RESEARCH COMMUNICATION

Association Between C1019T Polymorphism in the Connexin 37 Gene and *Helicobacter Pylori* Infection in Patients with Gastric Cancer

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Abstract

Objective: To investigate the association between the connexin 37 C1019T polymorphism and *Helicobacter pylori* infection in patients with gastric cancer. Methods: 388 patients with gastric cancer (GC), 204 with chronic superficial gastritis (CSG) were studied. *H. pylori* was detected by gastric mucosal biopsies biopsy dyeing method. Connexin 37 gene polymorphism 1019 site genotypes were determined by gene sequencing technology. Genotypes and alleles frequencies were compared. Results: (1) Connexin37 gene 1019 site distribution frequency (CC type, TC type, TT type) in the CSG group was 18.1%, 45.1% and 36.8%; in the stomach cancer group it was 35.1%, 45.9% and 19.%, conforming to the Hardy-Weinberg euilibrium. (2) In comparison with CSG group, the frequency of Connexin37 C allele was higher in the gastric cancer group (58.0% vs 40.7%, OR = 2.01, 95%CI = 1.58-2.57, P < 0.01). The prevalence of gastric cancer risk was significantly increased in the carriers of C allele (CC+TC) than in TT homozygote (OR = 2.47, 5%CI = 1.68- 3.610. (3) Gastric cancer patients complicated with Hp infection 211 cases, gastric cancer group of the male patients with HP positive patients with 187 cases, 40 cases of female patients with negative patients, 24 cases were HP positive, negative in 137 cases, control group male patients, 28 cases were Hp positive, negative in 95 patients, female patients with Hp positive 6 cases, 75 cases were negative. On hierarchical analysis, the male group OR value was 15.9 (95%CI to 9.22-27.3), and the female OR was 2.19 (95%CI 0.88-5.59), indicating a greater contribution in males (P <0.01). After elimination of gender effects, positive HP and gastric cancer were closely related (OR 8.82, 95% CI: 5.45-14.3). (4) The distribution frequency of C allele in patients with Hp infection was much higher than that in Hp negative cases in the GC group (64.5% vs 47.0%, OR = 2.05, 95%CI = 1.54-2.74, P < 0.01). Compared with TT homozygotes, (CC+TC) genotype prevalence of gastric cancer risk increased significantly (OR = 2.96, 5%CI = 1.76-2.99). Conclusion: The T allele in the connexin37 gene might not only be associated with gastric cancer but also with *H. pylori* infection.

Keywords: Stomach neoplasms - *Helicobacter pylori* - connexin 37 C1019T - polymorphism - risk factor

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Introduction

The gap junctional protein (Durlej et al., 2011; Xu, et al., 2012; Shaker et al., 2012) (connexin, Cx) are present in the cells that is responsible to exchange information and substance, which was channel protein and was essential for cell proliferation, differentiation and body growth and development of Cx. The Cx function is complex, in many physiological and pathological processes are involved, more meaningful, it is correlated with tumor development (Finegold et al., 2012), known as "the second kind of tumor suppressor gene". And Cx is a multigene family, all members of the gene sequence have a high degree of homology. At present, it had 13 sorts in human tissue, which were found. Cx is an integral membrane protein and has 6 dumbbell shaped Cx subunit oligomerization formed unilateral membrane channel, adjacent cell membrane channel connection formed intercellular membrane channel that gap junction channels, it was transmembrane hydrophilic channel structure. While organisms between adjacent cells, it passes ions, metabolites of small signaling molecules by the cell gap junction channels mainly, then it controlled the coordinated between cells growth. The activities are that gap junction-mediated intercellular substance, energy and information exchange as gap junctional intercellular communication (gap junctional intercellular communication, GJIC) (Tence et al., 2012). It is important to cell biology behavior regulation and

