

# Putative Association of *Fas* and *FasL* Gene Polymorphisms with Clinical Outcomes of Hepatitis B Virus Infection

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## Key Words

*Fas* · *FasL* · Polymorphism · Hepatitis B virus · Hepatocellular carcinoma

## Abstract

**Objective:** *Fas/FasL* polymorphisms, which are related to apoptosis, might influence the clearance of hepatitis B virus (HBV) infection and the occurrence of hepatocellular carcinoma (HCC). This study was performed to determine whether *Fas* and *FasL* promoter polymorphisms are associated with clinical outcome in chronic HBV infection. **Methods:** A total of 1,095 Korean subjects were prospectively allocated to two different groups: 'the chronic carrier group' (CC; n = 666), who were repeatedly hepatitis B surface antigen (HBsAg)-positive, and 'the spontaneous recovery group' (SR; n = 429), who were HBsAg-negative with antibodies to HBsAg and hepatitis B core antigen. In addition, the CC group was subcategorized into chronic hepatitis and HCC subgroups. *Fas* promoter polymorphisms at  $-1377G>A$  and  $-670A>G$  and the *FasL* promoter polymorphism at  $-844C>T$  were analyzed for and the genotype distributions of subjects were compared. **Results:** There were no significant as-

sociations between *Fas* or *FasL* promoter polymorphism with the HBV clearance and HBeAg clearance. However,  $-1377G>A$  in *Fas* promoter region showed protective effect to HCC occurrence (RH = 0.70, p = 0.03). **Conclusions:** *Fas*- $1377G>A$  polymorphisms might be involved in the pathogenesis of human HCC. Copyright © 2007 S. Karger AG, Basel

## Introduction

Hepatitis B virus (HBV) represents a global health problem as more than 350 million people are infected worldwide [1]. Although chronic HBV infection causes serious liver disease, the clinical course of HBV infection varies from spontaneous recovery after acute hepatitis to a persisting chronic infection that may progress to decompensated liver cirrhosis or hepatocellular carcinoma (HCC) [2]. The mechanisms underlying resolution of acute HBV infection or its progression to chronicity remain undetermined.

Age at infection has the most significant impact on the clinical outcome because chronic infection occurs in approximately 90% of infants infected at birth, in 25–50% of children infected between the ages of 1 and 5 years, and in less than 5% of those infected during adult life [2]. It is

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well known that the major mode of infection in HBV endemic areas, including Korea, is perinatal transmission [3, 4]. The mechanisms underlying resolution of acute HBV infection or its progression to chronicity in each age group remain undetermined. When determining the chronicity of HBV infection within a group of patients who are presumed to have been infected at the same age, i.e. perinatally in Korea, it is apparent that the outcome of the infection does not appear to be determined by variations in virulence of the viral strains [5], but that host factors are likely to influence disease outcome. Thus, it is conceivable that genetic differences play an additional role [6].

Cytotoxic T lymphocytes (CTLs) are involved in the immune clearance of HBV-infected hepatocytes and in the pathogenesis of chronic viral liver diseases [7]. Moreover, longstanding inflammatory activities may play important roles in the clinical course of HBV infection [8], and *Fas* and *Fas* ligand (*FasL*) are known to be involved in the T-cell-mediated apoptosis of infected hepatocytes [9]. *Fas* (also known as CD95 and APO-1) is a type I membrane protein that belongs to the tumor necrosis factor (TNF) receptor family, and is expressed in various tissues and cells [10], whereas *FasL* is a type II membrane protein that belongs to the TNF family and is predominantly expressed in activated T cells and natural killer cells [11]. Moreover, altered levels of *Fas* and/or *FasL* expression have been implicated in the pathogenesis of several liver diseases associated with immune regulation [12]. For example, it has been reported that *Fas* expression is up-regulated on hepatocytes in chronic hepatitis C [13], and *Fas* up-regulation and its correlation with degree of liver inflammation were demonstrated in chronic hepatitis B patients [14]. On the other hand, alterations of apoptosis-related genes, i.e. the evasion of apoptosis, are also likely to contribute to the pathogenesis of malignancies [15]. Indeed, human HCCs not expressing *Fas* have been reported to have a more aggressive biology than those expressing *Fas* [16].

