RESEARCH ARTICLE

The *KIF1B* (rs17401966) Single Nucleotide Polymorphism is not Associated with the Development of HBV-related Hepatocellular Carcinoma in Thai Patients

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Abstract

Hepatitis B virus (HBV) infection can become chronic and if left untreated can progress to hepatocellular carcinoma (HCC).Thailand is endemic for HBV and HCC is one of the top five cancers, causing deaths among Thai HBV-infected males. A single nucleotide polymorphism (SNP) at the *KIF1B* gene locus, rs17401966, has been shown to be strongly associated with the development of HBV-related HCC. However, there are no Thai data on genotypic distribution and allele frequencies of rs17401966. Thai HBV patients seropositive for HBsAg (n=398) were therefore divided into two groups: a case group (chronic HBV with HCC; n=202) and a control group (HBV carriers without HCC; n=196). rs17401966 was amplified by polymerase chain reaction (PCR) and analyzed by direct nucleotide sequencing. The genotypic distribution of rs174019660 for homozygous major genotype (AA), heterozygous minor genotype (AG) and homozygous minor genotype (GG) in the case group was 49.5% (n=100), 40.1% (n=81) and 10.4% (n=21), respectively, and in controls was 49.5% (n=97), 42.3% (n=83) and 8.2% (n=16). Binary logistic regression showed that rs17401966 was not statistically associated with the risk of HCC development in Thai chronic HBV patients (p-value=0.998, OR=1.00 and 95% CI=0.68-1.48). In conclusion, the *KIF1B* gene SNP (rs174019660) investigated in this study showed no significant association with HBV-related HCC in Thai patients infected with HBV, indicating that there must be other mechanisms or pathways involved in the development of HCC.

Key words: *KIF1B* - rs17401966 - chronic hepatitis B infection (HBV) - HCC - Thailand

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Introduction

Hepatitis B virus (HBV) is a major public health problem worldwide. In untreated chronic patients, HBV infection can cause liver cirrhosis and can progress to Hepatocellular Carcinoma (HCC) (Wong et al., 2006). In Thailand, HBV infection is the major cause of HCC: 65% of HCC patients are infected with HBV (Tangkijvanich et al., 1999), and patients with HBV are 20 times more likely to develop HCC (Tangkijavanich et al., 2003). More than 85% of primary liver cancers are HCC, and over 80% of these cancer patients are located in sub-Saharan Africa and Eastern Asia with China having the highest HCC prevalence (EI-Serag et al., 2007). HCC is also the most common cancer and cause of death in Thai males (Srivatanakul et al., 2004)

Recently, a genome-wide association study (GWAS) conducted in 5 different locations in China discovered that a single nucleotide polymorphism (SNP) known as rs17401966 was strongly associated with the development of HCC among chronic HBV patients (Zhang et al., 2010). This SNP is located within the 24th intron of the kinesin family member 1B (*KIF1B*) gene on human chromosome 1p36.22. In several types of cancers - including HCC - *KIF1B* has been deleted (Bagchi et al., 2008), which indicates that this gene may have tumor suppressor potential.

In order to understand the activity of *KIF1B* gene, its structure has been fully investigated. The gene encodes two spliced isoforms of kinesin protein, denoted as *KIF1B*α and *KIF1B*β, which play a role in organelle and vesicle transportation in many different cell types

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(Nangaku et al., 1994). Both *KIF1Bα* and *KIF1Bβ* share the same first 660 residues in the N-terminal motor domain but their C-terminal cargo-binding domains are completely different (Nangaku et al., 1994; Zhao et al., 2001). Aside from its transportation activity, *KIF1Bβ* has also been shown to suppress tumor in several cancers by interacting with inhibitors of the cell cycle or activators of apoptosis (Munirajan et al., 2004).