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play an important role in cell proliferation, differentiation, homeostasis, and the body of the new supersedes the old, growth and physiological processes. Cx regulates cell biology behavior of basic function its form gap junction intercellular GJIC, impact on a variety of activities of life, and is closely related to the occurrence and development of tumor. Green et al. (2001) and Yotti et al. (1379) found that in premalignant stages of tumor (formation stage) GJIC function inhibition might be important factors for cancer cell cloning of unrestricted proliferation. Vorderwulbecke et al. (2012) found some activation oncogenes can reduce gene expression of Cx and downregulation GJIC. Krutovskikh et al. (1997) established of liver cancer model in rats with carcinogenic agents induce and found that had no cell communication activities between Hepatocellular carcinoma and peritumoral tissue cells. At the same time, he also found that the same phenomenon also existed in human hepatocellular carcinoma tissues. Mesnil et al. (1990) reported that, in the transformation of rat liver epithelial cells in the process, communication function lost gradually. between it and non-transformed cell, which showed that Cx and cellular phenotype conveyed relationship closely. Formerly a large number of studies show that (Zhang et al., 2009), Cx gene in various tumor cell expression dropped or reduced, Cx were significantly decreased even disappeared, then GJIC function decreased or disappeared, then leaded to cell malignant transformation, tumor formation. While some studies have shown (Simon et al., 2009) some benign tumors, malignant tumors and transformed cell had CX gene stable expression. Another studies have shown that (Cocciadiferro et al., 2009) that some Cx gene did not express in normal cells but did in the tumors, even for high expression. Connexin37 gene C1019T polymorphism is the reason for gene coding region 1019 positional took place mutation (C - T) and leaded to encode 319 amino acid occurring missense mutations (proline serine P - S) and impact on connexin 37 three secondary structure formation directly. At the same time original phosphorylation site of the connexin37C end were changed (Functional area, bonded the specificity of protein kinases and protein ligands) because of the encoding of different amino acids, which make connexin37 gap junction function change. The 6 Connexin37 in the cell membrane is formed on the 1.5channels, and adjaced the other 1.5 channel in cell membrane, assembled and formed a complete channel, that is gap junctions, by it, adjacent cell small molecular substances (such as ions, small metabolites, second messenger molecules) exchanged information by passive diffusion and achieved the synchronous response of multicellular. Some studies showed that, Connexin37 gene (C1019T) polymorphisms can influenced the function of GJIC (Morel et al., 2010) and may play important role in the growth of tumor cells. Helicobacter pylori (Helicobacter pylori, Hp) infection is an important risk factor for gastric carcinoma, studies have found that Hp concentrated in epithelial cell junctions, changed the top connection compound structure and function and reduce the GJIC function (Amieva et al., 2003). This study was that patients with gastric cancer and chronic superficial gastritis group Connexin37 gene polymorphism were detected, explored the relation between the Connexin37 gene polymorphism and Hp infection.

Materials and Methods

Population

388 patients with gastric cancer were selected in this research who were excised by operation and examined of pathologically diagnosed from 2007 January to 2011 December in Zhejiang University Shaoxing hospital as gastric cancer group (GC) and 204 out-patients with chronic superficial gastritis (CSG) (mild) were as control group. GC group was a total of 388 patients, mean age 45.78±10.32 years (28~76), by the pathological detection, including 83 cases high differentiation adenocarcinoma, 67 cases of differentiated adenocarcinoma, 238 cases of poorly differentiated adenocarcinoma; in which 211 cases with Hp infection, 177 cases without Hp infection. CSG group was a total of 204 cases, mean age 46.70±5.73 (26~78), in which 72 cases, with Hp infection and 132 cases without Hp infection. All cases were unrelated, gastric malignancies history and family history of malignant neoplasm. At the same time, they did not take antibiotic, nonsteroidal anti-inflammatory drugs, proton pump inhibitors, gastric mucosal protective agent and anticancer drugs within 4 weeks. The study was agreed by Zhejiang University, Shaoxing Hospital of the medical ethics committee, and all patients signed informed consent. In the course of the study, patients could withdraw from the study without any reason.

