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TGF-β1 polymorphisms and familial aggregation of liver cancer in Guangxi, China

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ABSTRACT. The goal of present study was to investigate the relationship between polymorphisms of *TGF-β1* and familial aggregation of liver cancer in Guangxi Zhuang, Han, and Yao populations. We conducted a population-based case-control family study of liver cancer in Guanxi, China. A total of 214 individuals from 37 case families were surveyed for polymorphisms in *TGF-β1*. We genotyped six functional *TGF-β1* polymorphisms: rs1800469, rs2241715, rs2241716, rs11466345, rs8105161, and rs747857. Levels of TGF-β1, hepatitis B surface antigen, and anti-hepatitis C virus in all serum samples were detected using the enzyme-linked immunoassay method, and presence of hepatitis B virus (HBV) DNA was determined using polymerase chain reaction amplification. A standardized questionnaire was used to collect information from subjects, including alcohol consumption, smoking, eating, and water drinking habits. The results were compared with those from 214 control individuals. The results showed that the *TGF-β1* genotypes rs1800469, rs2241715, rs2241715, and rs8105161 were more frequent in patients than in controls. The risk factors for familial aggregation of liver cancer in Guangxi were determined, from high to low, to be: drinking sugared beverages > alcohol consumption > HBV

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DNA-positive > rs1800469 TT homozygous genotype > rs2241715 TT homozygous genotype. The results suggested that *TGF-β1* rs1800469 TT and rs2241715 TT homozygote genotypes represent the genetic factors underlying familial clustering of liver cancer in Guangxi, and that drinking water use, alcohol consumption, and testing positive for HBV DNA are the main environmental factors contributing to familial aggregation of liver cancer in Guangxi.

Key words: Transforming growth factor-β1; Gene polymorphism; Liver cancer; Familial aggregation; Risk factors; Chinese population

INTRODUCTION

In humans, the transforming growth factor-β1 (*TGF-β1*) gene is located on chromosome 19q13, and contains seven exons and six introns. The TGF-β1 polypeptide is a member of the TGF-β superfamily of cytokines. It is a secreted protein that performs many cellular functions, including the control of cell growth, cell proliferation, cell differentiation, and apoptosis. TGF-β1 plays an important role in controlling the immune system, and shows different activities on different types of cells, or on cells at different developmental stages.

Since the expression levels of TGF-β1 are assumed to be predominantly under genetic control, predisposition to disease might be associated with distinct allelic changes at the *TGF-β1* locus (Park et al., 2006; Shah et al., 2006a,b; Prasad et al., 2007; Sripriya et al., 2007; Thys et al., 2007; Toyoda et al., 2007; Weng et al., 2007). TGF-β1 gene polymorphism is associated with nasopharyngeal carcinoma (Hu et al., 2012), gastric cancer (Li et al., 2008; Lin et al., 2010; Guo et al., 2011), lung cancer (Kang et al., 2006; Helmig et al., 2009; Yuan et al., 2009; Teixeira et al., 2011; Togashi et al., 2011), head and neck cancer (Lundberg et al., 2010), liver cancer (Kim et al., 2003; Qi et al., 2009), bladder cancer (Castillejo et al., 2009), esophageal cancer (Wei et al., 2007a), pancreatic cancer (Wei et al., 2007a), and uterine and other cancers (Stanczuk et al., 2002). To our knowledge, few studies exist that have attempted to clarify the association between *TGF-β1* single nucleotide polymorphisms (SNPs) and familial aggregation of liver cancer. The Guangxi region in China has a high incidence of liver cancer (Wu et al., 2009). The analysis of familial aggregation of liver cancer is very complicated, and includes the potential contributions of environmental factors, including water pollution, aflatoxin exposure, and hepatitis B or C virus infection. We conducted the present case-control study to investigate the association between *TGF-β1* polymorphisms (rs1800469, rs2241715, rs2241716, rs11466345, rs8105161, and rs747857) and familial aggregation of liver cancer in Guangxi, China.

