Int J Clin Exp Med 2015;8(1):1247-1252
www.ijcem.com /ISSN:1940-5901/IJCEM0003231

*Original Article*

# rs17501976 polymorphism of CLDN1 gene is associated with decreased risk of colorectal cancer in a Chinese population

Jian-Jun Chen, Ming Zhong, Tong-Hai Dou, Zhi-Yong Wu, Wei-Jun Tang

*Department of General Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, Shanghai 200127, P.R. China*

Received October 18, 2014; Accepted January 7, 2015; Epub January 15, 2015; Published January 30, 2015

**Abstract:** The purpose of this study was to determine the relationship between polymorphisms in Claudin-1 (CLDN1) and the risk of colorectal cancer in a Chinese population. In this study, a case-control study was conducted in which polymorphisms in CLDN1 were analyzed in 50 patients with colorectal cancer (CRC) and 50 healthy individuals as controls. No rs16865344 and rs17429833 polymorphism were found among all analyzed samples. For the rs17501976 polymorphism, the TC genotype (OR = 0. 41, 95% CI = 0.18-0.91, and *P* = 0.045) was closely associated with the risk of colorectal cancer compared with the more common TT genotype. And the TC + CC genotypes (OR = 0.41, 95% CI = 0.18-0.91, and P = 0.045) were also significantly associated with the risk of CRC compared with the TT genotype. However, a C > T change of the rs17501976 polymorphism did not show a difference in transcription factor binding to the promoter region of CLDN1. For rs12696600 polymorphism, no significant difference was found in colorectal cancer risk between cases and controls in corresponding genotypes. Collectively, our data suggest that rs17501976 polymorphism significantly associated with a decreased susceptibility to CRC in a Chinese population.

**Keywords:** Colorectal cancer, Claudin 1, rs17501976, single nucleotide polymorphism

## Introduction

Colorectal cancer is one of the most frequent types of cancer leading to death in the developed countries. Many studies have indicated that the risk of CRC is increasing in Asian populations [1-3]. Genetic factors are involved in the development of CRC, such as single nucleotide polymorphism (SNP), one of the most common genetic polymorphisms that may lead to the individual's susceptibility to cancer [4]. So it is of great significance to find relevant genes and polymorphic loci of the disease for better diagnosis and treatment. CRC has been found associated with a variety of genetic polymorphisms, such as MLH1 gene [5], CDH1 (-160C > A) [6] and the promoter region of IL-18 [7], whose polymorphisms are closely related to susceptibility of CRC.

The claudin family of proteins, which consist of 24 closely related transmembrane proteins that are differentially expressed in various types of tissue, represents the principal tight junction proteins in the membrane structure [8, 9]. CLDN1 in particular has been shown to be crucial for the function of tight junctions and has been found abnormally expressed in a variety of tumors. At present, the CLDN1 expression was shown to be decreased in breast and prostate cancers and overexpressed in gastric, thyroid, pancreatic and cervical cancers [10]. In colorectal cancer, overexpression of CLDN1 in human CRC specimens was also observed [11-14]. However, the majority of studies were focused on annotation of CLDN1 functions in CRC, while researches in CLDN1 gene polymorphism of CRC have not yet reported.

For this reason, we have investigated whether the selected polymorphisms (rs17501976 and rs12696600) of CLDN1 gene contribute to CRC susceptibility by evaluating the association between each SNP and colorectal cancer risk in this case-control study.