Extraction of genomic DNA

The steps of extraction in DNA of tissue specimens were as the following: first, 10 pathological section about 10 μm thick were taken and were broken into 1.5mL centrifugal tube; which were then added 1mL xylene and bathed in 55 °C water for 15 minutes, 4000 r/min centrifugal for 5 min after abandoning the supernatant, which were repeated two times. Second, added anhydrous ethanol 1 mL, bathed in 55 °C water for 15 minutes, under room temperature centrifugation 4000 r/min and discarded supernatant after 5 minutes, then repeated 1 times and dried precipitation. Third, added 1 mL digestion buffer that included 100 mmol/L NaCl, 10 mmol/L Tris HCl, pH 8, 25 mmol/L EDTA, pH 8, 0.5% SDS and protease K 25 μL (20 mg/mL) and bathed in 55 °C water for the night. Then added protease K 25μL (20 mg/mL), bathed in 55°C water for the night and repeated 1 times. Fourth, added equal amount of phenol/chloroform (25:24), 4000 r/min centrifugal for 2 minutes and supernatant is were transferred to a new centrifugal pipe ,then repeated 1 times. Fifth, 330u L μL liquid phase were taken to several centrifuge tube and added 165 L7 5 mol/L ammonium acetate (1~3 L glycerol to protect the DNA) and 2-2.5 times volume ethanol under room temperature for two hours; centrifugated in 4 °C by 4000 r/min for 20 minutes, then the ethanol were abandoned and 20u L TE were added to dissolution precipitation and merged with a wax block source of DNA solution after room temperature overnight or incubated in 55 °C for 2 hours. Last, measured the absorbance of DNA solution (A) value

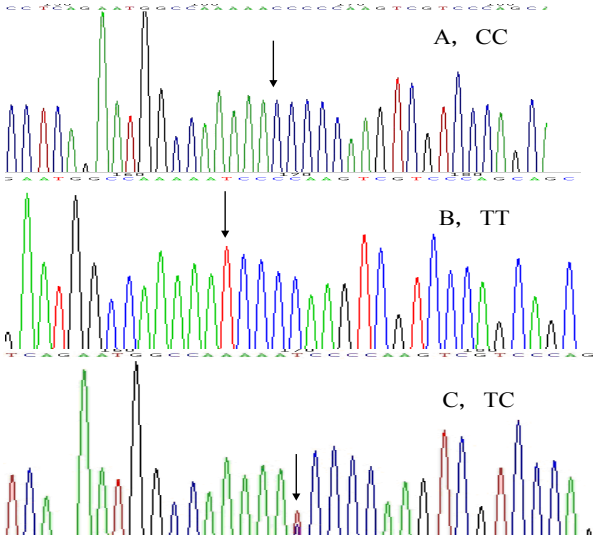

Figure 1. Gene Sequencing Diagram

Table 1. Comparison Clinical Data of Patients Between Two Groups

	CSG group (n=204)	GC group (n=388)
Gender (male / female)	123/81	227/161
Age (years)	46.70±5.73	45.78±10.32
Smoking history (yes / no)	96/108	154/234
Drinking history (yes / no)	135/83	253/121
Hs-CRP	6.23±2.76	11.27±23.65*
CA125(u/ml)	23.76±11.21	78.34±67.23*
CEA(ng/ml)	3.46±2.13	128.46±72.37*
Tumor necrosis factor (ng/ml)	1.21±0.78	2.39±1.22*
HP infection(positive / negative)	34/170	211/177*
Tumor classification		
High differentiation	-	83
Differentiation	-	67
Low differentiation	-	238

*That compared with CSG group, P < 0.05

Table 2. Analysis Gene Polymorphism of Test Results (%)

Groups	Genotype				Allelic gene	
	CC	TC	TT	CC+TC	C	T
GC group (n=388)	136(35.05)	178(45.88)	74(19.07)	314(80.93)	450(57.99)	326(42.01)
CSG group (n=204)	37(18.14)	92(45.10)	75(36.76)	129(63.24)	166(40.69)	242(59.31)
X^2			29.74	22.22	32.08	
P			<0.01	0.001[a]	<0.01	
OR (95%CI)				2.47(1.68~3.61)	2.01(1.58-2.57)	

Note: [a]compared with TT

by UV spectrophotometer.