MATERIAL AND METHODS

Subjects

This case-control study was carried out with the approval of the Ethics Committee of the First Affiliated Hospital of Guangxi Medical University. Subject recruitment and sample collection were performed only after obtaining the written informed consent of the participants. All patients resided in the Province of Guangxi, China, an area with a high incidence

of liver cancer. The patient cohort consisted of 214 individuals from 37 families. Patient results were compared with 214 control individuals. Volunteer subjects, predominantly family members, or recruited by word of mouth and able to provide informed, written consent for participation were recruited as controls. The patients and controls were interviewed using a standardized questionnaire, including: drinking alcohol, eating, and smoking habits, exposure to hepatitis B virus (HBV), family history of cancer, and, the relationship of the other family members with liver cancer to the proband.

Methods

Genomic DNA was extracted from peripheral blood using a Genomic DNA Extraction Kit (Invitrogen, Shanghai, China) following manufacturer instructions. Hepatitis B surface antigen (HBsAg) and anti-hepatitis C virus (HCV) antibodies were detected by ELISA kits (Shanghai Sangon Biological Engineering Technology & Services Co. Ltd., Shanghai, China) was performed according to the manufacturers' instructions. Presence of HBV DNA was screened by polymerase chain reaction (PCR). TGF-β1 rs1800469, rs2241715, rs2241716, rs11466345, rs8105161, and rs747857 genotypes were determined by PCR amplification, with conditions altered to 25 μL final volume and amplified using the 2X PFU Master Mix PCR system (Dongsheng Biotech Co., ShangHai, China). Final reaction mixtures contained 0.1 units/μL EconoTaq DNA Polymerase reaction buffer, pH 9.0, 400 μM dATP, 400 μM dGTP, 400 μM dCTP, 400 μM dTTP, 3 mM $MgCl_2$, 1 μM forward and reverse primers and a proprietary mix of PCR enhancer/stabilizer and blue and yellow tracking dyes (Dongsheng Biotech Corporation, ShangHai, China). We used 1 μL genomic DNA template for primary PCRs. The primers and probes for *TGF-β1* are described in Tables 1 and 2, respectively. Amplification was conducted using the following cycling conditions: 2 min initial denaturation at 95°C, followed by 25 cycles of 1 min at 95°C, 30 s annealing at 57°C, and 1 min at 72°C, with a 10-min final extension at 72°C. All PCR products were visualized on a 2% agarose gel containing 2 μL/100 mL ethidium bromide. The amplified products were analyzed by direct DNA sequencing using the SnapShop reaction (Invitrogen).

Table 1. Primers for *TGF-β1* single nucleotide polymorphism amplification.

Primer name	Primers (5'-3')	Length (bp)
rs1800469-UP	TTGAGTGACAGGAGGCTGCTTAG	318
rs1800469-LOW	GGTGATCCAGATGCGCTGTG	
rs2241715-UP	TGGGTCTCCTGGTTTTTGTC	210
rs2241715-LOW	TACTCAGCAAACCCCAAAGG	
rs8105161-UP	ACTGGCATGAGCCACCACAC	156
rs8105161-LOW	ATCTCTGAGGAGGTGATGTTCAGTC	
rs11466345-UP	CCATGTCCAAGGGTCAGTCT	243
rs11466345-LOW	ACAGCTGGCCTGATTTTTGT	
rs2241716-UP	GACATGAGCCAGAAGGAAGG	189
rs2241716-LOW	CAGCTTGGCAACAGAGTGAG	
rs747857-UP	GGGTGAAGGGGAGAAGAGAG	217
rs747857-LOW	GCGTCCCAAAGTGTTGGTAT	

Statistical analysis

Allele frequencies for each SNP were determined by gene counting. The genotype distribution of each SNP was analyzed for deviations from Hardy-Weinberg equilibrium using

χ^2 analyses. A χ^2 test and conditional logistic regression were performed to identify statistical associations between genotype and disease status. The Student *t*-test was used to evaluate differences among serum levels of TFG-β1. The statistical analysis was carried out using the SPSS version 17.0 statistical software package (SPSS, Inc., Chicago, IL, USA).

Table 2. Allele-specific probe sequences for *TGF-β1* single nucleotide polymorphisms.

