

RESEARCH ARTICLE

Association of PINX1 but not TEP1 Polymorphisms with Progression to Hepatocellular Carcinoma in Thai Patients with Chronic Hepatitis B Virus Infection

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Abstract

Hepatocellular carcinoma (HCC) is major health problem with high mortality rates, especially in patients with hepatitis B virus (HBV) infection. Telomerase function is one of common mechanisms affecting genome stability and cancer development. Recent studies demonstrated that genetic polymorphisms of telomerase associated genes such as telomerase associated protein 1 (TEP1) rs1713449 and PIN2/TERF1-interacting telomerase inhibitor 1 (PINX1) rs1469557 may be associated with risk of HCC and other cancers. In this study, 325 patients with HCC and 539 non-HCC groups [193 healthy controls, 80 patients with HBV-related liver cirrhosis (LC) and 266 patients with HBV-related chronic hepatitis (CH)] were enrolled to explore genetic polymorphisms of both SNPs using the allelic discrimination method based on MGB probe TaqMan real time PCR. We demonstrated that all genotypes of both genes were in Hardy-Wienberg equilibrium ($P > 0.05$). Moreover, there was no significant association between rs1713449 genotypes and HCC risk, HCC progression and overall survival ($P > 0.05$). Interestingly, we observed positive association of rs1469557 with risk of HCC when compared with the LC group under dominant (CC versus CT+TT, OR=1.89, 95% CI= 1.06-3.40, $P=0.031$) and allelic (C versus T alleles, OR=1.75, 95% CI=1.04-2.94, $P=0.033$) models, respectively. Moreover, overall survival of HCC patients with CC genotype of rs1469557 was significantly higher than non-CC genotype (Log-rank $P=0.015$). These findings suggest that PINX1 rs1469557 but not TEP1 rs1469557 might play a role in HCC progression in Thai patients with LC and be used as the prognosis marker to predict overall survival in HCC patients.

Keywords: TEP1 - PINX1 - hepatocellular carcinoma - chronic hepatitis B virus - telomerase - polymorphism

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Introduction

Hepatocellular carcinoma (HCC) is one of major lethal causes in worldwide, especially in patients with hepatitis B virus (HBV) infection (Chemin and Zoulim, 2009). In Thailand, HCC is the most common in male and the third in female (Somboon et al., 2014). Not only viral and immunological but also host genetic factors involve in pathogenesis of HCC and progression of tumor cells. HCC occurrence is multistep process beginning from chronic hepatitis, cirrhosis to HCC progression. Telomerase participates in cancer development by controlling telomere length and chromosomal stability as well as growth of tumor cells. Mutation of telomerase gene was reported as influence factor on the presence of liver cirrhosis and risk of HCC (Hartmann et al., 2011). Telomerase composes of 2 components, ribonucleoprotein complex (functional RNA component known as hTR or hTERC and

catalytic protein or hTERT) and some protein complex called telomerase associated proteins such as telomerase associated protein 1 (TEP1), P23/p90 and hGAR1 that play a role in telomerase function and regulation (Cong et al., 2002). Moreover, regulation of telomerase also relies on the control of tumor suppressor or telomerase inhibitor gene called PIN2/TERF1-interacting telomerase inhibitor 1 (PINX1) (Chang et al., 2012).

TEP1 gene locating at chromosome 14q11 is one part of the telomerase ribonucleoprotein complex playing a key role in telomere addition at the end of chromosome (Yan et al., 2014). Irregular function of this protein resulting from unbalanced gene expression or genetic variation may affect genome and chromosomal stability and promote cancer development. Previous findings of TEP1 polymorphisms were reported in many cancers such as prostate cancer (Gu et al., 2015), breast cancer (Varadi et al., 2009) and bladder cancer (Chang et al., 2012) as well

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as in male infertility (Yan et al., 2014). Recent study in Korea cohorts demonstrated that TEPI polymorphisms were associated with risk of HCC, especially in rs1713449 by using high-throughput SNPs genotyping assay (Jung et al., 2014). Moreover, this region was significantly associated with risk of male infertility resulting in sperm DNA fragmentation (Yan et al., 2014). However, other studies to confirm previous outcomes need to be conducted and no previous study describes this SNP in Thai patients with HCC.

