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MDM2 and *p53* polymorphisms are associated with the development of hepatocellular carcinoma in patients with chronic hepatitis B virus infection

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A single-nucleotide polymorphism (SNP) in the promoter region of *MDM2*, SNP 309, is associated with hepatocellular carcinoma (HCC) in patients with chronic hepatitis C virus infection. The effect of *p53* codon 72 polymorphism Arg72Pro on HCC risk remains inconsistent. This study evaluated the association of *MDM2* and *p53* polymorphisms with the presence and early onset of HCC in Korean patients with chronic hepatitis B virus (HBV) infection. In total, 583 consecutive patients with chronic HBV infection were classified according to the presence ($n = 287$) or absence ($n = 296$) of HCC. The *MDM2* SNP 309 and *p53* Arg72-Pro were genotyped using restriction fragment length polymorphism method. The *MDM2* G/G and *p53* Pro/Pro genotypes were more frequent in HCC group than in non-HCC group ($P < 0.001$ and $P = 0.004$, respectively). Multivariate analysis for the presence of HCC revealed that the odds ratio (OR) for *MDM2* G/G over T/T was 4.89 ($P < 0.001$) and that of *p53* Pro/Pro over Arg/Arg was 3.03 ($P = 0.006$). Combined *MDM2* G/G and *p53* Pro/Pro had a synergistic effect on HCC risk, with an OR of 20.78 ($P < 0.001$). The mean age of tumor onset in patients with *MDM2* G/G genotype was 50.9 years compared with 55.1 with T/T genotype ($P = 0.018$) and that with *p53* Pro/Pro was 49.7 years compared with 52.9 with Arg/Arg ($P = 0.040$). Thus, *MDM2* SNP 309 and *p53* Arg72Pro are associated with the early development of HCC in Korean patients with chronic HBV infection.

Introduction

Hepatocellular carcinoma (HCC) is the fifth most common tumor worldwide and accounts for >500 000 deaths each year (1,2). The incidence of HCC is not uniform throughout the world, but varies according to the prevalence of the underlying liver disease (3). It has been recognized that most important risk factor for the development of HCC is cirrhosis (4). Chronic infections with hepatitis B virus (HBV) and hepatitis C virus (HCV) are the most frequent causes of cirrhosis worldwide. In Asia, chronic HBV infection is the most important factor in the etiology of HCC (5,6). Virus factors such as HBV genotype, viral load and mutations in the basal core promoter (BCP) and the precore (PC) region of HBV have been associated with HCC development (5,7–9). Additional factors, such as age, gender, concurrent alcohol use and α-fetoprotein (AFP) levels, also contribute to the development of HCC (10). Finally, various genetic factors appear to affect the outcome of HBV infections (11–15).

MDM2 is an important negative regulator of *p53* that forms a negative autoregulatory feedback loop with *p53* by binding to its N-terminal transactivation domain, inhibiting its transcriptional activity (16). Recently, the 309T>G polymorphism [single-nucleotide polymorphism (SNP) 309, rs2279744], located in the intronic *p53*-responsive promoter of the *MDM2* gene, was found to increase Sp1 transcription factor binding and subsequent MDM2 protein levels (17). This increase in MDM2 results in the direct inhibition of *p53* transcriptional activity, enabling the damaged cells to escape the cell-cycle checkpoint control and become carcinogenic (18). *MDM2* SNP 309 is associated with the early onset of some malignancies, suggesting that it has a strong effect on tumorigenesis in humans (19). Furthermore, Dharel *et al.* (20) reported that *MDM2* SNP 309 was associated with HCC in patients with chronic HCV infection.

Because the loss of *p53* function plays a critical role in multistage hepatocarcinogenesis, the *p53* gene is a good candidate for modulating HCC risk (21). Genetic polymorphisms also exist in the *p53* gene, including the functionally significant 215G>C polymorphism at codon 72 (Arg72Pro, rs1042522) (22). Dumont *et al.* (23) reported that the Arg72 variant induced apoptosis more effectively than the Pro72 variant, which may influence cancer risk. The Pro72 variant has been identified as a risk factor for some malignancies, but the relationship between this polymorphism and the outcome of HBV infection has not yet been firmly established (11,24,25).

