Association of CHKA polymorphism (rs3794186) with α-fetoprotein levels in hepatocellular carcinoma

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Abstract. Choline kinase α (CHKA) has been identified to be associated with cancer development and progression. In this study, we investigated whether exonic single nucleotide polymorphisms (SNPs) of the CHKA gene are associated with hepatocellular carcinoma (HCC). Among all SNPs in the 3'-untranslated region (UTR), 5'-UTR and the coding region of CHA, only two SNPs (rs3794186 and rs11481) in the 3'-UTR had a heterozygosity above 0.1 and a minor allele frequency above 0.1. Therefore, we selected and assessed these two SNPs (rs3794186 and rs11481) in 189 HCC patients and 194 controls. Genetic data were analyzed using the SNPAnalyzer Pro, SNPStats and Haploview programs. No SNPs of the CHKA gene were found to be associated with the risk of HCC development. Upon analysis of the clinical characteristics of HCC, the genotypic frequency of rs3794186 was significantly associated with serum α-fetoprotein (AFP) levels (P=0.022 in the co-dominant 1 model, P=0.0045 in the dominant model and P=0.0052 in the log-additive model). A significant difference in the allelic frequency of rs3794186 was also observed between the high AFP (>200 ng/ml) group and the low AFP (≤200 ng/ml) group [P=0.009, odds ratio (OR) = 0.33, 95% confidence interval (95% CI) = 0.14-0.75]. The T allele frequency of rs3794186 was lower in the high AFP group (6.6%) compared to that in the low AFP group (17.8%). Our results suggest that CHKA SNPs (rs3794186 and rs11481) are not associated with HCC development; however, rs3794186 may correlate with serum AFP levels in HCC.

Introduction

Hepatocellular carcinoma (HCC) is the third leading cause of cancer mortality worldwide and appears at a high recurrence rate following surgical treatment, including liver transplantation (1-3). HCC causes a great epidemiological impact and is characterized by high incidence in patients with chronic liver diseases (4). A number of studies have been performed to analyze single nucleotide polymorphisms (SNPs) as a genetic marker of HCC. Chen *et al* (5) suggested that the polymorphisms in the promoter region of α-fetoprotein (AFP) may be pathologically significant in HCC. Kim *et al* (6) reported the association of EPH receptor B1 (EPHB1) polymorphisms with HCC.

Choline kinase is an enzyme which catalyzes the first reaction for phosphatidylcholine biosynthesis in the choline pathway. Phosphatidylcholine is important for facilitating the transport of cholesterol through the organism, acting as a substrate for the production of second messengers and as a co-factor for the activity of several membrane-related enzymes. Choline kinase plays a pivotal role in the regulation of cell growth and the signal transduction pathways related to mitogenesis. Thus, choline kinase is considered to be an important target for cancer (7). In humans, choline kinase has two isoforms, choline kinase α (CHKA) and choline kinase β (CHKB), α being the dominant isoform. Previous studies have described the oncogenic activity of CHKA. The expression of CHKA is related to the pathogenesis of several types of cancer. Eliyahu *et al* (8) reported that CHKA is overexpressed in breast cancer cells. Ramírez de Molina *et al* (9) demonstrated that the overexpression of choline kinase is a frequent feature in human tumor-derived cell lines and in lung, prostate and colorectal human cancers. However, the correlation between CHKA SNPs and HCC has not yet been studied.

In this study, we analyzed CHKA polymorphisms in order to explore their genetic correlation with HCC development and clinical characteristics in a Korean population.

Subjects and methods

Study subjects. A total of 189 HCC patients (162 males and 27 females) and 194 healthy individuals (145 males and 49 females) were enrolled in this study. HCC patients were recruited at the Kyung Hee University Medical Center, Seoul and Keimyung University Dongsan Medical Center, Daegu, Republic of Korea. HCC patients were divided into subgroups based on their clinical features, including tumor size, serum AFP levels, modified Union International Contre le Cancer (UICC) stage, radiological morphology and portal vein thrombosis (Table I). Patients with other types of cancer and severe diseases were excluded. The

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control subjects were recruited among participants receiving a general health check-up with no clinical evidence of HCC or other severe diseases. Informed consent was obtained from all individuals according to the Declaration of Helsinki guidelines. The study was approved by the Ethics Review Committee of The Medical Research Institute, Kyung Hee University Medical Center, Seoul, Republic of Korea.

