**Name of journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO: 19515**

**Manuscript Type:** **ORIGINAL ARTICLE**

***Observational Study***

***CD24* genetic variants contribute to overall survival in patients with gastric cancer**

Jia ZF *et al.* CD24 SNPs and gastric cancer

Zhi-Fang Jia, Li-Zhong Wang, Xue-Yuan Cao, Chuan Wang, Dong-Hui Cao, Xing Wu, Li-Li You, Mei-Shan Jin, Yin-Ping Wang, Bao-Sen Zhou, Jing Jiang

**Zhi-Fang Jia,** **Chuan Wang, Dong-Hui Cao, Xing Wu, Li-Li You, Jing Jiang,** Division of Clinical Epidemiology, First Hospital of Jilin University, Changchun 130021, Jilin Province, China

**Li-Zhong Wang,** Department of Genetics, University of Alabama at Birmingham, Birmingham, AL 35294, United States

**Xue-Yuan Cao,** Department of Gastrointestinal Surgery, First Hospital of Jilin University, Changchun 130021, Jilin Province, China

**Mei-Shan Jin, Yin-Ping Wang,** Division of Pathology, First Hospital of Jilin University, Changchun 130021, Jilin Province, China

**Zhi-Fang Jia,** **Bao-Sen Zhou,** Department of Epidemiology, School of Public Health, China Medical University, Shenyang 110112, Liaoning Province, China

**Author contributions**: Jia ZF, Wang LZ, Cao XY and Jiang J designed the research; Jia ZF, Wang C, Cao DH, Wu X, and You LL performed the research; Wang LZ, Cao XY, Jin MS, and Wang YP analyzed the data; Jia ZF and Jiang J wrote the manuscript; Wang LZ, Cao XY and Zhou BS revised the manuscript.

**Supported by** National Natural Science Foundation of China, No. 81373084 and No. 81273065; the Norman Bethune Program of Jilin University, No. 2013025; and the Youth Fund of the First Hospital of Jilin University, No. JDYY42013014.

**Institutional review board** **statement:** The study was reviewed and approved by the ethics committee of the First Hospital of Jilin University.

**Informed consent statement:** All the participants provided informed written consent prior to study enrollment.

**Conflict-of-interest statement:** All the authors declare no conflicts of interest.

**Data sharing statement:** Technical appendix, statistical code, and dataset available from the corresponding author at jingjiang19702000@jlu.edu.cn.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**Correspondence to****: Jing Jiang, MD, PhD,** Division of Clinical Epidemiology, First Hospital of Jilin University, 71 Xinmin Street, Changchun 130021, Jilin Province, China. jiangjing19702000@jlu.edu.cn

**Telephone:** +86-431-81875408

**Fax:** +86-431-85654528

**Received:** May 11, 2015

**Peer-review started:** May 12, 2015

**First decision:** July 19, 2015

**Revised:** August 12, 2015

**Accepted:** November 30, 2015

**Article in press:**

**Published online:**

**Abstract**

**AIM:** To investigate the role of single nucleotide polymorphisms (SNPs) in *CD24* genein susceptibility and overall survival of gastric cancer (GC).

**METHODS:** We genotyped 3 tagging SNPs of *CD24—*P-534 in the promoter region, P170 in the coding region of exon 2 and P1527 in the 3′ untranslated region (3′-UTR)—using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) in specimens from 679 histologically-confirmed GC cases, 111 gastric atrophy (GA) cases and 976 tumor-free controls. Serum immunoglobulin G antibodies to *Helicobacter pylori* of all subjects were detected by enzyme-linked immunosorbent assay. CD24 expression was evaluated by immunohistochemistry in 131 GC specimens. Correlations between SNPs and risk of GC or GA were shown by *P* values and odd ratios (ORs) with 95% confidence intervals (95%CI) compared with the most common genotype of each SNP using the unconditional logistic regression model after adjusting for age, sex and *H. pylori* infection. Survival within each SNP group was plotted by Kaplan-Meier method and compared by log-rank test (recessive model). Hazard ratios (HRs) with 95%CIs were computed by Cox regression model after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotherapy.

