Influence of polymorphisms in the Wnt/β-catenin pathway genes on hepatocellular carcinoma risk in a Chinese Han population

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Abstract

The Wnt/β-catenin pathway plays a vital role in initiating and sustaining hepatocellular carcinoma (HCC). However, few studies have investigated polymorphisms in the Wnt/β-catenin signaling pathway genes in the Chinese Han population. The aim of the present retrospective study was to investigate the correlations between polymorphisms of the Wnt/β-catenin signaling pathway genes (CTNNB1 and WNT2) and HCC susceptibility, development, and progression.

Twenty tagging single nucleotide polymorphisms were chosen from HapMap data and genotyped in 320 patients with HCC, 320 chronic hepatitis B virus (HBV)-infected patients without HCC (N-HCC, including 95 liver cirrhosis, 164 chronic hepatitis B, and 61 asymptomatic HBV carriers), and 320 healthy controls. Associations between polymorphisms in the 2 Wnt/β-catenin signaling pathway genes (CTNNB1 and WNT2) and HCC susceptibility, development, and progression were investigated.

Genotype AA ($P = 0.002$, odds ratio [OR] = 2.524) and allele A ($P = 0.0003$, OR = 1.613) of the WNT2 rs4730775 polymorphism were associated with HCC susceptibility compared with healthy controls. Genotype GA ($P = 0.001$, OR = 0.567) and allele A ($P = 0.002$, OR = 0.652) of rs3864004, and genotype AG ($P = 0.0004$, OR = 0.495) and allele G ($P = 0.001$, OR = 0.596) of rs11564475 in the CTNNB1 gene were correlated with HCC compared with N-HCC patients. These findings were consistent in dominant and recessive models. Multidimensionality reduction analysis revealed that interactions among rs3864004, rs11564475, and rs4730775 were significantly associated with HCC compared with N-HCC patients. The polymorphism rs4135385 of CTNNB1 genotype GA was associated with a higher risk for Stage III + IV HCC (modified Union for International Cancer Control) ($P = 0.001$, OR = 2.238).

Genetic polymorphisms in the WNT2 and CTNNB1 genes were closely associated with HCC risk and progression in a Chinese Han population.

Abbreviations: ALT = alanine aminotransferase, AST = aspartate aminotransferase, CHB = chronic hepatitis B, CVC = cross-validation consistency, HBV = hepatitis B virus, HC = healthy controls, HCC = hepatocellular carcinoma, MDR = multidimensionality reduction, N-HCC = chronic HBV-infected patients without HCC, OR = odds ratio, SNP = single nucleotide polymorphism, TBA = Testing balance accuracy, TBIL = total bilirubin, TgSNP = tagging SNP.

Keywords: CTNNB1, genetic polymorphism, hepatocellular carcinoma, WNT2

1. Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide, accounting for 70% to 85% of all liver cancers. About 75% to 80% of HCC cases are caused by chronic hepatitis B virus (HBV) or hepatitis C virus infection.[1] China is an endemic region for chronic HBV infection with 94 million patients, and approximately half of the global HCC patients are in China.[2,3] Overall, 2% to 3% of chronic HBV-infected patients develop HCC.[4] Male sex,[5,6] old age, inflammation,[7] and cirrhosis are known risk factors for HCC.[5,8,9] Moreover, single nucleotide polymorphisms (SNPs) of host genes have been found to be correlated with the development and progression of HCC.[10–12]

The Wnt/β-catenin pathway plays a vital role in initiating and sustaining HCC. It plays an important role in embryogenesis and in adult tissue homeostasis.[13] The CTNNB1 gene encodes β-catenin protein, which plays an important role in tissue regeneration, proliferation, differentiation, and development.[14,15] Wnt2 is a member of the Wnt/β-catenin pathway, and has been reported to be associated with multiple cancers.[16–19] Although the Wnt/β-catenin signaling pathway is important for HCC, few studies have investigated polymorphisms in the Wnt/β-catenin signaling pathway genes in the Chinese Han population.

The aim of the current retrospective study was to investigate the correlations between variants in 2 Wnt/β-catenin signaling pathway genes (CTNNB1 and WNT2) and HCC susceptibility and progression. SNP–SNP interactions were analyzed by multidimensionality reduction (MDR).