SNP selection and genotyping. The exonic SNPs of the CHKA gene were retrieved from the NIH SNP database (www.ncbi.nlm.nih.gov/SNP; dbSNP Build 135). Of the exonic SNPs in the 3'-untranslated region (UTR), 5'-UTR and the coding region of CHA, only two SNPs (rs3794186 and rs11481) in the 3'-UTR had a heterozygosity above 0.1 and a minor allele frequency above 0.1. Finally, two SNP (rs3794186 and rs11481) were selected. Genomic DNA was extracted using the Roche DNA Extraction kit (Roche, Indianapolis, IN, USA). Each DNA was amplified using the following primers: rs3794186 sense, 5'-TCC TTTTAATCTAGAGAAGGCA-3' and antisense, 5'-TCCGCT GCTCCAGCTTCAGCCA-3', 322 bp; and rs11481 sense, 5'-TGAAATGTCCTGCGGGATACAG-3' and antisense, 5'-AGGAGGGCTGATGATGGGGCCA-3', 298 bp. PCR products were sequenced using an ABI PRISM 3730XL analyzer (PE Applied Biosystems, Foster City, CA, USA) and sequence data were analyzed using SeqManII software (DNASTAR Inc., Madison, WI, USA).

Statistical analysis. SNPStats (http://bioinfo.iconcologia.net/index), SNPAnalyzer (ISTECH Inc., Goyang, Republic of Korea), and SPSS 18.0 statistical software (SPSS Inc., Chicago, IL, USA) were used to analyze the genetic data. Multiple logistic regression models (co-dominant 1, co-dominant 2, dominant, recessive and log-additive) were conducted to obtain the odds ratio (OR), 95% confidence interval (95% I) and P-value was adjusted for age and gender as co-variates. Hardy-Weinberg equilibrium (HWE) was evaluated using SNPStats. A linkage disequilibrium (LD) block was estimated using Haploview version 4.2 (Broad Institute, Cambridge, MA, USA). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Demographics and tumor characteristics. Demographic and clinical characteristics of the 189 HCCs are shown in Table I. HCCs consisted of tumors related to alcohol (n=22), hepatitis B virus (HBV, n=144), hepatitis C virus (HCV, n=15), and HBV and HCV (n=7). HCC with HBV accounted for 76.2% of the HCCs. HCC patients were divided into two subgroups according to the size of the tumor (<5 cm, 57.7%, n=109; ≥5 cm, 42.3%, n=80). The number of HCC patients with ≤200 ng/ml AFP was 131 (69.3%) and the number with >200 ng/ml AFP was 58 (30.7%). HCC patients were divided into four subgroups based on modified UICC stage [stage I (n=18), stage II (n=52), stage III (=55), stage IVa (n=38) and stage IVb (n=25)]. One patient with missing data was excluded. The number of HCC patients with nodular type was 127 (67.2%) and those with non-nodular type was 62 (32.8%). Portal vein thrombosis was identified in 66 patients (34.9%, compared to 122 HCC patients without portal vein thrombosis (65.1%). One patient with missing data was also excluded (Table I).

Table I. Demographic and clinical characteristics of the study population.

Characteristics	HCC	Control
Number of subjects	189	194
Age (mean ± SD)	58.2±10.8	51.8±10.8
Etiologies of HCC		
Alcohol	22	
HBV	144	
HCV	15	
HBV + HCV	7	
Tumor size		
<5 cm	109	
≥5 cm	80	
AFP		
≤200 ng/ml	131	
>200 ng/ml	58	
Modified UICC stage		
I	18	
II	52	
III	55	
IVa	38	
IVb	25	
Radiologic morphology		
Nodular	127	
Non-nodular	62	
Portal vein thrombosis		
Present	66	
Absent	122	

HCC, hepatocellular carcinoma; SD, standard deviation; HBV, hepatitis B; HCV, hepatitis C; AFP, α-fetoprotein, UICC, Union International Contre le Cancer.

