

# Effect of Fibroblast Growth Factor-2 and its Receptor Gene Polymorphisms on the Survival of Patients With Hepatitis B Virus-associated Hepatocellular Carcinoma

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**Abstract.** *Background/Aim:* Fibroblast growth factor (FGF), vascular endothelial growth factor, and hepatocyte growth factor play a critical role in the pathogenesis of hepatocellular carcinoma (HCC). *Materials and Methods:* We assessed nine single nucleotide polymorphisms (SNPs) in the *FGF1*, *FGF2*, *FGF receptor (FGFR)-2*, *Flt-1*, and *c-MET* genes in 245 HCC patients and 483 chronic hepatitis B virus (HBV) carriers without HCC. *Results:* Kaplan–Meier analysis showed that patients with the *FGF2 rs308447 TT* genotype had shorter overall survival than patients with the *CC* or *CT* genotype ( $p=0.016$ ) and that *FGF2 rs308379 A* allele carriers had shorter overall survival than patients with the *TT* genotype ( $p=0.020$ ). *Conclusion:* Multivariate Cox proportional analysis revealed that the *FGF2 rs308379 A* allele (hazard ratio(HR)=1.663,  $p=0.004$ ) and advanced tumor stage (HR=3.430,  $p<0.001$ ) were independent prognostic factors for overall survival in patients with HCC.

Hepatocellular carcinoma (HCC) is a devastating disease that is common worldwide and has the third highest mortality rate among cancers. Hepatitis B virus (HBV), hepatitis C virus, and heavy alcohol drinking are well known risk factors of HCC (1). Chronic HBV infection, in particular, accounts for 70-80% of HCC cases in HBV endemic areas such as Korea (2, 3). The pathogenesis of HCC involves a complex multistep process, including the activation of growth factor signaling pathways (4).

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HCC is a highly vascularized tumor. Among the widely recognized angiogenic factors, such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), angiopoietin, and hepatocyte growth factor (HGF) (5), VEGF is considered as the most potent angiogenic factor in HCC (6). VEGF-A and its receptor (FMS-like tyrosine kinase [FLT1]) have been identified as major mediators in VEGF signaling pathways (7). VEGF binds to its receptors, VEGFR1/FLT1 and VEGFR2, and subsequently activates several signaling pathways involved in the proliferation, migration, and invasion of endothelial cells (8).

FGF2 is also a potent angiogenic molecule that is involved in tumor progression (9, 10). Although VEGF is the key driver of tumor angiogenesis, there is a crosstalk between VEGF and FGF signaling in angiogenesis (11, 12), and FGF can act synergistically on VEGF signaling pathways to induce angiogenesis. HGF is a potent mitogen for hepatocytes. FGF and HGF control the proliferation and invasion of liver cancer cells (13, 14). HGF is the ligand for the MET receptor (15), the MET signaling pathway has been found to be activated in approximately 50% of advanced HCC cases (16). Overexpression of *c-met* defines a subgroup of HCC with poor prognosis and an aggressive phenotype (17).

Although the understanding of HCC pathogenesis has increased, the exact mechanism underlying tumor development and the host genetic factors determining prognosis are not yet defined. Genome-wide association studies have indicated that several polymorphic variants are associated with HCC (18). Recently, a single nucleotide polymorphism (SNP) 61\*G (rs4444903) in the epidermal growth factor gene was reported to be associated with increased epidermal growth factor expression and elevated risk of HCC development in cirrhotic Caucasian patients (19, 20). Another report suggested that the determination of VEGF and VEGFR

Table I. SNP information for genes analyzed in this study.

Gene			HWE	Major	Minor	Band	Role	MAF
FGF1	Int2	rs152524	0.577	C	T	5q31.3	Intron	0.245
FGF2	-9386C/T	rs308447	0.952	C	T	4q27	Promoter	0.082
FGF2	Int1	rs308379	0.991	T	A	4q27	Intron	0.109
FGFR2	V232V	rs1047100	0.654	G	A	10q26.13	Coding exon	0.043
FGFR2	Int2	rs2981578	0.426	G	A	10q26.13	Intron	0.073
FGFR2	Int2	rs1219648	0.415	T	C	10q26.13	Intron	0.297
FGFR2	Int2	rs2981582	0.372	C	T	10q26.13	Intron	0.250
FLT1	Int3	rs4771249	0.403	G	C	13q12.3	Intron	0.138
MET	D1286D	rs41736	0.121	C	T	7q31.2	Coding exon	0.014

SNP: Single nucleotide polymorphism; HWE: Hardy-Weinberg equilibrium; MAF: minor allele frequency; FGF: fibroblast growth factor; FGFR: fibroblast growth factor receptor; FLT: FMS-like tyrosine kinase.

genotypes might play a role in the prediction of clinical outcome of HCC patients receiving sorafenib (21).

There is evidence that SNPs in germ-line DNA may contribute to an individual's susceptibility to HCC or to the prognosis of the disease. The aim of this study was to determine whether SNPs in genes that encode for proteins involved in growth factor signaling pathways can influence the development or progression of HCC and the survival of patients with HBV-associated HCC.