2. Methods

2.1. Patients

In the present study, we obtained blood samples from unrelated Chinese Han patients at the Liaocheng People's Hospital, Liaocheng, Shandong Province, China between January 2012 and December 2015. A total of 960 individuals were selected for the study, including 320 chronic HBV-infected patients with HCC, 320 chronic HBV-infected patients without HCC (N-HCC, including 95 liver cirrhosis, 164 chronic hepatitis B [CHB], and 61 asymptomatic HBV carriers), and 320 healthy controls (HC). All the HCC and N-HCC cases showed more than 6 months of sero-positivitiy for hepatitis B surface antigen. We diagnosed HCC and liver cirrhosis patients according to clinical and biological criteria, which were confirmed by iconography examination (magnetic resonance imaging or computed tomography). Asymptomatic HBV carriers were defined as patients with chronic HBV infection and persistently normal liver biochemical parameters. CHB patients were defined as chronic HBV-infected patients with abnormal liver biochemical parameters (intermittent or persistent elevations of bilirubin or aminotransferases), and no evidence of HCC or liver cirrhosis. The HC group was defined as individuals with negative HBV sero-markers, no history of hepatitis, normal liver function, and no cancers or other disorders.

Each HC individual was matched with an HCC patient and an N-HCC patient by sex. The study protocol was approved by the ethics committee of Liaocheng People's Hospital. Written informed consent was obtained from all subjects.

2.2. Tagging SNP selection

Twenty tagging SNPs (tgSNPs) were chose from the CTNNB1 and WNT2 genes among data in the International HapMap Project, release27 (http://hapmap.ncbi.nlm.nih.gov) for the CHB population. The exclusion criteria were an r^2 value < 0.8 and a minimum allele frequency < 0.05. Nonsynonymous SNPs or functional SNPs in the literature or in the National Center for Biotechnology Information database were selected initially. The F-SNP program was used to determine the functional SNPs (http://compbio.cs.queensu.ca/F-SNP/).

2.3. DNA purification and SNP analysis

Wizard Genomic DNA Purification Kit (Promega; Madison, WI, USA) was used to obtain genomic DNA samples from peripheral blood leukocytes, according to the manufacturer's instructions. A NanoDrop spectrophotometer was used to measure the DNA concentration. DNA samples were diluted to 50 ng/μL and stored at −80°C for genotyping analysis. We used IplexGOLD chemistry on a SEQUENOM mass spectrometer (Sequenom Inc, San Diego, CA) to genotype the selected 20 tgSNPs. A negative control and duplicate samples were set in every 96-well plate for quality control.

2.4. SNP–SNP interaction analysis

Potential interactions among SNPs were tested by MDR (version 3.0.2), which is a nonparametric program. The program contains permutation testing and cross-validation. MDRpt (version 1.0_beta_2) was used for permutation testing to determine statistical significance. Significant interactions were defined according to $P < 0.05$. The best model is one that shows a cross-validation consistency (CVC) ≥ 9 and the largest testing balance accuracy (TBA). The MDRpt and MDR programs are freely available from http://www.epistasis.org.

2.5. Statistical analysis

HCC risk was investigated according to the genotypes and alleles of CTNNB1 and WNT2 in comparison to the control groups. We tested for Hardy–Weinberg equilibrium in the HC group with IHG program (http://ihg.gsf.de/cgi-bin/hw/hwa1.pl). The Mann–Whitney U test or χ^2 test was used to test differences in age, sex, or clinical characteristics between groups. We used recessive or dominant genetic models to evaluate the associations of each genotype with HCC. The relative risks of SNPs for HCC were investigated with binary logistic regression. Haploview software (v4.2) was used to identify haplotype frequencies. Bonferroni correction was used to correct P values for multiple testing ($0.05/20 = 0.0025$). Statistical calculations were carried out by SPSS 21.0 (Chicago, IL), and $P < 0.0025$ was considered to indicate statistical significance.

3. Results

Two SNPs (rs2293302 from CTNNB1 and rs733153 from WNT2) were excluded from the analysis owing to genotyping success rates $< 92\%$. The other 18 SNPs selected were all in accordance with Hardy–Weinberg equilibrium in the HC group.

3.1. Characteristics of the study population

Characteristics of the study population among the HCC, N-HCC, and HC groups are shown in Table 1. Sex and age did not differ significantly among the 3 groups ($P > 0.05$). The HCC group had significantly higher levels of HBV DNA, total bilirubin (TBIL), aspartate aminotransferase (AST), and alanine aminotransferase (ALT) compared with the HC group. However, the levels of HBV DNA, TBIL, AST, and ALT did not differ significantly between the N-HCC and HCC groups.

