Correlation Between CCND1 Gene Polymorphism and Genetic Susceptibility to Gastric Cancer in Chinese Population

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Research

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Abstract

Objective: The relationship between Cyclin D1 gene (CCND1) rs9344 polymorphism and susceptibility to gastric cancer (GC) is investigated.

Methods: In a case-control study, we selected 577 cases of GC from The People's Hospital Affiliated to Jiangsu University in China along with 678 normal controls. Blood DNA was extracted and PCR amplified, gene polymorphism was determined using Snapshot method.

Results: Analysis reveals significant difference in smoking between GC and control groups ($P=0.006$), however, not on polymorphism ($P>0.05$).

Conclusion: Smoking is associated with gastric cancer, whereas CCND1 rs9344 polymorphism does not implicate susceptibility of gastric cancer.

Introduction

Gastric cancer (GC) is one of the most common malignant tumors of the gastrointestinal tract and the second most common cause of cancer mortality worldwide. GC is a complex disease with multiple causes, including helicobacter pylori infection, environmental dietary factors, and genetic factors. Single Nucleotide Polymorphism (SNP), as a type of genetic factors, is essential genetic information that affects susceptibility of human diseases, plays an important role in pathogenesis of GC. Cyclin D1 (CCND1) gene located in chromosome 11q13 is the most important positive cell cycle regulator in the Cyclin family (Cyclin). A CCND1 forms a complex when binding to a cyclin-dependent kinase and stimulate cell transition from phase G1 to S. CCND1 gene decrease cell cycle through gene amplification and protein over-expression, leading to independent proliferation of tumor cells.

Studies suggest that polymorphism of CCND1 gene rs9344 affects risks and clinical outcomes of malignant tumors. Rs9344 polymorphism has been proved to be associated with breast cancer, rectal cancer, lung cancer, bladder cancer, but there is few work on its relationship with GC. This paper aims to provide evidence for early screening, diagnosis and treatment of GC.

Materials And Methods

During May 2013 and June 2017, a hospital based investigation was conducted involving 1255 random samples (577 gastric cancer patients and 678 controls). The subject group consisted of 394 male and 183 female Han Chinese aged at 61.34±11.097 years old. The control group of 678 healthy people were selected from the same survey area without blood relationship. The difference was not statistically significant in occurrence distribution of age or gender between case and control groups ($P>0.05$).

This research has been approved by the ethics review committee of Jiangsu University. A standardized training was conducted before study. Subjects who participated in this study were informed of significance.

DNA extraction, PCR amplification and Snapshot typing were used. Table 1 shows primer sequences of rs9344 locus were designed using software Primerpremier 5.0 software. Extension primers were designed. The PCR reaction conditions were pre-denaturation at 95°C for 3 minutes, denaturation at 94°C for 30 seconds, annealing at 56°C for 40 s, extension at 72°C for 40 s, a total of 30 cycles, and the final 72°C extension is for 5 min. PCR amplification products were purified with ExoI and FastAP for the extension reaction, and ABI3730XL sequencer was used for sequencing and genotyping detection.
Table 1 Single nucleotide polymorphism genotypic primers required

| PCR amplification primer: 5'-3' | Amplification products | Loci polymorphism | Extension of primers: 5'-3' | Extension direction | Extension production |
|---------------------------------|------------------------|-------------------|-----------------------------|---------------------|---------------------|
| F: TTCCAATCCGCCCTCCAT           | 249bp                  | [A/G]             | AAGGTTAGGCCGGAAGGTA         | reverse             | CT                  |
| R: CCCCCAACCTTTCACCTC           |                       |                   | GGGTTGGAACAGGGA            |                     | CAGGGA              |

Statistical method

Data were analyzed using software SPSS 20.0 (SPSS, Chicago, Illinois, USA). Hardy-Weinberg genetic balance was used to test the genotype distribution frequency. Comparing frequency distribution between gastric cancer and control groups by T-test and Chi-square test. Risk ratio, Odds ratio (OR) and 95% confidence interval (CI) were calculated from binary logistic regression. Both statistical tests were bilateral probability tests, with \( P < 0.05 \) indicating that the difference is statistically significant.

