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ORIGINAL ARTICLE

CCND2 polymorphisms associated with clearance of HBV Infection

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Cyclin D2s (CCND2s) are members of the D-type cyclin family. They interact and construct complexes with cyclin-dependent kinase (CDK)4 or 6. The cyclin D2/CDK4 or CDK6 complexes have key roles in controlling the progression of cell cycle from the Gap 1 (G1) phase to the synthesis (S) phase. Overexpression of cyclin D2 is associated with the development of tumors. In this study, we identified 16 sequence variants of *CCND2* polymorphisms through direct DNA sequencing in 24 individuals, and 5 common variants were selected for genotyping in larger-scale subjects (n=1100). Genetic associations of those polymorphisms with hepatitis B virus (HBV) clearance and hepatocellular carcinoma (HCC) outcome among patients with HBV were analyzed. Although no significant association was observed between the polymorphisms and HCC outcome among HBV patients, one common polymorphism in the 5′-untranslated region (that is, rs1049606) and the most common haplotype (*CCND-ht1 [T-C-T-A-T]*), however, were significantly associated with HBV clearance (odds ratio=0.69, P=0.0002, P^{corr}=0.001 and odds ratio=1.37, P=0.0009, P^{corr}=0.004, respectively). The minor allele frequency of rs1049606 among the spontaneously recovered (SR) group was significantly higher than that of the chronic carrier (CC) group (frequency=0.403 vs 0.336, P=0.0002). In contrast, the frequency of *CCND-ht1*was higher among the CC group than among the SR group (frequency=0.429 vs 0.374, P=0.0009). The information identified in this study might provide valuable insights into generating strategies for control of HBV.
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INTRODUCTION

Hepatitis B virus (HBV) infection is one of the most common viral infections among humans. It is particularly common in Asia, Africa, Southern Europe and Latin America,[1] and is the major cause of acute and chronic liver diseases. Continuous infection of HBV brings about liver cirrhosis and hepatocellular carcinoma (HCC).[2] HBV infection has various clinical courses from spontaneous recovery after hepatitis to chronic infection. Individuals who happen to be HBV chronic carriers (CCs) have a higher risk of developing liver cirrhosis or HCC than do uninfected individuals. Although the different outcomes of HBV infection do not seem to be determined by viral strains, allelic variants in the human genome, however, are likely to influence viral hepatitis progression after infection.[3] In addition to that, several previous studies have also reported associations between genetic polymorphisms and the risk of HCC and/or clearance of HBV.[3–7]

D-type cyclins are the main integral mediators associating the extracellular signaling environment with cell-cycle progression. They are divided into three subtypes: cyclin D1, D2 and D3. The cell cycle consists of a series of well-controlled events that drive DNA replication and cell division. These events are divided into specific phases: preparation for DNA synthesis (G1), DNA synthesis (S), a gap phase (G2) and mitosis (M). Transition between these phases requires tight control; the G1/S phase transition, in particular, includes many cell-cycle events. D-type cyclins are key sensors for mitogenic growth factors, and their expression levels seem to be rate limiting for cell-cycle G1 phase progression. D-type cyclins construct protein complexes by assembling with cyclin-dependent kinase (CDK)4 or 6, and bring about G1 phase progression through inactivation of the retinoblastoma protein (Rb) and related protein family members. The functions of the three D-type cyclins are interchangeable; therefore, differences in mechanism regulating the expression of each D-type cyclin are important to determine the roles each member has in the regulation of cell-cycle progression of various types of cells.[8]

Cyclin D2 (CCND2; MIM no. 123833), which is a member of the D-type cyclin family, forms a complex with and functions as a regulatory subunit of CDK4 or CDK6. This protein is known to interact with and be involved in the phosphorylation of the tumor-suppressor protein Rb. Knockout studies of the homologous gene in

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mice implicate the essential roles of this particular gene in ovarian granulosa and germcell proliferation. This is in line with the fact that high-level expression of this gene was observed in ovarian and testicular tumors.[9] In addition, a nominally statistically significant association between genotype and ovarian cancer survival has been observed for polymorphisms in *CCND2*.[10]

On the basis of those observations in cancer development, we hypothesized that polymorphisms in the CCND2 gene could influence the clearance of HBV and HCC progression among HBV-infected patients. We performed extensive screening of *CCND2* by direct sequencing to detect polymorphisms and examined genetic association with HBV clearance and HCC progression. In this study, we report 16 genetic polymorphisms identified in *CCND2* and the results of the genetic association study with HBV clearance and HCC progression in a Korean population (n=1100).