**Table 1.** Characteristics of the study population

| Variable | Cases (n = 50) | Controls (n = 50) |
|---|---|---|
| Sex (%) | | |
| Male | 25 (50.0%) | 25 (50.0%) |
| Female | 25 (50.0%) | 25 (50.0%) |
| Age (years ± SD) | 68.3 ± 10.40 | 60.9 ± 9.34 |
| Family history of cancer | | |
| Yes | 0 | 0 |
| No | 50 | 50 |
| Histology (%) | | |
| Tubular | 36 (72.0%) | - |
| Mucinous | 9 (18.0%) | - |
| Poorly differentiated or undifferentiated | 5 (10.0%) | - |

**Table 2.** Characteristics of selected polymorphisms

| SNPs | Genome region | Polymorphism | SNP location |
|---|---|---|---|
| rs17501976 | 5'-upstream | C/T | -402 |
| rs16865344 | 5'-upstream | C/G | -389 |
| rs12696600 | 5'-upstream | A/C | -212 |
| rs17429833 | 5'-upstream | C/G | -13 |

## Materials and methods

### *Study population*

A case-control study with 100 Chinese individuals, including 50 consecutive patients with colorectal cancer who underwent either elective or urgent surgery at Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University between May 2008 and August 2008, as well as 50 healthy individuals with no family history of malignant disease. The characteristics of the study population were shown in **Table 1**. A 5 ml sample of venous blood was collected from each subject for DNA extraction. Informed consent was obtained from all the subjects in this study.

### *SNP selection*

DNA was extracted from whole blood and tissue samples by the standard methods of the proteinase K digestion and phenol-chloroform extraction. DNA purity and concentrations were determined by spectrophotometric and electrophoresis measurement. CLDN1 gene polymorphisms were selected based on following principles: SNP located in the transcriptional regulatory regions or putative functional domains; SNP which identified functional effects or associations with disease in previously published literature; Minor allele frequency (MAF) of ≥ 5% or unknown in the HapMap and dbSNPs database. Ultimately, rs17501976, rs16865344, rs12696600 and rs17429833 were selected (**Table 2**). However, rs16865344 and rs17429833 polymorphisms were found in the Chinese population (Data not shown).

### *SNP genotyping*

Genotyping of polymorphisms of CLDN1 gene were performed by restriction fragment length polymorphisms analysis. To amplify DNA segment, the specific primer sequences were 5'-TGTCCTCACCTGCGACGAACAAC-3' (forward) and 5'-GAAGGCGAGAATGAAGCCCAACA-3' (reverse) that generated the 1.3-kb fragment. The amplification conditions consisted of an initial denaturation at 95°C for 5 min, followed by 30 cycles of 30 s at 94°C; 30 s at 58°C; 90 s at 72°C; and a final extension at 72°C for 6 min. After PCR, the product was run on in to 1% agarose gel in order to ensure a successful reproduction. Then, all genotypes of the polymorphisms were detected by direct sequencing.

### *Statistical analysis*

Genotype frequencies were determined by direct counting and Hardy-Weinberg equilibrium (HWE) was evaluated using a goodness-of-fit $\chi^2$ test by using the online software Finitti (http://ihg.gsf.de/cgi-bin/hw/hwal.pl) (**Table 3**). Unconditional logistic regression analysis was used to estimate the odds ratio (OR) and 95% confidence interval (CI) between polymorphisms and colorectal cancer. $P < 0.05$ was considered statistically significant. Reported $P$ values were two-sided. The data were analyzed using of SPSS 16.0 software.

## Results

### *Characteristics of study subjects*

This case-control study enrolled 50 CRC patients and 50 cancer-free controls. The demographic information on study subjects are shown in **Table 1**. The cases had an average

**Table 3.** MAF of selected polymorphisms in CRC cases and controls

| SNPs | Cases MAF | Controls MAF | MAF in Chinese population | HWE *P* value |
|---|---|---|---|---|
| rs17501976 | 0.230 | 0.340 | 0.354 | 0.354 |
| rs16865344 | - | - | - | - |
| rs12696600 | 0.180 | 0.220 | 0.151 | 1.000 |
| rs17429833 | - | - | - | - |