PINX1 gene locating at chromosome 8p23 is regulatory gene controlling telomerase function by inhibiting its activity and influencing on telomere length and chromosomal stability (Zhou, 2011). The 8p23 of PINX1 is reported as high frequency of loss heterozygosity (LOH) mostly found in HCC and other cancers such as gastric cancer and breast cancer (Zhou, 2011). Moreover, this region was previously reported as common site for HBV genome integration in HCC patients (Becker et al., 1996). The decrease of PINX1 expression promoted tumor growth and correlated with poor prognosis in various cancers such as ovarian carcinoma and breast cancer (Cai et al., 2010; Shi et al., 2015). Recent study demonstrated that genetic variation of PINX1 gene, especially SNP of PINX1 rs1469557 associated with risk of bladder cancer (Chang et al., 2012). Although there were previous studies reported the association of TEPI rs1713449 in HCC patients and PINX1 rs1469557 polymorphisms in bladder cancer, the genetic association of both genes and HCC outcomes in Thai and Asian populations with HCC

remains unclear and additional studies with different cohorts to confirm previous findings need to be elucidated. The purpose of this study was to investigate the association of TEPI rs1713449 and PINX1 rs1469557 polymorphisms and clinical outcomes in Thai patients with HCC.

Materials and Methods

Patient recruitment and specimen collection

Buffy coats were randomly selected from stored blood specimens of 325 patients with HCC and 539 non-HCC participants [193 healthy controls, 80 patients with HBV-related liver cirrhosis (LC) and 266 patients with HBV-related chronic hepatitis (CH)]. Each specimen was collected from seen and followed up patients at King Chulalongkorn Memorial Hospital (Bangkok, Thailand) between January 2011 and September 2015. LC was diagnosed by histopathology combination with clinical characteristics such as the presence of ascites or esophageal varices. CH was relied on prolonged elevation of alanine transaminase (ALT) levels and confirmed by histopathology. HCC diagnosis was confirmed by radio-imaging studies and histologic characterization (fine needle aspiration or surgical resection) following the guidelines of American Association for the Study of Liver Diseases (AASLD) (Bruix and Sherman, 2005). Tumor classification in HCC patients was based on the Barcelona Clinical Liver Cancer (BCLC) criteria (Llovet et al., 1999). Seropositive patients for hepatitis C virus (HCV) or human immunodeficiency virus (HIV) were

Table 1. Participant Characteristics in this Study

| | Healthy group (n=193) | CH group (n=266) | LC group (n=80) | HCC group (n=325) | P-value |
|------------------------|--------------------------|---------------------|--------------------|----------------------|--|
| Age (years) | 48.36 ± 5.68 | 50.28 ± 12.04 | 56.60 ± 11.60 | 58.66 ± 11.17 | <0.001 ^{*,§,†} ; 0.041 ^{**} ; 0.143 [§] |
| Gender | | | | | |
| Male (%) | 116 (60.10) | 177 (66.54) | 69 (86.25) | 271 (83.38) | |
| Female (%) | 77 (39.90) | 89 (33.46) | 11 (13.75) | 54 (16.62) | |
| AST (IU/L) | ND | 30.36 ± 18.49 | 38.92 ± 35.04 | 80.13 ± 96.17 | <0.001 ^{§,†} ; 0.042 [‡] |
| ALT (IU/L) | ND | 37.63 ± 39.71 | 41.43 ± 44.01 | 58.38 ± 73.00 | <0.001 [†] ; 0.473 [‡] ; 0.009 [§] |
| Albumin (g/dL) | ND | 4.32 ± 0.62 | 4.20 ± 0.60 | 3.61 ± 0.60 | <0.001 ^{§,†} ; 0.227 [‡] |
| ALP (IU/L) | ND | 75.91 ± 28.53 | 89.83 ± 51.44 | 148.89 ± 124.18 | <0.001 ^{§,†} ; 0.044 [‡] |
| AFP (IU/ml) | ND | 3.39 ± 3.47 | 74.80 ± 399.47 | 11669 ± 57928.97 | <0.001 [†] ; 0.001 [§] ; 0.189 [‡] |
| TB (mg/dL) | ND | 0.70 ± 3.68 | 0.98 ± 1.73 | 1.22 ± 0.87 | <0.001 [†] ; 0.124 [§] ; 0.251 [‡] |
| HBV DNA (logIU/ml) | ND | 5.69 ± 6.44 | 5.71 ± 6.23 | ND | 0.941 [‡] |
| Child-Pugh scoring (%) | | | | | |
| A | ND | ND | ND | 204 (62.77) | |
| B | ND | ND | ND | 72 (22.15) | |
| C | ND | ND | ND | 1 (0.31) | |
| No data | ND | ND | ND | 48 (14.77) | |
| BCLC staging (%) | | | | | |
| Early (0-A) | ND | ND | ND | 94 (28.92) | |
| Intermediate (B) | ND | ND | ND | 120 (36.92) | |
| Advance (C) | ND | ND | ND | 84 (25.85) | |
| Terminal (D) | ND | ND | ND | 2 (0.62) | |
| No data | ND | ND | ND | 25 (7.69) | |

HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis; AST, aspartate aminotransferase; ALT, alanine aminotransferase; TB, total bilirubin; ALP, alkaline phosphatase; AFP, alpha-fetoprotein; ND, no data; ^{*}The P-value between healthy and LC; ^{**}The P-value between healthy and CH; [‡]The P-value between healthy and HCC; [§]The P-value between LC and CH; [†]The P-value between LC and HCC; [‡]The P-value between CH and HCC

excluded from this study.

Patient information such as laboratory record of aspartate aminotransferase (AST), ALT, total albumin, Total bilirubin (TB), alkaline phosphatase (ALP), alpha-fetoprotein (AFP) and HBV DNA level were recorded. This study was conducted in accordance with the good clinical practice (GCP) guidelines, following the Declaration of Helsinki statements. All patients were read and signed inform consent before enrolling in this study. The protocol of this study was approved by the Institutional Review Board, Faculty of Medicine, Chulalongkorn University (Bangkok, Thailand; IRB No. 427/58).

Isolation of genomic DNA

Genomic DNA was isolated from 200 µl of buffy coat using FavorPrep™ Blood Genomic DNA Extraction Mini Kit (Favorgen, Taiwan) by following instruction manual. Concentration and purity of DNA was determined using Nanodrop spectrophotometer (NanoDrop 2000c, Thermo scientific, USA) and kept at -20°C until analyzed.

Genotyping of TEP1 gene (rs1713449) and PINX1 (rs1469557)

The SNPs of TEP1 rs1713449 and PINX1 rs1469557 were genotyped using allelic discrimination method based on MGB probe TaqMan real time PCR with a StepOne Plus Real time PCR machine (Applied Biosystems, USA). The 1 µl (50-500 ng/µl) of genomic DNA was amplified

with 5 µl of 2X TaqMan Genotype Mastermix (Applied Biosystems, USA), 0.5 µl of each 20X TaqMan Genotype Assay Mix (rs1713449; ID: C_8921353_20 or rs1469557; ID: C_8340019_30)(Applied Biosystems, USA) and 3.5 µl of DNase and RNase free water. PCR condition was performed by following the instruction manual and fluorescent signals of VIC and FAM were collected at the end of each cycle following by data analysis with StepOne™ software (Version 2.3, Applied Biosystems, USA). Positive and negative controls of each SNPs were included in each experiment to validate the quality of the assay.