Zhang *et al.* (18) tested the gene–gene interactions between *MDM2* SNP 309 and *p53* Arg72Pro in lung cancer and showed that these two polymorphisms increased the risk of lung cancer in a supermultiplicative manner. The combined influence of these two polymorphisms was also tested in hereditary non-polyposis colorectal cancer, and the results indicated that *MDM2* SNP 309, alone or in combination with *p53* Arg72Pro, did not influence the age at diagnosis of non-polyposis colorectal cancer patients (26).

To clarify the association of the *MDM2* SNP 309 and *p53* Arg72-Pro, alone or in combination, with the risk of HCC in patients with chronic HBV infection, we analyzed the association of these SNPs with the presence of HCC and age at the time of diagnosis.

Abbreviations: AFP, α-fetoprotein; BCP, basal core promoter; CI, confidence interval; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HWE, Hardy–Weinberg equilibrium; OR, odds ratio; PC, precore; PCR, polymerase chain reaction; SNP, single-nucleotide polymorphism.

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Patients and methods

Clinical data

Five hundred and eighty-three consecutive patients with chronic HBV infection who visited Severance Hospital between December 2004 and October 2006 were enrolled. All patients were ethnic Koreans and were stratified into two groups according to the presence ($n = 287$) or absence ($n = 296$) of HCC. Only newly diagnosed HCC patients were included; patients with a prior history of HCC or other cancers were excluded. All patients tested positive twice for the hepatitis B surface antigen (Abbott, Wiesbaden, Germany) over a 6-month interval and tested negative for the HCV antibody (Green Cross Medical Science Corp., Yongin-shi, Korea). This study was approved by the Institutional Review Board of the Severance Hospital, and written informed consent was obtained from all study subjects. HCC was diagnosed either histologically or by typical HCC imaging patterns, using angiography, computed tomography and/or magnetic resonance imaging, sometimes combined with serum AFP analysis. Liver cirrhosis was determined by histology, imaging or clinical indications, such as esophageal varices or ascites. Age, gender and serum AFP were recorded. Heavy alcohol intake was defined as ethanol intake ≥80 g/day for >10 years.

SNP analysis

Genomic DNA was extracted from a 200 μl peripheral blood sample using a QIAamp blood kit (Qiagen, Chatsworth, CA) according to the manufacturer's instructions. For *MDM2* genotyping, DNA was amplified by polymerase chain reaction (PCR) using the following primers: 5′-GATTTCGGACGGC-TCTCGCGGC-3′ (sense) and 5′-CATCCGGACCTCCCGCGCTG-3′ (antisense). In the antisense primer, a PstI restriction site was created by

introducing a mismatched A in the place of a G at a site 2 bp from the polymorphic site. The 121 bp PCR product was then digested overnight with 5 U PstI (New England BioLabs, Beverly, MA). The DNA fragments were separated by electrophoresis on 3% NuSieve agarose gels. The wild-type T allele produced a single 121 bp fragment, and the polymorphic G allele produced fragments of 104 and 17 bp. The *p53* codon 72 was genotyped using the primers 5′-TTGCCGTCCCAAGCAATGGATGA-3′ (sense) and 5′-TCTGGGAAGGGACAGAAGATGAC-3′ (antisense). The 199 bp PCR product was digested with AccII (New England BioLabs) for 2 h at 37°C. AccII digestion of the amplified fragment identified two alleles: the Arg allele produced 113 and 86 bp fragments and the Pro allele produced a 199 bp fragment.

Quantification of HBV DNA

HBV DNA was measured using hc2 HBV DNA Test (Digene, Gaithersburg, MD) as described in the manufacturer's protocol. The minimum quantifiable concentration was 1.4×10^5 copies/ml. HBV DNA $\geq 1.4 \times 10^5$ copies/ml was considered HBV DNA positive in this study. Samples with undetectable HBV DNA, which were considered to have HBV DNA levels $< 1.4 \times 10^5$ copies/ml, were defined as HBV DNA negative.