Table 1

Demographic and clinical characteristics of the study population.

Characteristics	HC (n = 320)	N-HCC (n = 320)	HCC (n = 320)	P (HC vs HCC/N-HCC vs HCC)
Male/female	238/82	238/82	238/82	1.00/1.00
Age	53.57 ± 13.93	52.29 ± 15.35	54.63 ± 13.28	0.50/0.07
Mean ALT, IU/L (range)	18.9 (2.1–37.2)	92.1 (13.2–1340.2)	88.2 (24.1–1451.2)	<0.01/>0.05
Mean AST, IU/L (range)	21.4 (4.2–38.5)	87.8 (14.3–1355.3)	86.6 (12.4–1428.6)	<0.01/>0.05
Mean TBIL, μmol/L (range)	7.8 (1.6–17.8)	69.3 (5.9–320.7)	59.4 (5.2–611.2)	<0.01/>0.05
Mean HBV-DNA, IU/L (range)	18.8 (2.2–37.1)	3.21E4 (0–6.21E7)	3.45E4 (0–2.72E7)	<0.01/>0.05

Categorical variables as n (%), continuous variables as mean ± standard or median (range).
ALT = alanine aminotransferase, AST = aspartate aminotransferase, HBV = hepatitis B virus, HC = healthy controls, HCC = hepatocellular carcinoma, N-HCC = none HCC patients including asymptomatic carrier, chronic hepatitis, and liver cirrhosis, TBIL = total bilirubin.

Table 2

Association of WNT2 (rs4730775) in HCC risk.

Genotype rs4730775	HC (%)	N-HCC (%)	HCC (%)	P (OR) HC vs HCC	N-HCC vs HCC
GG	211 (66.14)	205 (64.06)	176 (55.34)	—	—
GA	89 (27.90)	80 (25.00)	102 (32.08)	0.073 (1.374)	0.029 (1.485)
AA	19 (5.96)	35 (10.94)	40 (12.58)	0.002 (2.524)	0.259 (1.331)
G	511 (80.09)	490 (76.56)	454 (71.38)	—	—
A	127 (19.91)	150 (23.44)	182 (28.62)	0.0003 (1.613)	0.035 (1.310)
Dominant model	—	—	—	0.005 (1.576)	0.025 (1.438)
Recessive model	—	—	—	0.005 (2.272)	0.520 (0.854)

HC = healthy controls, HCC = hepatocellular carcinoma, N-HCC = none HCC, OR = odds ratio.

Table 3

Association of CTNNB1 (rs3864004 and rs11564475) in HCC risk.

Genotype	HC (%)	N-HCC (%)	HCC (%)	P (OR) HC vs HCC	N-HCC vs HCC
rs3864004					
GG	199 (62.38)	176 (55.35)	218 (68.34)	—	—
GA	103 (32.29)	121 (38.05)	85 (26.65)	0.108 (0.753)	0.001 (0.567)
AA	17 (5.33)	21 (6.60)	16 (5.01)	0.675 (0.859)	0.161 (0.615)
G	501 (78.53)	473 (74.37)	521 (81.66)	—	—
A	137 (21.47)	163 (25.63)	117 (18.34)	0.161 (0.821)	0.002 (0.652)
Dominant model	—	—	—	0.114 (0.768)	0.001 (0.574)
Recessive model	—	—	—	0.858 (0.938)	0.393 (0.747)
rs11564475					
AA	240 (75.00)	216 (67.71)	256 (80.00)	—	—
AG	75 (23.44)	87 (27.27)	51 (15.94)	0.026 (0.638)	0.0004 (0.495)
GG	5 (1.56)	16 (5.02)	13 (4.06)	0.095 (2.437)	0.326 (0.686)
A	555 (86.72)	519 (81.35)	563 (87.97)	—	—
G	85 (13.28)	119 (18.65)	77 (12.03)	0.501 (0.893)	0.001 (0.596)
Dominant model	—	—	—	0.131 (0.750)	0.0005 (0.524)
Recessive model	—	—	—	0.065 (2.668)	0.563 (0.802)

HC = healthy controls, HCC = hepatocellular carcinoma, N-HCC = none HCC, OR = odds ratio.