## Materials and Methods

**Study subjects.** The case-control population included a total of 728 Korean patients who were admitted at the outpatient clinic of the Gastroenterology Department of Ajou University Hospital (Suwon, South Korea) between June 2000 and February 2006. They were divided into three groups according to their HBV infection status, clinical data, and serological profile: the chronic hepatitis B group, the HBV-associated liver cirrhosis group, and the HBV-associated HCC group. The chronic hepatitis B group included 293 patients with chronic hepatitis B (male patients=214; female patients=79; mean age, 38.0 years). One hundred and ninety patients were diagnosed as having liver cirrhosis (male patients=144; female patients=46; mean age, 45.8 years), based on the typical morphological findings on computed tomography or ultrasonography, and on evidence of portal hypertension or corresponding laboratory features. The HCC group included 245 HCC patients (male patients=185; female patients=60; mean age, 53.9 years). Patients were defined as having HCC if they had a tumor with a maximum diameter greater than 2 cm, serum  $\alpha$ -fetoprotein (AFP) level greater than 200 ng/ml, and typical features of HCC observed using a dynamic imaging technique. If the findings were not characteristic or if the vascular profile was not typical, a biopsy was performed (22). All the subjects were of a single ethnic group, that is, Korean. Informed consent was obtained from each subject, and the Institutional Review Board of Human Research of Ajou University Hospital approved the study protocol (GN3-08-030).

**Genotyping.** SNPs at nine polymorphic sites were assessed: the *FGF1* gene at position rs152524 (C to T); the *FGF2* gene at positions rs308447 (C to T) and rs308379 (T to A); the *FGFR2* gene at positions rs1047100 (G to A), rs2981578 (G to A),

rs1219648 (T to C), and rs2981582 (C to T); the *FLT1* gene at position rs4771249 (G to C); and the *MET* gene at position rs41736 (C to T) (Table I). Genotyping was performed using a Golden gate genotyping assay kit according to a standard protocol (Illumina Inc., San Diego, CA, USA) as previously described (23). Briefly, 250 ng of genomic DNA were mixed with oligomers, and allele-specific extension was carried out by ramping the temperature from 70°C to 30°C over 16 h. Specific extension products were then used in a polymerase chain reaction (PCR) consisting of 34 cycles for 35 sec at 95°C, 35 sec at 56°C and 2 min at 72°C. PCR products were purified using 96-well filter plates (Millipore, Billerica, MA, USA). For hybridization, all samples were transferred to a 384-well microplate. The Sentrix array matrix chip and purified PCR products were hybridized at 60°C for 30 min and then at 45°C for 16 h. The Sentrix array matrix chip was then washed and imaged at a resolution of 0.8 mm using a BeadArray Reader (Illumina Inc.). Genotyping analysis was performed using Illumina's BEADSTUDIO software (Version 3.0.22). All other reagents were purchased from Sigma-Aldrich (St. Louis, MO, USA).

**Statistical analysis.** Statistical testing was performed using SPSS version 22.0 (SPSS, Chicago, IL, USA). To check for deviations from the Hardy-Weinberg equilibrium,  $\chi^2$  tests were performed. Genetic models for the association tests were divided into co-dominant, dominant, and recessive models. Associations between genotypes and HCC development were analyzed using a multiple logistic regression model after controlling for age and gender as covariates. Chronic HBV carriers (chronic hepatitis B and HBV-associated liver cirrhosis groups) were regarded as a control group compared to an HBV-associated HCC group for analyzing association between genotypes and HCC development. A linkage disequilibrium (LD) block of SNPs was confirmed using the HAPLOVIEW software (version 4.0; <http://www.broad.mit.edu/mpg/haploview>). Individual haplotypes were inferred by the EM algorithm using the SAS haplotype procedure. Haplotype analysis was performed by multiple logistic regressions. Within the HBV-associated HCC group, associations between tumor characteristics and genotypes were also analyzed using a multiple logistic regression model. Association between liver transplantation-free overall survival of patients with HCC and genotypes were analyzed using a multivariate Cox regression analysis. For all statistical tests, significance was set at  $p < 0.05$ . For more precise estimates,