HBV genotyping

HBV genotypes were analyzed by restriction fragment mass polymorphism using matrix-assisted laser desorption/ionization time of flight mass spectrometry as described previously (27). The assay was based on the mass measurement of oligonucleotides having genotypic variations of the S gene.

Sequencing of BCP and PC regions of HBV

Sequencing of BCP and PC regions of HBV genome was performed by amplification of a nucleotide using nested PCR and direct sequencing of the purified PCR fragments. The primers were designed to flank nucleotide positions 1660–1919 of the BCP and PC regions of HBV DNA. External primers were 5′-CATAAGATGGACTCTTGGACT-3′ (sense, positions 1653–1672) and 5′-GGAAAGAAATCAGAAGGCA-3′ (antisense, positions 1974–1956). Internal primers were 5′-GGACTCTTGGACTCTCTCAGCAA-3′ (sense, positions 1660–1680) and 5′-TCCACAGAAGCTCCAAATTTCTTT-3′ (antisense, positions 1941–1919). The sequencing of target PCR fragments was determined using the primers of the second round PCR with a 3100 Automatic Sequencer (Applied Biosystems, Foster City, CA). A dual mutation in BCP region involving an A to T substitution at nucleotide 1762 and a G to A substitution at nucleotide 1764 were examined. Also, a mutation in PC region involving a G to A substitution at nucleotide 1896 was identified.

Statistical analysis

Statistical analysis including Hardy–Weinberg equilibrium (HWE) was performed using Statistical Analysis System Genetics 9.13 (SAS Institute, Cary, NC). The characteristics of HCC and non-HCC patients were compared using the two-sample *t*-test for continuous variables and the χ^2 test for categorical variables. The χ^2 test was used to compare genotype frequencies among subjects. The association between HCC and the genotypes was estimated based on an odds ratio (OR) and a 95% confidence interval (CI) using a multivariate logistic regression model. The ages at the diagnosis of HCC between genotypes were compared using analysis of variance with least significance difference method. For all tests, $P < 0.05$ was considered significant.

Results

The characteristics of the study population are presented in Table I. There were no significant differences of gender, HBV DNA positive and PC 1896 between HCC and non-HCC patients. However, all the other variables including age, cirrhosis, heavy alcohol intake, AFP $\geq$20 ng/ml, HBV genotype C and BCP 1762/1764 were significantly different between two groups. As for HBV genotype, genotype non-C patients were all proved to be genotype A.

Tables II and III present the genotype distributions of *MDM2* SNP 309 and *p53* Arg72Pro, respectively. In the non-HCC group, the *P*-values of HWE for the genotype frequencies of *MDM2* SNP 309 and *p53* Arg72Pro were 0.063 and 0.978, respectively. Also, *P*-value of HWE for *MDM2* SNP 309 in HCC group was 0.233. However, the genotype frequencies of *p53* Arg72Pro in HCC group deviated significantly from those expected under the HWE ($P < 0.001$).

In the HCC group, the proportions of the T/T, T/G and G/G genotypes were 15.7, 43.5 and 40.8%, respectively, which were significantly different from those observed in the non-HCC group ($P < 0.001$). In comparison with the T/T genotype, the crude ORs

Table I. Characteristics of the study population

Variable	HCC (N = 287), n (%)	Non-HCC (N = 296), n (%)	P-value
Gender			
Male	228 (79.4)	236 (79.7)	0.931
Female	59 (20.6)	60 (20.3)	
Age (years)[a]	52.3 ± 9.5	36.7 ± 11.9	<0.001
Cirrhosis	210 (73.2)	48 (16.2)	<0.001
Heavy alcohol intake	52 (18.1)	24 (8.1)	<0.001
HBV DNA positive	140 (48.8)	130 (43.9)	0.239
AFP ≥20 ng/ml	179 (62.4)	27 (9.1)	<0.001
HBV genotype C	284 (99.0)	284 (95.9)	0.022
BCP 1762/1764			
Wild-type	103 (35.9)	131 (44.3)	0.039
T1762/A1764 mutant	184 (64.1)	165 (55.7)	
PC 1896			
Wild-type	218 (76.0)	234 (79.1)	0.371
A1896 mutant	69 (24.0)	62 (20.9)	

[a]Age (years) is shown as mean ± SD, calculated by a two-sample *t*-test.