3.2. Correlations of SNPs with HCC

Compared with the HC group, the rs4730775 genotype AA ($P=0.002$, odds ratio [OR] = 2.524) and allele A ($P=0.0003$, OR = 1.613) from the *WNT2* gene showed significant differences in the HCC group. Significant associations of this SNP with HCC susceptibility were also detected in the dominant and recessive genetic models ($P=0.005$, OR = 1.576; $P=0.005$, OR = 2.272) (Table 2).

Compared with the N-HCC group, the rs3864004 genotype GA ($P=0.001$, OR = 0.567) and allele A ($P=0.002$, OR = 0.652) and the rs11564475 genotype AG ($P=0.0004$, OR = 0.495) and allele G ($P=0.001$, OR = 0.596) of the *CTNNB1* gene showed significant differences in the HCC group. Significant associations of these SNPs with HCC were also detected in the dominant and recessive genetic models ($P=0.001$, OR = 0.574; $P=0.0005$, OR = 0.524) (Table 3).

3.3. Correlations of SNPs with HCC tumor stage

Genotype GA of the rs4135385 polymorphism of *CTNNB1* was associated with a higher risk for Stage III+IV HCC (modified Union for International Cancer Control) ($P=0.001$, OR = 2.238). This finding was confirmed in the dominant model ($P=0.001$, OR = 2.207). We did not find any other SNPs associated with HCC tumor stage (Table 4).

3.4. MDR for SNP–SNP interactions

MDR was performed to analyze the SNP–SNP interactions. Since rs4730775, rs3864004, and rs11564475 were associated with HCC, we carried out MDR for these 3 SNPs with HCC as the case group and HC or N-HCC as the control group. In this analysis, we found 1-way (rs3864004), 2-way (rs11564475 and rs4730775), and 3-way (rs3864004, rs11564475, and rs4730775) interactions in the HCC versus N-HCC comparison (Table 5). The best model was that for the 3-way interaction among rs3864004, rs11564475, and rs4730775 (TBA of 0.5938 and CVC 10/10; $P<0.001$) (Fig. 1).

Table 4

Association of CTNNB1 rs4135385 in HCC tumor stage.

Genotype	Stage I+II	Stage III+IV	P (OR)
GG	94 (53.41)	56 (39.16)	—
GA	69 (39.20)	71 (49.65)	0.001 (2.238)
AA	13 (7.39)	16 (11.19)	0.077 (2.066)
G	257 (73.01)	183 (63.99)	—
A	95 (26.99)	103 (36.01)	0.003 (1.682)
Dominant model	—	—	0.001 (2.207)
Recessive model	—	—	0.397 (1.394)

HCC = hepatocellular carcinoma, OR = odds ratio.

3

MDR interaction analysis between SNPs in N-HCC versus HCC.

MDR models	TBA	CVC	P*
rs3864004	0.5109	6	0.6380
rs11564475–rs4730775	0.5750	10	0.000–0.001
rs3864004–rs11564475–rs4730775	0.5938	10	0.000–0.001

CVC = cross-validation consistency, HCC=hepatocellular carcinoma, MDR = multidimensionality reduction, N-HCC=none HCC, SNP = single nucleotide polymorphism, TBA = testing balance accuracy.

* P values as calculated after 1000 permutations.

4. Discussion

In the present study, 20 tgSNPs from the *WNT2* and *CTNNB1* genes were genotyped in a Chinese Han population. One SNP of the *WNT2* gene (rs4730775) and 2 SNPs of the *CTNNB1* gene (rs11564475 and rs3864004) were found to be correlated with HCC. Moreover, rs4135385 of the *CTNNB1* gene was associated with HCC tumor stage. Interactions among the 3 SNPs (rs3864004, rs11564475, and rs4730775) showed an association with HCC risk.

Accumulating evidence has revealed that Wnt/β-catenin signaling plays an important role in hepatic carcinogenesis.[20] Polymorphisms of *CTNNB1* were reported to be associated with prostate cancer,[21] breast cancer,[22] colorectal cancer,[23] and gastric cancer.[24] In a Korean population, *CTNNB1* polymorphisms were suggested to be involved in tumor development and survival of HBV-associated HCC patients.[25] However, there are no previous studies related to polymorphisms in *CTNNB1* with HBV-related HCC patients in the Chinese Han population. In the present study, we found that genetic polymorphisms of *CTNNB1* (rs11564475 and rs3864004) were associated with HCC development. rs11564475 is located in the intron region (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=11564475) and rs3864004 is located in the promoter region (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=3864004) of *CTNNB1*. The functional prediction scores (F-SNP) for the 2 SNPs were 0.268 (rs11564475) and 0.065 (rs3864004). rs3864004 was reported to affect CTNNB1 expression levels in Korean asthma patients.[26] We speculate that both rs11564475 and rs386400 have functional effects and could influence the CTNNB1 expression level. However, direct experiments are needed to validate the functional consequences of these SNPs.