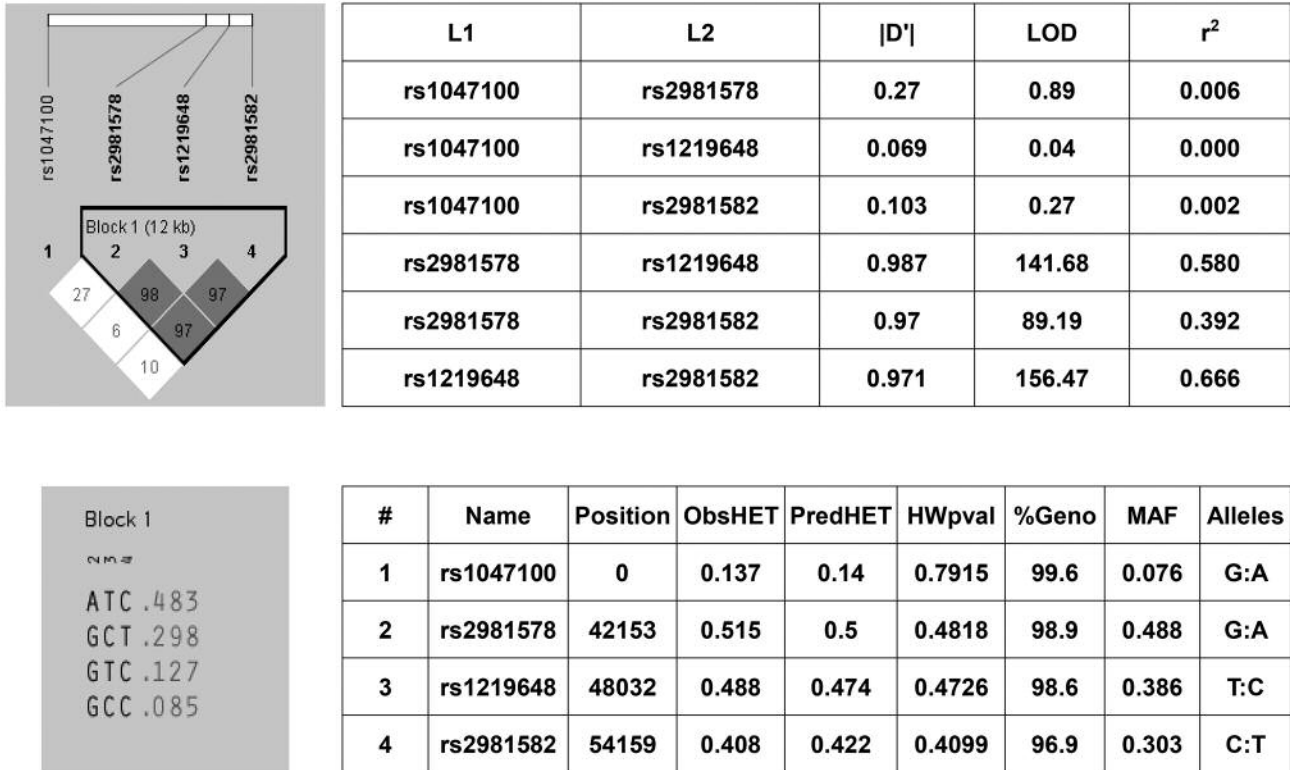


Figure 1. Linkage disequilibrium blocks and haplotypes of the fibroblast growth factor receptor-2 gene.

statistical computation was used to determine the *p*-values of Fisher’s exact test by Monte Carlo simulation (number of iterations=100,000).

**Results**

*Patient characteristics.* Genotypic and clinical data obtained from 728 subjects (483 chronic HBV carriers and 245 HBV-associated HCC) were analyzed. Proportion of male gender was similar between patients who were chronic HBV carriers without HCC and those with HBV-associated HCC (74.3% vs. 75.9%, *p*=0.640). The age of patients with HCC was greater than that of patients who were chronic HBV carriers without HCC (53.83±10.34 vs. 41.13±10.31 years; mean±standard deviation, *p*<0.001). The baseline patient characteristics and clinicopathological features of the tumors in the enrolled HCC patients are summarized in Table II. Approximately one-third of the patients had serum AFP levels greater than 400 ng/ml. One hundred and nine (44.5%) of the 245 tumors had maximum diameters, greater than 5 cm in size. Seventy-eight (3.18%) patients had vascular invasion. Advanced tumor stage (modified union for international cancer control stages III+IV) was observed in 132 (53.8%) patients.

Table II. Clinical characteristics of the enrolled patients with hepatocellular carcinoma.

Parameters	N=245
Age (mean±standard deviation, years)	53.83±10.34
Male gender	186 (75.9%)
Alpha fetoprotein	
≤400 ng/ml	157 (64.1%)
>400 ng/ml	88 (35.9%)
Tumor size	
≤5 cm	136 (55.5%)
>5 cm	109 (44.5%)
Major vessel invasion	78 (31.8%)
Tumor stage (modified UICC)	
I	28 (11.4%)
II	85 (34.7%)
III	88 (35.9%)
IV	44 (18.0%)

UICC: Union for International Cancer Control.

*Genotype distribution and haplotype construction.* The observed genotype frequencies of all nine SNPs in the patients with HCC and chronic HBV carriers without HCC were in Hardy-Weinberg equilibrium. The SNPs in the

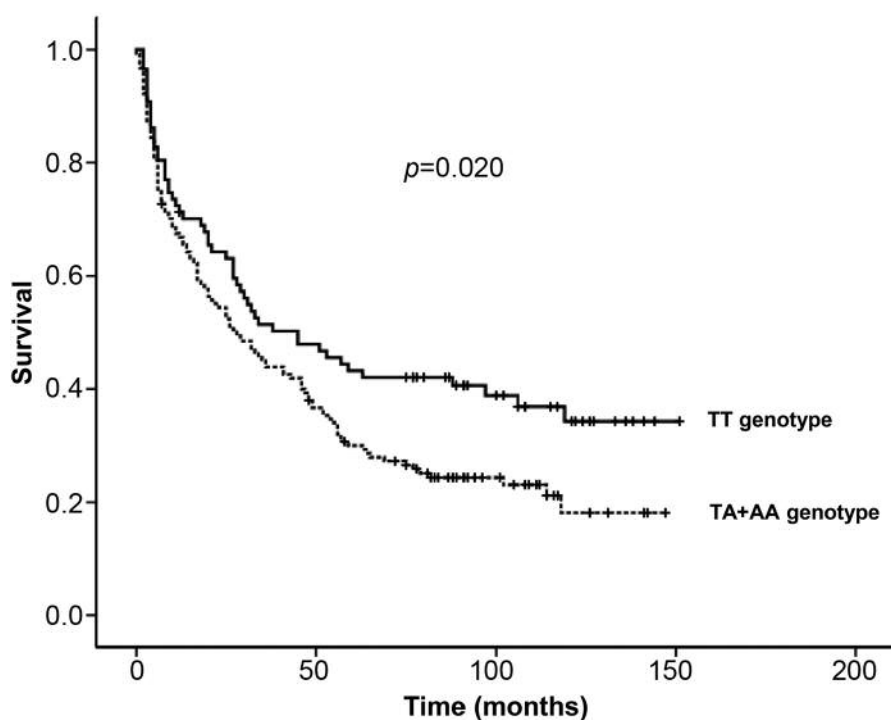


Figure 2. Fibroblast growth factor-2 rs308379 A allele carriers had shorter overall survival than those with the TT genotype ( $p=0.020$ ).