The major allele (A) of rs17401966 has been reported to be associated with HBV-related HCC and occurs at a frequency of 71% whereas its minor allele (G) is protective against the development of HBV-related HCC and has a frequency of 29% (Zhang et al., 2010). In contrast, two other studies involving Koreans, Japanese, Hong Kong Chinese and Saudi Arabians (Al-Qahtani et al., 2012; Sawai et al., 2012) showed that there was no significant association between rs1740966 and the development of HBV-related HCC.

Currently, it is unclear why two separate studies conducted in China showed different results. However, because of this discrepancy - and lack of data in Thais - the authors decided to investigate the genotypic distributions and allele frequencies of rs1740966 in Thai HBV patients with and without HCC. In addition, the authors assessed whether rs1740966 is associated with the development of HBV-related HCC. The information obtained from this study will help with the management of surveillance programs of HBV-related HCC patients.

Materials and Methods

Study population

HBV-infected patients from Chulalongkorn King Memorial Hospital, Thailand between January 2012 and December, 2012 were divided into two groups: case group (with HCC) and control group (without HCC). There were 202 and 197 patients in the case and control groups, respectively. All patients, regardless of group, were HBs antigen positive. HCC was diagnosed based on clinical symptoms, ultrasonogram and/or computed tomography and an alpha fetoprotein concentration of more than 400 ng/ml. Patients who had any evidence of liver mass by ultrasonography were excluded from the control group. This study was approved by the Institutional Review Board of the Faculty of Medicine, Chulalongkorn University (IRB number 534/54). Informed consent was obtained from all patients before any procedures were performed.

Sample collection

Blood samples were collected from HBV patients with and without liver cancer. For each patient, 2-3 ml of blood was collected into an EDTA tube. PBMCs were obtained by centrifugation and put in a separation medium (Wisent Inc., Quebec, Canada). Red blood cells were removed using Red Cell Lysis Buffer (1M Tris-HCl pH 7.6, 5 M NaCl, 1 M $MgCl_2$). PBMCs were re-suspended in Phosphate Buffer Saline (PBS) at pH 7.4 and stored at -20˚C until further analysis.

DNA extraction

DNA was extracted from 100 µL of PBMC suspension from each patient. The cells were lysed with lysis buffer (proteinase-K/SDS in Tris buffer) followed by phenol/chloroform extraction and ethanol precipitation. The pellet was dissolved in 30 µL sterile distilled water and stored at -20˚C until further analysis.

Amplification of the KIF1B gene flanking rs17401966 region

The DNA region flanking rs17401966 of the *KIF1B* gene was amplified by polymerase chain reaction (PCR) using specific primers. The forward primer was rs17401966_F: 5'-ATTCATCCCACTTTAGTCATTGCAAG-3' and the reverse primer was rs17401966_R: 5'-CACAACATACTATGACTTCAGCAACT-3'. The PCR reaction was composed of 0.5 μL of 10 µM forward and reverse primers (0.2 µM final concentration for each), 10μL of 2.5X PerfectTaq Plus MasterMix (5 PRIME Inc., Hamburg, Germany), 1μL of DNA sample and distilled water up to a final volume of 25 µL. The PCR reaction was performed under the following thermal conditions: initial denaturation at 95˚C for 5 minutes; followed by 35 cycles of denaturation at 94˚C for 30 seconds, annealing at 60˚C for 30 seconds and extension at 72˚C for 45 seconds; then ending with the final extension at 72˚C for 7 minutes. To assess the size of the PCR product, electrophoresis was performed on a 2% w/v agarose gel stained with ethidium bromide. The desired PCR product size of 292 base pairs was confirmed and visualized on a UV transilluminator.

DNA purification and nucleotide sequencing

The PCR products of interest were purified from the gel using the GelExtract Mini Kit (5PRIME, Hamburg,Germany) and were used as a template for nucleotide sequencing. DNA sequencing was performed by FirstBASE Laboratories Sdn Bhd (Selangor Darul Ehsan, Malaysia). Nucleotide sequences of the samples were analyzed by using Chromas Lite (Technelysium Pty Ltd) and BLAST (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

Statistical analysis

The association between SNP rs17401966 and the risk of developing HCC were assessed by using binary logistic regression. Statistical significant difference was accepted when p-value was below 0.05. The odds ratio was considered if both upper and lower 95% CI did not cover 1. Statistical parameters were calculated using SPSS for Windows v13.0 (SPSS Inc., Chicago, IL).

