcoholic cirrhosis.

There is accumulating genetic evidence on the progression of HCC, which involves mutations in p53 or β-catenin,[41-43] loss of heterozygosity,[43-45] and hypermethylation of the 14-3-3 sigma gene.[43,46] However, none of these factors fully explain its pathogenesis, due to the multistep and heterogeneous nature of hepatocarcinogenesis. Recently, genetic polymorphisms of the uridine 5'-diphosphate-glucuronosyltransferase (UGT1A7) gene were found to be associated with HCC in Germany.[47]

The serum albumin, total bilirubin, and alanine aminotransferase levels, and the prothrombin time and platelet count affected the presence of HCC in the univariate analysis. However, these parameters did not remain factors in the multivariate model, due to the marked confounding effect of the parameter 'presence of cirrhosis'. Although alcohol intake is well known to increase the risk of HCC, we did not find any association between alcohol intake and HCC. This may be because there were fewer alcoholics in our cohort compared to other studies.[39,48]

In our final multivariate logistic regression model, the odds ratios of covariates other than IL-1β-31, AFP, presence of cirrhosis, sex, and age, were similar to those of previous studies.[36-38,49,50] When we introduced interaction terms between IL-1β-31 and the other covariates in our model, none of them significantly improved the fit of the model, indicating that the IL-1β-31 genotype has an independent effect on the presence of HCC.

Some TNF-α polymorphisms enhance the production of TNF-α, and are associated with a variety of infectious diseases and inflammatory disorders.[19] However, we could not find any association between TNF-α polymorphisms and the presence of HCC. Therefore, we concluded that IL-1β-31 genotype T/T and IL-1β-511/-31 haplotype C-T were associated with susceptibility to HCC in Japanese patients with chronic HCV infection.

Despite the limitations of a cross-sectional study, our analyses showed an obviously prominent effect of the IL-1β-31 T/T genotype and IL-1β-511/-31 haplotype C-T on the risk of HCC. Since many patients are referred to our hospital for the treatment of HCC, our study population may be biased towards having HCC or cirrhosis. However, our multivariate model included most of the previously reported risk factors for HCC plus IL-1β polymorphism. This implies that our result can be generalized to the Japanese population. The uncertainty of the odds ratios arising from the study design might be resolved in a subsequent controlled trial. Nonetheless, it is noteworthy that the association between genetic polymorphism and HCC was completely independent of the presence of cirrhosis. This IL-1β-31 genotype host factor might be used as a marker to identify a subgroup at higher risk of HCC in Japanese patients with chronic HCV infection.

## REFERENCES


1. World Health Organization 1998 Press releases. http://www.who.int/inf-pr-1998/en/pr98-36.html.
2. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcomes after transfusion-associated hepatitis C. N Engl J Med 1995; 332: 1463-1466.
3. Poynard T, Bedossa P, Opolon P for the OBSVIRC, METAVIR, CLINIVIR, and DOSVIRC groups. Natural history of liver fibrosis progression in patients with chronic hepatitis C. Lancet 1997; 349: 825-832.
4. Saito I, Miyamura T, Ohbayashi A, Harada H, Katayama T, Kikuchi S, Watanabe Y, et al. Hepatitis C virus infection is associated with the development of hepatocellular carcinoma. Proc Natl Acad Sci USA 1990; 87: 6547-6549.
5. Shiratori Y, Shiina S, Imamura M, Kato N, Kanai F, Okudaira T, Teratani T, et al. Characteristic difference of hepatocellular carcinoma between hepatitis B- and C- viral infection in Japan. HEPATOLOGY 1995; 22: 1027-1033.
6. Takano S, Yokosuka O, Imazeki F, Tagawa M, Omata M. Incidence of hepatocellular carcinoma in chronic hepatitis B and C: a prospective study of 251 patients. HEPATOLOGY 1995; 21: 650-655.
7. Taylor-Roibinson SD, Foster GR, Arora S, Hargreaves S, Thomas HC. Increase in primary liver cancer in the UK, 1979-94. Lancet 1997; 350:1142-1143.
8. Deuffic S, Poynard T, Buffat L, Valleron AJ. Trends in primary liver cancer. Lancet 1998; 351: 214-215.
9. El-Serag HB, Mason AC. Rising incidence of hepatocellular carcinoma in the United States. N Engl J Med 1999; 340: 745-750.
10. Tarao K, Rino Y, Ohkawa S, Shimizu A, Tamai S, Miyakawa K, Aoki H, et al. Association between high serum alanine aminotransferase levels and more rapid development and higher rate of incidence of hepatocellular carcinoma in patients with hepatitis C virus-associated cirrhosis. Cancer 1999; 86: 589-595.
11. Yoshida H, Shiratori Y, Moriyama M, Arakawa Y, Ide T, Sata M, Inoue O, et al. for the IHIT study group. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and noncirrhotic patients with chronic hepatitis C in Japan. Ann Intern Med 1999; 131: 174-181.
12. Koziel MJ. Cytokines in viral hepatitis. Semin Liver Dis 1999; 19: 157-169.
13. Losser MR, Payen D. Mechanisms of liver damage. Semin Liver Dis 1996; 16: 357-367.
14. Dinarello CA. Biologic basis for interleukin-1 in disease. Blood 1996; 87: 2095-2147.
15. Vassali P. The pathophysiology of tumor necrosis factors. Annu Rev Immunol 1992; 10: 411-452.
16. El-Omar EM. The importance of interleukin 1b in Helicobacter pylori-associated disease. Gut 2001; 48: 743-747.
17. Tilg H, Wilmer A, Vogel W, Herold M, Nölchen B, Judmaier G, Huber C. Serum levels of cytokines in chronic liver diseases. Gastroenterology 1992; 103: 264-274.
18. Kishihara Y, Hayashi J, Yoshimura E, Yamaji K, Nakashima K, Kashiwagi S. IL-1β and TNF-α produced by peripheral blood