*FGF2* and *FGFR2* genes were analyzed for LD, and haplotypes were constructed. LD coefficients ( $|D'|$  and  $r^2$ ) among SNPs were calculated based on the genotypes of the study subjects. Two polymorphic sites in the *FGF2* gene had no linkage. Three (rs2981578, rs1219648, and rs2981582) of four polymorphic sites in the *FGFR2* gene were closely linked together (Figure 1). Using the EM algorithm, four common haplotypes [ht1 (A-T-C), ht2 (G-C-T), ht3 (G-T-C), and ht4 (G-C-C)] of rs2981578, rs1219648 and rs2981582 were inferred from those SNPs in the *FGFR2* gene and used for haplotype association analysis.

*Association between the genotype/haplotype frequencies and the development of HCC.* When we compared the genotype frequencies between the patients who were chronic HBV carriers (chronic hepatitis B and HBV-associated liver cirrhosis) and the patients with HCC, none of the SNPs showed a significant association with HCC development. Similarly, haplotype frequencies in the *FGFR2* gene were not associated with HCC development (data not shown).

*Association between the genotype/haplotype frequencies and the severity of HCC.* Table III summarizes the association between tumor characteristics and nine SNPs of

the analyzed genes. The *FGF2* rs308379 A allele was associated with small tumor size ( $p=0.042$  in a co-dominant model and  $p=0.028$  in a dominant model) and early tumor stage ( $p=0.010$  in a co-dominant model and  $p=0.003$  in a recessive model). Furthermore, the *FGF2* rs308379 A allele was found to be significantly associated with a lower likelihood of vascular invasion ( $p=0.034$  in a recessive model) in patients with HCC (Table III). With haplotype analyses, *FGFR2* ht3 (G-T-C) was found to be in marginal association with small tumor size in a co-dominant model ( $p=0.041$ ).

The *Flt-1* rs4771249 SNP C allele was associated with low AFP levels (AFP<400 ng/ml) in a dominant model ( $p=0.036$ ; Table III).

*Association between the genotype frequencies and the overall survival in patients with HCC.* The genotype frequencies and the median survival time of patients with HCC are depicted in Table IV. Among the nine SNPs evaluated, two SNPs in the *FGF2* gene and one SNP in the *FGFR2* gene were significantly associated with patient overall survival. Kaplan–Meier analysis showed that patients with the *FGF2* rs308447 TT genotype had shorter survival rates than patients with the CC genotype ( $p=0.021$ ) or the CT genotype ( $p=0.008$ ). Similarly, patients with the *FGF2* rs308447 TT genotype showed lower survival rates than

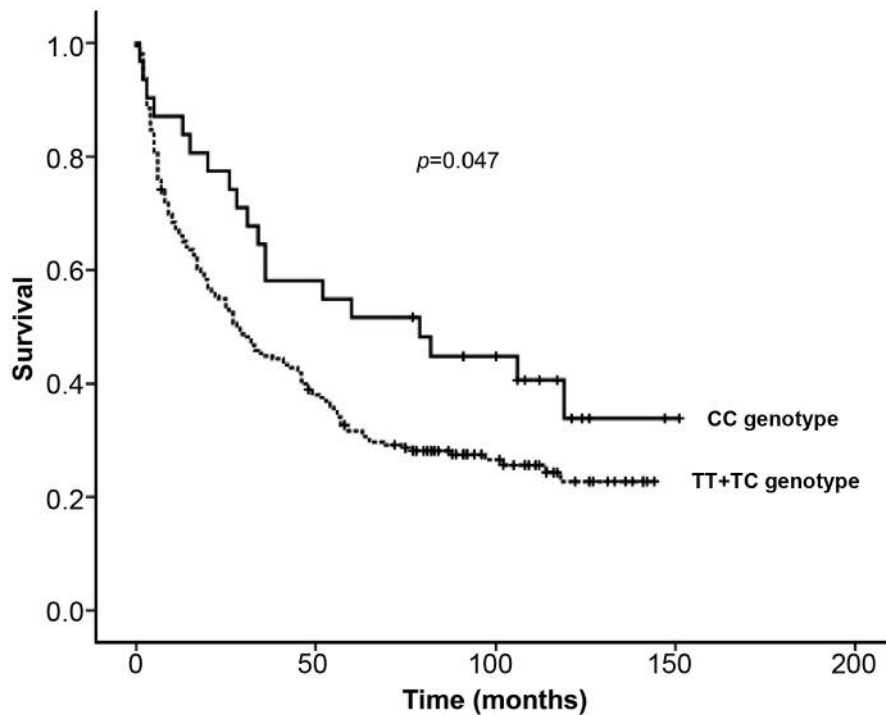


Figure 3. The fibroblast growth factor receptor-2 rs1219648 CC genotype was significantly associated with increased overall survival ( $p=0.047$ ).

patients with the CC or CT genotypes ( $p=0.016$ ). The FGF2 rs308379 A allele carriers had a shorter survival than those with the TT genotype ( $p=0.020$ ; Figure 2), which was contradictory considering the association of the A allele with early tumor stage. The FGFR2 rs1219648 CC genotype was significantly associated with increased overall survival rates ( $p=0.047$ ; Figure 3).

No significant association between the SNPs of the *FGF1*, *FLT1*, and *MET* genes and overall survival were observed in patients with HCC.