**Table 4.** The genotype and allele frequencies of rs17501976 among cases and controls

| Genotypes | Cases | Controls | *P* value | Crude OR (95% CI) |
|---|---|---|---|---|
| | N = 50 (%) | N = 50 (%) | | |
| T Allele | 77 (77) | 66 (66) | 1 (ref) | ... |
| C Allele | 23 (23) | 34 (34) | 0.117 | 0.58 (0.31-1.08) |
| TT | 31 (62) | 20 (40) | 1 (ref) | ... |
| TC | 15 (30) | 26 (52) | 0.035 | 0.37 (0.16-0.87) |
| CC | 4 (8) | 4 (8) | 0.704 | 0.65 (0.15-2.90) |
| CC + TC | 19 (38) | 30 (60) | 0.045 | 0.41 (0.18-0.91) |
| TC + TT vs CC | 46 (92) | 46 (92) | 1 | 1.00 (0.24-4.24) |

OR: odds ratio; CI: confidence interval. Values in parentheses indicate percentage values. The bold number represents the *P*-values with significant differences.

**Table 5.** The genotype and allele frequencies of rs12696600 among cases and controls

| Genotypes | Cases | Controls | *P* value | Crude OR (95% CI) |
|---|---|---|---|---|
| | N = 50 (%) | N = 50 (%) | | |
| A Allele | 82 (82) | 78 (78) | 1 (ref) | ... |
| C Allele | 18 (18) | 22 (22) | 0.596 | 0.78 (0.39-1.56) |
| AA | 33 (66) | 30 (60) | 1 (ref) | ... |
| AC | 16 (32) | 18 (36) | 0.674 | 0.81 (0.35-1.86) |
| CC | 1 (2) | 2 (4) | 0.608 | 0.46 (0.04-5.27) |
| CC + AC | 17 (34) | 20 (40) | 0.679 | 0.77 (0.34-1.74) |
| AC + AA vs CC | 49 (98) | 48 (96) | 1.00 | 0.49 (0.04-5.58) |

OR: odds ratio; CI: confidence interval. Values in parentheses indicate percentage values. The bold number represents the *P*-values with significant differences.

age of 68.3 ± 10.40 and 50 healthy controls with average age of 60.9 ± 9.34. No significant associations were found between cases and controls in sex and age distribution (P > 0.05).

*Association between CLDN1 polymorphisms and colorectal cancer*

The genotype frequencies of rs17501976 and rs12696600 in the cases and controls are presented in **Tables 4**, **5**. Genotype and allele frequencies were in Hardy-Weinberg equilibrium in two groups (**Table 3**). There was no significant difference between the cases and the controls in the distribution of the alleles. We further evaluated the association between rs17501976 and rs12696600 polymorphism and CRC risk. The distributions of the genotypes for rs17501976 were 62% (TT), 30% (TC), and 8% (CC) in the cases and 40% (TT), 52% (TC), and 8% (CC) in control subjects. Compared to the TT genotype, the TC + CC genotypes (OR = 0.41, 95% CI = 0.18-0.91, and *P* = 0.045) were significantly associated with the risk of CRC. Additionally, the TC genotype was also associated with a decreased risk of CRC (OR = 0.37, 95% CI = 0.16-0.87, and *P* = 0.035) compared with the TT genotype (**Table 4**). However, no statistical significance was observed between the rs12696600 polymorphism and the risk of CRC (**Table 5**).

*The effect of rs17501976 polymorphism on transcriptional binding sites*

To further investigate whether rs17501976 polymorphism has an implication on the promoter region where exists binding sites for transcription factor of CLDN1, we analyzed the potential transcription factor binding to the region around rs17501976 loci by the DNAssist software (26 bp before and after the SNP loci, SNP loci at the 26 bp). As shown in **Figure 1**, AP-2 alphaA, AP-2 alphaB, gammaCAC1, gammaCAC2 and CACCC-binding factor binding sites are in presence around transcription vicinity of the SNP loci. However, rs17501976 polymorphism did not add new or change the original transcription binding sites.