mononuclear cells before and during interferon therapy in patients with chronic hepatitis C. Dig Dis Sci 1996; 41: 315-321.

19. Bidwell JL, Keen LJ, Gallagher G, Kimberly R, Huizinga T, McDermott MF, Oksenberg J, et al. Cytokine Gene Polymorphism in Human Disease. On-line Databases. http://www.pam.bris.ac.uk/services/GAI/cytokine4.htm
20. El-Omar EM, Carrington M, Chow W-H, McColl, KEL, Bream JH, Young HA, Herrera J, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2001; 412(6842): 99.
21. El-Omar EM, Carrington M, Chow W-H, McColl, KEL, Bream JH, Young HA, Herrera J, et al. Interleukin-1 polymorphisms associated with increased risk of gastric cancer. Nature 2000; 404: 398-402.
22. Santtila S, Savinainen K, Hurme M. Presence of the IL-1RA allele 2 (IL1RN*2) is associated with enhanced IL-1beta production in vitro. Scand J Immunol 1998; 47: 195-198.
23. Mansfield JC, Holden H, Tarlow JK, KiGiovine F, McDowell TL, Wilson AG, Holdsworth CD, et al. Novel genetic association between ulcerative colitis and the anti-inflammatory cytokine interleukin-1 receptor antagonist. Gastroenterology 1994; 106: 637-642.
24. Herebach D, Alizadeh M, Dabadie A, Le Berre N, Colombel JF, Yaouanq J, Bretagne JF, et al. Significance of interleukin-1β and interleukin-1 receptor antagonist genetic polymorphism in inflammatory bowel diseases. Am J Gastroenterol 1997; 92: 1164-1169.
25. Blakemore AIF, Tarlow JK, Cork MJ, Gordon C, Emery P, Duff GW. IL-1 receptor antagonist gene polymorphism as a disease severity factor in systemic lupus erythematosus. Arthritis Rheum 1994; 37: 1380-1385.
26. Perrier S, Coussediere C, Dubost JJ, Albuisson E, Sauvezie B. IL-1 receptor antagonist (IL1RA) gene polymorphism in Sjögren's syndrome and rheumatoid arthritis. Clin Immunol Immunopathol 1998; 87: 309-313.
27. Wilson AG, Symons JA, McDowell TL, McDevitt HO, Duff GW. Effects of a polymorphism in the human tumor necrosis factor a promoter on transcriptional activation. Proc Natl Acad Sci USA 1997; 94: 3195-3199.
28. Negoro K, Kinouchi Y, Hiwatashi N, Takahashi S, Takagi S, Satoh J, Shimosegawa T, et al. Crohn's disease is associated with novel polymorphisms in the 5'-flanking region of the tumor necrosis factor gene. Gastroenterology 1999; 117: 1062-1068.
29. Cookson S, Constantini PK, Clare M, Underhill JA, Bernal W, Czaja AJ, Donaldson PT. Frequency and nature of cytokine gene polymorphisms in type 1 autoimmune hepatitis. HEPATOLOGY 1999; 30: 851-856.
30. Rosen HR, Lentz JJ, Rose SL, Rabkin J, Corless CL, Taylor K, Chou S. Donor polymorphism of tumor necrosis factor gene. Relationship with variable severity of hepatitis C recurrence after liver transplantation. Transplantation 1999; 68: 1898-1902.
31. Knight JC, Udalova I, Hill AVS, Greenwood BM, Peshu N, Marsh K, Kwiatkowski D. A polymorphism that affects OCT-1 binding to the TNF promoter region is associated with severe malaria. Nat Genet 1999; 22: 145-150.
32. Desmet VJ. Histopathology of chronic hepatitis. In: Liaw YF, ed. Chronic Hepatitis. Amsterdam: Elsevier, 1986: 27-44.
33. Scheuer PJ, Ashrafzadeh P, Sherlock S, Brown D, Dusheiko GM. The pathology of hepatitis C. HEPATOLOGY 1992; 15: 567-571.
34. Togo G, Toda N, Kanai F, Kato N, Shiratori Y, Kishi K, Imazeki F, et al. A transforming growth factor β type II receptor gene mutation common in sporadic cecum cancer with microsatellite instability. Cancer Res 1996; 56: 5620-5623.
35. Hsieh FY, Bloch DA, Larsen MD. A simple method of size calculation for linear and logistic regression. Stat Med 1998; 17: 1623-1634.
36. Tsukuma H, Hiyama T, Tanaka S, Nakao M, Yabuuchi T, Kitamura T, Nakanishi K, et al. Risk factors for hepatocellular carcinoma among patients with chronic liver disease. N Engl J Med 1993; 328: 1797-1801.
37. Ikeda K, Saitoh S, Arase Y, Chayama K, Suzuki Y, Kobayashi M, Tsubota A, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1,643 patients using statistical bias correction with proportional hazard analysis. HEPATOLOGY 1999; 29: 1124-1130.
38. Shuto T, Hirohashi K, Ikebe T, Mikami S, Yamamoto T, Kubo S, Wakasa K, et al. Additional hepatocellular carcinomas undetectable before surgery. World J Surg 2000; 24: 1566-1569.
39. Takamatsu M, Yamauchi M, Maezawa Y, Saito S, Maeyama S, Uchikoshi T. Genetic polymorphisms of interleukin-1β in association with the development of alcoholic liver disease in Japanese patients. Am J Gastroenterol 2000; 95: 1305-1311.
40. Tian Z, Shen X, Feng H, Gao B. IL-1β attenuates IFN-αβ-induced antiviral activity and STAT1 activation in the liver: involvement of

