CTNND₁ 755 T>G Promoter Polymorphism and Risk of Pancreatic Carcinoma in Chinese

Wei Wang,^{1a} Yang Fei,^{1a*} and Sheng-li Liu²

¹Department of General Surgery, Baiyi Hospital Affiliated Nanjing University of Chinese Medicine/The 81st Hospital of P.L.A., Nanjing, China

²Department of General Surgery, ZhongDa Hospital, The Affiliated Hospital of Southeast University, Nanjing, China

Objective: To investigate the relationship between 755 T>G polymorphisms in the CTNND₁ gene, which is associated with the risk of pancreatic carcinoma in Chinese. **Methods:** CTNND₁ 755 T>G genotypes were determined by PCR-RFLP in 122 pancreatic carcinoma patients and 180 healthy controls matched for age and sex, who did not receive radiotherapy or chemotherapy for newly diagnosed and histopathologically confirmed pancreatic carcinoma. **Results:** In control subjects, the frequency of T/T and G/T genotypes, and T and G alleles was 79.4%, 17.2%, 88.1%, and 11.9%, respectively. The distribution of genotypes and allelotypes in the pancreatic carcinoma patients was significantly different from that in the controls

($P = 0.007$, $P = 0.012$). Combined GG and GT genotypes were found to have a higher OR in male pancreatic carcinoma patients and the group under the age of 70 years (males: OR, 1.409; 95%CI, 0.912~1.921; under 70 years: OR 1.626; 95% CI, 0.878~2.312). This study also showed a distinct difference in the distribution of P120ctn and single nucleotide polymorphisms (SNPs) between Chinese and Canadian (11.9% vs. 3.9%, $P = 0.008$). **Conclusion:** CTNND₁ 755 T>G polymorphism may be a stratification marker to predict the susceptibility to pancreatic carcinoma, at least in Chinese. CTNND₁ promoter SNPs is diverse in ethnic populations. J. Clin. Lab. Anal. 31:e22055, 2017. © 2016 Wiley Periodicals, Inc.

Key words: CTNND₁; gene polymorphism; pancreatic carcinoma

INTRODUCTION

Pancreatic carcinoma is a relatively rare tumor, and the lifetime risk of dying from pancreatic carcinoma is between 1% and 2% of the general population. In China, the incidence of pancreatic cancer has been rising during the past years, currently varying from 0.47/100,000 in the Hunan Province to 7.21/100,000 in Shanghai, representing the sixth leading cause of death from malignant disease (1). Because symptoms typically remain absent until late in the course of disease, most cancers are detected at an advanced stage when prognosis is poor (2). Therefore, it is important to investigate the genetic and epigenetic variation in susceptibility to pancreatic carcinogenesis and identify the markers that will facilitate identification of individuals at risk of pancreatic carcinogenesis.

DNA methylation is a major epigenetic modification involving the addition of a methyl group to the 5' position of a cytosine in a CpG dinucleotide.

A number of studies suggested that aberrant DNA cytosine methylation may play an important role in carcinogenesis (3, 4). CTNND₁ is required for the establishment and maintenance of genomic methylation patterns and proper murine development (5, 6). The gene is down-regulated to different degrees in some malignancies, including colon carcinoma, prostate carcinoma, and pancreatic carcinoma (7–9). Recently, several candidate single nucleotide polymorphisms (SNPs), in the CTNND₁ gene have been deposited in public databases. Although the functional effects of these polymorphisms have not been

^aCo-first author.

*Correspondence to: Department of General Surgery, Baiyi Hospital Affiliated Nanjing University of Chinese Medicine/The 81st Hospital of P.L.A., Nanjing 21002, China. E-mail: fei_yanggood@163.com

Received 14 June 2016; Accepted 6 July 2016

DOI 10.1002/jcla.22055

Published online in Wiley Online Library (wileyonlinelibrary.com).

elucidated, some studies showed that some of these variants may influence the CTNND₁ activity on DNA methylation, thereby modulating the susceptibility to breast cancer, liver cancer, and prostate carcinoma (10). The CTNND₁ gene contains a single T>G SNP in the transcription start site of the promoter region (-755 bp from exon 3), and this probably affects gene function. Some studies suggested that CTNND₁ -755 T>G may modify susceptibility to tumors (11). Although conflicting results have been reported in different tumor types, the heterozygous genotypes have a significantly reduced risk of colon cancer and breast cancer (7, 11). However, no report on the allele of CTNND₁ is available. This study was to investigate the polymorphism of CTNND₁ in pancreatic carcinoma.