Results

Age and sex ratio between case and control groups

In this study, there were a total of 398 HBV-infected

patients. All of the patients were confirmed to be HBsAg positive and were later divided into 2 groups based on whether they had HCC or not. There were 202 HBV-infected patients with HCC (case group) and 196 asymptomatic HBV carriers (control group). The mean age and standard deviation for case and control groups were 59.8±12.3 years and 46.3±9.9 years, respectively. The sex ratio (female/male) in the case group was 44/158 whereas in control group it was 65/131 (Table 1).

Genotypic distributions of rs17401966 and its association to HBV-related HCC

The genotypic distributions of rs174019660 between both groups were comparable (Table 2, Figure 1A). In the case group, 49.5% (n=100), 40.1% (n=81) and 10.4% (n=21) had homozygous major genotype (AA), heterozygous minor genotype (AG) and homozygous minor genotype (GG), respectively. Similarly, in the control group, 49.5% (n=97), 42.3% (n=83) and 8.2% (n=16) had homozygous major genotype (AA), heterozygous minor genotype (AG) and homozygous minor genotype (GG), respectively. rs17401966 was not significantly associated with the development of HCC for the heterozygous genotype (AG) [odd ratio (95% CI)=0.95 (0.79-1.31) with p=0.795], homozygous minor genotype (GG) [odd ratio (95% CI)=1.27 (0.63-2.58) with p=0.503] or heterozygous (AG)+homozygous (GG) genotypes [odd ratio (95% CI)=1.00 (0.68-1.48) with p=0.998] (Table 2).

Table 1. Basic Demographic Data of the Patients in this Study

Characteristics	Case	Controls
Total	202	196
Age		
Mean (Standard Deviation)	59.8 (12.3)	46.3 (9.9)
Sex		
Female, n (%)	44 (21.8)	65 (33.2)
Male, n (%)	158 (78.2)	131 (66.8)
Ratio (female/male)	44/158	65/131

Allele frequencies of rs17401966 and its association to HBV-related HCC

The frequencies of the major (A) and minor (G) alleles were comparable between both groups. In the case group, 69.6% and 30.4% had major (A) and minor (G) alleles, respectively. In the control group, 70.7% and 29.3% had major (A) and minor (G) alleles, respectively (Table 3, Figure 1B). After adjusting for variables such as age and sex in the logistic regression analysis, minor allele (G) of rs17401966 was not significantly associated with the development of HCC [odd ratio (95% CI)=0.95 (0.70-1.28) with p=0.733] (Table 3).

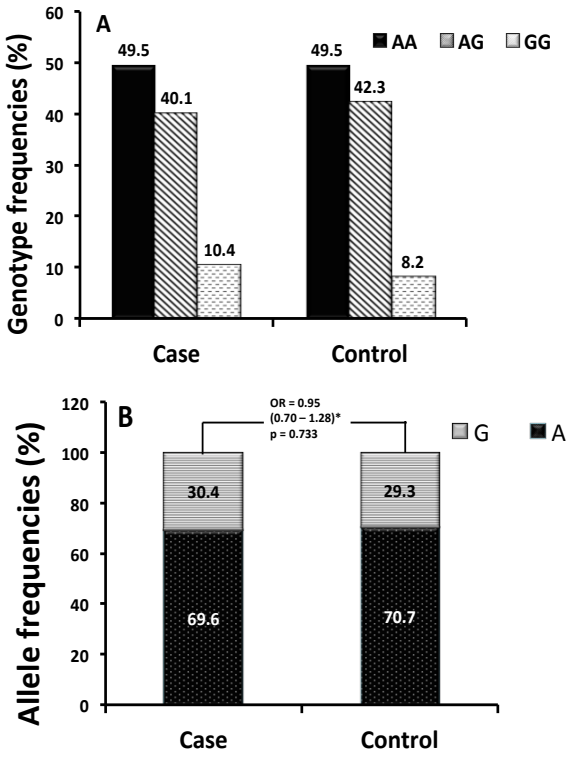

Figure 1. Genotype (A) and Allele (B) Frequencies for rs17401966 between Case and Control Groups. *95%CI (lower-upper)