Clinical outcomes in cases of HBV infection do not appear to be determined by viral strains, rather allelic variants in the human genome are viewed as being more likely to affect viral hepatitis progression after infection [17]. Frequently, single nucleotide polymorphism (SNP) analysis is used in association studies to map genetic variations, determine the frequencies of given SNPs, and to verify their associations with disease [18]. Moreover, several SNPs have been identified in the promoter regions of the *Fas* and *FasL* genes, and have been linked to the differential expressions of these two genes [19, 20]. Thus, we

hypothesized that *Fas* and *FasL* polymorphisms known to be related to apoptosis might influence the clearance of HBV infection and the occurrence of HCC.

As part of our ongoing efforts to discover polymorphisms in genes implicated in HBV progression, we studied SNPs in the *Fas* and *FasL* promoter regions. Specifically, the present study was undertaken to investigate the relationship between known SNPs of *Fas* and *FasL* and the clearance of HBV infection, HBeAg seroclearance, and the risk of HCC occurrence in patients with chronic HBV infection.

## Materials and Methods

### Patients

A total of 1,095 Korean subjects with either present or past evidence of HBV infection, were prospectively enrolled at the Outpatient Clinic of the Liver Unit or at the Center for Health Promotion at Seoul National University Hospital between January 2001 and August 2003. Subjects were allocated to two different groups according to serologic markers, i.e., the chronic carrier (CC) group or the spontaneous recovery (SR) group, which consisted of 666 and 429 subjects, respectively (table 1). Diagnoses of CC or SR were established by repeated seropositivity for hepatitis B surface antigen (HBsAg) (Enzygnost® HBsAg 5.0; Dade Behring, Marburg, Germany) over a 6-month period, and for both anti-HBs (Enzygnost® Anti-HBs II; Dade Behring) and anti-HBc (AB-Corek; DiaSorin Srl, Saluggia, Italy) of the IgG type without HBsAg, respectively. We excluded subjects positive for anti-HBs but not for anti-HBc, and those positive for anti-HCV or anti-HIV (HCV® 3.2; Dong-A Pharmaceutical Co., Seoul, Genedia®; Greencross Life Science Corp., Yongin-shi, Korea). Patients with other types of liver disease, e.g., autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis, or Budd-Chiari syndrome were also excluded. No patient had a previous history of immunosuppressive or antiviral treatment.

A diagnosis of hepatic cirrhosis was based on previous liver biopsy, or on clinical, biochemical and ultrasonographic findings. A diagnosis of HCC was based on American Association for the Study of Liver Disease (AASLD) guidelines [21]. Informed consent was obtained from all subjects, and the Institutional Review Board of Human Research at Seoul National University Hospital approved the study protocol. The clinical parameters of subjects are summarized in table 1.

### Genotyping Using Fluorescence Polarization Detection

Two polymorphisms in *Fas*, i.e. -1377G>A and -670A>G, and a single polymorphism in *FasL* at -844C>T were genotyped. Amplifying primers and probes were designed for TaqMan® for genotyping these three polymorphic sites. Primer Express (Applied Biosystems) was used to design both PCR primers and MGB TaqMan probes. One allelic probe was labeled with FAM dye and the other with fluorescent VIC dye. Distilled water and duplicated samples were used for negative and positive control of TaqMan assay. Typically, PCR was run in a TaqMan Universal Master mix without UNG (Applied Biosystems) at a primer concentration of

**Table 1.** Clinical profiles of study subjects

| Clinical profiles        | SR           | CC           |              |
|--------------------------|--------------|--------------|--------------|
|                          |              | CH or LC     | HCC          |
| Subjects                 | 429          | 339          | 327          |
| Mean age (range)         | 54.9 (28–79) | 49.9 (22–85) | 58.3 (25–79) |
| Male/female              | 240/189      | 274/65       | 277/50       |
| HBeAg (positive rate, %) | 0            | 33.5         | 19.6         |
| HBeAb (positive rate, %) | 38.2         | 47.3         | 63.8         |
| HBsAg (positive rate, %) | 0            | 100          | 100          |
| HBsAb (positive rate, %) | 100          | 0            | 0            |

900 nm and a TaqMan MGB probe concentration of 200 nM. Reactions were performed in a 384-well format in a total reaction volume of 5 µl using 20 ng of genomic DNA in a thermal cycler (PE 9700, Applied Biosystems) and heated for 2 min at 50°, 10 min at 95°, followed by 40 amplification cycles of 95° for 15 s and 60° for 1 min. TaqMan assay plates were then transferred to a Prism 7900HT instrument (Applied Biosystems) and the fluorescence intensity of each well was read. Fluorescence data files of each plate were analyzed using SDS 2.1 software. Information regarding the primers using is available on our website ([http://www.snp-genetics.com/reference/Supplementary information to HBV.doc](http://www.snp-genetics.com/reference/Supplementary%20information%20to%20HBV.doc)).