Statistical analysis

Clinical data were presented as mean ± standard deviation (SD) for quantitative variables and as percentages for categorical variables. Comparison of either quantitative or categorical variables among or between groups was based on Student t-test, F-test or Chi-square test. Hardy-Weinberg equilibrium (HWE) of each SNP was explored using online software based on Pearson's goodness-of-fit chi-square (Institute of Human Genetics, Germany; <https://ihg.gsf.de/ihg/snps.html>). Association study of SNPs and risk for HCC were assessed under allelic, additive, dominant and recessive models by calculating odd ratios (ORs) with 95% confidence intervals (CIs) with MedCalc statistical software Version 16.2 (https://www.medcalc.org/calc/odds_ratio.php) (Chanthra et al., 2015). The

Table 2. Hardy-Wienberg Equilibrium (HWE) analysis in TEP1 rs1713449 and PINX1 rs1469557 Genotypes among Groups in this Study

| Gene | Group of subject | Genotype | Observed amount | Expected amount | P-value [†] |
|--------------------|------------------|----------|-----------------|-----------------|----------------------|
| TEP1 rs1713449 | HCC | CC | 142 | 141.60 | 0.915 |
| | | CT | 145 | 145.90 | |
| | | TT | 38 | 37.60 | |
| | LC | CC | 40 | 41.30 | 0.463 |
| | | CT | 35 | 32.30 | |
| | | TT | 5 | 6.30 | |
| | CH | CC | 118 | 121.80 | 0.286 |
| | | CT | 124 | 116.40 | |
| | | TT | 24 | 27.80 | |
| | Healthy | CC | 79 | 84.90 | 0.058 |
| | | CT | 98 | 86.20 | |
| | | TT | 16 | 21.90 | |
| | All participants | CC | 379 | 389.40 | 0.110 |
| | | CT | 402 | 381.30 | |
| TT | | 83 | 93.40 | | |
| HCC | CC | 215 | 212.80 | 0.435 | |
| | CT | 96 | 100.30 | | |
| | TT | 14 | 11.80 | | |
| LC | CC | 63 | 62.10 | 0.352 | |
| | CT | 15 | 16.70 | | |
| | TT | 2 | 1.10 | | |
| PINX1 rs1469557 | CH | CC | 183 | 179.50 | 0.141 |
| | | CT | 71 | 78.00 | |
| | | TT | 12 | 8.50 | |
| | Healthy | CC | 129 | 131.00 | 0.324 |
| | | CT | 60 | 56.00 | |
| | | TT | 4 | 6.00 | |
| All participants | CC | 590 | 585.10 | 0.252 | |
| | CT | 242 | 251.80 | | |
| | TT | 32 | 27.10 | | |

[†]P-value based on Pearson's goodness-of-fit chi-square; HCC, hepatocellular carcinoma; LC, liver cirrhosis; CH, chronic hepatitis

relationship of gene polymorphisms and overall survival in HCC patients was tested using Kaplan-Meier method with the log-rank analysis. All statistical analysis was evaluated with SPSS version 23 (IBM, USA). P-values lower than 0.05 were stated as statistically significant.

Results

Characteristics of participants in this study

The clinical data of all participants were presented in Table 1. The age of participants in the HCC, LC and CH groups was significantly higher than healthy controls

(P<0.05, respectively). No significant difference was found in age between the LC and HCC groups (P=0.143). All participants were mostly male more than female. Chemical parameters such as ASL, ALT, ALP, AFP and TB were significantly higher in HCC than other groups (p<0.05). Most HCC patients were clustered in Child-Pugh score A (62.77%) and in intermediate stage (B) of HCC (36.92%) according to BCLC staging system. However, some HCC patients could not be classified in both parameters due to lacking of some clinical data for interpretation.