Probe name	Allele	Probe sequence (5'-3')
rs1800469-45 bp	C/T	ttttttttttttttttttttttttttTCCTGACCCTTCCATCC
rs11466345-50 bp-FX	C/T	ttttttttttttttttttttttttttttttttttGCTGGGATTACGGG
rs747857-65 bp-FX	A/G	ttttttttttttttttttttttttttttttttttttttttttttttttTCTCTCCATTTCTGC
rs2241715-65 bp	G/T	ttttttttttttttttttttttttttttttttttttttttttttttttTTTCTCCTCCACGGTCC
rs8105161-70 bp	C/T	ttttttttttttttttttttttttttttttttttttttttttttttttttCCTTAACTCCTAAAGCAGTG
rs2241716-75 bp-FX	C/T	ttttttttttttttttttttttttttttttttttttttttttttttttttttttttGGTTTGTGTTCTTCTATC

RESULTS

A total of 418 individuals, including 214 individuals from families with a high incidence of liver cancer and 214 healthy volunteers, were recruited from Guangxi Province for this study. Patients comprised a total of 214 Guangxi Zhuang, Han, and Yao individuals, 154 men and 60 women, with a mean ± standard error age of 34.5 ± 5.4 years.

Association of *TGF-β1* gene polymorphisms with familial aggregation of liver cancer

TGF-β1 rs1800469 polymorphism

The rs1800469 genotypes were in Hardy-Weinberg equilibrium for both patient (χ^2 = 0.742, P > 0.05) and control groups (χ^2 = 0.172, P > 0.05). There were differences in the group distribution for the CC and TT genotypes [χ^2 = 6.832, P = 0.009, odds ratio (OR) = 0.497, 95% confidence interval (CI), 0.292-0.845; and χ^2 = 8.159, P = 0.004, OR = 1.763, 95%CI = 1.193-2.605], and the distribution of the T/C allele was different between the two groups (χ^2 = 11.909, P = 0.001, OR = 1.631, 95%CI = 1.234-2.154) (Table 3).

Table 3. Comparison of rs1800469 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	101	88	25	290	138
Controls	72	97	45	241	187
χ^2	8.159	0.771	6.832	11.909	
P value	0.004	0.380	0.009	0.001	
OR	1.763	-	0.497	1.631	
95%CI	1.193-2.605	-	0.292-0.845	1.234-2.154	

OR = odds ratio; CI = confidence interval.

TGF-β1 rs2241715 polymorphism

The rs2241715 genotype distribution had significant differences between high incidence family liver cancer and control groups (χ^2 = 11.905, P = 0.003), and there were signifi-

cant differences in the distribution of CC and TT genotypes between groups (χ^2 = 8.082, P = 0.004, OR = 0.497, 95%CI = 0.277-0.797; and χ^2 = 8.191, P = 0.004, OR = 1.716, 95%CI = 1.195-2.613, respectively). The distribution of the T/G allele was different between the two groups (χ^2 = 12.841, P = 0.000, OR = 1.660, 95%CI = 1.257-2.192) (Table 4). The genotype distributions of the high liver cancer incidence family and control groups were in Hardy-Weinberg equilibrium (χ^2 = 0.569, P > 0.05; χ^2 = 0.043, P > 0.05).

Table 4. Comparison of rs2241715 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	100	89	25	289	139
Controls	71	96	47	238	190
χ^2	8.191	0.467	8.082	12.841	
P value	0.004	0.000	0.004	0.000	
OR	1.716		0.497	1.660	
95%CI	1.195-2.613		0.277-0.797	1.257-2.192	

OR = odds ratio; CI = confidence interval.

TGF-β1 rs8105161 polymorphism

The rs8105161 genotypes were significantly different between high incidence liver cancer family and control groups (χ^2 = 9.361, P = 0.009), and there were differences in the distribution of CC and TT genotypes between groups (χ^2 = 4.081, P = 0.043, OR = 1.738, 95%CI = 1.012-2.984; and χ^2 = 7.952, P = 0.005, OR = 0.564, 95%CI = 0.378-0.841, respectively). The distribution of the T/C allele was significantly different between the two groups (χ^2 = 9.031, P = 0.004, OR = 1.525, 95%CI = 1.157-2.009) (Table 5). The rs8105161 genotypes were in Hardy Weinberg equilibrium for both patient and control groups (χ^2 = 0.569, P > 0.05; χ^2 = 0.043, P > 0.05).