MATERIALS AND METHODS

Subjects and outcomes

A total of 1100 Korean subjects having either present or past evidence of HBV infection were extracted from the outpatient clinic of the liver unit and from the Center for Health Promotion of Seoul National University Hospital from January 2001 to August 2003. Subjects were divided into two groups according to serologic markers: the CC and the spontaneously recovered (SR). The CC and SR cohorts consisted of 667 and 433 subjects, respectively (Table 1). Hepatitis B surface antigen (HBsAg)-positive patients (CC group) were those who were HBsAg positive over a 6-month period and were followed up for disease progression at least every 6 months. The diagnoses of the CC and SR subjects were established by repeated seropositivity for the HBsAg (Enzygnost HBsAg 5.0; Dade Behring, Marburg, Germany) over a 6-month period, and for both anti-HBs (Enzygnost Anti-HBs II) and anti-HBc (AB-Corek; DiaSorin s.r.l., Saluggia, Italy) of the IgG type without HBsAg, respectively. We excluded subjects who were positive only for anti-HBs and not for anti-HBc, and those positive for anti-HCV (hepatitis C virus) or anti-HIV (human immunodeficiency virus) (GENEDIA; Greencross Life Science, Yongin, Korea, HCV3.2; Dong-A Pharmaceutical, Seoul, Korea). Patients who had any other types of liver disease such as autoimmune hepatitis, toxic hepatitis, primary biliary cirrhosis or the Budd–Chiari syndrome were also excluded. None of the patients had a previous history of immunosuppression or antiviral treatment.

Informed consent was obtained from each patient. The Institutional Review Board of Human Research at the Seoul National University Hospital approved the study protocol. Liver cirrhosis was either diagnosed pathologically or by clinical evidences of portal hypertension, such as visible collateral vessels on the abdominal wall, esophageal varices on esophagogastroscopy, palpable splenomegaly and sonographically definite findings of cirrhotic liver or ascites. HCC was diagnosed as described previously.[11] The clinical parameters are summarized in Table 1.

Table 1 Clinical profiles of study subjects

		Chronic carrier (CC)	
	Spontaneously recovered (SR)	*Chronic hepatitis (CH) or liver cirrhosis (LC)*	*Hepatocellular carcinoma (HCC)*
No. of subjects	433	342	325
Age (mean (range))	54.95 (28–79)	49.78 (22–82)	58.43 (25–79)
Sex (male/female)	243/190	277/65	278/47
HBeAg (positive rate, %)	0	33.33	19.69
HBeAb (positive rate, %)	0	47.25	64.25
HBsAg (positive rate, %)	38.13	100	100
HBsAb (positive rate, %)	100	0	0
Urine albumin (positive rate, %)	0	8.42	14.47
Urine blood (positive rate, %)	28.54	14.49	24.75

Abbreviations: HBeAb, antibody to hepatitis B e antigen; HBeAg, hepatitis B e antigen; HBsAb, hepatitis B surface antibody; HBsAg, hepatitis B surface antigen.

Sequencing analysis of *CCND2*

Using the ABI PRISM 3730 DNA analyzer (Applied Biosystems, Foster City, CA, USA), we sequenced all exons, including exon–intron boundaries and promoter regions ($\sim$1.5 kb), to discover polymorphisms using DNA samples of 24 unrelated healthy Korean individuals. With the 24 samples (48 chromosomes), it was expected that $>$90% of single-nucleotide polymorphisms (SNPs) with frequencies $>$0.05 would be detected[12] and that the overall linkage disequilibrium (LD) pattern, despite the deficiency, could be defined. In all, 20 primer sets for amplification and sequencing analysis were designed based on the GenBank sequences (NT_009759.15) (Supplementary Table 2). Sequence variants were verified by chromatograms.

Genotyping with fluorescence polarization detection

To genotype for polymorphic sites in our study, amplifying primers and probes were designed for TaqMan.[13] One allelic probe was labeled with the FAM dye and the other with the fluorescent VIC dye. Genotyping probe information is presented in Supplementary Table 3.