#### Statistical Analysis

$\chi^2$  tests were used to compare observed numbers of each genotype with those expected for the population based on compliance with the Hardy-Weinberg equilibrium.

We examined Lewontin's  $D'$  ( $|D'|$ ) and LD coefficient  $r^2$  between all pairs of biallelic loci [22]. Haplotypes of each individual were inferred using the PHASE algorithm developed by Stephens et al. [23], which uses a Bayesian approach incorporating a priori expectations of a haplotypic structure based on population genetic and coalescent theory. Genetic effects of inferred haplotypes were analyzed in the same way as SNPs. Logistic regression models were used to calculate odds ratios (95% confidence interval) and corresponding  $p$  values after controlling for age (a continuous variable) and sex (male = 0, female = 1) as covariates. In our analysis of HCC occurrence, LC (LC = 1, no LC = 0) and HBeAg (negative = 0, blank = 1, positive = 2) were also used as covariates. Throughout the study, a  $p$  value <0.05 was considered statistically significant.

## Results

This study included 666 chronic hepatitis B carrier patients (CC; subdivided into CH, LC and HCC) and 429 spontaneously recovered subjects (SR). Genotype distributions of *Fas* promoter polymorphisms at positions  $-1377G>A$  and  $-670A>G$  and of the *FasL* promoter polymorphism at position  $-844C>T$  were not significantly different between the SR and CC groups. Moreover, the

genotypes of the *Fas* and *FasL* promoters in SR and CC subjects did not deviate from Hardy-Weinberg predicted values. Table 2 shows the association between spontaneous HBV infection clearance and polymorphisms in the *Fas* and *FasL* genes. In further haplotype analysis, *Fas-haplotype1* ( $-1377G, -670A$ ; abbreviated as *Fas-ht1*) and *-haplotype2* ( $-1377A, -670G$ ; abbreviated as *Fas-ht2*) were almost equivalent to *Fas-670A>G* and *Fas-1377G>A*, respectively, and the frequency of *ht4* was insignificant (frequency 0.001). Thus, haplotypes with either equivalent to single polymorphisms or with frequencies less than 3% were excluded from the statistical analysis. After applying logistic regression analysis and adjusting for age and sex, no statistically significant association was observed between the *Fas* and *FasL* promoter polymorphisms and susceptibility to chronic HBV infection according to co-dominant, dominant, or recessive genetic models (table 2).

We also analyzed the association between the *Fas* and *FasL* polymorphisms and the clearance of HBeAg; all HBeAg-positive (CC group;  $n = 666$ ) patients were included in this analysis. However, no significant association was observed between these polymorphisms and HBeAg clearance using logistic regression analysis after adjusting for age and sex (table 3). Analyses of progression to HCC from CH or LC as functions of *Fas* and *FasL* SNPs and haplotypes were performed using logistic regression models. Age (continuous value), male sex (male = 0, female = 1) and LC (LC = 1, no LC = 0) were all found to be highly associated with the occurrence of HCC (all  $p < 0.0001$ , data not shown). All HBeAg-positive (CC group;  $n = 666$ ) patients were included in this analysis. No significant difference in genotype frequencies was observed between HCC patients and controls (CH and LC subgroups without HCC) for any of the three polymorphisms (table 4).