Table 3. Genotype and Allele Frequencies of SNPs TEP1 rs1713449 and PINX1 rs1469557 on Risk of HCC Development

| SNPs Genotype and Allele | HCC (n=325) | LC (n=80) | CH (n=266) | Healthy (n=193) | HCC vs non-HCC* P-value | OR (95% CI) | HCC vs Healthy P-value | OR (95% CI) | HCC vs LC P-value | OR (95% CI) | HCC vs CH P-value | OR (95% CI) |
|--------------------------|-------------|-------------|-------------|-----------------|-------------------------|-------------------|------------------------|------------------|-------------------|------------------|-------------------|------------------|
| TEP1 rs1713449 | | | | | | | | | | | | |
| Additive model | | | | | | | | | | | | |
| CC | 142 (43.69) | 40 (50.00) | 118 (44.36) | 79 (40.93) | - | 1 | - | 1 | - | 1 | - | 1 |
| CT | 145 (44.62) | 35 (43.75) | 124 (46.62) | 98 (50.78) | 0.687 | 0.94 (0.70-1.26) | 0.310 | 0.82 (0.56-1.20) | 0.552 | 1.17 (0.70-1.94) | 0.870 | 0.97 (0.69-1.37) |
| TT | 38 (11.69) | 5 (6.25) | 24 (9.02) | 16 (8.29) | 0.160 | 1.41 (0.873-2.28) | 0.398 | 1.32 (0.69-2.52) | 0.134 | 2.14 (0.79-5.80) | 0.342 | 1.31 (0.75-2.32) |
| Allelic model | | | | | | | | | | | | |
| Major (C) | 429 (66.00) | 115 (72.00) | 360 (68.00) | 256 (66.00) | - | 1 | - | 1 | - | 1 | - | 1 |
| Minor (T) | 221 (34.00) | 45 (28.00) | 172 (32.00) | 130 (34.00) | 0.438 | 1.08 (0.88-1.33) | 0.916 | 1.01 (0.78-1.32) | 0.157 | 1.32 (0.90-1.93) | 0.544 | 1.08 (0.85-1.37) |
| Dominant model | | | | | | | | | | | | |
| CC | 142 (43.69) | 40 (50.00) | 118 (44.36) | 79 (40.93) | - | 1 | - | 1 | - | 1 | - | 1 |
| CT+TT | 183 (56.31) | 40 (50.00) | 148 (55.64) | 114 (59.07) | 0.936 | 1.01 (0.77-1.33) | 0.539 | 0.89 (0.62-1.28) | 0.310 | 1.29 (0.79-2.10) | 0.870 | 1.03 (0.74-1.42) |
| Recessive model | | | | | | | | | | | | |
| CC+CT | 287 (88.31) | 75 (93.75) | 242 (90.98) | 177 (91.71) | - | 1 | - | 1 | - | 1 | - | 1 |
| TT | 38 (11.69) | 5 (6.25) | 24 (9.02) | 16 (8.29) | 0.107 | 1.45 (0.92-2.29) | 0.223 | 1.46 (0.79-2.70) | 0.164 | 1.99 (0.75-5.22) | 0.293 | 1.34 (0.78-2.29) |
| PINX1 rs1469557 | | | | | | | | | | | | |
| Additive model | | | | | | | | | | | | |
| CC | 215 (66.15) | 63 (78.75) | 183 (68.80) | 129 (66.84) | - | 1 | - | 1 | - | 1 | - | 1 |
| CT | 96 (29.54) | 15 (18.75) | 71 (26.69) | 60 (31.09) | 0.382 | 1.15 (0.84-1.56) | 0.837 | 0.96 (0.65-1.42) | 0.044† | 1.87 (1.02-3.46) | 0.450 | 1.15 (0.80-1.66) |
| TT | 14 (4.31) | 2 (2.50) | 12 (4.51) | 4 (2.07) | 0.329 | 1.44 (0.69-2.97) | 0.199 | 2.10 (0.67-6.52) | 0.350 | 2.05 (0.45-9.26) | 0.986 | 0.99 (0.45-2.20) |
| Allelic model | | | | | | | | | | | | |
| Major (C) | 525 (81.00) | 141 (88.00) | 437 (82.00) | 318 (82.00) | - | 1 | - | 1 | - | 1 | - | 1 |
| Minor (T) | 124 (19.00) | 19 (12.00) | 95 (18.00) | 68 (18.00) | 0.241 | 1.16 (0.90-1.50) | 0.551 | 1.10 (0.80-1.53) | 0.033† | 1.75 (1.04-2.94) | 0.583 | 1.09 (0.80-1.46) |
| Dominant model | | | | | | | | | | | | |
| CC | 215 (66.15) | 63 (78.75) | 183 (68.80) | 129 (66.84) | - | 1 | - | 1 | - | 1 | - | 1 |
| CT+TT | 110 (33.85) | 17 (21.25) | 83 (31.20) | 64 (33.16) | 0.278 | 1.18 (0.88-1.58) | 0.873 | 1.03 (0.71-1.50) | 0.031† | 1.89 (1.06-3.40) | 0.495 | 1.13 (0.80-1.59) |
| Recessive model | | | | | | | | | | | | |
| CC+CT | 311 (95.69) | 78 (97.5) | 254 (95.49) | 189 (97.93) | - | 1 | - | 1 | - | 1 | - | 1 |
| TT | 14 (4.31) | 2 (2.50) | 12 (4.51) | 4 (2.07) | 0.382 | 1.38 (0.67-2.84) | 0.189 | 2.13 (0.69-6.56) | 0.463 | 0.57 (0.13-2.56) | 0.904 | 0.95 (0.43-2.10) |