Table 5. Comparison of rs8105161 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	63	111	40	237	191
Controls	91	98	25	280	148
χ^2	7.952	1.580	4.081	9.031	
P value	0.005	0.209	0.043	0.004	
OR	0.564		1.738	1.525	
95%CI	0.378-0.841		1.012-2.984	1.157-2.009	

OR = odds ratio; CI = confidence interval.

TGF-β1 rs747857 polymorphism

The rs747857 genotypes were significantly different between high incidence liver cancer family and control groups (χ^2 = 10.505, P = 0.005), and there were differences in the distribution of GG and AA genotypes between groups (χ^2 = 5.849, P = 0.016, OR = 2.028, 95%CI = 1.134-3.625; and χ^2 = 7.237, P = 0.027, OR = 1.025, 95%CI = 1.003-1.048, respectively). The distribution of the C/T allele was significantly different between the two groups

(χ^2 = 8.416, P = 0.004, OR = 0.451, 95%CI = 0.260-0.781) (Table 6). The genotypic distribution in high liver cancer incidence family and control groups were in Hardy-Weinberg equilibrium (χ^2 = 4.94, P > 0.05; χ^2 = 0.51, P > 0.05)

Table 6. Comparison of rs747857 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	177	32	5	386	42
Controls	194	20	0	408	20
χ^2	5.849	3.152	7.237	8.416	
P value	0.0016	0.076	0.027	0.004	
OR	2.028	0.586	1.025	0.451	
95%CI	1.134-3.625	0.324-1.062	1.003-1.048	0.260-0.781	

OR = odds ratio; CI = confidence interval.

TGF-β1 rs2241716 polymorphism

The rs2241716 genotype had no significant distribution differences between high incidence liver cancer family and control groups (χ^2 = 4.516, P = 0.105; χ^2 = 1.977, P = 0.160) (Table 7). The genotypic distributions in high liver cancer incidence family and control groups were in Hardy-Weinberg equilibrium (χ^2 = 0.465, P > 0.05; χ^2 = 2.179, P > 0.05).

Table 7. Comparison of rs2241716 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	134	75	5	343	85
Controls	126	74	14	326	102
χ^2	0.627	0.01	4.461	1.977	
P value	0.428	0.919	0.035	0.16	
OR	1.17	1.021	0.342	0.16	
95%CI	0.793-1.725	0.686-1.519	0.121-0.966	0.912-1.748	

OR = odds ratio; CI = confidence interval.

TGF-β1 rs11466345 polymorphism

The rs11466345 genotype showed no significant differences in distribution between high liver cancer incidence family and control groups (χ^2 = 3.655, P = 0.161) (Table 8). The genotypic distributions in high liver cancer incidence family and control groups were in Hardy-Weinberg equilibrium (χ^2 = 0.741, P > 0.05; χ^2 = 0.808, P > 0.05).

Table 8. Comparison of rs11466345 genotype data between patients and controls.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Patients	30	92	92	152	276
Controls	21	83	110	125	303
χ^2	1.803	0.783	3.038	3.891	
P value	0.179	0.376	0.081	0.049	
OR	1.498	1.19	0.713	1.335	
95%CI	0.828-2.712	0.809-1.751	0.487-1.044	1.001-1.779	

OR = odds ratio; CI = confidence interval.

Distribution of HBV DNA between patients and controls

A group of 39 of the 59 HBsAg (+) individuals from the patient group were positive for HBV DNA as well, accounting for 18.2% of individuals from these families. The number of HBV DNA-positive individuals was significantly different between patients and controls (χ^2 = 12.207, P = 0.000, OR = 2.975, 95%CI = 1.576-5.547) (Table 9).

Table 9. Distribution of HBV-DNA between patients and controls.

Group	HBV-DNA (+)	HBV-DNA (-)	Total
Patients	39	157	214
Controls	15	199	214
χ^2	12.207		
P value	0		
OR	2.957		
95%CI	1.576-5.547		

OR = odds ratio; CI = confidence interval.