Statistics

LD was inferred using the algorithm (Haploview) developed by the Broad Institute (Cambridge, MA, USA), which searches for a spine of strong $|D'|$ and LD coefficient r^2 running from one marker to another.[14] Haplotypes of each individual were determined using the algorithm (PHASE, version 2.0) developed by Stephens *et al*.[15] Subjects with missing genotypes were omitted from the analysis of individual SNPs and haplotypes. The omission of a few number of individuals was unlikely to result to any bias in the analysis as the genotyping success rate was $>$99%. For analysis of viral clearance as an outcome, logistic regression models were used for calculating odds ratios (95% confidential interval) and corresponding P-values controlling for age (continuous value) and sex (male=0, female=1) as covariates. Cox models were used for calculating relative hazards and P-values controlling for sex and status of the subject with liver cirrhosis among the CC group.

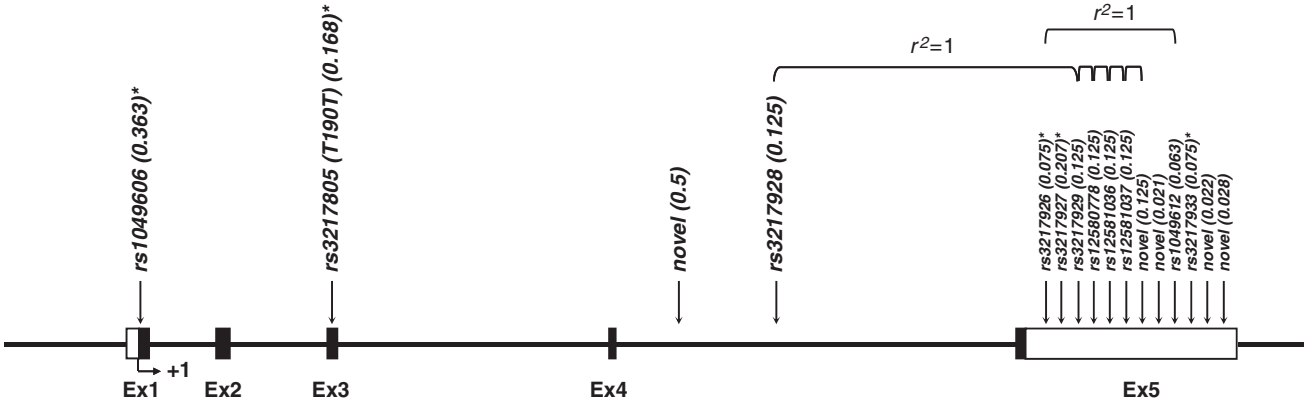
The effective number of independent marker loci in *CCND2* was calculated to correct for multiple testing, using the SNPSpD software (Genetic Epidemiology, Molecular Epidemiology and Queensland Statistical Genetics Laboratories, http://genepi.qimr.edu.au/general/daleN/SNPSpD/), which is based on the SpD (spectral decomposition) of matrices of pairwise LD between SNPs.[16] The resulting number of independent marker loci was applied to check for multiple testing.

RESULTS

By directly sequencing 24 individuals, we identified 16 sequence variants in the CCND2 gene: 2 in the coding regions of exons, 2 in introns and 12 in the 3′-untranslated region (UTR) (Figure 1a). Pairwise comparisons among all 16 polymorphisms revealed two sets of markers in absolute LD with each other ($|D'|$=1 and r^2=1, Figure 1a). Among the 16 variants, 5 common polymorphisms (namely rs1049606, rs3217805, rs3217926, rs3217927 and rs3217933) were selected for larger-scale genotyping (n=1100) based on location, minor allele frequency ($>$0.05) and LD. The minor allele frequencies of the five genotyped polymorphisms were 0.363 (rs1049606), 0.168 (rs3217805), 0.075 (rs3217926), 0.207 (rs3217927) and 0.075 (rs3217933) (Figure 1a). No significant deviations from the Hardy–Weinberg equilibrium were observed in all polymorphisms ($P>0.05$, Supplementary Table 1). Five major haplotypes showed frequencies $>$0.05 and accounted for $>$81.6% of distribution (Figure 1b).