Table II. Genotype frequencies of *MDM2* SNP 309

Polymorphism	HCC, n (%)	Non-HCC, n (%)	Crude OR (95% CI) for HCC presence	Adjusted OR (95% CI) for HCC presence[a]
Genotype				
T/T	45 (15.7)	83 (28.0)	1.00	1.00
T/G	125 (43.5)	132 (44.6)	1.75 (1.13–2.71)*	3.45 (1.71–6.94)*
G/G	117 (40.8)	81 (27.4)	2.67 (1.68–4.22)*	5.61 (2.67–11.74)*
Recessive model				
T/T + T/G	170 (59.2)	215 (72.6)	1.00	1.00
G/G	117 (40.8)	81 (27.4)	1.83 (1.29–2.59)*	2.46 (1.42–4.27)*
Dominant model				
T/T	45 (15.7)	83 (28.0)	1.00	1.00
T/G + G/G	242 (84.3)	213 (72.0)	2.10 (1.40–3.15)*	4.27 (2.23–8.20)*

[a]Adjusted for all variables listed in Table I.
*$P < 0.05$.

Table III. Genotype frequencies of *p53* Arg72Pro

Polymorphism	HCC, n (%)	Non-HCC, n (%)	Crude OR (95% CI) for HCC presence	Adjusted OR (95% CI) for HCC presence[a]
Genotype				
Arg/Arg	110 (38.3)	124 (41.9)	1.00	1.00
Arg/Pro	111 (38.7)	135 (45.6)	0.93 (0.65–1.33)	1.08 (0.61–1.89)
Pro/Pro	66 (23.0)	37 (12.5)	2.01 (1.25–3.24)*	3.73 (1.73–8.02)*
Recessive model				
Arg/Arg + Arg/Pro	221 (77.0)	259 (87.5)	1.00	1.00
Pro/Pro	66 (23.0)	37 (12.5)	2.09 (1.35–3.25)*	3.59 (1.77–7.31)*
Dominant model				
Arg/Arg	110 (38.3)	124 (41.9)	1.00	1.00
Arg/Pro + Pro/Pro	177 (61.7)	172 (58.1)	1.16 (0.83–1.62)	1.53 (0.91–2.57)

[a]Adjusted for all variables listed in Table I.
*$P < 0.05$.

(95% CI) for the presence of HCC were 1.75 (1.13–2.71; $P = 0.012$) for the T/G genotype and 2.67 (1.68–4.22; $P < 0.001$) for the G/G genotype. The ORs (95% CI), which were adjusted for all the variables listed in Table I, were 3.45 (1.71–6.94; $P = 0.001$) for T/G genotype and 5.61 (2.67–11.74; $P < 0.001$) for G/G genotype. Subjects carrying the G/G genotype had an elevated adjusted OR (95% CI) of 2.46 (1.42–4.27; $P = 0.001$) as compared with T/T or T/G carriers. The heterozygous or homozygous variant forms (T/G or G/G) had an adjusted OR (95% CI) of 4.27 (2.23–8.20; $P < 0.001$) as compared with the homozygous T/T genotype (Table II).

The genotype frequencies of *p53* Arg72Pro in HCC and non-HCC patients are presented in Table III, and a significant difference in genotype distribution was observed ($P = 0.004$). In contrast to the Arg/Arg genotype, the Pro/Pro genotype increased the risk of HCC with a crude OR (95% CI) of 2.01 (1.25–3.24; $P = 0.004$) and an adjusted OR (95% CI) of 3.73 (1.73–8.02; $P = 0.001$). In addition, individuals with the Pro/Pro genotype had an elevated adjusted OR (95% CI) of 3.59 (1.77–7.31; $P < 0.001$) as compared with those with the Arg/Arg or Arg/Pro genotype.

Table IV presents the ORs (95% CI) for these HCC risk factors after multivariate logistic regression. In comparison with the reference T/T genotype of *MDM2* SNP 309, the ORs (95% CI) for T/G and G/G were 3.19 (1.57–6.48) and 4.89 (2.30–10.41), respectively. The Pro/Pro genotype of *p53* Arg72Pro caused a 3-fold increase in risk as compared with the Arg/Arg genotype (OR = 3.03, 95% CI = 1.38–6.63). However, the heterozygous Arg/Pro genotype did not increase HCC risk.