Risk factor analysis

After testing for association, we identified that the *TGF-β1* genotypes rs1800469, rs2241715, rs2241715, and rs8105161 were found more frequently in patients than in controls. We identified risk factors by conditional logistic regression (Table 10). The risk factors suggested for familial aggregation of liver cancer in Guangxi were, from high to low: drinking sugared beverages > alcohol consumption > presence of HBV DNA > rs1800469 TT homozygous genotype > rs2241715 TT homozygous genotype.

Table 10. Conditional logistic regression model analysis.

Variable	Beta	SE	Wald	df	P value	Exp (Beta)
Alcohol consumption	1.772	0.422	17.655	1	0.000	5.881
Drinking water	2.580	1.085	5.652	1	0.017	13.192
HBV-DNA positive	0.690	0.382	3.270	1	0.071	1.994
rs1800469	0.187	0.831	0.051	1	0.822	1.206
rs2241715	0.133	0.829	0.026	1	0.872	1.142
rs8105161	-0.297	0.135	4.834	1	0.028	0.743
rs6747857	-0.699	0.368	3.609	1	0.057	0.497

SE = standard error; Wald = Wald Chi-squared test; df = degrees of freedom.

Stratified analysis

Stratified analysis of the rs1800469 polymorphism

In accordance with blood kinship to the proband, the population was divided into a hepatocellular carcinoma group (including the probands) and an unrelated liver cancer group (non-consanguineous relatives + control subjects in the same family). There were significant differences between these groups in rs1800469 genotype distribution (χ^2 = 13.333, P = 0.001). There were also statistical differences in the distribution of TT and CC genotypes between

groups (χ^2 = 9.850, P = 0.002, OR = 1.865, 95%CI = 1.261-2.756; and χ^2 = 8.301, P = 0.004, OR = 0.458, 95%CI = 0.267-0.785, respectively), and the distribution of the C/T allele was different between the two groups (χ^2 = 14.415, P = 0.000, OR = 1.716, 95%CI = 1.297-2.269) (Table 11).

Table 11. Distribution of rs1800469 genotypes between kinship and unrelated groups in liver cancer.

Subjects	Genotype			Allele	
	TT	CT	CC	T	C
Kinship group	100	85	23	285	131
Unrelated group	73	100	47	246	194

From our analysis of individuals stratified by blood kinship, the result suggested that the TT genotype and the T allele in all levels of blood relatives showed no statistical difference in their overall distribution (χ^2 = 3.927, P = 0.416; χ^2 = 6.200, P = 0.185); however, the distribution frequencies decreased with with the TGF1 beta blood levels of the proband (Table 12).

Table 12. Distribution of rs1800469 genotypes in families with high incidence liver cancer.

Subjects	Genotype			Allele	
	CC	CT	TT	C	T
Proband	1	3	6	5	15
First-degree relatives	9	42	52	60	146
Second-degree relatives	10	24	27	44	78
Third-degree relatives	6	13	15	25	43
Unrelated individuals	2	3	1	7	5

Stratified analysis of the rs2241715 polymorphism

The rs2241715 distribution was significantly different between kinship and unrelated groups of liver cancer patients (χ^2 = 15.482, P = 0.000), and there were significant differences between the distribution of TT and GG genotypes between groups (χ^2 = 9.853, P = 0.002, OR = 1.867, 95%CI = 1.262-2.762; and χ^2 = 11.280, P = 0.001, OR = 0.402, 95%CI = 0.234-0.692, respectively). The distribution of the T/G allele was different between the two groups (χ^2 = 16.493, P = 0.000, OR = 1.780, 95%CI = 1.346-2.354) (Table 13).

Table 13. Distribution of rs2241715 genotypes between kinship and unrelated groups with liver cancer.

Subjects	Genotype			Allele	
	TT	GT	GG	T	G
Kinship group	99	87	22	285	131
Unrelated group	72	98	50	242	198

From the stratified analysis of kinship groups, our results suggested that the rs2241715 TT genotype and the T allele in all levels of blood relatives showed no statistical difference in the overall distribution (χ^2 = 0.300, P = 0.348; χ^2 = 7.054, P = 0.133), but the frequency distribution decreased with the blood levels of the proband (Table 14).

Table 14. Distribution of rs2241715 genotypes in families with high incidence liver cancer.