**Table 2.** Logistic analysis of the *Fas* and *FasL* polymorphisms with respect to HBV infection clearance

| Gene        | Locus      | Genotype       | CC          | SR          | Co-dominant      |      | Dominant         |      | Recessive        |      |
|-------------|------------|----------------|-------------|-------------|------------------|------|------------------|------|------------------|------|
|             |            |                |             |             | OR (95% CI)      | p    | OR (95% CI)      | p    | OR (95% CI)      | p    |
| <i>Fas</i>  | -1377G>A   | GG             | 225 (34.5%) | 146 (34.1%) | 0.95 (0.79–1.13) | 0.54 | 0.98 (0.75–1.29) | 0.89 | 0.85 (0.61–1.18) | 0.34 |
|             |            | AG             | 313 (48.0%) | 200 (46.7%) |                  |      |                  |      |                  |      |
|             |            | AA             | 114 (17.5%) | 82 (19.2%)  |                  |      |                  |      |                  |      |
|             | -670A>G    | AA             | 194 (30.0%) | 126 (29.5%) | 0.97 (0.81–1.16) | 0.75 | 0.97 (0.73–1.28) | 0.80 | 0.96 (0.71–1.30) | 0.78 |
|             |            | AG             | 309 (47.8%) | 204 (47.8%) |                  |      |                  |      |                  |      |
|             |            | GG             | 144 (22.3%) | 97 (22.7%)  |                  |      |                  |      |                  |      |
|             | <i>ht3</i> | -/-            | 575 (90.4%) | 389 (91.3%) | 1.17 (0.76–1.80) | 0.47 | 1.11 (0.71–1.73) | 0.66 |                  | 0.98 |
|             |            | -/ <i>ht3</i>  | 57 (9.0%)   | 37 (8.7%)   |                  |      |                  |      |                  |      |
|             |            | <i>ht3/ht3</i> | 4 (0.6%)    | 0 (0.0%)    |                  |      |                  |      |                  |      |
| <i>FasL</i> | -844C>T    | CC             | 346 (55.9%) | 221 (52.3%) | 0.92 (0.75–1.13) | 0.44 | 0.85 (0.65–1.10) | 0.21 | 1.15 (0.69–1.90) | 0.60 |
|             |            | CT             | 226 (36.5%) | 173 (40.9%) |                  |      |                  |      |                  |      |
|             |            | TT             | 47 (7.6%)   | 29 (6.9%)   |                  |      |                  |      |                  |      |

Logistic regression models were used to calculate odds ratios (95% confidential interval) and the corresponding p values of co-dominant models for SNP sites and haplotypes, controlling age and sex as covariates. *Fas-ht1* and *-ht2* were almost equivalent to *Fas-670A>G* and *Fas-1377G>A*, respectively. Haplotypes with either equivalence to single polymorphisms or with frequencies of <3% were excluded from the statistical analysis.

**Table 3.** Analysis of *Fas* and *FasL* polymorphisms with respect to HBsAg clearance

| Gene        | Locus      | Genotype       | HBsAg       |             | Co-dominant      |      | Dominant         |      | Recessive         |      |
|-------------|------------|----------------|-------------|-------------|------------------|------|------------------|------|-------------------|------|
|             |            |                | positive    | negative    | OR (95% CI)      | p    | OR (95% CI)      | p    | OR (95% CI)       | p    |
| <i>Fas</i>  | -1377G>A   | GG             | 68 (38.6%)  | 77 (31.6%)  | 0.91 (0.69–1.20) | 0.50 | 0.79 (0.52–1.20) | 0.27 | 1.03 (0.62–1.71)  | 0.91 |
|             |            | AG             | 75 (42.6%)  | 120 (49.2%) |                  |      |                  |      |                   |      |
|             |            | AA             | 33 (18.8%)  | 47 (19.3%)  |                  |      |                  |      |                   |      |
|             | -670A>G    | AA             | 59 (33.3%)  | 66 (27.3%)  | 0.88 (0.67–1.16) | 0.38 | 0.76 (0.50–1.17) | 0.22 | 0.96 (0.59–1.56)  | 0.87 |
|             |            | AG             | 81 (45.8%)  | 120 (49.6%) |                  |      |                  |      |                   |      |
|             |            | GG             | 37 (20.9%)  | 56 (23.1%)  |                  |      |                  |      |                   |      |
|             | <i>ht3</i> | -/-            | 163 (92.6%) | 222 (92.1%) | 0.80 (0.40–1.60) | 0.52 | 0.76 (0.35–1.62) | 0.47 | 1.13 (0.07–18.54) | 0.93 |
|             |            | -/ <i>ht3</i>  | 12 (6.8%)   | 18 (7.5%)   |                  |      |                  |      |                   |      |
|             |            | <i>ht3/ht3</i> | 1 (0.6%)    | 1 (0.4%)    |                  |      |                  |      |                   |      |
| <i>FasL</i> | -844C>T    | CC             | 89 (52.4%)  | 132 (56.4%) | 1.06 (0.77–1.46) | 0.73 | 1.19 (0.79–1.79) | 0.40 | 0.71 (0.31–1.62)  | 0.42 |
|             |            | CT             | 71 (41.8%)  | 84 (35.9%)  |                  |      |                  |      |                   |      |
|             |            | TT             | 10 (5.9%)   | 18 (7.7%)   |                  |      |                  |      |                   |      |