**RESULTS:** All of the three loci were in Hardy-Weinberg equilibrium in the control group. Median follow-up time for the 600 GC patients included in the survival analysis was 36.2 mo (range: 2.1-66.7 mo; 95%CI: 34.3-36.5 mo). Patients with the P-534 A/A genotype had significantly shorter survival (HR = 1.38, 95%CI: 1.01-1.88; *P* = 0.042) than did the C/C or C/A genotype carriers after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotherapy. This trend was more evident in patients who lived longer than 2.5 years (HR = 7.55, 95%CI: 2.16-26.32, *P* = 0.001). The P170 T/T genotype was associated with a shorter lifespan than the non-T/T genotypes, but not significantly so. None of the three genetic variants was found to be associated with risk of GC (including tumor stage, grade and distant metastasis) or with risk of gastric atrophy. Furthermore, no difference of CD24 expression was found among the genotypes.

**CONCLUSION:** The P-534 site in *CD24* gene affects the overall survival of gastric cancer and may serve as a prognostic marker for gastric cancer.

**Key words:** Gastric cancer; *CD24*; Single nucleotide polymorphisms; Gastric atrophy; Overall survival

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**Core tip**: We evaluated the role of three genetic variants of *CD24* in gastric cancer (GC) risk and prognosis using 679 GC cases and 976 controls. We observed that GC cases with the A/A genotype of P-534 (which lies in the *CD24* promoter) had a significantly shorter survival (hazard ratio, 1.38) especially among patients who lived longer than 2.5 years (hazard ratio, 7.55) after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotherapy. Our study provides the first evidence that P-534 site in *CD24* may serve as a prognostic marker for gastric cancer.

Jia ZF, Wang LZ, Cao XY, Wang C, Cao DH, Wu X, You LL, Jin MS, Wang YP, Zhou BS, Jiang J. *CD24* genetic variants contribute to overall survival in patients with gastric cancer. *World J Gastroenterol* 2015; In press

# INTRODUCTION

Gastric cancer (GC) is one of the most common malignancies and the third cause of cancer-related death worldwide[1]. *Helicobacter pylori* (*H. pylori*) infection has been established to cause GC and was classified as carcinogenic to humans (Group 1) by IARC in 1994[2]. Although over half of the world’s population are estimated to be infected with *H. pylori*[3], relatively few develop GC, and gastric damages induced by *H. pylori* infection vary widely, which together imply a role for host genetic factors in response to chronic *H. pylori* infection and subsequent GC development.

CD24 is a glycosylphosphatidylinositol (GPI)-anchored cell-surface glycoprotein with functions in signal transduction and cell adhesion. It is expressed in a large variety of human malignancies. Over-expression of CD24 results in a more aggressive malignant phenotype with greater proliferative and cell migration capabilities, whereas its down-expression shows a less malignant phenotype[4-6]. CD24 is reportedly associated with tumor growth, invasion, metastasis, recurrence and treatment response in various cancers, including breast cancer[7,8], prostate cancer[9], colorectal cancer[10,11], hepatocellular carcinoma[12], esophageal squamous cell carcinoma[13] and GC[14,15]. CD24 is also a potential marker of cancer stem cells, which possess capabilities for tumorigenesis, self-renewal and producing differentiated progeny[16-18].

In GC, CD24 can mediate carcinogenesis and promote GC progression[14,15]. CD24 expression gradually increases in the progression of normal gastric mucosa, non-atrophic chronic gastritis, chronic atrophic gastritis (CAG), CAG with intestinal metaplasia, dysplasia and finally, GC[14]. Mice with normal *CD24* expression show more gastric inflammation, parietal cell atrophy and gland hyperplasia following *Helicobacter felis* infection compared with *CD24*-null mice which have the genetic background of inbred *CD24*-normal mice[19].

Given the important role of CD24 in GC, we tested whether single nucleotide polymorphisms (SNPs) in the *CD24* gene are associated with genetic susceptibility to GC tumor progression and prognosis in a Chinese population.