Subjects	Genotype			Allele	
	GG	GT	TT	G	T
Proband	1	2	7	4	16
First-degree relatives	7	46	50	60	146
Second-degree relatives	9	23	29	41	81
Third-degree relatives	5	16	13	26	42
Unrelated individuals	2	3	1	7	5

Association of serum levels of TGF-β1 with familial aggregation of liver cancer

The serum levels of TGF-β1 in the high incidence liver cancer family group was significantly higher than those in control group (28.495 ± 17.495 ng/mL *vs* 20.24 ± 7.56 ng/mL, P = 0.000). Levels of TGF-β1 in the liver cancer kinship group were significantly higher than those in liver cancer unrelated group (39.445 ± 7.445 ng/mL *vs* 21.4 ± 1.6 ng/mL, P = 0.000) (Table 15).

Table 15. TGF-β1 serum levels among different groups.

Subjects	Level
High incidence family	28.495 ± 17.495
Non-cancer control	20.240 ± 7.560
Unrelated group	21.400 ± 1.600
Kinship group	39.445 ± 7.445

According to our analysis of serum levels of TGF-β1, those corresponding to the rs2241715 TT genotype were significantly higher than those of the GT (30.330 ± 15.660 ng/mL *vs* 23.550 ± 11.450 ng/mL, P = 0.000) and the GG genotypes (30.330 ± 15.660 ng/mL *vs* 20.000 ± 10.000 ng/mL, P = 0.000) (Table 16). Comparison of the rs2241715 TT genotype distribution between the non-cancer control and high-risk liver cancer groups demonstrated that the distribution in the latter was significantly higher than that in the former (18.815 ± 4.145 ng/mL *vs* 38.995 ± 6.995 ng/mL, P = 0.000) (Table 17).

Table 16. Comparison of TGF-β1 serum levels between rs2241715 and rs1800469 genotypes.

Genotype	N	Level
rs2241715		
TT	171	30.330 ± 15.660
GT	185	23.550 ± 11.450
GG	72	20.000 ± 10.000
rs1800469		
TT	173	39.445 ± 7.445
CT	185	26.330 ± 13.650
CC	70	26.245 ± 13.555

The TGF-β1 plasma levels corresponding to rs1800469 TT genotype were significantly higher than those of the CT genotype (39.445 ± 7.445 ng/mL *vs* 26.330 ± 13.650 ng/mL, P = 0.000) and CC genotype (39.445 ± 7.445 ng/mL *vs* 26.245 ± 13.555 ng/mL, P = 0.000).

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However, there were no differences between the CT and CC genotypes in serum TGF-β1 levels (26.330 ± 13.650 ng/ml *vs* 26.245 ± 13.555, P = 0.347) (Table 16). Comparison between non-cancer control and high-risk liver cancer group for the rs1800469 TT genotype demonstrated that the latter was significantly higher than the former (43.395 ± 3.495 ng/mL *vs* 35.950 ± 3.950 ng/mL, P = 0.000) (Table 17).

Table 17. Comparison of TGF-β1 serum levels between patients and controls with rs2241715 TT and rs1800469 TT genotypes.

Genotype	N	Level
rs2241715 TT		
Controls	71	18.815 ± 4.145
Patients	100	38.995 ± 6.995
rs1800469 TT		
Controls	72	35.950 ± 3.950
Patients	101	43.395 ± 3.495

DISCUSSION

TGF-β1 is a member of the TGF-β superfamily, and is the regulating factor of the polymorphic cell growth related to the growth of many tumors, and is also involved in the body's immune surveillance mechanisms (Tucker et al., 1984). During immune imbalance, TGF-β1 levels are increased, which destroys the function of immune surveillance and causes the body to lose its normal immune response to foreign pathogens or tumor cells, leading to the occurrence of disease or tumors (Li et al., 2006). To date, there has been no correlative research between *TGF-β1* gene polymorphisms and familial aggregation of liver cancer.