Although WNT2 is reported to be associated with multiple cancers, few studies have investigated polymorphisms of *WNT2* with HBV-related HCC. In the present study, a polymorphism of *WNT2* (rs4730775) was found to be correlated with HCC susceptibility. This is the first study to reveal that rs4730775 is associated with HBV-related HCC. rs4730775 is located in the 3'-untranslated region (http://www.ncbi.nlm.nih.gov/projects/SNP/snp_ref.cgi?rs=4730775) of *WNT2*. The functional prediction score (F-SNP) for rs4730775 was 0.5. We speculate that rs4730775 might have functional consequences and could affect the WNT2 expression level. However, direct experiments are needed to validate its function.

MDR analysis revealed that rs3864004 (*CTNNB1*), rs11564475 (*CTNNB1*), and rs4730775 (*WNT2*) might interact in HBV-related HCC development. Both gene products (*CTNNB1* and *WNT2*) are involved in the Wnt/β-catenin signaling pathway. Therefore, all 3 SNPs might affect the expression of *CTNNB1* and *WNT2*, resulting in HBV-related HCC development.
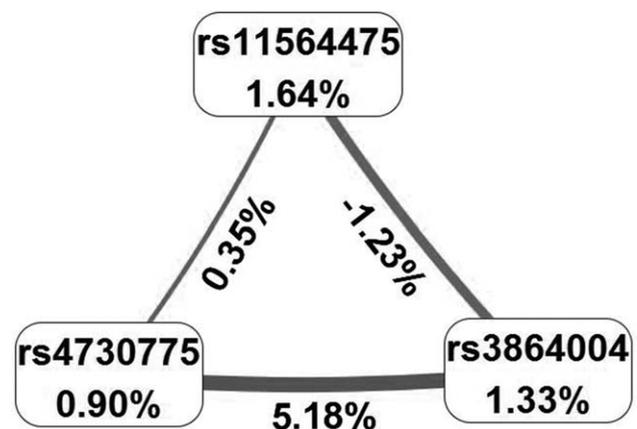
Figure 1. Interactions among SNPs in relation to HCC development. Interactions among SNPs in HCC versus N-HCC. Values in nodes represent the main effect. Values between nodes represent the interaction effect. HCC = hepatocellular carcinoma, N-HCC=chronic HBV-infected patients without HCC, SNP = single nucleotide polymorphism.

A previous study showed that mutation in the *CTNNB1* gene could affect ß-catenin activity, which is correlated with liver tumor progression.[27] This is consistent with our finding that rs4135385 of the *CTNNB1* gene was associated with HCC tumor stage. rs4135385 is located in the intron of *CTNNB1* (http://www.ncbi.nlm.nih.gov/proje cts/SNP/snp_ref.cgi?rs=4135385), and the functional prediction score (F-SNP) for rs4135385 was 0.242. Introns play a vital role in the regulation of transcription or post-transcription processes to affect gene expression.[28,29] Therefore, SNPs in the intron region could affect this regulation. We speculate that rs4135385 might be a functional SNP to affect the expression of *CTNNB1*.

4.1. Limitations

There are 3 limitations of this study that should be taken into consideration. First, all included individuals (HC, N-HCC, and HCC patients) were recruited from only 1 hospital, which might result in selection bias. However, the distributions of all genotypes were in accordance with Hardy–Weinberg equilibrium in the HC group, except for 2 SNPs (rs2293302 from *CTNNB1* and rs733153 from *WNT2*), which revealed that our subjects might be a good representation of the general population. Second, our study had a relatively small sample size, which might reduce the ability to find differences among groups in statistical analysis. Third, our study was a retrospective study, which had a less power to assess the risk of HCC development than a prospective study, in particular in cirrhotic HBV-infected patients (at high risk for liver cancer).

5. Conclusion

In conclusion, we found that polymorphisms in the Wnt/β-catenin signaling pathway genes (*CTNNB1* and *WNT2*) are associated with HCC susceptibility and progression. In the future, more studies (especially prospective study) in multiple centers with larger sample sizes are needed to validate our findings that genetic polymorphisms in *WNT2* and *CTNNB1* are closely associated with HCC risk.

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