Genetic association between CHKA SNPs and HCC. As shown Table II, the genotypic frequencies of rs3794186 in the control group were 74.7% for CC, 21.6% for CT and 3.6% for TT. In the HCC group, the genotypic frequencies of rs3794186 were 76.0% for CC, 19.3 % for CT and 4.7% for TT. The genotypic frequencies of rs11481 in the control group were 51.6% for CC, 38.4 % for CT and 10.0% for TT, while those of rs11481 were 51.2% for CC, 37.8 % for CT and 11.0% for TT. In this study, there was no significant difference observed in the rs3794186 and rs11481 CHKA SNPs between the HCC group and the control group.

The LD block between rs3794186 and rs11481 was not constructed ($D'=0.671$ and $r^2=0.18$). Therefore, we did not analyze the haplotypes consisting of rs3794186 and rs11481. The two tested SNPs were in HWE ($P>0.05$, data not shown).

Genetic association between CHKA SNPs and clinical characteristics of HCC. The correlations between CHKA SNPs and clinical characteristics of HCC were investigated. We found that rs3794186 in CHKA was significantly associated with the serum levels of AFP (Table III). In the low AFP group (≤200 ng/ml), the genotypic frequencies of rs3794186

Table II. Genotypic and allelic frequencies of CHKA polymorphisms in HCC patients and control subjects.

	Control		HCC				
SNP	n	%	n	%	Model	OR (95% CI)	P-value
rs3794186 3'-UTR							
Genotype							
C/C	145	74.7	130	76.0	Co-dominant 1	1.02 (0.58-1.81)	0.94
C/T	42	21.6	33	19.3	Co-dominant 2	1.55 (0.49-4.86)	0.46
T/T	7	3.6	8	4.7	Dominant	1.10 (0.65-1.87)	0.73
					Recessive	1.54 (0.49-4.81)	0.46
					Log-additive	1.13 (0.74-1.72)	0.58
Allele							
C	332	85.6)	293	85.7		1	
T	56	14.4)	49	14.3		0.99 (0.66-1.50)	0.97
rs11481 3'-UTR							
Genotype							
T/T	98	51.6	84	51.2	Co-dominant 1	0.96 (0.58-1.58)	0.87
T/A	73	38.4	62	37.8	Co-dominant 2	1.25 (0.58-2.73)	0.57
A/A	19	10.0	18	11.0	Dominant	1.02 (0.64-1.62)	0.94
					Recessive	1.28 (0.60-2.71)	0.52
					Log-additive	1.06 (0.75-1.50)	0.73
Allele							
T	269	70.8	230	70.1		1	
A	111	29.2	98	29.9		1.03 (0.75-1.43)	0.85

CHKA, choline kinase α; HCC, epatocellular carcinoma; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval.

Table III.Genotypic and allelic frequencies of CHKA polymorphisms in HCC patients with AFP levels of ≤200 and >200.

	AFP ≤200		AFP >200					
SNP	n	%	n	%	Model	OR (95% CI)	P-value	P-value[a]
rs3794186 3'-UTR								
Genotype								
C/C	83	70.3	47	88.7	Co-dominant 1	0.30 (0.11-0.85)	**0.023**	**0.022**
C/T	28	23.7	5	9.4	Co-dominant 2	0.22 (0.03-1.86)	0.16	0.26
T/T	7	5.9	1	1.9	Dominant	0.28 (0.11-0.73)	**0.0045**	
					Recessive	0.27 (0.03-2.27)	0.16	0.44
					Log-additive	0.36 (0.16-0.81)	**0.0052**	
Allele								
C	194	82.2	99	93.4		1		
T	42	17.8	7	6.6		0.33 (0.14-0.75)	**0.009**	
rs11481 3'-UTR								
Genotype								
T/T	59	51.3	25	51.0	Co-dominant 1	1.08 (0.52-2.23)	0.83	
T/A	43	37.4	19	38.8	Co-dominant 2	0.84 (0.26-2.65)	0.76	1.00
A/A	13	11.3	5	10.2	Dominant	1.02 (0.52-2.02)	0.95	
					Recessive	0.81 (0.27-2.46)	0.71	1.00
					Log-additive	0.97 (0.59-1.60)	0.90	
Allele								
T	161	70.0	69	70.4		1		
A	69	30.0	29	29.6		0.98 (0.59-1.65)	0.94	