# MATERIALS AND METHODS

***Subjects***

From July 2008 to December 2012, patients with histologically diagnosed GC who underwent tumorectomies at the Department of Gastric and Colorectal Surgery of the First Hospital of Jilin University were invited to join this study. Patients with gastric atrophy (GA) and controls with no tumor history were recruited at the Physical Examination Center of the same hospital during the same period. A total of 1766 individuals, 679 GC cases, 111 GA cases and 976 controls signed the informed consent forms and agreed to participate in the study. The study protocol was reviewed and approved by the Ethics Committee of the First Hospital of Jilin University.

Gastric cancer cases were followed-up by telephone calls three months, six months, and one year after each patient’s tumorectomy and every one year thereafter until the end of the study or the death of the patients. Cases would not be included in the survival analysis if (1) they were lost to follow-up by the first telephone interview; or (2) they died of surgical complications in the perioperative period. Survival time was defined as the duration from the date of surgery to the date of death if the patients died, or to the date of the last successful interview if the patients were lost to follow-up or alive until the end of the study. Survival time was right-censored except for patients who died of GC.

Treatment information after surgery was also collected during the follow-up period. Post-operational chemotherapy is defined as at least 3 cycles of chemotherapy received after surgery. One third of the GC patients received this type of therapy. The treatment was classified into three regimens: FOLFOX-4 (combination of 5-fluorouracil, leucovorin and oxaliplatin); XELOX (capecitabine and oxaliplatin) and “other” (such as capecitabine or 5-fluorouracil alone).

***Genotyping***

Blood samples were collected in EDTA tubes and stored at -80 °C until DNA extraction. Genomic DNA was isolated following the protocol provided by the manufacturer (Axygen Biosciences, USA).

The full length of the *CD24* gene was first identified by our previous study and was mapped to Chromosome 6q21 by fluorescence *in situ* hybridization (submitted to NCBI database, accession number FJ226006)[20]. Although SNP data on *CD24* was unavailable in HapMap project or dbVar database, we have identified three linkage disequilibrium (LD) blocks that cover the promoter region and exons 1-2 of the *CD24* locus[20]. Haplotype tagging SNPs (tag SNPs) were identified from the three LD blocks with the pairwise *r*2 ≥ 0.9 and miner allele frequency (MAF) > 0.05.

Three tagging SNPs, P-534C/A, P170C/T and P1527TG/del were genotyped to evaluate the association of *CD24* and genetic susceptibility to GC. P-534C/A is located in the promoter region of *CD24* and is 534bp away from the translation-starting site. P170C/T is located in the coding region of exon 2 and its C-to-T transition leads to an alanine to valine substitution at codon 57 of the CD24 protein. P1527TG/del, 1527bp down from the translation-starting site, is located in the 3’ untranslated region (3’-UTR).

Genotypes of the selected sites were determined by polymerase chain reaction- restriction fragment length polymorphism (PCR-RFLP) as described by Li *et al*[21] and our previous study[20] (Primers used are listed in Table 1 and Figure 1 and were synthesized by Takara, Dalian, China). Briefly, the PCR products of P-534 were digested with endonuclease *Bsr*FI overnight at 37 °C and the restriction site indicated the presence of the C allele (126 bp and 83 bp) (All restriction endonucleases were bought from New England Biolabs, USA). For P170 and P1527, a nested PCR in which one of the primers for the first PCR mapped to an intron was performed to increase specificity as the exon 2 of *CD24* shows high homology with the intronless pseudogenes in Chromosomes 1, 15 and Y[22]. The second PCRs were amplified independently for P170 and P1527 using the 1000-fold-diluted products of the first PCR as templates (All reagents for PCR were from Tiangen, Beijing, China). These PCR products were then digested overnight with the restriction enzyme *Bst*XI (for P170, 37 ℃) and *Bsr*I (for P1527, 65 ℃). The T allele of P170 produced two fragments，275 bp and 129 bp; the TG allele of P1527 produced 645 and 202 bp fragments. All products were separated by electrophoresis on 1.5% agarose gels with ethidium bromide staining and scanned on gel imaging system (Gel DocTM XR+ system, Bio-Rad, USA). Fifty samples were randomly selected to be genotyped by direct sequencing to confirm the validity of PCR-RFLP; three of them with different genotypes were used as positive controls for each PCR-RFLP run. The overall concordance was 100%.