Relationship between *TGF-β1* gene polymorphisms and familial aggregation of liver cancer

Our preliminary study showed that there was no relationship between the *TGF-β1* rs1800469 locus and familial aggregation of liver cancer (Tan et al., 2012). The rs1800469TT genotype was shown to be one of the risk factors of high incidence liver cancer family members, conferring 1.763 fold that of the control group. According to the kinship study, carrying the rs1800469TT genotype confers a risk factor of 1.865 fold to individuals who are blood relatives of a liver cancer patient in a high incidence liver cancer family, as compared with someone unrelated. In accordance with the kinship distance stratification analysis, the rs1800469TT genotype distribution in probands is quite high, but decreases with the decrease of shared genetic relationship. This analysis further indicated that the rs1800469TT polymorphism is related to the occurrence of familial aggregation of hepatocellular carcinoma in Guangxi. The results of this study differ from those of previous studies, in that: 1) the population size was increased compared to the previous study; and 2) previous studies did not consider environmental factors, such as smoking, drinking, and water drinking habits, along with HBV DNA status. The mechanisms underlying liver cancer are complex and involve many factors. In this study, based on single and multivariate analyses, we obtained statistically significant results. However, we also determined that the risk factors of familial aggregation appeared comprehensive in liver cancer; this question needs further study and analysis.

Chow et al. (2005) and Healy et al. (2009) presented analyses of the *TGF-β1* promoter, wherein the rs1800469 promoter was found to interfere with the identification of functional transcription factors, which influenced the gene expression levels of *TGF-β1*. The rs1800469 locus containing a cytosine base promoted selective binding of AP1, which lowered the binding potential of other transcriptional activating factors, which led to lower plasma TGF-β1 levels (Shah et al., 2006a). The plasma levels of TGF-β1 associated with the CC genotype of rs1800469 were significantly lower than those of the TT genotype (Guo et al., 2011), and the individual plasma TGF-β1 levels of the *TGF-β1* rs1800469TT genotype was 2 times that of non-TT individuals (Grainger et al., 1999). The results of this study showed that the plasma TGF-β1 levels associated with the rs1800469TT genotype were significantly higher than those of the CT and CC genotypes (P = 0.000, P = 0.000), and the rs1800469 polymorphism is the genetic factor that affects the expression of TGF-beta1. However, analysis of the different groups showed that the rs1800469TT genotype conferred higher overall serum TGF-β1 expression levels to the high liver cancer incidence family group than the overall cancer-free controls (P = 0.000, P = 0.000). Therefore, the rs1800469 gene polymorphism appears not to be the only genetic factor affecting plasma TGF-β1 expression; it may interact with other related components of familial liver cancer aggregation, or play a role with other susceptibility genes segregating in familial liver cancer.

TGF-β1 inflammation-related cytokine production, and *TGF-β1* gene expression or dysfunction are associated with liver fibrosis and hepatocellular carcinoma, as is chronic HBV infection (Gewaltig et al., 2002; Kim et al., 2003; Wang, 2003). Gong et al. (2008) reported on the relationship between *TGF-β1* gene rs2241715 polymorphism and hepatocellular carcinoma, wherein they found that the rs2241715TT genotype frequencies in patients with liver cirrhosis and hepatocellular carcinoma were higher than those of a control group; however, to date there is no related report on rs2241715 gene polymorphism and familial clustering of hepatocellular carcinoma. This study found that the distributions of the rs2241715TT genotype and the T allele were significantly higher in high liver cancer incidence families than in control families without cancer (χ^2 = 8.191, P = 0.004; χ^2 = 12.841, P = 0.000). Examination of the association between the gene polymorphism and drinking, smoking, and drinking water habits, HBsAg levels, and presence of HBV-DNA by logistic regression analysis found that when rs2241715TT was entered into the model, the corresponding regression coefficient was positive. This finding suggested that the *TGF-β1* rs2241715 gene polymorphism is a risk factor for familial aggregation of liver cancer and that carrying the rs2241715TT genotype is one of the cancer predisposing risk factors for high liver cancer incidence family members, with a risk 1.716 times higher than that of the control group. Subsequent analysis based on the level of kinship suggested that carrying the rs2241715TT genotype represents the genetic risk factor predisposing blood relatives of a liver cancer patient in high incidence families to liver cancer, with the risk factor of liver cancer being 1.867 times that of an unrelated person. These results serve to further illustrate that the rs2241715 polymorphism is related to familial clustering of liver cancer in Guangxi.