Results And General Features Of Samples

Results in Table 2 showed that there was no statistically significant difference between two groups' age \( (P=0.065) \). There was no statistically significant difference in gender frequency between the case and control groups \( (P=0.635) \). Smoking ratio in case group was 34.49% higher than control group 27.29%. Alcohol ratio was 21.49% in case lower than 23.30% in control group, alcohol consumption was not statistically significant difference between two groups \( (P=0.443) \).

Table 2 Distribution of selected demographic variables and risk factors in cases and controls

| Variable          | Overall Cases (n=577) | Overall Controls (n=678) | \( P \) |
|-------------------|-----------------------|--------------------------|--------|
|                   | \( n (%) \)           | \( n (%) \)              |        |
| Age (years)       | 61.34±11.097          | 62.31±7.549              | 0.065  |
| < 62              | 268 (46.45)           | 324 (47.79)              |        |
| \( \geq 62 \)     | 309 (53.55)           | 354 (52.21)              | 0.635  |
| Sex               |                       |                          |        |
| Male              | 394 (68.28)           | 456 (67.26)              |        |
| Female            | 183 (31.72)           | 222 (32.74)              | 0.698  |
| Smoking status    |                       |                          |        |
| Never             | 378 (65.51)           | 493 (72.71)              |        |
| Ever              | 199 (34.49)           | 185 (27.29)              | \( 0.006 \) |
| Alcohol use       |                       |                          |        |
| Never             | 453 (78.51)           | 520 (76.70)              |        |
| Ever              | 124 (21.49)           | 158 (23.30)              | 0.443  |
Primary information for gene CCND1 rs9344 Polymorphisms

Table 3 shows that rs9344 is located in 11 chromosome, category is protein coding, minor allele frequency (MAF) for Chinese in genecard database is 0.442 and in our controls is 0.451. Hardy-Weinberg equilibrium test in our controls is 0.295 ($P>0.05$), which means that our sample population is representative. We use Snapshot method as genotyping and the percentage of successful tests is 99.20%.

Table 3 Primary information for gene CCND1 rs9344 Polymorphisms

| GenotyPed SNPs | Gene  | Chr Pos (NCBI Build 38) | Category       | MAF$^a$ for Chinese in database | MAF in our controls (n = 678) | P value for HWE$^b$ test in our controls | Genotyping method | Genotyping value (%) |
|---------------|-------|-------------------------|----------------|----------------------------------|-------------------------------|------------------------------------------|------------------|---------------------|
| rs9344        | CCND1 | 11:69648142             | Protein Coding | 0.442                            | 0.451                         | 0.295                                    | Snapshot         | 99.20               |

$^a$ MAF: minor allele frequency;

$^b$ HWE: Hardy–Weinberg equilibrium;

GeneCards Database: https://www.genecards.org/

Table 4 Hardy-Weinberg equilibrium test: There was no statistically significant difference between the case and control groups ($P>0.05$). Genotype frequency conforms to the law of genetic balance, the selected samples are representative.

Table 4 Hardy–Weinberg equilibrium analysis for rs9344

| Group     | rs9344 |   |   |   |   |
|-----------|--------|---|---|---|---|
|           | AA     | AG | GG | $\chi^2$ | $P$ |
| Case      | Observed | 168 | 302 | 107 | 1.983 | 0.159 |
|           | Expected | 176.4 | 285.3 | 115.4 | 0.620 | 0.431 |
| Control   | Observed | 182 | 343 | 143 | 187.1 | 332.9 | 148.1 | 0.620 | 0.431 |

$P$ value $\leq 0.05$ means statistically significant difference; $\alpha$ takes two sides check; $df=1$.

Table 5 shows: The A allele frequency in case group is higher than control group (47.43% $>$ 45.07%); The G allele distribution in case group is lower than control group (52.57% $<$ 54.93%), yet this difference is no statistically significant between the two groups ($P=0.237$).

Table 5 Analysis of rs9344 A allele and G allele between cases and controls
The results of Table 6 showed that occurrence of AG was slightly higher in case than in control (52.34% >51.35%) with no statistical significance ($P=0.722$). There was still no statistically significant difference ($P=0.787$) after being logistics regression test.