## Discussion

In this case-control study, rs17501976 and rs12696600 in the CLDN1 gene were analyzed to establish the relationship between these nucleotide changes and CRC risk. Our findings

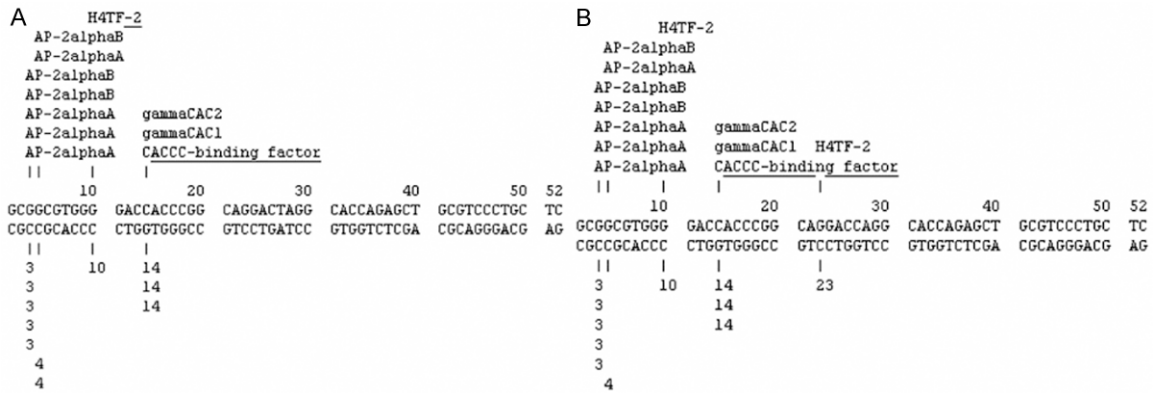


**Figure 1.** The effect of rs17501976 polymorphism on transcriptional binding sites of CLDN1. DNAssist analyzed results for potential transcription factor binding sites in genotype TT (A) and genotype CC (B).

showed a pronounced association between rs17501976 polymorphism of the CLDN1 gene with risk of colorectal cancer in a Chinese population.

Claudin belongs to a class of transmembrane proteins, molecular weights from 17~27 kDa, and constitute the tight junctions between cells combined with occludin and junctional adhesion molecule [15]. Abnormal expression of these Claudins have been considered to be an important step in loss of intercellular adhesion and metastasis of tumor cells [16, 17]. CLDN1 is a member of Claudin family, many studies have revealed its relationship with CRC from both protein and mRNA levels. CLDN1 has been proved associates with the depth of tumor invasion [18] and its high expression may promotes tumor development [11, 13, 19-21]. SNPs of Claudin family have been reported in a number of different diseases. Wu *et al* found that SNP of CLDN5 associated with positive symptoms of schizophrenia [22]; mutation in CLDN1 gene is closely associated with hereditary cholestasis [23]; Wilcox et al observed CLDN14 mutations in the recessive deafness [24]; And mutations in CLDN14 resulted in destroyed tight junctions, which ultimately contribute to malabsorption of magnesium and calcium, thus causing familial hypomagnesemia [25]. Given the critical roles of CLDN1 in CRC and SNPs in other Claudins, we hypothesized whether there is any polymorphism of CLDN1 affect CRC.

We genotyped four CLDN1 SNPs and found a SNP site located in -402 bp of 5' flanking region, rs17501976, which genotype frequencies were significantly different between the cases and controls group. Association analyses revealed that whether TC genotype or TC + CC genotypes of rs17501976 showed a decreased risk of CRC compared with the more common TT genotype. Although these results indicate a dominant effect of the C allele, CC genotype did not show a significant relationship between CRC cases and controls compared with TT genotype. The variation seen between studies may mainly due to small sample size in the subgroups. Besides, a C > T change of the rs17501976 polymorphism did not show a difference in transcription factor binding to the promoter region of CLDN1. Furthermore, whether rs17501976 polymorphism display a role in the binding activity of specific transcription factor remains to be further demonstrated. For rs12696600, we observed no statistical significance between its polymorphism and the risk of CRC. To our knowledge, this is the first study focused on the relationship between CLDN1 polymorphism and the risk of CRC. However, the limitation of the small sample size may decrease the statistical power, a larger sample size are needed to confirm the association between these loci and CRC.