**Multivariate analysis for overall survival using the Cox proportional hazard model.** We performed multivariate analysis of the effects of genotype on survival, with the Cox proportional hazard models, which included all the SNPs evaluated and other clinically significant covariates such as age, sex, AFP levels, and tumor stage (stages I and II and stages III and IV). In multivariate analyses using a forward stepwise selection method, FGF2 rs308379 and tumor stage remained significant. The FGF2 rs308379 A allele (HR=1.663, 95% confidence interval=1.171–2.361,  $p=0.004$ ) and advanced tumor stage (hazard ratio=3.430, 95% CI=2.414–4.875,  $p<0.001$ ) were independent poor prognostic factors for overall survival in patients with HBV-associated HCC (Table V).

## Discussion

We demonstrated that the SNPs in the FGF2 and FGFR2 genes were significantly associated with overall survival in patients with HBV-associated HCC. Furthermore, multivariate analysis showed that the FGF2 rs308379 SNP A allele was independently a poor prognostic factor for overall survival. To our knowledge, this is the first study to show the association between SNPs in FGF-related genes and survival of HCC patients.

Tumor progression might depend on the close network of signaling pathways rather than just a single pathway. Previous studies have reported a crosstalk between the FGF and VEGF signaling pathways, indicating a possible synergistic effect on angiogenesis (11, 12). Moreover, FGF signaling is also implicated in the development of resistance to drugs targeting VEGF (24). Therefore, we attempted to investigate the effect of SNPs in the key genes related to various growth factor signaling pathways implicated in the pathogenesis of HCC.

The FGF signaling pathway has an established role in the molecular pathogenesis of HCC (4). In HCCs, the expression of FGF1 and FGF2 has been found to increase sinusoidal capillarization, suggesting a role for FGF signaling in tumor angiogenesis (25). FGF2 stimulates HCC

Table III. Association between genotype frequencies and tumor characteristics.

		AFP		p-Value		Vascular invasion		p-Value		Tumor size		p-Value		Tumor stage		p-Value								
		>400 ng/ml		<400 ng/ml		Yes		No		≤5 cm		>5 cm		I+II		III+IVA+IVB								
		n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%							
FGF1 rs152524	Co-dominant	CC	74	47.4%	39	44.8%	0.609	77	46.4%	36	46.8%	0.970	67	49.3%	46	43.0%	0.597	56	49.6%	57	43.8%	0.434		
		CT	67	42.9%	36	41.4%	70	42.2%	33	42.9%	49	45.8%	43	38.1%	60	46.2%	14	12.4%	13	10.0%	13	10.0%	0.554	
	Recessive	CC+CT	141	90.4%	75	86.2%	0.320	147	88.6%	69	89.6%	95	88.8%	0.964	99	87.6%	117	90.0%	117	90.0%	117	90.0%	0.373	
		TT	15	9.6%	12	13.8%	19	11.4%	8	10.4%	15	11.0%	12	11.2%	14	12.4%	13	10.0%	14	12.4%	13	10.0%	0.161	
	Dominant	CC	74	47.4%	39	44.8%	0.696	77	46.4%	36	46.8%	46	43.0%	0.330	56	49.6%	57	43.8%	57	50.4%	73	56.2%	0.501	
		CT+TT	82	52.6%	48	55.2%	89	53.6%	41	53.2%	61	57.0%	61	57.0%	57	50.4%	73	56.2%	73	56.2%	19	14.8%	0.257	
FGF2 rs308447	Co-dominant	CC	121	79.6%	70	83.3%	0.548	127	79.9%	64	83.1%	0.081	101	78.9%	90	83.3%	0.701	84	77.8%	107	83.6%	0.161		
		CT	30	19.7%	13	15.5%	32	20.1%	11	14.3%	26	20.3%	17	15.7%	24	22.2%	19	14.8%	24	22.2%	19	14.8%	0.501	
	Recessive	CC+CT	151	99.3%	83	98.8%	1.000	159	100.0%	75	97.4%	107	99.1%	1.000	108	100.0%	126	98.4%	126	100.0%	126	98.4%	0.501	
		TT	1	0.7%	1	1.2%	0	0.0%	2	2.6%	1	0.8%	1	0.9%	0	0.0%	2	1.6%	0	0.0%	2	1.6%	0.257	
	Dominant	CC	121	79.6%	70	83.3%	0.485	127	79.9%	64	83.1%	0.552	101	78.9%	90	83.3%	0.388	84	77.8%	107	83.6%	0.257		
		CT+TT	31	20.4%	14	16.7%	32	20.1%	13	16.9%	27	21.1%	18	16.7%	24	22.2%	21	16.4%	24	22.2%	21	16.4%	0.010	
FGF2 rs308379	Co-dominant	TT	56	36.1%	31	35.6%	0.997	60	36.4%	27	35.1%	0.071	40	29.9%	47	43.5%	0.042	38	33.9%	49	37.7%	0.010		
		TA	76	49.0%	43	49.4%	75	45.5%	44	57.1%	69	51.5%	50	46.3%	49	43.8%	70	53.8%	49	43.8%	70	53.8%	0.003	
	Recessive	AA	23	14.8%	13	14.9%	30	18.2%	6	7.8%	25	18.7%	11	10.2%	25	22.3%	11	8.5%	25	22.3%	11	8.5%	0.543	
		TA+TA	132	85.2%	74	85.1%	0.983	135	81.8%	71	92.2%	0.034	109	81.3%	97	89.8%	0.066	87	77.7%	119	91.5%	0.003		
	Dominant	TT	56	36.1%	31	35.6%	0.938	60	36.4%	27	35.1%	0.845	40	29.9%	47	43.5%	0.028	38	33.9%	49	37.7%	0.543		
		TA+AA	99	63.9%	56	64.4%	105	63.6%	50	64.9%	94	70.1%	61	56.5%	74	66.1%	81	62.3%	74	66.1%	81	62.3%	0.704	
FGFR2 rs1047100	Co-dominant	GG	133	84.2%	79	89.8%	0.112	141	83.9%	71	91.0%	0.329	114	83.8%	98	89.1%	0.395	97	85.1%	115	87.1%	0.704		
		GA	25	15.8%	8	9.1%	26	15.5%	7	9.0%	21	15.4%	12	10.9%	16	14.0%	17	12.9%	16	14.0%	17	12.9%	0.463	
	Recessive	AA	0	0.0%	1	1.1%	1	0.6%	0	0.0%	1	0.7%	0	0.0%	1	0.9%	0	0.0%	1	0.9%	0	0.0%	0.463	
		GG+GA	158	100.0%	87	98.9%	0.358	167	99.4%	78	100.0%	1.000	135	99.3%	110	100.0%	1.000	113	99.1%	132	100.0%	132	100.0%	0.645
	Dominant	AA	0	0.0%	1	1.1%	1	0.6%	0	0.0%	1	0.7%	0	0.0%	1	0.9%	0	0.0%	1	0.9%	0	0.0%	0.645	
		GG	133	84.2%	79	89.8%	0.223	141	83.9%	71	91.0%	0.133	114	83.8%	98	89.1%	0.234	97	85.1%	115	87.1%	115	87.1%	0.461
FGFR2 rs2981578	Co-dominant	GA+AA	25	15.8%	9	10.2%	27	16.1%	7	9.0%	0.950	30	22.1%	22	20.6%	0.541	27	23.7%	25	19.4%	25	19.4%	0.461	
		GG	38	24.2%	14	16.3%	0.314	36	21.6%	16	21.1%	0.950	30	22.1%	22	20.6%	0.541	27	23.7%	25	19.4%	0.461		
	Recessive	AA	82	52.2%	52	60.5%	91	54.5%	43	56.6%	71	52.2%	63	58.9%	64	56.1%	70	54.3%	64	56.1%	70	54.3%	0.256	
		AA	37	23.6%	20	23.3%	40	24.0%	17	22.4%	35	25.7%	22	20.6%	23	20.2%	34	26.4%	23	20.2%	34	26.4%	0.414	
	Dominant	AA	37	23.6%	20	23.3%	40	24.0%	17	22.4%	0.787	101	74.3%	85	79.4%	0.345	91	79.8%	95	73.6%	95	73.6%	0.256	
		GG	38	24.2%	14	16.3%	0.150	36	21.6%	16	21.1%	0.929	30	22.1%	22	20.6%	0.777	27	23.7%	25	19.4%	25	19.4%	0.414
	GA+AA	119	75.8%	72	83.7%	131	78.4%	60	78.9%	106	77.9%	85	79.4%	87	76.3%	104	80.6%	87	76.3%	104	80.6%	0.414		