*Non-HCC included healthy, LC and CH; HCC, hepatocellular carcinoma; CH, chronic hepatitis; LC, liver cirrhosis; OR, odd ratios; CI, confidence interval; †P-value represented statistically different.

Association of TEP1 rs1713449 and HCC risk

The genotypes of TEP1 rs1713449 in all groups were in HWE ($P>0.05$) indicating that there was no bias of sample collection for polymorphism analysis (Table 2). After analyzing the association of TEP1 rs1713449 genotypes and HCC risk, the results revealed that there was no statistical difference among genotype frequencies between the HCC versus non-HCC groups, the HCC versus healthy groups, the HCC versus LC groups and HCC versus CH groups in additive, dominant and recessive models (CC versus TT and CC versus CT, CC versus CT+TT and TT versus CC+CT, respectively; $P>0.05$). Moreover, the allele frequencies of TEP1 rs1713449 analyzed with allelic model among all groups of study did not represent statistically different (C versus T alleles, $P>0.05$), which corresponded to the findings of genotype analysis (Table 3). Next, we investigated the correlation of TEP1 rs1713449 and HCC stages. Our results illustrated that there was no significantly different between TEP1 genotypes and HCC stages (CC versus non-CC, $P=0.987$) (Table 4). These pieces of evidence suggested that TEP1 rs1713449 might not be associated with HCC occurrence and severity of disease in Thai patients.

Table 4. Association between TEP1 rs1713449 and PINX1 rs1469557 polymorphisms on HCC classification

| | Early stage (0-A) | Intermediate (B) | Late stage* (C-D) | P-value [†] |
|-----------------|----------------------|---------------------|----------------------|----------------------|
| TEP1 rs1713449 | | | | |
| CC (n=131) | 41 (31.30) | 53 (40.46) | 37 (29.24) | 0.987 |
| non-CC (n=169) | 53 (31.36) | 67 (39.64) | 49 (29.00) | |
| PINX1 rs1469557 | | | | |
| CC (n=199) | 61 (30.66) | 80 (40.20) | 58 (29.14) | 0.932 |
| non-CC (n=101) | 33 (32.67) | 40 (39.60) | 28 (27.73) | |

The number in parenthesis represented stage of HCC based on BCLC staging system; *Late stage referred to BCLC stage C and stage D; Patients with absence of BCLC data were excluded from this analysis; [†]P-value of Chi-square test

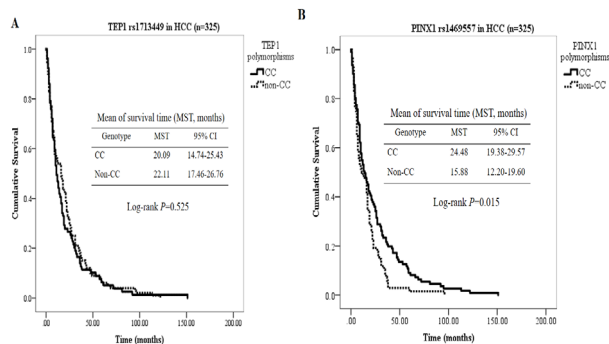


Figure 1. Association of SNPs TEP1 rs1713449 (CC and non-CC Genotypes) on Mean Survival Time, MST (A) and PINX1 rs1469557 (CC and non-CC genotypes) on Mean Survival Time, MST (B) in Thai Patients with HBV-Related HCC