PCR amplification

Amplification genomic DNA that has been extracted Connexin 37C1019T locus primer sequence were as follows: The upstream primer, 5'-CCTCCTCAGACCCTTACACGG-3' and downstream primer, 5'-CATCCCAGGCAGCCAGACT-3' (designed and produced by Ying-Jun biological company in Shanghai). A 20μl reaction volume was used in PCR containing 10 μl 2x mix(including Mg^+, dNTPs, Taq DNA polymerase), 1.0 μl upstream primer (10 pmol), 1.0 μl downstream primer (10 pmol), 2 μl genomic DNA template (add to 4.0 μl according to the concentration) and 6 μl ddH_2O. The reaction begins with denaturation at 94 °C for 5 min, followed by 35 cycles of denaturation at 95 °C (30s), annealing at 60 °C (30 s), extension at 72 °C (30s), and a final extension at 72 °C (7 min). Genotype analysis was performed using DNA sequencing, which was completed in Ying-Jun biological company in Shanghai.

Statistical analysis

Allele frequencies were calculated by allele counting. Analyses for possible deviations of the genotype distribution from that expected for a population in Hardy-Weinberg equilibrium were done with the χ^2 test. Data were given as mean (SD) or number (proportion). Continuous variables with a Gaussian distribution, as determined by the Shapiro-Wilk test, were compared by one-way analysis of variance (ANOVA) or t test; categorical values were compared by the χ^2 test. Continuous variables with a non- Gaussian distribution were compared by the Mann-Whitney U test. Analyses were done using statistical software SPSS (Version 13.0, SPSS Inc, Chicago, IL, USA). A P-value of less than 0.05 was considered significant.

Results

General information

The study included 592 patients. By endoscopic and operation resection and pathological examination, all patients were divided into two groups, GC group (n=388) and CSG group (n=204). Two groups in gender, age, smoking, and alcohol drinking history, C reactive protein, tumor markers (CA125, CEA), tumor necrosis factor had no difference (Table 1).

Genetic equilibrium degree detection (DF (Degrees of freedom) γ=1)

CSG group (a=37, b=92, c=75, $\chi2$=0.88, P>0.05)

GC group (a=136, b=178, c=74, $\chi2$=1.32, P>0.05)

According to $\chi^2=(a+b+c)(b^2-4ac)^2/(2a+b)^2(b+2c)^2$, DF γ= Gene number - the number of alleles (a is CC genotype observed value, b is TC genotypes observed value, and C is TT genotypes observed value), if P > 0.05, it meant that gene type frequency conformed to Hardy- Weinberg genetic equilibrium, which showed the representativeness of the sample would be worthy of trust.

Analysis of Connexin37 genotype

According to sequence of Connexin37 genotype gene, a total of three types: type CC, type TT and type TC (Figure 1).

Table 3. Connexin37gene C1019T Polymorphism Distribution in GC Group Hp Infection and Non-infection

Group	Genotype				Allelic gene	
	CC	CT	TT	CC+TC	C	T
HP Positive (n=211)	87(41.23)	98(46.44)	26(12.32)	185(87.68)	272(64.45)	150(35.55)
HP Negative	41(23.16)	84(47.46)	52(29.38)	125(70.62)	166(46.89)	188(53.11)
X^2			23.48	17.43	24.15	
P			<0.01	0.001[a]	<0.01	
OR (95%CI)				2.96(1.76~2.99)	2.05(1.54-2.74)	

Note: [a]compared with TT

comparison Connexin37gene C1019T polymorphism distribution between GC group and CSG group

There were significant differences in the three Connexin 37 C1019T genotype frequencies distributing in the two groups (χ^2 =29.74, P<0.01). Allele frequency of C gene in GC group was significantly higher than that of CSG group (57.99% vs 40.69%, OR=2.01, 95%CI=1.58-2.57, P<0.01). The C allele carriers (CC+TC) in GC group and CSG group were 80.93% and 63.24%, respectively (P=0.001). Compared with TT homozygotes, (CC+TC) genotype prevalence of gastric cancer risk increased significantly (OR = 2.47, 95%CI =1.68-3.61, Table 2).

Compared HP infection of Gender differences in patients with GC and CSG group.