Table III. Continued

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	AFP		p-Value		Vascular invasion		p-Value		Tumor size		p-Value		Tumor stage		p-Value						
	>400 ng/ml		>400 ng/ml		No		Yes		<=5 cm		>5 cm		I+II		III+IVA+IVB						
	n	%	n	%	n	%	n	%	n	%	n	%	n	%	n	%					
FGFR2 rs1219648	TT	56	35.7%	35	41.7%	0.480	62	37.6%	29	38.2%	0.950	51	38.1%	40	37.4%	0.937	38	33.9%	53	41.1%	0.169
	TC	82	52.2%	37	44.0%		81	49.1%	38	50.0%		65	48.5%	54	50.5%		55	49.1%	64	49.6%	
	CC	19	12.1%	12	14.3%		22	13.3%	9	11.8%		18	13.4%	13	12.1%		19	17.0%	12	9.3%	
	TT+TC	138	87.9%	72	85.7%	0.629	143	86.7%	67	88.2%	0.748	116	86.6%	94	87.9%	0.767	93	83.0%	117	90.7%	0.076
	CC	19	12.1%	12	14.3%		22	13.3%	9	11.8%		18	13.4%	13	12.1%		19	17.0%	12	9.3%	
	TT	56	35.7%	35	41.7%	0.360	62	37.6%	29	38.2%	0.931	51	38.1%	40	37.4%	0.914	38	33.9%	53	41.1%	0.253
FGFR2 rs2981582	TC+CC	101	64.3%	49	58.3%	0.412	103	62.4%	47	61.8%	0.728	83	61.9%	67	62.6%	0.698	43	43.0%	69	53.9%	0.262
	CC	68	45.9%	44	55.0%		72	47.4%	40	52.6%		59	48.8%	53	49.5%		45	45.0%	47	36.7%	
	CT	64	43.2%	28	35.0%		64	42.1%	28	36.8%		51	42.1%	41	38.3%		45	45.0%	47	36.7%	
	TT	16	10.8%	8	10.0%		16	10.5%	8	10.5%		11	9.1%	13	12.1%		12	12.0%	12	9.4%	
	CC+CT	132	89.2%	72	90.0%	0.849	136	89.5%	68	89.5%	1.000	110	90.9%	94	87.9%	0.453	88	88.0%	116	90.6%	0.522
	TT	16	10.8%	8	10.0%		16	10.5%	8	10.5%		11	9.1%	13	12.1%		12	12.0%	12	9.4%	
FLT1 rs4771249	CC	68	45.9%	44	55.0%	0.192	72	47.4%	40	52.6%	0.454	59	48.8%	53	49.5%	0.907	43	43.0%	69	53.9%	0.102
	CT+TT	80	54.1%	36	45.0%		80	52.6%	36	47.4%		62	51.2%	54	50.5%		57	57.0%	59	46.1%	
	GG	70	44.6%	51	58.6%	0.107	79	47.6%	42	53.8%	0.491	60	44.4%	61	56.0%	0.087	53	46.9%	68	51.9%	0.731
	GC	74	47.1%	30	34.5%		75	45.2%	29	37.2%		66	48.9%	38	34.9%		51	45.1%	53	40.5%	
	Cc	13	8.3%	6	6.9%		12	7.2%	7	9.0%		9	6.7%	10	9.2%		9	8.0%	10	7.6%	
	GG+GC	144	91.7%	81	93.1%	0.699	154	92.8%	71	91.0%	0.635	126	93.3%	99	90.8%	0.467	104	92.0%	121	92.4%	0.923
MET rs41736	CC	13	8.3%	6	6.9%		12	7.2%	7	9.0%	0.362	60	44.4%	61	56.0%	0.074	53	46.9%	68	51.9%	0.435
	GG	70	44.6%	51	58.6%	0.036	79	47.6%	42	53.8%		75	55.6%	48	44.0%		60	53.1%	63	48.1%	
	GC+CC	87	55.4%	36	41.4%		87	52.4%	36	46.2%	0.278	32	26.2%	25	22.9%	0.154	29	28.2%	28	21.9%	0.173
	CC	40	27.0%	17	20.5%	0.525	35	22.7%	22	28.6%		73	59.8%	58	53.2%		60	58.3%	71	55.5%	
	CT	82	55.4%	49	59.0%		93	60.4%	38	49.4%	0.339	105	86.1%	83	76.1%	0.053	89	86.4%	99	77.3%	0.079
	TT	26	17.6%	17	20.5%		26	16.9%	17	22.1%		17	13.9%	26	23.9%		14	13.6%	29	22.7%	
Recessive	CC+CT	122	82.4%	66	79.5%	0.585	128	83.1%	60	77.9%	0.331	105	86.1%	83	76.1%	0.562	29	28.2%	28	21.9%	0.271
	TT	26	17.6%	17	20.5%		26	16.9%	17	22.1%		17	13.9%	26	23.9%		14	13.6%	29	22.7%	
	CC	40	27.0%	17	20.5%	0.268	35	22.7%	22	28.6%	0.331	32	26.2%	25	22.9%	0.562	29	28.2%	28	21.9%	0.271