Association of PINX1 rs1469557 and HCC risk

The genotypes of PINX1 rs1469557 in all groups were in HWE ($P>0.05$) (Table 2). In this study, we found that there was no significant difference of rs1469557 genotypes between the HCC versus non-HCC groups, HCC versus healthy groups and HCC versus CH in additive, dominant and recessive models (CC versus TT and CC versus CT, CC versus CT+TT and TT versus CC+CT, respectively), which was corresponded with allelic model (C versus T alleles) (all $P>0.05$). However, we found the significantly different in genotype distribution and allele frequencies between HCC and LC groups in additive model (CC versus CT, OR=1.87, 95% CI= 1.02-3.46, $P=0.044$), dominant model (CC versus CT+TT, OR=1.89, 95% CI=1.06-3.40, $P=0.031$) and allelic model (C versus T, OR=1.75, 95% CI=1.04-2.94, $P=0.033$) (Table 3). Subsequently, we investigated the association of PINX1 genotypes and HCC stages as presented in Table 4 and found that there was no association between PINX1 genotypes and HCC classification (CC versus non-CC, $P=0.936$).

Overall survival of HCC patients in each genotype of TEP1 and PINX1 SNPs

We next analyzed the association of each genotypes of TEP1 rs1713449 and PINX1 rs1469557 SNPs on survival time of HCC patients as shown in Figure 1 (A and B, respectively). Our results illustrated the longest mean survival time (MST) was found in CC genotype of TEP1 rs1713449 up to 151 months. In addition, MST in HCC patients with non-CC genotype of TEP1 rs1713449 was longer than CC genotype but no statistical difference was found (20.09 months, 95% CI=14.74-25.43 versus 22.11 months, 95% CI=17.46-26.76, Log-rank $P=0.525$). Interestingly, we found the statistical difference of MST between CC and non-CC genotypes of PINX1 rs1469557 in HCC patients (24.48 months, 95% CI=19.38-29.57 versus 15.88 month, 95% CI=12.20-19.60, respectively, Log-rank $P=0.015$).

Discussion

Chromosomal and genome instability is one of hallmarks of cancer, which is manipulated by telomerase (the protein complex that adds telomeres at the end of genome). Different expression and genetic variation of both telomerase and telomerase component genes involve in cancer development including HCC. In this study, we investigated the genetic variation in case of genetic polymorphisms of TEP1 rs1713449 and PINX1 rs1469557 and the effect of these variations on clinical outcomes in HCC patients. To our knowledge, this is the first study inventing both SNPs in Thai patients with HCC, especially in PINX1 polymorphism which has not ever been reported in HCC. Moreover, we categorized the groups of participants thoroughly into the HCC, LC, CH and healthy groups, which has not been classified in previous studies. According to our findings, genotype distribution of both gene were in HWE indicating that there is no bias of sample sizes in each group and all genotypes independently occur. Moreover, it will support

the reliable results in our study.