MATERIALS AND METHODS

Study Population

This case-control study included 122 pancreatic carcinoma patients and 180 healthy controls. Pancreatic carcinoma was histopathologically confirmed in the 122 patients during surgery at the department of general surgery of our hospitals. The control subjects were selected from cancer-free subjects who visited the same hospital for a regular physical examination and volunteered to participate in the epidemiology survey during the same period. We defined a healthy subject as a person free of disease (including no history of cancer) at health check-up. The controls were matched for age and sex with the patients (Table 1). All patients and controls were ethnically Chinese and resided in Jiangsu Province or in its surrounding regions. The study protocol conformed to the guidelines by the institutional ethical committee. All patients gave their informed consent to be included in the study. The research was carried out according to the principles of The Declaration of Helsinki.

TABLE 1. Distribution of Selected Variables in Pancreatic Carcinoma Patients and Control Subjects *n* (%)

Variables	Case patients (<i>n</i> = 122)	Control subjects (<i>n</i> = 180)	<i>P</i> -value
Age (years)			
<70	53 (43.4)	71 (39.4)	0.096
>70	69 (56.6)	109 (60.6)	
Sex			
Male	79 (64.8)	102 (56.7)	0.137
Female	43 (35.2)	78 (43.3)	

DNA Extract

Five milliliters of venous blood was drawn from each subject into vacuum tubes containing EDTA and stored at 4°C. Genomic DNA was extracted within 1 week after sampling by proteinase K digestion and salted out as previously described (12).

CTNND₁ Genotyping

Transition from G to T of the CTNND₁ SNP creates a *Mva*I restriction site which can be exploited for genotyping by PCR and subsequent restriction fragment length polymorphism (RFLP) analysis. PCR was performed in a volume of 25 containing 100 ng of DNA template, 10 × PCR master mix (Promega, Madison, WI), and 10 pmol/L each of sense primer (5'-GAGGTCTCGCATGCCTAGG-3') and antisense primer (5'-GGGA GCTCACCTTCTAGAAA-3'). For PCR amplification, an initial denaturation at 94°C for 5 min was followed by 30 cycles at 94°C for 30 sec, at 57°C for 30 sec, at 72°C for 30 sec, and a final extension at 72°C for 7 min. The PCR products were digested overnight with five units of *Mva*I (New England Biolabs, Beverly, MA) at 37°C and separated on 2% agarose gels. RFLP bands were visualized under UV light with ethidium bromide staining. The CTNND₁ T/T genotype was expected to show two DNA bands at the positions of 132 bp and 93 bp, whereas the G/G genotype was expected to show a single band (225 bp), and the heterozygote was expected to have three bands (225 bp, 132 bp, 93 bp). For quality control, genotyping analysis was performed blindly with respect to case-control status and repeated twice for all subjects.

DNA Sequencing Analysis

To confirm the genotyping results, selected PCR-amplified DNA samples were examined by DNA sequencing. The PCR fragments were recovered from agarose gel followed by purification with a DNA clean-up kit (Wizard SV Gel and PCR Clean-up System, Promega). DNA sequences of the PCR products were determined using the PCR sense primer with an Applied Biosystems model 377 sequencer (PE Applied Biosystems, Warrington, UK). The results of genotyping were 100% concordant.

Statistical Analysis

Patients and controls were compared using Student's *t*-test for continuous variables and chi square test for categorical variables. Hardy-Weinberg equilibrium

Fig. 1. PCR-RFLP analysis of -755 T>G CTNND₁ polymorphism. lane M: DNA ladder; lane 1: TT genotype; lane 2: TG genotype; lane 3: GG genotype.

was tested with a goodness-of-fit chi square test with one degree of freedom to compare the observed genotype frequencies with the expected genotype frequencies among the subjects. Comparison of the CTNND₁ genotype and allelotype distribution in the study groups was performed by means of contingency tables using chi square test or Fisher's exact test. The odds ratio (OR) and 95% confidence interval (CI) were calculated using an unconditional logistic regression model and adjusted for age and gender accordingly. $P < 0.05$ was considered statistically significant.

RESULTS

The demographics of the cases and controls enrolled in this study are shown in Table 1. There were no significant differences in the mean age and sex distribution between cases and controls, suggesting that the matching based on these two variables was adequate. There was no evidence of a deviation from Hardy-Weinberg equilibrium among the cases or controls. The mean age of the patients and controls was 69.6 years (± 7.3 years; range, 52–84 years) and 63.2 years (± 8.1 years; range, 55–85 years), respectively. All the patients and controls were successfully genotyped for the CTNND₁ polymorphism (Fig. 1). The genotyping by PCR-RFLP analysis was

completely confirmed by DNA sequencing analysis, and the results of PCR-RFLP genotyping and sequencing analysis were also 100% concordant (Fig. 2). The distribution of CTNND₁ 755 T>G polymorphism was in Hardy-Weinberg equilibrium. The frequency of G allele in control subjects (11.9%) was different from that in the previous study among Canadian (3.9%). The distributions of CTNND₁ 755 T>G genotypes in Chinese and Canadian are shown in Table 2.