The incidences reported of liver cancer are closely related to degree of blood relationship (Chen et al., 1998), with the highest incidence among first-degree relatives, followed by second-degree relatives, third-degree relatives, and the general population. Stratified analysis by kinship distance with the proband demonstrated that the rs2241715TT genotype and the T allele have a very high frequency distribution in the probands, which decreases in first-, second-, and third-degree relatives. Therefore, the rs2241715TT genotype and the T allele may

be one of the genetic factors underlying the occurrence of familial clustering of hepatocellular carcinoma occurrence in Guangxi, wherein the distribution and effect of this polymorphism decreases with decreasing relative level.

The quantity of serum TGF-β1 expression in individuals with the rs2241715TT genotype was higher than those with genotypes TG or GG (P = 0.000, P = 0.000). This suggested that rs2241715 gene polymorphism is related to serum TGF-β1 expression level, and is also one of the determining genetic factors of TGF-β1 expression levels. However, the grouping analysis showed that in the high liver cancer incidence family group, the genotype rs2241715TT plasma TGF-β1 expression levels were higher overall than in the control cancer free family group (P = 0.000). Therefore, the rs2241715 gene polymorphism is not the only genetic factor affecting plasma TGF-β1 expression; it may interact with other related factors contributing to liver cancer familial aggregation, or play a role in conjunction with other susceptibility genes involved in familial clustering of liver cancer.

The rs2241716 and rs2241715 polymorphisms demonstrate complete linkage, but there was no difference in distribution of the rs2241716 genotype and allele between high liver cancer incidence family and non-cancer control groups ($\chi^2 = 4.516$, P = 0.105; $\chi^2 = 1.977$, P = 0.160). There was no obvious correlation between the rs2241716 polymorphism and familial clustering of liver cancer, rs2241715 played a more important role in familial clustering of HCC occurrence. There have been no reports of rs11466345 association with liver cancer and this study found that there was no obvious correlation between the rs11466345 gene polymorphism and familial clustering of liver cancer. There have also been no reports of rs8105161 or rs747857 association with liver cancer and this study found that these two loci had no obvious correlation with familial clustering of liver cancer in Guangxi.

Relationship between HBV DNA and family aggregation of liver cancer

HBV infection is one of the risk factors for liver cancer, but its mechanism is not well understood. Studies (Sheng et al., 2009) have shown that familial clustering of liver cancer is related to positive HBsAg status. In this study, HBV-DNA positive status was found to be a risk factor of liver cancer familial aggregation (B = 0.690, Wald value 0.382). Because the HBV DNA status of patients with HBSAg is generally positive while HBV-negative patients are rarely HBSAg positive, it is more exact to state that being HBV-DNA (+) is a risk factor for liver cancer familial aggregation.

This is the first study to analyze blood TGF-β1 expression level by family relationship or kinship. The overall TGF-β1 levels in the high incidence liver cancer family group were higher than those in the control non-cancer family group (P = 0.000), and blood TGF-β1 levels were higher in the liver cancer-related group than in the liver cancer unrelated group (P = 0.000). Therefore, the overall levels of TGF-β1 and rs1800469TT, rs2241715TT genotype frequency distributions are in agreement. These results further suggest that the rs1800469TT and rs2241715TT genotypes are risk factors for liver cancer familial aggregation in Guangxi.

This study also took into consideration environmental factors such as gender, age, nationality, and HBSAg status. The relationship among *TGF-β1* gene polymorphisms, presence of HBV-DNA, and liver cancer family aggregation was considered by single analysis, and the comprehensive lifestyle factors of smoking, alcohol consumption, and drinking water habits were considered by multiple factors analysis. Our results showed that the rs1800469TT and rs2241715TT genotypes are genetic risk factors of liver cancer familial aggregation in

Guangxi, while alcohol consumption, quality of drinking water, and HBV DNA-positive status are environmental factors that contribute to the risk of family aggregation liver cancer in Guangxi. The largest effect on family aggregation of cancer in Guangxi is alcohol consumption, followed by the quality of drinking water. We are currently investigating what kinds of carcinogens are present in sugared beverages related to the high incidence of liver cancer. In summary, both environmental and genetic factors may impact the familial aggregation of liver cancer in Guangxi Zhuang, Han, and Yao populations.

Conflicts of interest

The authors declare no conflict of interest.

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