TEP1 is the component of telomerase complex that participates the role in adding new telomeres at the end of chromosomes to maintain stability of genome. In recent study, there were many TEP1 SNPs studied in CHB related HCC such as rs1713449, rs1760904, rs872072, rs1760898 and rs1760897 (Jung et al., 2014). In this study, we selected rs1713449, the nonsynonymous SNP (V2241I) according to the strongly positive association with HCC in Korean cohort (Jung et al., 2014). Moreover, this SNP was significantly associated with risk of male infertility by representing the high level of DNA fragmentation (Yan et al., 2014). Our findings showed that no significant correlation was found between TEP1 rs1713449 and HCC occurrence in all analyzed models, which contradicts with previous study investigated in Korea cohort by Jung and his co-workers. These may from the different genetic backgrounds of participants between our and previous cohorts. Moreover, we also found no association between TEP1 rs1713449 and stages of HCC. These findings imply that TEP1 rs1713449 is not influence on HCC progression in Thai patients although the SNP locates in exon that alters protein structure and function. However, we cannot rule out that TEP1 gene is not related to HCC development because we focused on only one position of polymorphism in this gene. We continued this study by exploring the association of different two genotypes (CC versus non-CC) and overall survival time (MST) of HCC patients. We divided our HCC group into two different genotypes in accordance with the previous finding arguing that non-CC increased risk of HCC (Jung et al., 2014). Our results revealed that there was no significantly different of MST between CC and non-CC genotypes ($P=0.525$) although the results showed the longer time survival in patient with CC than non-CC groups (151 months and 122 months, data not shown). These findings oppose to previous study mentioning the significantly longer MST in CC than non-CC participants (Jung et al., 2014). The dissimilar results between our and previous cohorts may be from the difference in races, a number of participants and the disease progression during our cross-sectional retrospective study.

PINX1 is the member of telomere-telomerase genes acting as regulatory gene that controls the telomerase function by binding with telomeric repeat binding factor-1 (TRF-1). Due to the location of the gene on chromosome 8p23 presenting high frequency of loss of heterozygosity, genetic variations of this gene involve in many genetic diseases including HCC and this site was the common hotspot for HBV genome integration (Becker et al., 1996; Zhou, 2011). In some cancer such as ovarian carcinoma and breast cancer, low expression of PINX1 gene correlated with tumor progression and promoted metastasis of cancer cells when compared with normal expression of this gene (Cai et al., 2010; Shi et al., 2015). Therefore, PINX1 can also acts as tumor suppressor gene. In this study, we selected PINX1 rs1469557 (locating at intergenic region) as candidate SNP to explore the association with HCC risk, HCC progression and survival time because prior study in bladder cancer mentioned the correlation of this SNP and risk of cancer development.

Our results demonstrated that there was no association between genotype distribution and risk of HCC in the HCC versus non-HCC groups, HCC versus healthy groups and HCC versus CH groups ($P>0.05$). Surprisingly, our findings represented the significant difference in genotype and allele frequencies between HCC and LC in additive model (CC versus CT, $P=0.044$), dominant model (CC versus CT+TT, $P=0.031$) and allelic model (C versus T allele, $P=0.033$), respectively. We therefore imply from our observation that PINX1 rs1469557 has not an impact on risk for HCC in CH patients but may be influence on the progression of disease from LC to HCC by presenting the high risk in cirrhosis in patients with T alleles. Moreover, we can also suggest CC genotype as protective genotype and prognosis marker in cirrhosis patients. Then, we analyzed the SNP results with BCLC classification system and found no association between stages of HCC and genotype frequencies which may reveal that PINX1 rs1469557 has no impact on risk and progression of HCC. However, like TEP1 rs1713449, we cannot exclude that PINX1 gene is not associated with HCC occurrence because in this study, we focus on one SNP only. We finally investigated the correlation between PINX1 polymorphism and survival time of patients with HCC and presented that patients with CC genotype have the longer time survival than non-CC genotypes (MST=24.48 months versus MST=15.88 months, Log rank $P=0.015$). This may support that CC genotype of PINX1 rs1469557 is protective genotype and can be used as the prognosis marker in order to predict survival time in HCC patients.

In conclusion, our data suggest that PINX1 rs1469557 is correlated with HCC progression in patients with LC and overall survival. These findings can fulfill the role of telomerase in HCC pathogenesis. Additionally, PINX1 rs1469557 may be also used as genetic marker for predicting the occurrence and survival of HCC patients. Due to our limitation of this study about the sample size, larger cohort of enrolled participants need to be investigated to validate previous results. Moreover, the functional study of both TEP1 and PINX1 on HCC are also necessary to explore for future development of genetic and protein markers for diagnosis, prognosis and anticancer drugs.

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