The distributions of CTNND₁ 755 T>G genotypes in controls and patients are shown in Table 3. The genotype distributions of the polymorphisms in the controls were in Hardy-Weinberg equilibrium. Significant deviation was observed in the genotype distributions of both polymorphisms between overall pancreatic carcinoma patients and controls. The TT genotype was found to be the most frequent genotype in both cases (58.2%) and controls (79.4%). Frequencies of the GG genotype and the G allele were significantly higher among patients than controls ($P = 0.007$, $P = 0.012$). Then, we stratified the results by sex and age. Patients and controls were found to be different with respect to the genotype distribution (Table 4). Combined GG and GT genotypes were found to have a higher OR in male pancreatic carcinoma patients and the group under the age of 70 years (males: OR, 1.409; 95% CI, 0.912–1.921; under 70 years: OR 1.626; 95% CI, 0.878–2.312). Moreover, combined GG and GT genotypes showed significant association between CTNND₁ 755 T>G polymorphism and the risk of pancreatic carcinoma.

DISCUSSION

Single nucleotide polymorphisms (SNP) are the most common form of genetic variants of the human genome some of which might have functional effects on the

Fig. 2. Sequencing results for each of the PCR products from different genotypes. The SNP sites are indicated by the arrowhead. The results were completely matched to the corresponding results derived from PCR-RFLP genotyping.

TABLE 2. CTNND₁ Genotype and Allele Frequency in Chinese and Canadian

	TT	GT	GG	G allele frequency (%)
Chinese	143 (79.4)	31 (17.2)	6 (3.4)	11.9*
Canadian	152 (92.1)	11 (6.7)	2 (1.2)	3.9*

*A significant ($P < 0.05$) test value.

TABLE 3. CTNND₁ Genotype and Allele Frequency in Patients and Control Subjects and Their Association with Pancreatic Carcinoma n (%)

	Case patients ($n = 122$)	Control subjects ($n = 180$)	OR(95% CI)
CTNND ₁ 755T>G			
TT	71 (58.2)	143 (79.4)	
GT	28 (23.0)	31 (17.2)	1.572 (1.166–2.491)*
GG	23 (18.8)	6 (3.3)	1.855 (1.225–2.233)*
P	0.007		
Relative allele frequencies			
T allele (%)	170 (69.7)	317 (88.1)	1.696
G allele (%)	74 (30.3)	43 (11.9)	(1.038–2.275)*
P	0.012		

P , the significance level of comparison between cases and controls (χ^2 test); OR, odds ratio; 95% CI, 95% confidence interval; *, a significant ($P < 0.05$) test value.

TABLE 4. Stratification Analysis of CTNND₁ 755T>G Genotype Frequencies in Pancreatic Carcinoma Patients and Controls, Adjusted OR (95% CI)

Variable	TT genotype Case-control	GT+GG genotype Case-control	OR and 95% CI of GT+GG genotype
Age			
<70	39/56	14/15	1.626 (0.878–2.312)*
>70	32/87	37/22	1.298 (0.619–1.566)*
Sex			
Male	43/76	36/26	1.409 (0.912–1.921)*
Female	28/67	15/11	1.138 (0.761–1.883)*

OR, odds ratio; 95% CI, 95% confidence interval; *, a significant ($P < 0.05$) test value.

susceptibility to the development of human cancers by modifying the transcriptional activation. Studies suggested that some variants in the promoter region of genes may affect either the expression or activity levels of enzymes and therefore may be mechanistically associated with cancer risk (4, 13, 14). It has been recently shown that SNP of the CTNND₁ promoter 755 T>G (from exon 3 transcription start site) decreases the susceptibility of an individual to colon cancer and breast cancer (7, 11), suggesting that CTNND₁ promoter 755 G>T polymorphism can be used as a risk factor for

cancer to evaluate the population susceptible to tumors. However, it was also reported that there is no association between polymorphism of 755 T>G and prostate carcinoma. However, to the best of our knowledge, the relative significance of SNP in the genetic susceptibility to pancreatic carcinoma has not yet been disclosed. In this study, we investigated the influence of CTNND₁ polymorphisms on the risk of pancreatic carcinoma in a hospital-based case-control study.