Table 2. Genotypic Distribution of rs17401966 and Its Association with HBV-related HCC

SNPs	Genotype	Cases (n=202)	Controls (n=196)	OR*	95%CI	p-values*
rs17401966	AA	100 (49.5%)	97 (49.5%)	1**	–	–
	AG	81 (40.1%)	83 (42.3%)	0.95	0.63-1.43	0.795
	GG	21 (10.4%)	16 (8.20%)	1.27	0.63-2.58	0.503
	AG+GG	102 (50.5%)	99 (50.5%)	1.00	0.68-1.48	0.998

*Cases compare with controls; ** Reference

Table 3. Allele Frequencies of rs17401966 and Association to HBV-related HCC

SNP	Allele	Cases (2n=404)	Controls (2n=392)	OR*	95%CI	p-values*
rs17401966	Major (A)	281 (69.6%)	277 (70.7%)	1**	-	-
	Minor (G)	123 (30.4%)	115 (29.3%)	0.95	0.70-1.28	0.733

*Cases compare with controls; **Reference

Table 4. Comparison of Genotypic Distribution of rs17401966 among Different Populations

Populations	Case, n (%)						Control, n (%)						OR	95%CI	p-value	Reference
	Total	AA	AG	GG	A	G	Total	AA	AG	GG	A	G				
Chinese (Total)	2310	1497 (64.8)	736 (31.9)	77 (3.3)	3730 (80.7)	890 (19.3)	1789	936 (52.3)	715 (40.0)	138 (7.7)	2587 (72.3)	991 (27.7)	1.61	1.45-1.78	<0.001	Zhang et al., 2010
Japanese (Total)	321	196 (61.0)	107 (33.3)	18 (5.6)	499 (77.7)	143 (22.3)	1020	609 (59.7)	352 (34.5)	59 (5.8)	1570 (77.0)	470 (23.0)	0.94	0.76-1.16	0.572	Sawai et al., 2012
Korean	164	88 (53.6)	59 (36.0)	17 (10.4)	235 (71.6)	93 (28.4)	144	74 (51.4)	55 (38.2)	15 (10.4)	203 (70.5)	85 (29.5)	1.06	0.75-1.50	0.751	Sawai et al., 2012
Hong Kong Chinese	93	44 (46.8)	39 (41.5)	10 (10.6)	127 (68.3)	59 (31.7)	187	94 (50.3)	80 (42.8)	13 (6.9)	268 (71.7)	106 (28.3)	0.85	0.58-1.25	0.409	Sawai et al., 2012
Thai	202	100 (49.5)	81 (40.1)	21 (10.4)	281 (69.6)	123 (30.4)	196	97 (49.5)	83 (42.3)	16 (8.2)	277 (70.7)	115 (29.3)	0.95	0.70-1.28	0.733	This study
Saudi Arabian	183	127 (69.4)	47 (25.7)	9 (4.9)	301 (82.2)	65 (17.8)	72	47 (65.3)	22 (30.6)	3 (4.2)	116 (80.6)	28 (19.4)	1.12	0.68-1.83	0.657	Al-Qahtani et al.,2012

Discussion

There are no data on the genotypic distributions and allele frequencies of rs17401966 in Thai HBV-infected patients, and this study is the first to report such data in Thais. The genotypic distributions between both groups were comparable for the homozygous major genotype (AA), heterozygous minor genotype (AG) and homozygous minor genotype (AA). Similar results were also observed in Japanese, Korean, Hong Kong Chinese and Saudi Arabian populations (Al-Qahtani et al., 2012; Sawai et al., 2012). The genotypic distributions of rs17401966 among different populations are summarized in Table 4.