The genotype frequencies of both the CC and the SR groups for each polymorphism were analyzed using a logistic regression model (Table 2), controlling for age and sex as covariates in our Korean HBV

a **Map of *CCND2* (cyclin D2) on 12p13 (33.5 kb)**



b **Haplotypes in *CCND2***

Hap.	*rs1049606*	*rs3217805*	*rs3217926*	*rs3217927*	*rs3217933*	Freq.
ht1	T	C	T	A	T	0.408
ht2	T	C	T	G	T	0.123
ht3	C	C	T	A	T	0.121
ht4	C	G	T	A	T	0.107
ht5	T	C	T	A	C	0.059
Others.	.	.	.	.	.	0.184

c **LDs among *CCND2* polymorphisms**

		|D'|				
		rs1049606	*rs3217805*	*rs3217926*	*rs3217927*	*rs3217933*
r^2	*rs1049606*	-	0.822	0.267	0.014	0.402
	rs3217805	0.245	-	1	0.174	0.808
	rs3217926	0.010	0.017	-	1	0.967
	rs3217927	0.000	0.024	0.021	-	1
	rs3217933	0.008	0.011	0.006	0.022	-

Figure 1 Gene maps and haplotypes of the CCND2 gene. (**a**) Polymorphisms identified in CCND2. Coding exons are marked by shaded blocks and 5′- and 3′-UTR by white blocks. Asterisks (*) indicate SNPs that were genotyped in the larger population. The LD coefficients (r^2) are based on the genotypes of 24 Korean samples. (**b**) Haplotypes of CCND2 in the Korean population. Only those with frequencies >0.05 are shown. (**c**) LD coefficients (|*D*'| and r^2) among selected SNPs based on the genotypes of whole study subjects in this study (n=1100).

Table 2 Logistic analysis for HBV clearance with SNPs and haplotypes in the CCND2 gene in Korean subjects (n=1100)

					Codominant			*Dominant*			*Recessive*		
rs No.	*Position*	*Amino acid*	*CC (n=667)*	*SR (n=433)*	*OR (95% CI)*	*P-value*	$P^{corr\ a}$	*OR (95% CI)*[a]	*P-value*	$P^{corr\ a}$	*OR (95% CI)*	*P-value*	$P^{corr\ a}$
rs1049606	Exon1		0.336	0.403	0.69 (0.57–0.84)	**0.0002**	**0.001**	0.61 (0.46–0.79)	**0.0003**	**0.001**	0.65 (0.45–0.94)	0.02	0.114
rs3217805	Exon3	Thr190Thr	0.155	0.190	0.75 (0.58–0.95)	0.02	NS	0.74 (0.56–0.97)	0.03	NS	0.56 (0.24–1.32)	0.18	NS
rs3217926	Exon5		0.078	0.069	1.04 (0.74–1.47)	0.82	NS	1.02 (0.71–1.47)	0.92	NS	1.66 (0.32–8.64)	0.55	NS
rs3217927	Exon5		0.199	0.220	0.83 (0.67–1.03)	0.09	NS	0.78 (0.60–1.01)	0.06	NS	0.89 (0.51–1.58)	0.70	NS
rs3217933	Exon5		0.079	0.071	1.10 (0.78–1.55)	0.60	NS	1.13 (0.78–1.62)	0.52	NS	0.66 (0.11–3.98)	0.65	NS
ht1			0.429	0.374	1.37 (1.14–1.64)	**0.0009**	**0.004**	1.21 (0.93–1.58)	0.16	NS	2.26 (1.57–3.27)	**0.00001**	**0.0001**
ht2			0.118	0.129	0.86 (0.66–1.12)	0.26	NS	0.87 (0.65–1.18)	0.38	NS	0.58 (0.24–1.41)	0.23	NS
ht3			0.114	0.132	0.87 (0.66–1.14)	0.31	NS	0.87 (0.65–1.18)	0.37	NS	0.63 (0.20–1.94)	0.42	NS
ht4			0.094	0.126	0.68 (0.51–0.91)	0.01	0.05	0.68 (0.50–0.93)	0.02	NS	0.37 (0.10–1.35)	0.13	NS
ht5			0.065	0.050	1.31 (0.88–1.96)	0.18	NS	1.29 (0.86–1.95)	0.22	NS		0.98	NS