This is the first study of CTNND₁ polymorphism in pancreatic carcinoma. We investigated the influence of 755 T>G polymorphism in the CTNND₁ gene on the risk of pancreatic carcinoma. Individuals carrying G allele in the CTNND₁ gene were found to have a nearly consistent risk of pancreatic carcinoma compared with those carrying T allele. Then, we stratified the results by sex and age, patients and controls.

Combined GG and GT genotypes showed significant association between CTNND₁ 755 T>G polymorphism and risk of esophagus cancer, suggesting that 755 T>G polymorphism in the CTNND₁ gene can be used as a marker of genetic susceptibility to pancreatic cancer. Our study showed that CTNND₁ polymorphism was associated with the risk of pancreatic carcinoma, and other studies reported a increased risk of colon and breast cancer in those harboring G allele. Since the different variants of CTNND₁ may alter catalytic activity and are expressed in a tissue-specific manner and the repression of CTNND₁ activity does not result in re-expression of all hypermethylated tumor suppressor genes in some cell systems, it is therefore important to explore the complex interplay of CTNND₁ in different tumor types.

In this study, a distinct difference was found in the distribution of CTNND₁ SNP between Chinese and Canadian. Additionally, the frequency of GT and GG genotype in Chinese was higher than that in Canadian. The great diversity in CTNND₁ SNP distribution in different ethnic populations remains unknown.

In conclusion, the CTNND₁ gene may be involved in the development of pancreatic carcinoma. Further studies with a larger sample are required to confirm our findings, to understand the role of CTNND₁ polymorphisms in determining the risk of pancreatic carcinoma, and to clarify the association of CTNND₁ polymorphism with pancreatic carcinoma in different ethnic populations.

REFERENCES

- Guo X, Cui Z. Current diagnosis and treatment of pancreatic cancer in China. *Pancreas* 2005;31:13–22.
- Nakayama KI. Ubiquitin ligases: Cell-cycle control and cancer. *Nat Rev Cancer* 2006;6:369–381.

3. Beaulieu N, Morin S, Chute IC. An essential role for DNA methyltransferase DNMT3B in cancer cell survival. *Biol Chem* 2002;277:28176–28181.
4. Momparler RL, Bovenzi V. DNA methylation and cancer. *J Cell Physiol* 2011;183:145–154.
5. Stefano A, Anna MS. p120 catenin and phosphorylation: Mechanisms and traits of an unresolved issue. *Biochim Biophys Acta* 2007;1773:47–58.
6. Reynolds AB, Roesel DJ, Kanner SB, et al. Transformation specific tyrosine phosphorylation of a novel cellular protein in chicken cells expression oncogenic variants of the avian cellular src gene. *Mol Cell Biol* 1989;9:629–638.
7. Bellocq DI, Bates RC, Muzikansky A, et al. Altered localization of p120 catenin during epithelial to mesenchymal transition of colon carcinoma is prognostic for aggressive disease. *Cancer Res* 2005;65:10938–10945.
8. Vanli G, Peltzer N, Dubuis G, et al. The activity of the anti-apoptotic fragment generated by the caspase-3/p120 RasGAP stress-sensing module displays strict Akt isoform specificity. *Cell Signal* 2014;26:2992–2997.
9. Fei Y, Cheng ZJ, Liu SL, et al. Expression and clinical significance of P120 catenin mRNA and protein in pancreatic carcinoma. *Bosn J Basic Med Sci* 2009;9:192–197.
10. Marín N, Zamorano P, Carrasco R, et al. S-Nitrosation of β -catenin and p120 catenin: A novel regulatory mechanism in endothelial hyperpermeability. *Circ Res* 2012;111:553–563.
11. Mastracci TL, Tjan S, Bane AL. E-cadherin alterations in atypical lobular hyperplasia and lobular carcinoma in situ of the breast. *Mod Pathol* 2005;18:741–751.
12. Varanda EA, Varella SD, Rampazo RA, et al. Mutagenic and cytotoxic effect of planifolin: A naphthopyranone dimer isolated from *Paepalanthus planifolius*. *Toxicol In Vitro* 2006;20:664–668.
13. Shen H, Wang L, Spitz MR, et al. A novel polymorphism in human cytosine DNA-methyltransferase-3B promoter is associated with an increased risk of lung cancer. *Cancer Res* 2002;62:4992–4995.
14. Lubeck BA, Lapinski PE, Oliver JA, et al. Cutting edge: Codeletion of the Ras GTPase-activating proteins (RasGAPs) neurofibromin 1 and p120 RasGAP in T cells results in the development of T cell acute lymphoblastic leukemia. *J Immunol* 2015;195:31–35.