Logistic regression models were used to calculate odds ratios (95% confidential interval) and corresponding p values for each SNP site and haplotype after controlling for age and sex as covariables using SAS. p values of co-dominant, dominant and recessive models are also given. Age (continuous variable), and sex (male = 0, female = 1) were adjusted for by including them in the logistic analysis as co-variables. All patients included in study were HBsAg-positive (chronic hepatitis).

The roles of SNPs and haplotypes of the *Fas* and *FasL* genes were analyzed using a Cox relative hazards model in the CC group. By Cox analysis of the relation between age and HCC, *Fas-1377G>A* showed a protective effect on HCC occurrence in a recessive model (RH = 0.70, p = 0.03; table 5) and this was reflected by Kaplan-Meier survival curve analysis (fig. 1).

## Discussion

In the present study, we focused on genetic variations of the *Fas* and *FasL* genes to clarify whether variations in these apoptosis-related genes influence the outcome of HBV infection. In our analysis, the presence of *Fas-670G* allele (*Fas-670A/G* or *G/G*), *Fas-1377A* allele (*Fas-1377G/A* or *A/A*), or *FasL-844T* allele (*FasL-844C/T* or *T/T*)

**Table 4.** Analysis of HCC occurrence with *Fas* and *FasL* polymorphisms

| Gene          | Locus          | Genotype    | HCC         | CH/LC       | Co-dominant      |      | Dominant         |      | Recessive        |      |
|---------------|----------------|-------------|-------------|-------------|------------------|------|------------------|------|------------------|------|
|               |                |             |             |             | OR (95% CI)      | p    | OR (95% CI)      | p    | OR (95% CI)      | p    |
| <i>Fas</i>    | -1377G>A       | GG          | 103 (33.0%) | 119 (35.7%) | 0.86 (0.66–1.14) | 0.29 | 0.91 (0.61–1.35) | 0.63 | 0.71 (0.43–1.17) | 0.18 |
|               |                | AG          | 155 (49.7%) | 156 (46.9%) |                  |      |                  |      |                  |      |
|               | -670A>G        | AA          | 54 (17.3%)  | 58 (17.4%)  | 0.88 (0.67–1.14) | 0.33 | 0.74 (0.49–1.12) | 0.16 | 0.97 (0.62–1.53) | 0.90 |
|               |                | AA          | 98 (31.4%)  | 93 (28.4%)  |                  |      |                  |      |                  |      |
|               |                | AG          | 140 (44.9%) | 168 (51.2%) |                  |      |                  |      |                  |      |
|               | <i>ht3</i>     | GG          | 74 (23.7%)  | 67 (20.4%)  | 1.02 (0.56–1.88) | 0.94 | 1.14 (0.58–2.21) | 0.71 |                  | 0.98 |
| -/-           |                | 280 (91.5%) | 289 (89.5%) |             |                  |      |                  |      |                  |      |
| -/ <i>ht3</i> |                | 26 (8.5%)   | 30 (9.3%)   |             |                  |      |                  |      |                  |      |
|               | <i>ht3/ht3</i> | 0 (0.0%)    | 4 (1.2%)    |             |                  |      |                  |      |                  |      |
| <i>FasL</i>   | -844C>T        | CC          | 168 (55.8%) | 173 (55.6%) | 1.33 (0.97–1.81) | 0.08 | 1.35 (0.91–2.02) | 0.14 | 1.74 (0.83–3.66) | 0.14 |
|               |                | CT          | 105 (34.9%) | 119 (38.3%) |                  |      |                  |      |                  |      |
|               |                | TT          | 28 (9.3%)   | 19 (6.1%)   |                  |      |                  |      |                  |      |

Logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding p values for each SNP site and haplotype after controlling for age, sex, and the presence of liver cirrhosis (LC) as co-variables using SAS. p values of co-dominant, dominant and recessive models are also given. Age (continuous variable), sex (male = 0, female = 1), LC (LC = 1, no LC = 0) and HBeAg (negative = 0, blank = 1, positive = 2) were adjusted for by including them in the logistic analysis as co-variables. All patients included in study were HBsAg-positive (chronic hepatitis).