Male patients with positive HP patients in GC group were 187 cases, 40 cases with negative patients, female patients with HP positive were 24 cases and negative was 137 cases. Male patients in CSG group, 28 cases were HP positive, 95 patients was HP negative, female patients with Hp positive 6 cases and negative was 75 cases. Stratified analyses indicated that the male group OR value were:15.86, 95%CI 9.22-27. 28 female OR 2.19, 95%CI 0.88-5.59,which Hp positive was a risk factor for male patients occurred gastric cancer , and gender consistency of OR P < 0.01, Hp positive hints male gastric cancer risk was significantly higher than that of female. Elimination of gender effects, positive HP and gastric cancer are closely related, OR 8.82, 95%CI 5.45-14.28.

Overall Polymorphism Findings with Reference to HP infection.

A comparison of HP positive and negative groups is given in Table 3.

Discussion

This study based on 592 cases of patients with Connexin 37 C1019T gene polymorphism detection, 388 Cases in GC group, 204 cases in CSG group. The results showed that C gene frequency in GC group patients was enhanced and the T gene frequency was significantly lower, which was more pronounced in men. Correction of gender factors, found the Connexin 37 C1019T C allele frequency in Helicobacter pylori positive gastric cancer patients increased while T gene frequency was significantly reduced. In recent years, epidemiologic and experimental animal studies showed that chronic Hp. Hp infection played an important role in gastric cancer. However, molecular mechanisms are still not clear (Kuo et al., 2012). Some studies have shown that intermittently connected intercellular communication is the important regulation of cell proliferation and differentiation mechanism. Other Studies have shown that (Nakamura et al., 2005), Hp may directly reduced gastric epithelial cells by intermittent junctional intercellular communication, induce gastric epithelial cell hyperplasia, accelerate cellular DNA synthesis, make DNA impaired increase, obtain gastric mucosal cell gene mutation or activation and lead to uncontrolled proliferation. At the same time, intermittent junctional intercellular communication suppression can make cancerous starting cells avoiding the surrounding normal cell regulation and continue or malignant proliferation, then culminating in the formation of cancer. This study, extracting DNA from diseased tissues, showed that the Connexin 37 C1019T C allele gene frequency in Hp positive gastric cancer patients increased while T gene frequency was significantly reduced.

Gastric cancer is a multifactorial disease, its pathogenesis, genetic factors and environmental factors are closely related, and there is a certain degree of sex difference. In order to reduce the effects of gender, we took gender stratified analysis for two groups, CC+TC gene frequencies in Hp positive gastric cancer in male were significantly increased. Then correcting for gender effects, Connexin 37 C1019T C allele frequency in Hp positive gastric cancer patients increased and T gene frequency decreased obviously.

Intermittent connection intercellular communication can be used for small molecules (such as ions, small metabolites, second messenger molecules) with passive diffusion form in multicellular synchronous exchanging of information among cells, which is one of the most popular means of communication, and connexins is a large class of composition of gap junction channel structure protein. The Connexin 37 gene inhibited the growth of tumor cells, occured mutation rarely in tumor genomes, and enhanced the expression by gene transfection or elicitor treatment (Eghbali et al., 1991; Rose et al., 1993; Mesnil et al., 1995; Duflot-Dancer et al., 1997). Connexin 37 C1019T polymorphism loci (Derouette et al., 2009) is the result that gene coding region of 1019positions took place mutation (C-T), leaded to encode 319th amino acid occurring missense mutations (proline -serine), and directly affected the gap junction protein structure of the three forms. In addition, since the encoded amino acid were changed that may change the phosphorylation site and also affect the protein synthesis, transport, processing, and signal transduction processes. Studies showed that the frequency of Connexin37C allele in endothelial cells increased

apparently, and affected the growth of endothelial cells, proliferation, senescence and regeneration after injury. This study showed that Connexin 37 C1019T gene polymorphism and Hp positive gastric carcinoma occurrence had correlation. The possible mechanisms included collaborative Hp inhibition of intermittent junctional intercellular communication and increasing after Hp infection of gastric epithelial uncontrolled proliferation. However, the exact mechanisms are to be further studied.

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