Occurrence of GG in case group was lower than in control group (18.54%<21.40%), but the difference was not statistically significant ($P=0.208$). GG were still not statistically different ($P=0.189$) after being logistics regression test.

In dominant model, mutation AG+GG frequency was lower in case group than control group (70.88% <72.75%), but difference was not statistically significant ($P=0.464$). There was still no statistical difference between the two groups ($P=0.496$) after logistic regression analysis. For recessive model dominated, GG/(AA+AG) with no statistical difference ($P=0.209$).

Table 6 Logistic regression analysis of CCND1 gene rs9344 Polymorphism between cases and controls

| Genotype | GC Cases (n=577) | Controls (n=678) | Crude OR (95% CI) | $P$ | Adjusted OR$^a$ (95% CI) | $P$ |
|----------|-----------------|-----------------|------------------|-----|--------------------------|-----|
| rs9344   |                 |                 |                  |     |                          |     |
| AA       | 168             | 182             | 1.00             |     |                          |     |
| AG       | 302             | 343             | 0.95(0.74-1.24)  | 0.722| 0.96(0.74-1.25)          | 0.787|
| GG       | 107             | 143             | 0.81(0.59-1.12)  | 0.208| 0.90(0.76-1.06)          | 0.189|
| AG+GG    | 409             | 486             | 0.91(0.71-1.17)  | 0.464| 0.99(0.85-1.09)          | 0.496|
| GG       | 107             | 143             | 0.84(0.63-1.11)  | 0.209| 0.91(0.79-1.04)          | 0.173|
| AA+AG    | 470             | 525             | 1.00             |     |                          |     |

Table 7 shows that stratified analysis, we analyzed stratification according to gender, age, smoking and drinking factors. The results showed that differences were not statistically significant in heterozygous mutants, homozygous mutants, dominant models or recessive models.

Table 7 Stratified analyses between rs9344 Polymorphism and risk by sex, age, BMI, smoking status and alcohol consumption
| Variable | (case/control) | Adjusted OR (95% CI); P |
|----------|----------------|------------------------|
|          |                | AA | AG | GG | AA | AG | GG | (AG+GG)VSAA | GGVS(AA+AG) |
| gender   |                |    |    |    |    |    |    |             |             |
| Male     |                | 119/119 | 200/233 | 75/96 | 1.00 | 0.86(0.63-1.18); | 0.78(0.53-1.16); | 0.84(0.62-1.13); | 0.84(0.60-1.18); | 0.344 | 0.220 | 0.242 | 0.304 |
| Female   |                | 49/63 | 102/110 | 32/47 | 1.00 | 1.19(0.75-1.89); | 0.88(0.49-1.57); | 1.10(0.71-1.70); | 0.78(0.43-1.29); | 0.454 | 0.655 | 0.678 | 0.329 |
| Age      |                |    |    |    |    |    |    |             |             |
| <62      |                | 68/91 | 151/160 | 49/66 | 1.00 | 1.26(0.86-1.86); | 0.99(0.61-1.61); | 1.18(0.82-1.71); | 0.85(0.56-1.28); | 0.234 | 0.979 | 0.367 | 0.442 |
| ≥62      |                | 100/91 | 151/183 | 58/77 | 1.00 | 0.75(0.53-1.07); | 0.69(0.44-1.07); | 0.73(0.52-1.03); | 0.82(0.56-1.20); | 0.115 | 0.095 | 0.069 | 0.314 |
| Smoking  |                |    |    |    |    |    |    |             |             |
| Never    |                | 106/136 | 205/246 | 67/102 | 1.00 | 1.07(0.78-1.46); | 0.08(0.57-1.26); | 1.00(0.74-1.35); | 0.81(1.14-0.57); | 0.077 | 0.401 | 0.985 | 0.219 |
| Ever     |                | 62/46 | 97/97 | 40/41 | 1.00 | 0.74(0.46-1.19); | 0.72(0.41-1.29); | 0.74(0.47-1.15); | 0.88(0.54-1.43); | 0.217 | 0.273 | 0.181 | 0.601 |
| Alcohol  |                |    |    |    |    |    |    |             |             |
| Never    |                | 128/145 | 241/257 | 84/109 | 1.00 | 1.06(0.79-1.43); | 0.87(0.60-1.27); | 1.01(0.76-1.33); | 0.84(0.61-1.15); | 0.689 | 0.473 | 0.967 | 0.280 |
| Ever     |                | 40/37 | 61/86 | 23/34 | 1.00 | 0.66(0.38-1.14); | 0.63(0.31-1.25); | 0.65(0.38-1.10); | 0.82(0.46-1.49); | 0.135 | 0.184 | 0.105 | 0.520 |