Abbreviations: CC, chronic carrier; CI, confidential interval; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; NS, not significant; OR, odds ratio; SNP, single-nucleotide polymorphism; SR, spontaneously recovered.
Logistic regression models were used for calculating ORs (95% CI) and corresponding *P*-values for each SNP site and haplotypes controlling age and sex as covariables using SAS. *P*-values of codominant, dominant and recessive models are also given. Age (continuous value) and sex (male=0, female=1) were adjusted by including in logistic analysis as covariables. All patients included in study were HBsAg-positive (chronic hepatitis).
Values in bold indicate the cases $P<0.05$.
[a]To achieve optimal correction for multiple testing of SNPs in linkage disequilibrium (LD) with each other, the effective number of independent marker loci (4.85) in *CCND2* was calculated using the software SNPSpD (http://genepi.qimr.edu.au/general/daleN/SNPSpD/), on the basis of the spectral decomposition (SpD) of matrices of pairwise LD between SNPs.

study (667 CC vs 433 SR). Among the five polymorphisms examined, one common 5′-UTR polymorphism (rs1049606) emerged to be significantly associated with HBV clearance, for example, higher frequency of 'C' in rs1049606 (*CCND-171T>C*) among the SR group was significantly higher compared with the result from the CC group (odds ratio=0.69, frequency=0.403 vs 0.336, P^{corr}=0.001).

Table 3 Cox relative hazards analysis for onset age of HCC as functions of SNPs and haplotypes

		Codominant			*Dominant*			*Recessive*		
rs No.	n/*Event*	χ^2	*RH*	P-*value*	χ^2	*RH*	P-*value*	χ^2	*RH*	P-*value*
rs1049606	627/295	0.85	0.93	0.36	0.18	0.95	0.67	1.57	0.80	0.21
rs3217805	650/307	1.95	0.85	0.16	2.17	0.83	0.14	0.06	0.90	0.81
rs3217926	647/304	2.28	1.26	0.13	0.97	1.18	0.33	7.20	3.40	0.007
rs3217927	651/310	1.15	0.90	0.28	0.69	0.91	0.41	1.07	0.77	0.30
rs3217933	655/310	0.12	1.05	0.73	0.12	1.05	0.73	0.01	1.10	0.93
ht1	651/307	0.00	1.00	0.96	0.06	0.97	0.81	0.04	1.03	0.84
ht2	651/307	0.18	0.95	0.67	0.00	0.99	0.96	1.14	0.66	0.29
ht3	651/307	0.69	1.11	0.41	0.47	1.10	0.49	0.70	1.46	0.40
ht4	651/307	1.56	0.83	0.21	1.52	0.83	0.22	0.13	0.70	0.72
ht5	651/307	0.40	1.11	0.53	0.39	1.11	0.53	0.03	1.18	0.87

Abbreviations: HBeAg, hepatitis B e antigen; HBsAg, hepatitis B surface antigen; HCC, hepatocellular carcinoma; HBV, hepatitis B virus; LC, liver cirrhosis; SNP, single-nucleotide polymorphism; RH, relative hazard.
Cox models (codominant model) were used for calculating relative hazards and *P*-values for SNPs and haplotypes controlling for sex, adjusted age (age<40, adjage=0; 40<=age<60, adjage=1; age>60, adjage=2), LC (LC=0, no LC=1), and HBeAg (negative=0, positive=1) by SAS. All patients included in this table were HBsAg-positive (chronic HBV).

In addition, the most common haplotype (*CCND-ht1 [T-C-T-A-T]*) was also significantly associated with HBV clearance (odds ratio=1.37, P=0.0009, P^{corr}=0.004). The *ht1 [T-C-T-A-T]* showed the strongest genetic effects in recessive model (odds ratio=2.26, P^{corr}=0.0001) (Table 2).

The role of *CCND2* polymorphisms in the onset age of HCC was also analyzed by the Cox relative hazards model among the CC group. No significant associations were observed (Table 3).

DISCUSSION

D-type cyclins have a major role in cell-cycle regulation. Their abnormal or untimely expression can disrupt the cell cycle, thus turning them into proliferation-promoting genes or oncogenes. Although D-type cyclins are overexpressed in a great number of tumors, not all family members of cyclin Ds, however, are overexpressed in the same tumor. Furthermore, three cyclins have been differentially linked to poor outcome of the disease. A previous study has shown that overexpression of cyclin D2 in gastric cancer correlates with disease progression and poor prognosis.[17]