Statistical gene–gene interactions between the *MDM2* and *p53* polymorphisms were also examined (Table V). Crude and adjusted ORs (95% CI) for the presence of HCC were obtained by logistic regression. The presence of both *MDM2* G/G and *p53* Pro/Pro was associated with a higher risk of HCC (adjusted OR = 20.78, 95% CI = 5.25–82.36). The combination of T/G and Arg/Pro, T/G and Pro/Pro and G/G and Arg/Arg also increased the risk of HCC after adjustment for all variables listed in Table I.

The role of *MDM2* SNP 309 and *p53* Arg72Pro in HCC development was analyzed via analysis of variance with least significance difference method (Table VI). The mean ages of tumor diagnosis for the *MDM2* SNP 309 T/T, T/G and G/G genotypes were 55.1, 53.4 and 50.9 years, respectively. In comparison with the T/T genotype, a significant acceleration in the development of HCC was apparent in patients with the G/G genotype ($P = 0.013$).

The same analysis was applied to *p53* Arg72Pro. The mean ages of tumor diagnosis for the Arg/Arg, Arg/Pro and Pro/Pro genotypes were 52.9, 54.2 and 49.7 years, respectively. Significant early onset of HCC was noted in patients with the Pro/Pro genotype as compared with Arg/Arg ($P = 0.028$) genotype.

Finally, the same analysis was applied to the combinations of the *MDM2* and *p53* genotypes. Significant early onset of HCC was noted in subjects with both the *MDM2* G/G and *p53* Pro/Pro genotypes compared with those with T/T and Arg/Arg genotype (mean age = 48.5, $P = 0.001$). Also, subjects with G/G and Arg/Arg genotype revealed significant early onset of HCC compared with those with T/T and Arg/Arg genotype. In these subjects, the mean age of tumor diagnosis was 46.7 years.

Table IV. Multivariate analysis of risk factors for the presence of HCC

Risk factor	Status	OR (95% CI)	*P*-value
Gender	Female	1.00	
	Male	1.58 (0.81–3.07)	0.181
Age (years)	≤40	1.00	
	>40	11.19 (5.76–21.75)	<0.001
Cirrhosis	Absent	1.00	
	Present	8.61 (4.98–14.87)	<0.001
Heavy alcohol intake	Absent	1.00	
	Present	1.45 (0.66–3.15)	0.355
HBV DNA	Negative	1.00	
	Positive	0.74 (0.42–1.32)	0.307
AFP (ng/ml)	<20	1.00	
	≥20	26.47 (13.18–53.19)	<0.001
HBV genotype	A	1.00	
	C	1.78 (0.26–4.27)	0.554
BCP 1762/1764	Wild-type	1.00	
	T1762/A1764	2.32 (1.26–4.27)	0.007
PC 1896	Wild-type	1.00	
	A1896	0.69 (0.35–1.37)	0.285
MDM2 SNP 309	T/T	1.00	
	T/G	3.19 (1.57–6.48)	0.001
	G/G	4.89 (2.30–10.41)	<0.001
p53 Arg72Pro	Arg/Arg	1.00	
	Arg/Pro	1.10 (0.62–1.97)	0.740
	Pro/Pro	3.03 (1.38–6.63)	0.006

Discussion

This study demonstrated an association between genetic polymorphisms in *MDM2* and/or *p53* and the development of HCC in patients with HBV infection.