In conclusion, nucleotide polymorphism in CLDN1 may play an important role in CRC. In this study, we found no association between rs12696600 polymorphism and CRC, but the rs17501976 polymorphism was associated with a decreased risk in a Chinese population. Additional large-scale, well-designed studies that include clinical pathological parameters and follow-up are required to further validate the role of CLDN1 gene polymorphisms in CRC risk.

## Acknowledgements

We are especially grateful to all of the patients participated in this study.

## Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Wei-Jun Tang, Department of General Surgery, Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University, 1630 Dongfang Road, Shanghai 200127, P.R. China. Tel: +86-21- 68383985; E-mail: drtangweijun@aliyun.com

## References

[1] Ji BT, Devesa SS, Chow WH, Jin F and Gao YT. Colorectal cancer incidence trends by subsite in urban Shanghai, 1972-1994. Cancer Epidemiol Biomarkers Prev 1998; 7: 661-666.

[2] Yee YK, Tan VP, Chan P, Hung IF, Pang R and Wong BC. Epidemiology of colorectal cancer in Asia. J Gastroenterol Hepatol 2009; 24: 1810-1816.

[3] Yiu HY, Whittemore AS and Shibata A. Increasing colorectal cancer incidence rates in Japan. Int J Cancer 2004; 109: 777-781.

[4] Fearnhead NS, Wilding JL, Winney B, Tonks S, Bartlett S, Bicknell DC, Tomlinson IP, Mortensen NJ and Bodmer WF. Multiple rare variants in different genes account for multifactorial inherited susceptibility to colorectal adenomas. Proc Natl Acad Sci U S A 2004; 101: 15992-15997.

[5] Tao WP, Hu S, Feng JP and Xie YS. [Association of MLH1 gene 415G/C polymorphism with colorectal cancer in Chinese]. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2009; 26: 314-317.

[6] Pittman AM, Twiss P, Broderick P, Lubbe S, Chandler I, Penegar S and Houlston RS. The CDH1-160C>A polymorphism is a risk factor for colorectal cancer. Int J Cancer 2009; 125: 1622-1625.

[7] Haghshenas MR, Hosseini SV, Mahmoudi M, Saberi-Firozi M, Farjadian S and Ghaderi A. IL-18 serum level and IL-18 promoter gene polymorphism in Iranian patients with gastrointestinal cancers. J Gastroenterol Hepatol 2009; 24: 1119-1122.

[8] Hewitt KJ, Agarwal R and Morin PJ. The claudin gene family: expression in normal and neoplastic tissues. BMC Cancer 2006; 6: 186.

[9] Krause G, Winkler L, Mueller SL, Haseloff RF, Piontek J and Blasig IE. Structure and function of claudins. Biochim Biophys Acta 2008; 1778: 631-645.

[10] Sappayatosok K and Phattarataratip E. Overexpression of Claudin-1 is Associated with Advanced Clinical Stage and Invasive Pathologic Characteristics of Oral Squamous Cell Carcinoma. Head Neck Pathol 2014; [Epub ahead of print].

[11] Grone J, Weber B, Staub E, Heinze M, Klaman I, Pilarsky C, Hermann K, Castanos-Velez E, Ropcke S, Mann B, Rosenthal A and Buhr HJ. Differential expression of genes encoding tight junction proteins in colorectal cancer: frequent dysregulation of claudin-1, -8 and -12. Int J Colorectal Dis 2007; 22: 651-659.