In this study, we showed that one common polymorphism in 5′-UTR (rs1049606) and *CCND2-ht1 [T-C-T-A-T]* were associated with HBV clearance. Although it was difficult to determine as to which polymorphism is causal, the association signal of *ht1 [T-C-T-A-T]* was equivalent to the effect of rs1049606 as >90% of *ht1 [T-C-T-A-T]* were tagged by rs1049606 (Figure 1b). The alternative functions mediated by the polymorphisms in 5′-UTR would be expected to influence several aspects, such as alternative transcription by separated multiple promoters, transcription initiation at different sites within one promoter or alternative splicing of mRNA. Polymorphisms or disease-causing mutations in DNA sequences of 5′-UTR can also deregulate the regulatory effect of 5′-UTR by inappropriate expression of regulatory proteins or by changing the expression and activity of rate-limiting translation factors.[18] In addition, a recent study showed that nucleotide A insertion at position 193 of the 5′-UTR of HCV could affect spontaneous HCV clearance by acute hepatitis B superinfection during liver transplantation.[19]

To date, no study has shown the association between CCND2 and HBV infection or clearance. However, previous studies have suggested the involvement of other proteins in HBV-related HCC. The abnormal expression of both cyclin D1 (CCND1), a member of the cyclin D family, and p53 protein, the inducer of cell-cycle progression inhibitory protein (p21) has been reported to be associated with HBV-related HCC.[20] Furthermore, we failed to find any significant association between the CCND2 gene and age at HBV infection in our study. However, associations of other several genes with the onset age of HCC after HBV infection in previous studies have been reported. The variants (*−1082A>G*, *−592A>C* and *IL10-ht2 [A-C-C-T]*) in the IL10 (interleukin 10) gene were associated with age of HCC occurrence among the CC group.[5] In addition, a promoter polymorphism in the HDAC10 (histone deacetylase 10) gene, *−589C>T* was also associated with the onset age of HCC.[3]

CCND2 is known to be involved in immune responses. CCND2 is first induced in T and B lymphocytes and controls the proliferation of lymphocytes by regulating the cell cycle. It has also been shown that the CCND2 promoter is regulated by transcription factors, such as signal transducer and activator of transcription 5 and SP1.[21]

A previous study has suggested the association between *CCND2* polymorphisms and another human disease. Three polymorphisms of *CCND2* (namely rs3217862, rs3217901 and rs3217933) were significantly associated with ovarian cancer survival. Of the three polymorphisms, the rs3217862 polymorphism was associated with reduced hazard of ovarian cancer (hazard ratio=0.85, P=0.043), whereas both rs3217901 and rs3217933 were associated with increased hazard of ovarian cancer (hazard ratio=1.14, P=0.024 and hazard ratio=1.16, P=0.020, respectively).[10] The genetic effect of rs3217862 in the previous study was similar to the function of rs1049606 observed in our study in terms of their protective effect on human disease. In contrast, the rs3217901 and rs3217933 polymorphisms in the previous study and the *CCND2-ht1 [T-C-T-A-T]* in our study showed a similar genetic effect in terms of granting susceptibility to human diseases.

In summary, we identified 16 genetic variants in the human CCND2 gene. Five common polymorphic sites were selected for genotyping in our HBV cohort, and statistical analyses proved that one polymorphism (rs1049606) in the 5′-UTR and *CCND2-ht1 [T-C-T-A-T]* were associated with HBV clearance. Our findings will provide an approach to elucidate the molecular mechanisms of HBV clearance.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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1 Lin, C. L. & Kao, J. H. Hepatitis B viral factors and clinical outcomes of chronic hepatitis B. *J. Biomed. Sci.* **15,** 137–145 (2008).
2 Lin, C. L., Liao, L. Y., Wang, C. S., Chen, P. J., Lai, M. Y., Chen, D. S. *et al.* Basal core-promoter mutant of hepatitis B virus and progression of liver disease in hepatitis B e antigen-negative chronic hepatitis B. *Liver Int.* **25,** 564–570 (2005).
3 Park, B. L., Kim, Y. J., Cheong, H. S., Lee, S. O., Han, C. S., Yoon, J. H. *et al.* HDAC10 promoter polymorphism associated with development of HCC among chronic HBV patients. *Biochem. Biophys. Res. Commun.* **363,** 776–781 (2007).
4 Shin, H. D., Park, B. L., Cheong, H. S., Yoon, J. H., Kim, Y. J. & Lee, H. S. SPP1 polymorphisms associated with HBV clearance and HCC occurrence. *Int. J. Epidemiol.* **36,** 1001–1008 (2007).