For CCND1 gene rs9344 locus, genotype was detected successfully 99.20% in 1255 samples.

**Discussion**

The early symptoms of GC are not obvious. Patients have often reached middle or late stages when diagnosed clearly, with a low 5 years survival rate of 30%[15,16]. Current treatments for GC includes surgery, chemotherapy, drug targeted therapy, etc. Finding specific biomarkers is beneficial for early screening diagnosis, and clinical targeted drug therapy.

This project is based on the relationship between polymorphism in CCND1 gene and GC, with few similar studies currently reported. In 2010, Kuo et al [17] studied 358 GC patients and 358 healthy people and found that rs9344 locus polymorphism was associated with gastric cancer risk, and that subjects carrying variants AG and GG genotypes had lower risk than
carrying AA genotypes. Rs9344 genotype maybe a useful biomarker for detection of early GC. Kuo et al also investigated the interaction between rs9344 genotype and individual smoking status on GC risk and found that there was a synergistic effect between gene and smoking, which may increase the develop risk of GC. Another Yokoyama et al. [18] suggested that there was no correlation between CCND1 rs9344 gene polymorphism and risk of GC in Japanese population in 2001. Our study suggest that there is no significant association between CCND1 rs9344 polymorphism and gastric cancer besides that smoking is a risk factor.

Alcohol drinking in this study was not related to gastric cancer, which is consistent with the results from Yokoyama et al, but differs from the research by Inoue M [19]. According to Inoue M research, drinking alcohol may be a potential risk factor for gastric cancer: the reason why drinking alcohol as risk factor may be that occurrence of gastric cancer is a slow process under joint action of multiple factors. Long-term heavy drinking can cause damage to gastric mucosa itself. On the other hand, alcohol intake in wine can stimulate gastric mucosa to reduce barrier function and increase gastric mucosa permeability, resulting in increased absorption of carcinogens. Another study by Tahara et al. [20-21] showed that CCND1 rs9344 polymorphism was associated with high methylation risk of CpG island promoter in gastric cancer, which was the first report to show a potential association between CCND1 hypermethylation status in gastric cancer. A meta analysis [22] showed that analysis shows the relationship between site polymorphism and gastric cancer is different between Asian and Caucasian populations. Rs9344 polymorphism increases risk of GC in Caucasians but not in Asian.

The occurrence and development of gastric cancer is affected by various factors, in addition to genetic background, other factors that should be taken into consideration, may include environment, lifestyle, race, ethnicity, eating habits, genetic heterogeneity, sample size, etc. Therefore, the relationship between CCND1 gene and gastric cancer susceptibility needs further verification, larger samples, and elaborate design in the further.

**Abbreviations**

GC: gastric cancer; CCND1: Cyclin D1

**Declarations**

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**Authors’ Contributions**

Xuyu Gu is the co-first author. Huiwen Pan and Xuyu Gu wrote and edited the manuscript. Yu Fan, Keping Chen provided direction and guidance throughout the preparation of this manuscript. Huiwen Pan finished the data analysis. Chen Zou revised the manuscript for important intellectual content. All authors read and approved the final manuscript.

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**Availability of data and materials**

Data sharing is not applicable to this article as no datasets were generated or analysed during the current study.

**Ethics approval and consent to participate**
The study was approved by the Institutional the Affiliated People's Hospital of Jiangsu University. The patients consented to participate.

Consent for publication

Written informed consent for research and publication from the patients was obtained.

Competing interests

The authors declare that they have no competing interests

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