***Testing for H. pylori infection and diagnosis of gastric atrophy***

Serum immunoglobulin G (IgG) antibodies to *H. pylori* were detected by enzyme-linked immunosorbent assay (ELISA) using *H. pylori*-IgG ELISA kits (Biohit, Finland) according to the manufacturer’s protocol. Titers higher than the cut off value of 30 EIU were considered positive for *H. pylori* infection. The inter-day coefficient variations (CV) of the negative and the positive control samples were 4.5% and 1.4%, respectively.

Diagnosis of GA was described elsewhere[23]. Briefly, serum pepsinogen I (PGI) and II (PGII) were quantified by ELISA kits (Biohit, Finland). Individuals with PGI < 82.3 ng/mL and PGI/PGII < 6.05 were diagnosed as GA.

***Immunohistochemistry of CD24***

CD24 expression was assessed in tumor tissue of 131 gastric cancer patients by immunohistochemistry (IHC) method. The detailed procedure is described elsewhere[24]. Briefly, the 4-μm-wide tissue sections were deparaffinized and stained using a streptavidin-biotin immunoperoxidase technique. All slides were then incubated with anti-human CD24 polyclonal antibody (1:100 diluted, sc-7034, Santa Cruz, USA) and developed by 3, 3-diaminobenzidine (DAB). As negative controls, the slides were treated with the IgG isotypes in place of primary antibodies and all negative controls demonstrated negligible background staining. The stained slides were independently evaluated by two pathologists (MSJ and YPW) who were blinded to clinical data and outcomes. The HSCORE system was used to assess the staining results and was calculated by a following equation: (I = 0, 1, 2, 3, Pi = 0-100). The i means the intensity of staining (no staining: 0; weak staining: 1; moderate staining: 2; and strong staining: 3). Pi represents percentages (0-100) of stained cells with intensities. The HSCORE ranges from 0 to 300.

***Statistical analysis***

Continuous data were summarized as medians (25th to 75th percentiles) and compared by Mann-Whitney *U* test or Kruskal-Wallis test. Categorical variables were described as frequencies and percentages and compared using χ2-test. Correlations between SNPs and risk of GC or GA were demonstrated by *P* values and odd ratios (ORs) with 95% confidence intervals (95%CI) compared with the most common genotype of each SNP. The *P* values and ORs with 95%CIs were calculated using the unconditional logistic regression model after adjusting for age, sex and *H. pylori* infection. Survival functions of the GC patients within each SNP were plotted by Kaplan-Meier method and compared by log-rank test using the recessive model. Hazard ratios (HRs) with 95%CIs were used to quantify the influence of genotypes of each SNP on overall survival and were calculated with Cox regression model after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotherapy. For haplotypes with frequencies > 1%, their associations with risk of GC or GA were assessed compared to the most common haplotype using the logistic regression model with the HAPSTAT software 3.0[25]. Unless otherwise stated, analyses were performed in SAS 9.1.3 software (SAS Institute Inc, USA). A two-tailed *P* value < 0.05 was considered to be statistically significant.

# RESULTS

***Subject characteristics***

A total of 679 GC cases, 111 GA cases and 976 tumor-free controls were included in the study. The baseline characteristics of the subjects are summarized in Table 2. The GC group was oldest in the three group and the control group was youngest (median age: 61.0 *vs* 50.0 *vs* 48.5 years, *P* < 0.001 for all pairwise comparisons). And there were more males in the cancer group (71.7% *vs* 59.5% *vs* 59.2%, *P* < 0.001 for comparisons to the atrophy cases or the controls). In the GC group, 67.8% were positive for *H. pylori* infection, significantly higher than the control group (49.7%, *P* < 0.001) but non-significantly lower than the GA group (75.7%, *P* = 0.097). Therefore, comparisons of genotype distribution below were adjusted by age, sex and *H. pylori* infection.

The GC cases were mainly of tubular adenocarcinoma type (82.2%), with low-grade differentiation (68.4%), at TNM stage II (35.1%) or III (34.6%). One third of the cases received chemotherapy after operation (32.8%); 14.7% received FOLFOX-4 and 6.9% received XELOX.