CHKA, choline kinase α; AFP, α-fetoprotein; SNP, single nucleotide polymorphism; OR, odds ratio; 95% CI, 95% confidence interval. Values in bold indicate statistical significance. [a]From Fisher's exact test.

were 70.3% for CC, 23.7% for CT and 5.9% for TT. In the high AFP group, the genotypic frequencies of rs3794186 were 88.7% for CC, 9.4% for CT and 1.9% for TT. The genotypic frequency of rs3794186 was associated with the AFP levels in the co-dominant 1 (CT vs. CC, P=0.023, Fisher's exact test P=0.022, OR = 0.30, 95% CI = 0.11-0.85), dominant (CT/TT vs. CC, P=0.0045, OR = 0.28, 95% CI = 0.11-0.73), and log-additive models (P=0.0052, OR = 0.36, 95% CI = 0.16-0.81). The frequencies of genotypes (CT and TT) containing the T allele were lower in the high AFT group (9.4 and 1.9%) than those in the low AFP group (23.7 and 5.9%). In the analysis of allelic frequency, rs3794186 was associated with AFP levels (P=0.009, OR = 0.33, 95% CI = 014-0.75), and the frequency of the T allele was decreased in the high AFP group (6.6%) compared to the low AFP group (17.8%). These results suggest that the T allele of rs3794186 may be a protective factor for the expression of AFP in HCC. The two examined SNPs (rs3794186 and rs11481) were not associated with other clinical characteristics of HCC, including tumor size, modified UICC stage, radiological morphology and portal vein thrombosis.

Sample power. The sample power was calculated to verify the data using a genetic power calculator (http://pngu.mgh.harvard.edu/~purcell/gpc/cc2.html). In this study, the sample power of each SNP was 0.743 for rs3794186 (α=0.05, genotype relative risk = two-fold, number of cases for 70% power = 170), 0.815 for rs11481 (α=0.05, genotype relative risk = two-fold, number of cases for 70% power = 142). Therefore, our results have relative statistical significance.

Discussion

During the last decade, a number of studies have investigated the genetic association between SNPs of candidate genes and several diseases. Although accumulating evidence has demonstrated that CHKA is important for cancer development and progression (8-10), there have been no studies on CHKA polymorphisms in cancer. Only two studies have published the genetic association between CHKA SNPs and spina bifida (11) or infertile women with endometriosis (12). Enaw *et al* (11) reported that the genotypes (AC and CC) with at least one C allele in an intronic CHKA SNP (rs7928739) were associated with a reduced risk of spina bifida in a Californian population (OR = 0.60, 95% CI = 0.38-0.94). Another study by Szczepańska *et al* (12) demonstrsted that rs7928739 was not associated with infertile women with endometriosis in a Polish population. In this study, we analyzed two SNPs (rs3794186 and rs11481) on the 3'-UTR of the CHKA gene. The two examined SNPs (rs3794186 and rs11481) were associated with the levels of AFP, but not associated with the development of HCC. The 3'-UTR is known to play an important role in the translation, localization and stability of mRNA. Therefore, it is important in the development and progression of various diseases (13).

AFP has been used as a tumor marker. Focusing on HCC, Forner *et al* (14) demonstrated the usefulness of AFP for screening and diagnosis of HCC. Nomura *et al* (15) found that AFP levels have not only a diagnostic, but also a prognostic value. Ho *et al* (16) reported that high AFP levels (>200 ng/ml) are a risk factor for HCC recurrence. In our study, an exonic CHKA SNP, rs3794186, was associated with AFP levels. The T allele frequency of rs3794186 was lower in the high AFP group (6.6%) than in the low AFP group (17.8%), suggesting the the T allele of rs3794186 may have a protective effect on the expression of AFP in HCC. Considering the correlation between HCC and AFP, the rs3794186 CHKA SNP may affect AFP expression.

In conclusion, rs3794186 was significantly associated with AFP levels in HCC. In particular, the T allele frequency of rs3794186 was decreased in HCC patients with high AFP levels. Additional studies on different populations or other SNPs of CHKA are required to confirm our results.

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