MDM2 is an E3 ubiquitin ligase that suppresses the activity of p53 through both ubiquitinization and direct protein binding (28). *MDM2* SNP 309, which is a T-to-G substitution at position 309 in the first intron of the *MDM2* gene, results in higher levels of *MDM2* messenger RNA and protein (29). Increased MDM2 levels inhibit p53, which enables damaged cells to escape the cell-cycle checkpoint and become carcinogenic (18). Previous studies have investigated the association between *MDM2* SNP 309 and various malignancies, including soft tissue sarcoma, lung cancer, esophageal squamous cell carcinoma, colorectal cancer, head and neck squamous cell carcinoma, breast cancer, gastric carcinoma and Li–Fraumeni syndrome (16–19,29–36), but the results of these studies were inconsistent. Dharel *et al.* (20) reported that *MDM2* SNP 309 was associated with

Table V. Risk of HCC for combinations of *MDM2* and *p53* genotypes

MDM2 SNP 309	*p53* Arg72Pro	HCC, *n* (%)	Non-HCC, *n* (%)	Crude OR (95% CI)	Adjusted OR (95% CI)[a]
T/T	Arg/Arg	23 (8.0)	40 (13.5)	1.00	1.00
T/T	Arg/Pro	18 (6.3)	38 (12.8)	0.85 (0.40–1.81)	0.51 (0.16–1.66)
T/T	Pro/Pro	4 (1.4)	6 (2.0)	1.16 (0.30–4.54)	1.23 (0.15–10.10)
T/G	Arg/Arg	48 (16.7)	56 (18.9)	1.49 (0.79–2.83)	1.57 (0.56–4.37)
T/G	Arg/Pro	58 (20.2)	53 (17.9)	1.90 (1.01–3.59)*	4.12 (1.45–11.68)*
T/G	Pro/Pro	19 (6.6)	23 (7.8)	1.44 (0.65–3.18)	3.94 (1.09–14.27)*
G/G	Arg/Arg	39 (13.6)	28 (9.5)	2.42 (1.20–4.91)*	3.82 (1.27–11.49)*
G/G	Arg/Pro	35 (12.2)	45 (15.2)	1.35 (0.69–2.66)	2.15 (0.70–6.58)
G/G	Pro/Pro	43 (15.0)	8 (2.7)	9.35 (3.75–23.28)*	20.78 (5.25–82.36)*

[a]Adjusted for all variables listed in Table I.
*$P < 0.05$.

Table VI. Ages[a] at the diagnosis of HCC based on *MDM2*, *p53* and combination of these genotypes

	p53 Arg72Pro		
	Arg/Arg	Arg/Pro	Pro/Pro
MDM2 SNP 309	52.9 ± 10.0	54.2 ± 8.4	49.7 ± 10.2[b]
T/T	T/T + Arg/Arg	T/T + Arg/Pro	T/T + Pro/Pro
55.1 ± 9.8	56.1 ± 10.1	54.9 ± 7.2	47.5 ± 16.3
T/G	T/G + Arg/Arg	T/G + Arg/Pro	T/G + Pro/Pro
53.4 ± 9.0	53.8 ± 9.6	53.2 ± 8.4	52.9 ± 9.9
G/G	G/G + Arg/Arg	G/G + Arg/Pro	G/G + Pro/Pro
50.9 ± 9.9[c]	46.7 ± 9.6[d]	55.6 ± 9.0	48.5 ± 9.7[d]

[a]Ages are expressed as mean ± SD.
[b]$P < 0.05$ compared with Arg/Arg genotype.
[c]$P < 0.05$ compared with T/T genotype.
[d]$P < 0.05$ compared with T/T + Arg/Arg genotype.

HCC in patients with chronic hepatitis C infection. In this study, the frequency of the G/G genotype was 34.0% (198/583), which was much higher than that observed in Caucasians (12%) and slightly higher than that observed in Japanese (27%) and Chinese (25%) subjects in previous studies (19,20,32). These differences in genotype frequency may be the result of differences in the study populations. The G/G genotype was more frequent in HCC patients than in non-HCC patients, which is consistent with the results of a previous report by Dharel *et al.* (20). This report examined a larger study population and demonstrated a clear association between *MDM2* SNP 309 and both HBV infection and the early onset of HCC.