[12] Miwa N, Furuse M, Tsukita S, Niikawa N, Nakamura Y and Furukawa Y. Involvement of claudin-1 in the beta-catenin/Tcf signaling pathway and its frequent upregulation in human colorectal cancers. Oncol Res 2001; 12: 469-476.

[13] Dhawan P, Singh AB, Deane NG, No Y, Shiou SR, Schmidt C, Neff J, Washington MK and Beauchamp RD. Claudin-1 regulates cellular transformation and metastatic behavior in colon cancer. J Clin Invest 2005; 115: 1765-1776.

[14] Tang W, Dou T, Zhong M and Wu Z. Dysregulation of Claudin family genes in colorectal cancer in a Chinese population. Biofactors 2011; 37: 65-73.

[15] Kohler K and Zahraoui A. Tight junction: a coordinator of cell signalling and membrane trafficking. Biol Cell 2005; 97: 659-665.

[16] Tsukita S and Furuse M. Pores in the wall: claudins constitute tight junction strands containing aqueous pores. J Cell Biol 2000; 149: 13-16.

[17] Cereijido M, Shoshani L and Contreras RG. Molecular physiology and pathophysiology of tight junctions. I. Biogenesis of tight junctions and epithelial polarity. Am J Physiol Gastrointest Liver Physiol 2000; 279: G477-482.

[18] Swisshelm K, Macek R and Kubbies M. Role of claudins in tumorigenesis. Adv Drug Deliv Rev 2005; 57: 919-928.

[19] Mees ST, Mennigen R, Spieker T, Rijcken E, Senninger N, Haier J and Bruewer M. Expression of tight and adherens junction proteins in ulcerative colitis associated colorectal carcinoma: upregulation of claudin-1, claudin-3, claudin-4, and beta-catenin. Int J Colorectal Dis 2009; 24: 361-368.

[20] Kinugasa T, Huo Q, Higashi D, Shibaguchi H, Kuroki M, Tanaka T, Futami K, Yamashita Y, Hachimine K, Maekawa S, Nabeshima K, Iwasaki H and Kuroki M. Selective up-regulation of claudin-1 and claudin-2 in colorectal cancer. Anticancer Res 2007; 27: 3729-3734.

[21] Shiou SR, Singh AB, Moorthy K, Datta PK, Washington MK, Beauchamp RD and Dhawan

P. Smad4 regulates claudin-1 expression in a transforming growth factor-beta-independent manner in colon cancer cells. Cancer Res 2007; 67: 1571-1579.

[22] Wu N, Zhang X, Jin S, Liu S, Ju G, Wang Z, Liu L, Ye L and Wei J. A weak association of the CLDN5 locus with schizophrenia in Chinese case-control samples. Psychiatry Res 2010; 178: 223.

[23] Hadj-Rabia S, Baala L, Vabres P, Hamel-Teillac D, Jacquemin E, Fabre M, Lyonnet S, De Prost Y, Munnich A, Hadchouel M and Smahi A. Claudin-1 gene mutations in neonatal sclerosing cholangitis associated with ichthyosis: a tight junction disease. Gastroenterology 2004; 127: 1386-1390.

[24] Wilcox ER, Burton QL, Naz S, Riazuddin S, Smith TN, Ploplis B, Belyantseva I, Ben-Yosef T, Liburd NA, Morell RJ, Kachar B, Wu DK, Griffith AJ, Riazuddin S and Friedman TB. Mutations in the gene encoding tight junction claudin-14 cause autosomal recessive deafness DFNB29. Cell 2001; 104: 165-172.

[25] Muller D, Kausalya PJ, Claverie-Martin F, Meij IC, Eggert P, Garcia-Nieto V and Hunziker W. A novel claudin 16 mutation associated with childhood hypercalciuria abolishes binding to ZO-1 and results in lysosomal mistargeting. Am J Hum Genet 2003; 73: 1293-1301.