	CT+TT	108	73.0%	66	79.5%		119	77.3%	55	71.4%		90	73.8%	84	77.1%		74	71.8%	100	78.1%	

AFP: Alpha fetoprotein; FGF: fibroblast growth factor; FLT: FMS-like tyrosine kinase.

Table IV. Overall survival according to genotype frequencies using Kaplan-Meier analysis.

		Total Death MST (95% CI) p-Value							Total Death MST (95% CI) p-Value				
FGF1 rs152524 C>T	Co-dominant	CC	113	74	35 (19.2-50.7)	0.845	FGFR2 rs1219648 T>C	Co-dominant	TT	90	62	33 (15.5-50.4)	0.129
		CT	102	79	29 (18.2-39.7)	0.234			TC	119	92	27 (17.8-36.1)	0.023
		TT	27	19	52 (18.0-85.9)				CC	31	19	79 (18.0-139.9)	
	Recessive	CC+CT	215	153	31 (21.5-40.4)	0.495		Recessive	TT+TC	209	154	29 (20.6-37.3)	0.047
		TT	27	19	52 (18.0-85.9)				CC	31	19	79 (18.0-139.9)	
Dominant	CC	113	74	35 (19.2-50.7)	0.247	Dominant	TT	90	62	33 (15.5-50.4)	0.665		
	CT+TT	129	98	30 (21.4-38.5)			TC+CC	150	111	31 (23.4-38.5)			
FGF2 rs308447 C>T	Co-dominant	CC	190	137	29 (20.0-37.9)	0.021	FGFR2 rs2981582 C>T	Co-dominant	CC	111	78	25 (13.4-36.5)	0.208
		CT	43	30	45 (24.4-65.5)	0.008			CT	92	70	30 (14.8-45.1)	0.171
		TT	2	2	3				TT	24	16	52 (7.5-96.4)	
	Recessive	CC+CT	233	167	31 (20.3-41.6)	0.016		Recessive	CC+CT	203	148	28 (20.0-37.9)	0.179
		TT	2	2	3				TT	24	16	52 (7.5-96.4)	
Dominant	CC	190	137	29 (20.0-37.9)	0.772	Dominant	CC	111	78	25 (13.4-36.5)	0.687		
	CT+TT	45	32	41 (16.0-65.9)			CT+TT	116	86	35 (20.0-49.9)			
FGF2 rs308379 T>A	Co-dominant	TT	87	54	45 (20.6-69.3)	0.270	FLT1 rs4771249 G>C	Co-dominant	GG	120	88	25 (16.0-33.9)	0.870
		TA	118	91	25 (12.8-37.1)	0.398			GC	104	72	46 (30.6-61.3)	0.405
		AA	36	27	49 (19.6-78.4)				CC	19	14	29 (13.0-44.9)	
	Recessive	TT+TA	205	145	30 (22.7-37.2)	0.975		Recessive	GG+GC	224	160	32 (20.8-43.1)	0.758
		AA	36	27	49 (19.6-78.4)				CC	19	14	29 (13.0-44.9)	
Dominant	TT	87	54	45 (20.6-69.3)	0.020	Dominant	GG	120	88	25 (16.0-33.9)	0.191		
	TA+AA	154	118	28 (18.0-37.9)			GC+CC	123	86	41 (29.0-52.6)			
FGFR2 rs1047100 G>A	Co-dominant	GG	212	151	31 (19.9-42.0)	0.570	MET rs41736 C>T	Co-dominant	CC	56	39	27 (22.2-39.7)	0.922
		GA	32	23	41 (24.4-57.5)	0.496			CT	131	96	31 (2.2-55.7)	0.833
		AA	1	1	25				TT	43	32	29 (13.0-44.9)	
	Recessive	GG+GA	244	174	32 (21.3-42.6)	0.560		Recessive	CC+CT	187	135	30 (13.0-44.9)	0.854
		AA	1	1	25				TT	43	32	29 (13.0-44.9)	
Dominant	GG	212	151	31 (19.9-42.0)	0.888	Dominant	CC	56	39	27 (1.9-52.0)	0.919		
	GA+AA	33	24	41 (18.5-63.4)			CT+TT	174	128	30 (20.7-39.2)			
FGFR2 rs2981578 G>A	Co-dominant	GG	52	36	46 (24.7-67.2)	0.974							
		GA	134	101	27 (19.4-34.5)	0.114							
		AA	56	35	41 (20.3-61.6)								
	Recessive	GG+GA	186	137	31 (23.9-38.0)	0.230							
		AA	56	35	41 (20.3-61.6)								
Dominant	GG	52	36	46 (24.7-67.2)	0.279								
	GA+AA	190	136	29 (18.9-39.0)									