The *p53* tumor suppressor gene is critical for the regulation of the cell cycle and the maintenance of cell integrity (13). The *p53* gene is polymorphic, with 13 described variants (37). The codon 72 polymorphism in exon 4 results in an arginine-to-proline substitution. This polymorphism is located in a proline-rich region of the p53 protein, which is required for growth suppression and apoptosis mediated by p53. The association between *p53* Arg72Pro and the risk of cancer has been investigated in many organs, including the lung, esophagus, stomach, breast, nasopharynx, prostate and liver (24–26,38–44). However, these studies did not provide consistent results to support the association between the *p53* polymorphism and HCC; furthermore, Zhu *et al.* (25) reported that *p53* Arg72Pro was not correlated with HCC risk in patients with chronic HBV infection. However, this study demonstrated that *p53* Arg72Pro is associated with a risk of HCC in HBV-infected patients; in particular, homozygosity for the Pro allele of *p53* was associated with an elevated risk of HCC.

The major etiological factors for HCC are infection with HBV or HCV, excessive alcohol intake and aflatoxin B1 exposure (45–47). However, the most important risk factor for the development of HCC is cirrhosis, and other factors, such as age, gender and AFP, are thought to contribute to the relative risk (10). Virus factors such as HBV genotype, viral load and mutations in the BCP and/or PC regions of HBV have been associated with HCC development (5,7–9). Most of these factors were included for analysis in this study. Gender, HBV DNA positive and mutations in PC 1896 were not different between two groups. After multivariate analysis, heavy alcohol intake and HBV genotype were not significant variables for HCC presence. AFP $\geq$20 ng/ml was the most striking risk factor (OR = 26.47, 95% CI = 13.18–53.19). The sensitivity and specificity of AFP for HCC detection at patient enrollment were 62.4 and 91.9%, respectively, and these results were not much different from previous report (48). As one would expect, age and cirrhosis were also strikingly significant factors with an OR of 11.19 and 8.61, respectively. The multivariate analysis demonstrated that the *MDM2* G/G and *p53* Pro/Pro genotypes caused 4.89- and 3.03-fold increased risk for HCC, respectively.

Previous research has demonstrated an interaction between *MDM2* and *p53* at the molecular level (49), and the combined effects of *MDM2* SNP 309 and *p53* Arg72Pro have been examined in lung cancer, Li–Fraumeni syndrome and non-polyposis colorectal cancer, with conflicting results (18,26,38). In this study, these two polymorphisms produced a combined effect on HCC risk in patients with chronic HBV infection. *MDM2* SNP 309 and *p53* Arg72Pro were not statistically independent, and the presence of both the G/G and Arg/Arg genotypes resulted in a positive synergistic effect on the risk of HCC, with an adjusted OR (95% CI) of 20.78 (5.25–82.36). The presence of both T/G and Pro/Pro revealed significant risk of HCC with an adjusted OR (95% CI) of 3.94 (1.09–14.27). Five combinations (T/T and Pro/Pro, T/G and Arg/Arg, T/G and Arg/Pro, G/G and Arg/Arg and G/G and Arg/Pro) had one genotype of significant risk for HCC. The combination of T/T and Pro/Pro had small number of patients and were not significant for HCC presence. The remaining four combinations included *MDM2* genotype of significant risk for HCC presence and *p53* genotype was not associated with HCC risk. The two of these four combinations were associated with the presence of HCC and the other two combinations were not.

In addition, analysis of variance with least significance difference method was used to demonstrate that the *MDM2* G/G and *p53* Pro/Pro genotypes were both associated with earlier age at HCC diagnosis. However, previous reports have not tested or shown this result (13,20,25). Subjects with G/G and Pro/Pro genotypes exhibited a significant acceleration in the development of HCC compared with T/T and Arg/Arg genotypes, respectively. The combination of both G/G and Pro/Pro demonstrated further early onset of HCC compared with combination of both T/T and Arg/Arg. Significant acceleration was also noted in subjects with both G/G and Arg/Arg genotype.

This study has a number of limitations. The study design was cross-sectional. Furthermore, since the study population was hospital based, healthy subjects were not evaluated as a control group.

Despite these limitations, this study revealed an association of *MDM2* SNP 309 and/or *p53* Arg72Pro with the risk of HCC. Furthermore, these polymorphisms were associated with an earlier age at HCC diagnosis. These results suggest that *MDM2* SNP 309 and *p53* Arg72Pro are involved in HCC development and may be useful as predictive markers of HCC in patients with chronic HBV infection.

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