proteasome-dependent pathway. J Immunol 2000; 165: 3959-3965.

41. Vautier G, Bomford AB, Portmann BC, Metivier E, Williams R, Ryder SD. P53 mutations in British patients with hepatocellular carcinoma: clustering in genetic hemochromatosis. Gastroenterology 1999; 117: 154-160.
42. De la Coste A, Romagnolo B, Billuart P, Renard CA, Buendia MA, Soubrane O, Fabre M, et al. Somatic mutations of the beta-catenin gene are frequent in mouse and human hepatocellular carcinomas. Proc Natl Acad Sci 1998; 95(15): 8847-8851.
43. Feitelson MA, Sun B, Tufan NLS, Liu J. Pan JB, Lian ZR. Genetic mechanisms of hepatocarcinogenesis. Oncogene 2002; 21: 2593-2604.
44. Laurent-Puig P, Legoix P, Bluteau O, Belghiti J, Franco D, Binot F, Monges G, et al. Genetic alterations associated with hepatocellular carcinomas define distinct pathways of hepatocarcinogenesis. Gastroenterology 2001; 120: 1763-1773.
45. Yeh SH, Chen PJ, Shau WY, Chen YW, Lee PH, Chen JT, Chen DS. Chromosomal allelic imbalance evolving from liver cirrhosis to hepatocellular carcinoma. Gastroenterology 2001; 121: 699-709.
46. Iwata N, Yamamoto H, Sasaki S, Itoh F, Suzuki H, Kikuchi T, Kaneto H, et al. Frequent hypermethylation of CpG islands and loss of expression of the 14-3-3 sigma gene in human hepatocellular carcinoma. Oncogene 2000; 19(46): 5298-5302.
47. Vogel A, Kneip S, Barut A, Ehmer U, Tukey RH, Manns MP, Strassburg CP. Genetic link of hepatocellular carcinoma with polymorphisms of the UDP-glucuronosyltransferase UGT1A7 gene. Gastroenterology 2001; 121: 1136-1144.
48. Lieber CS. Alcohol and the liver: 1994 update. Gastroenterology 1994; 106: 1085-1105.
49. Bruno S, Silini E, Crosignani A, Borzio F, Leandro G, Bono F, Asti M, et al. Hepatitis C virus genotypes and risk of hepatocellular carcinoma in cirrhosis: a prospective study. HEPATOLOGY 1997; 25: 754-758.
50. Chen THH, Chen CJ, Yen MF, Lu SN, Sun CA, Huang GT, Yang PM, et al. Ultrasound screening and risk factors for death from hepatocellular carcinoma in a high risk group in Taiwan. Int. J. Cancer 2002; 98: 257-261.