***Association of SNPs with risk of gastric cancer or gastric atrophy***

All of the three SNPs were in Hardy-Weinberg equilibrium in the control group (P-534: *P* = 0.612; P170 *P* = 0.413; P1527: *P* = 0.423). Distributions of genotypes and alleles are listed in Table 3. Compared with the most common genotype of each SNP, no difference was observed for the distributions of the three loci between the GC and control groups after adjusting for age, sex and *H. pylori* infection. No allele or haplotype was associated with risk of GC. Similar negative results were obtained for GA risk (Table 3). Moreover, no associations were observed between SNPs and risk of *H. pylori* infection (data not shown).

***Association of SNPs with clinicopathologic parameters of GC***

Genotypic distributions of SNPs were analyzed by clinicopathologic parameters such as histological type, tumor differentiation, TNM stage and distant metastasis in GC cases. However, no significant association was observed (Table 4).

***Association of SNPs with survival of gastric cancer***

Follow-up information was available for 610 of the 679 GC patients (89.8%). Ten patients died of postoperative complications within 30 d at the beginning of the study period (range: 0-29 d, median: 15.5 d) and these cases were excluded from analyses of effects of SNPs on survival. The median follow-up time for the remaining 600 GC patients was 36.2 mo (range: 2.1-66.7 mo; 95%CI: 34.3-36.5 mo). Two hundred and sixty patients (43.3%) died from GC during the follow-up, 272 patients (45.3%) lived and 68 (11.3%) died of other causes or were lost to follow up.

Survival curves were plotted and compared according to genotypes of each SNP using the recessive model. The patients who carried the A/A genotype of P-534 had shorter survival than those carrying C/C or C/A after adjusting for age, sex, histological type, tumor differentiation, clinical stage and post-operational chemotherapy (HR = 1.38, 95%CI: 1.01-1.88; *P* = 0.042, Table 5, Figure 2A). This trend was more evident in patients who lived longer than 2.5 years (HR = 7.55, 95%CI: 2.16-26.32, *P* = 0.001). Similarly, the P170 T/T carriers tended to have shorter survival time than the C/C or C/T carriers, although not significantly so (Figure 2B). No meaningful correlation could be observed between the variation of “TG” deletion in P1527 and GC survival (*P* = 0.799).

Multivariate Cox regression analysis showed that three other factors—degree of differentiation, TNM stage and post-operational chemotherapy—were associated with the prognosis of GC. Patient whose GC had low-grade differentiation or advanced clinical stage or who did not received post-operational chemotherapy had shorter survival time (Table 5).

***CD24 expressions in tissues of GC***

To assess whether SNPs were associated production of CD24 protein, CD24 expression was evaluated in cancerous tissue of 131 GC cases using IHC. Genotypic distributions of the three SNPs in selected cancer cases were similar to non-selected cases (data not shown). CD24 expression was seen mainly in membranes of tumor cells (Figure 3). However, CD24 expression did not observably differ among genotypes of each SNP (Figure 3, Table 3).

# DISCUSSION

In this study, we explored the association between variants of *CD24* gene and GC. We found that patients who harbored the P-534 A/A genotype tended to have shorter survival than those who carry P-534 non-A/A genotypes.

This is the first study on the association between SNPs of *CD24* gene and GC, as no *CD24* SNPs were included in any genome-wide association studies (GWAS) of GC[26,27]. Distribution of the P-534 genotypes of *CD24* differs slightly from that of Caucasian populations. The minor allele C of P-534 in Caucasian population (37.2%[20]) was the major allele (52.8%) in Han Chinese in our study. Distributions of P170 and P1527 were similar to those of other ethnicities[28,29].

Numerous studies have reported that SNPs of *CD24* gene are correlated with risk of various autoimmune diseases, such as systemic lupus erythematosus (SLE)[28-31]，multiple sclerosis[28,32-35] and inflammatory bowel disease[20,36]. Li *et al*[21] reported that P170 and P1527 of *CD24* affected risk and progression of chronic hepatitis B infection and Sheng *et al*[37] showed that the T/T genotype of P170 correlated with a 2.96-fold increased of risk of hepatocellular carcinoma. In our study, however, we did not observe any influence of *CD24* polymorphisms on risk of *H. pylori* infection (data not shown, but available on request), gastric atrophy, precancerous lesions of GC that are induced partly by *H. pylori* infection[38,39], or GC (Table 3).