**Table 5.** Cox relative hazards analysis for age at HCC occurrence as functions of *Fas* and *FasL* polymorphisms

| Gene        | Locus      | n/event | Co-dominant |      |      | Dominant |      |      | Recessive |      |      |
|-------------|------------|---------|-------------|------|------|----------|------|------|-----------|------|------|
|             |            |         | $\chi^2$    | RH   | p    | $\chi^2$ | RH   | p    | $\chi^2$  | RH   | p    |
| <i>Fas</i>  | -1377G>A   | 645/305 | 2.25        | 0.88 | 0.13 | 0.13     | 0.95 | 0.71 | 4.84      | 0.70 | 0.03 |
|             | -670A>G    | 640/305 | 1.20        | 0.92 | 0.27 | 0.33     | 0.93 | 0.57 | 1.61      | 0.84 | 0.20 |
|             | <i>Ht3</i> | 629/299 | 1.52        | 1.30 | 0.22 | 1.68     | 1.32 | 0.20 | 0.00      | 0.00 | 0.98 |
| <i>FasL</i> | -844C>T    | 612/294 | 2.14        | 1.15 | 0.14 | 1.38     | 1.16 | 0.24 | 1.73      | 1.33 | 0.19 |

Cox models were used for calculating relative hazards and p values for SNPs and haplotypes after controlling for sex and adjusted age (age <40, adj. age = 0; age ≤60, adj. age = 1; age >60, adj. age = 2), LC (LC = 0; no LC = 1) and HBeAg (negative = 0, blank = 1, positive = 2) by SAS.

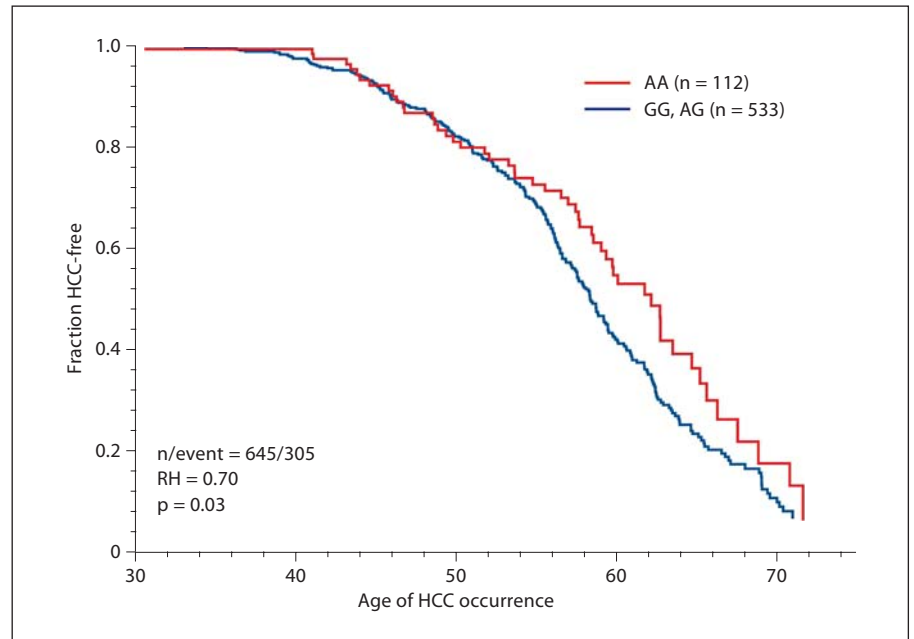
variants was not found to be associated with outcome in cases of HBV infection. However, *Fas*-1377G>A was found to be associated with late onset HCC (p = 0.03).

It has been shown that the G>A transition at position -1377 and the A>G transition at position -670 in the promoter region of the *Fas* gene disrupt SP-1 (stimulatory protein 1) and STAT-1 (signal transducer and activator of transcription 1) protein-binding element, respectively, and thus diminish promoter activity and reduce *Fas* gene expression [19]. With regard to the *FasL* gene, position -844 in its promoter region is located in a binding motif for another transcription factor, CAAT/enhancer-binding protein [20]. Actually, a higher basal expression of *FasL* has been associated with the presence of the *FasL*

-844C allele rather than the *FasL*-844T allele [20]. We hypothesized that *Fas* and *FasL* polymorphisms, which are known to be related to apoptosis, might influence the clearance of HBV infection. However, no association was found between these three polymorphisms and the resolution of HBV infection. Paradoxically, these results place an emphasis on the importance of non-cytopathic antiviral mechanisms, such as those involving antigen-non-specific cytokines, in viral clearance during acute viral hepatitis B [24].