5 Shin, H. D., Park, B. L., Kim, L. H., Jung, J. H., Kim, J. Y., Yoon, J. H. *et al.* Interleukin 10 haplotype associated with increased risk of hepatocellular carcinoma. *Hum. Mol. Genet.* **12,** 901–906 (2003).
6 Kim, Y. J., Yoon, J. H., Kim, C. Y., Kim, L. H., Park, B. L., Shin, H. D. *et al.* IGF2 polymorphisms are associated with hepatitis B virus clearance and hepatocellular carcinoma. *Biochem. Biophys. Res. Commun.* **346,** 38–44 (2006).
7 Park, B. L., Kim, Y. J., Cheong, H. S., Kim, L. H., Choi, Y. H., Lee, H. S. *et al.* Association of common promoter polymorphisms of MCP1 with hepatitis B virus clearance. *Exp. Mol. Med.* **38,** 694–702 (2006).
8 Kida, A., Kakihana, K., Kotani, S., Kurosu, T. & Miura, O. Glycogen synthase kinase-3beta and p38 phosphorylate cyclin D2 on Thr280 to trigger its ubiquitin/proteasome-dependent degradation in hematopoietic cells. *Oncogene* **26,** 6630–6640 (2007).
9 Susaki, E., Nakayama, K. & Nakayama, K. I. Cyclin D2 translocates p27 out of the nucleus and promotes its degradation at the G0-G1 transition. *Mol. Cell. Biol.* **27,** 4626–4640 (2007).
10 Song, H., Hogdall, E., Ramus, S. J., Dicioccio, R. A., Hogdall, C., Quaye, L. *et al.* Effects of common germ-line genetic variation in cell cycle genes on ovarian cancer survival. *Clin. Cancer. Res.* **14,** 1090–1095 (2008).
11 Bruix, J., Sherman, M., Llovet, J. M., Beaugrand, M., Lencioni, R., Burroughs, A. K. *et al.* Clinical management of hepatocellular carcinoma. Conclusions of the Barcelona-2000 EASL conference. European Association for the Study of the Liver. *J. Hepatol.* **35,** 421–430 (2001).
12 Eberle, M. A. & Kruglyak, L. An analysis of strategies for discovery of single-nucleotide polymorphisms. *Genet. Epidemiol.* **19**(Suppl 1), S29–S35 (2000).
13 Livak, K. J. Allelic discrimination using fluorogenic probes and the 5′ nuclease assay. *Genet. Anal.* **14,** 143–149 (1999).
14 Barrett, J. C., Fry, B., Maller, J. & Daly, M. J. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* **21,** 263–265 (2005).
15 Stephens, M., Smith, N. J. & Donnelly, P. A new statistical method for haplotype reconstruction from population data. *Am. J. Hum. Genet.* **68,** 978–989 (2001).
16 Nyholt, D. R. A simple correction for multiple testing for single-nucleotide polymorphisms in linkage disequilibrium with each other. *Am. J. Hum. Genet.* **74,** 765–769 (2004).
17 Mermelshtein, A., Gerson, A., Walfisch, S., Delgado, B., Shechter-Maor, G., Delgado, J. *et al.* Expression of D-type cyclins in colon cancer and in cell lines from colon carcinomas. *Br. J. Cancer.* **93,** 338–345 (2005).
18 Smith, L. Post-transcriptional regulation of gene expression by alternative 5′-untranslated regions in carcinogenesis. *Biochem. Soc. Trans.* **36,** 708–711 (2008).
19 Sergi, C., Arnold, J. C., Rau, W., Otto, H. F. & Hofmann, W. J. Single nucleotide insertion in the 5′-untranslated region of hepatitis C virus with clearance of the viral RNA in a liver transplant recipient during acute hepatitis B virus superinfection. *Liver* **22,** 79–82 (2002).
20 Choi, Y. L., Park, S. H., Jang, J. J. & Park, C. K. Expression of the G1-S modulators in hepatitis B virus-related hepatocellular carcinoma and dysplastic nodule: association of cyclin D1 and p53 proteins with the progression of hepatocellular carcinoma. *J. Korean Med. Sci.* **16,** 424–432 (2001).
21 White, P. C., Shore, A. M., Clement, M., McLaren, J., Soeiro, I., Lam, E. W. *et al.* Regulation of cyclin D2 and the cyclin D2 promoter by protein kinase A and CREB in lymphocytes. *Oncogene* **25,** 2170–2180 (2006).

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