MST: Median survival time; CI: confidence interval; FGF: fibroblast growth factor; FGFR: fibroblast growth factor receptor; FLT: FMS-like tyrosine kinase.

proliferation through an autocrine mechanism and plays a key role in HCC invasion and induction of angiogenesis (9). In clinical samples, high preoperative serum FGF2 levels predicted an invasive HCC phenotype with shorter overall survival after surgical resection (10). FGFR2 expression was also reported to be a potential prognostic indicator of postsurgical survival in Korean patients with HCC (26). Taken together, it can be assumed that the expression levels of FGF2 and FGFR2 could be used as prognostic markers in HCC patients.

Interestingly, Kaplan–Meier and Cox multivariate analyses showed that the FGF2 rs308379 SNP A allele was a prognostic factor for poor overall survival, even though the FGF2 rs308379 A allele was associated with small tumor size, less vascular invasion, and early tumor stage in patients with HCC. If we consider that both tumor status and liver

dysfunction contribute to prognosis in patients with HCC, these discrepant results can be acceptable. Another explanation is that the cross-sectional study design of the present study might be a cause of the false association between tumor characteristics and SNPs in the *FGF2* gene.

Considering its location within the intron, the functional effect of the FGF2 rs308379 SNP on the transcript is unpredictable. Given the multiple splicing variants of FGFs and FGFRs, dysregulation of mRNA splicing can result in altered FGF signaling (27). The F-SNP database used to predict the functional effects of the analyzed polymorphisms showed that the intron-located FGF2 rs308379 polymorphism may predict changes in transcriptional activity (<http://compbio.cs.queensu.ca/F-SNP/>). However, it is unclear how the FGF2 rs308379 SNP has biological effects on the clinical outcomes of patients with HCC.

Overexpression of VEGF, as well as VEGFR1 and VEGFR2, frequently correlates with increased microvasculature and metastasis in HCC (7). Increased serum VEGF levels are correlated with disease progression and tumor recurrence in



Table V. Multivariate analysis of SNPs using forward stepwise selection for overall survival.

Parameter	Multivariate analysis		p-Value
	Hazard ratio	95%CI	
Tumor stage (I+II <i>versus</i> III+IV)	3.430	2.414-4.875	<0.001
FGF2 rs308379 (TT <i>versus</i> TA + AA)	1.663	1.171-2.361	0.004

SNP: Single nucleotide polymorphism; CI: confidence interval; FGF: fibroblast growth factor.

patients with HCC undergoing surgical resection (6, 28). Previous results established that p53 can differentially stimulate transcription in the case of a polymorphic variant of the flt-1 promoter (29). The FLT1 rs4771249 C allele is associated with colon cancer with a TP53 mutation, which is one of the most frequent mutations in HCC (30). In our study, the Flt-1 rs4771249 SNP C allele was associated with low AFP levels, but showed no association with overall survival in patients with HCC.

In conclusion, *FGF2* and *FGFR2* SNPs were significantly associated with overall survival in patients with HBV-associated HCCs. Furthermore, the FGF2 rs308379 SNP was an independent prognostic factor for overall survival in multivariate analysis. Functional analysis of this polymorphism is required to clarify the usefulness of FGF2 genotyping in clinical practice.

### Conflicts of Interest

There are no conflicts of interest from any of the Author regarding the data and contents of this manuscript.

### Authors' Contributions

Sung Won Cho and Jae Youn Cheong designed the study. Soon Sun Kim and Jae Youn Cheong wrote the draft of the manuscript. Jung Woo Eun, Hyo Jung Cho, Hyun-Young Lee and Chul Won Seo conducted and analyzed the experimental and clinical data. Choong Kyun Noh, Sung Jae Shin and Kee Myung Lee supervised the overall study and revised the manuscript.

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