In this study, the frequency of homozygous major genotype (AA) was 49.5% for both groups while frequency of heterozygous minor genotype (AG) was 40.1% and 42.3% for the case and control groups, respectively, and the frequency of homozygous minor genotype (GG) was 10.4% and 8.2% for case and control groups, respectively. The distribution of rs17401966 genotypes in the Thai population was closely related to the Hong Kong Chinese population but highly different from those found in Saudi Arabians. The genotypic distributions of rs17401966 between the case and control group were significantly different in Chinese populations from Guangxi, Beijing, Jiangsu, Guandong and Shanghai (Zhang et al., 2010). The (AA) homozygous major genotype in the case group was significantly higher than the control group, whereas the (AG) heterozygous and (GG) heterozygous minor genotypes in the case group were lower than those found in the control group. This discrepancy indicates that there are other factors aside from ethnicity that are involved in the development of HCC. It is been previously shown that factors such as age, sex, alcohol abuse, folate intake, aflatoxin B1, mutations of *p53*, other viral factors, viral mutation and different genotypes of HBV may be associated with HCC development (Montesano et al., 1997; Lee et al, 2007).

In addition, based on binary logistic regression analysis, rs17401966 was not significantly associated with the development of HCC in Thai patients chronically infected with HBV (p-value=0.998, OR=1.00 and 95% CI=0.68-1.48). This finding is in agreement with results reported in Japanese, Korean, Hong Kong Chinese and Saudi Arabian populations (AI-Qahtani et al., 2012; Sawai et al., 2012). Although the study conducted by Zhang et al. indicated otherwise, this may be due to other factors as previously mentioned.

Thus, it is likely that a combination of other factors contribute to the development of HCC. For example, age has been shown to be positively correlated with HCC. Likewise, males are 4-5 times more likely to develop HCC compared to females (Tangkijvanich et al., 1999). However these factors cannot be controlled for, unlike alcohol and folate consumption. Alcohol abuse will increase the risk of HCC development by 2-4 folds (Fattovich et al., 2004; Morgan et al., 2004) whereas high folate intake may help reduce the risk of developing alcohol-associated HCC (Person et al., 2013). The correlation among these factors indicates the complexity of hepato-carcinogenesis. Another factor that may affect the development of HCC is aflatoxin B1, which is a carcinogen associated with R249S mutation in the *p53* tumor suppressor gene (Groopman et al., 2005). The *p53* mutation is extremely prevalent in sub-Saharan Africa and China because of high exposure to aflatoxin (Montesano et al., 1997). Other factors that may also contribute to the development of HCC are certain genotypes and mutations of HBV. For example, HBV genotype C was positively correlated with HCC incidence. The risk of HCC development increases if patients with HBV genotype C also have a double Basal Core Promoter mutation (Yang et al., 2008). The prevalence of HBV genotype C in Korea, Japan, Thailand and China are 100%, 82.3%, 73% and 50.99%, respectively (Tangkijvanich et al., 2005; Kim et al., 2007; Matsuura et al., 2009) while Saudi Arabians are predominantly infected with genotype D (Abdo et al., 2006). Thus, the different viral genotypes that predominate in each country are associated with the development of HCC in patients. Therefore, not only the allele frequency is responsible for the differences in association between rs17401966 and the risk of HBV-related HCC development. The authors speculate that there must be several factors aside from rs17401966 interacting with each other to

cause discrepancies among the studies. Furthermore, the difference of mean age between case and control groups may be a limitation for the comparison of genotype and allele frequency. In conclusion, SNP rs17401966 was not associated with the risk of developing HCC in Thai HBV-infected patients. This indicates that there must be other mechanisms or pathways involved in the development of HCC.

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