HBeAg seroclearance in chronic hepatitis B patients is associated with a reduction in inflammatory activity and an improved clinical outcome [25]. Moreover, the immune-mediated apoptosis of infected hepatocytes is

**Fig. 1.** Kaplan-Meier survival curves demonstrating age at HCC onset versus *Fas*-1377G>A: *Fas*-1377A/A, G/G and G/A. Number of patients (n), p values and relative hazard ratios calculated using the Cox proportional hazards model are given.



known to be related to spontaneous HBeAg clearance [26]. Thus, we considered that *Fas/FasL* system-mediated apoptosis might be related to spontaneous HBeAg clearance, and that polymorphisms of *Fas* and *FasL* might be associated with outcome. However, no association was found between the *Fas* and *FasL* polymorphisms examined and HBeAg clearance, which implies that non-genetic factors make a more dominant contribution to HBeAg seroclearance. Actually, a recent report suggested that viral factors correlate with the development of sustained HBeAg seroconversion or loss [27].

The *Fas/FasL* system also plays an important role in HCC. It has been suggested that the persistent necroinflammation of hepatocytes is a strong risk factor of virus-induced hepatocarcinogenesis [28], and it has also been suggested that reduced *Fas* expression and/or increased *FasL* expression play important roles in the development and progression of cancer [29, 30]. Several studies have shown that genetic polymorphisms in these death pathway genes appear to play a role in the developments and progressions of other cancers [31, 32]. Thus, it was considered that *Fas* and *FasL* polymorphisms might contribute to the pathogenesis of HCC. Several studies have concluded that the *Fas/FasL* system might be implicated in hepatocarcinogenesis [33–35], and candidate genes have been vigorously studied. Polymorphisms in genes that metabolize hormones or xenobiotics have been main targets of these studies, and polymorphisms in *CYP2E1*,

*CYP1A1*, and *NAT2* have been found to be associated with HCC occurrence [36]. In addition, polymorphisms of some related cytokines were recently studied by Kim et al. [37] who found that the  $[-509C>T; L10P]$  haplotype (a high TGF- $\beta_1$ -producing genotype) is associated with a reduced risk of HCC in chronic hepatitis B patients. In addition, Shin et al. [38] showed that the interleukin-10 (IL-10) polymorphism, IL-10-ht2, is associated with increased IL-10 production and that this accelerates the progression of chronic HBV infection, especially to HCC development. On the other hand, the effects of the *Fas* and *FasL* polymorphisms studied in the present study were not found to be significantly associated with HCC occurrence. However, the observed associations between HCC age at onset among chronic hepatitis B carriers might be noteworthy. The effects of *Fas* genetic polymorphisms on the late onset of HCC were not dramatic in the present study. Therefore, it may be argued that Bonferroni correction should be applied to the p values obtained. If Bonferroni corrections were strictly adopted, associated p values could not retain the significances (the threshold of significance would be 0.003 (4 polymorphisms and 3 analysis models)). However, although there is a chance of type 1 error due to multiple comparisons, when considering the fact that the comparisons were not totally independent of each other due to tight LDs among SNPs/haplotype and related phenotypes, the significance of associations might be acceptable. Further biological

and/or functional evidence is needed to confirm the possibility of associations between *Fas* and *FasL* polymorphisms and HCC.

HBV DNA levels have been regarded as important factors in the HBV clearance and the development of HCC. However, the HBV DNA levels are fluctuating during the follow-up in the majority of our HBV cohort. Therefore, logistic models for HBV clearance were adjusted by only age and sex, and Cox models were controlled by HBeAg status, which can represent HBV DNA levels grossly [39], in addition to age, sex and LC status.

Summarizing, in the present study, we genotyped *Fas*-1377G>A, *Fas*-670A>G, and *FasL*-844C>T polymor-

phisms in Korean chronic hepatitis B carriers and in subjects who spontaneously recovered, and then analyzed overall hepatitis B infection outcomes. It was found that the *Fas*-1377G>A is putatively associated with late onset of HCC.

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