Polymorphisms of CD24 have been related to prognosis in several cancers. In breast cancer, CD24 expression was associated with adverse prognosis[7,8] and *CD24* P170 polymorphism could predict response to chemotherapy[40-42]. In esophageal cancer, P170 of *CD24* was involved in regional lymph node metastasis[43]. In our study, we found that P-534 of *CD24* affected long-term survival of GC, as P-534 A/A genotype carriers have a 7.5-fold increased mortality risk compared wirh non-A/A carriers among patients who lived longer than 2.5 years (Figure 2A). P170 T/T carriers also tended to have shorter survival than did non-T/T carriers, although not significantly so (Figure 2B).

The P-534, which is located in the promoter region of CD24, may influence transcriptional activity. Our previous work showed that a hypermorphic haplotype that contained the C allele of P-534 was associated with risk of multiple sclerosis and this haplotype was involved in higher transcriptional activity and increased expression of CD24 in peripheral blood lymphocytes (PBL)[20]. The non-synonymous variant P170 may alter the quantity and quality of CD24. Zhou *et al* found that the P170T/T genotype expressed more cell-surface CD24 than did the C/T or C/C genotypes using flow cytometry, which showed an increased risk and more rapid progression of multiple sclerosis[32]. In our study, we evaluated CD24 expression using IHC and found that CD24 was expressed mainly in the membranes of GC cells. However, we did not observe any differences of CD24 expression among genotypes of P-534, P170 or P1527.

Three limitations should be noted in our study. The first one is that some baseline characteristics such as age are different among the three study groups, and we cannot rule out the possibility that individuals in the control group could develop GC when they are older. However, we control the potential influences to the greatest extent by adjusting for these factors in the multivariate analysis. The second is that the follow-up time of the GC cases seems insufficient because most of cases on the right side of the survival plots are censored. This may explain that although individuals carrying P-534 A/A genotype tended to have shorter observable survival time, *P* value from log-rank tests is not significant. However, when we adjust the potential influencing factors and divide the patients to subgroups who live shorter than 2.5 years and those who live longer than 2.5 years, the association of P-534 with long-term survival is statistically significant (HR 7.5, 95% CI 2.2-26.3). Nonetheless, longer follow-up time is needed to re-evaluate the role of *CD24* SNPs in prognosis of GC. The last one is that we semi-quantified CD24 expression in tissue using IHC and the influence of SNPs on CD24 might be offset, as protein production can be regulated by factors known and unknown *e.g.*, regulations of transcription, post-transcription and translation. Therefore, more rigorous design should be applied in our future study.

In summary, we find that polymorphisms of the *CD24* gene affect the overall survival of GC, as patients who bear the P-534 A/A genotype tend to have shorter survival than do patients with P-534 non-A/A genotypes. However, we do not observe any associations between *CD24* SNPs and risk of *H. pylori* infection, GA or GC. More studies with larger samples and longer follow-up time are needed to clarify the role of CD24 in GC.

# ACKNOWLEDGEMENTS

The authors would like to thank all of those who participated in this study, especially to Ying Song for her work on following-up of subjects. The authors would also like to thank the Biospecimen Bank of the First Hospital of Jilin University for their kindness in supplying the specimens to our research.

# COMMENTS

***Background***

CD24 expression as a potential biomarker is associated with poor prognosis in patients with gastric cancer (GC) but its genetic basis still remains to be elucidated.

***Research frontiers***

GC is one of the most common malignancies and the third cause of cancer-related death worldwide. CD24 mediates gastric carcinogenesis and promotes GC progression. Elucidation of the association between CD24 genetic variants and risk and prognosis of GC may provide novel biomarkers to discriminate individuals with higher risk of GC or to predict prognosis for GC cases.

***Innovations and breakthroughs***

This study for the first time evaluates the effect of CD24 genetic variations in GC carcinogenesis and prognosis. Its results indicate that CD24 variants may serve as a marker for GC prognosis, but not for carcinogenesis.

***Applications***

P-534 site of *CD24* might be used as a prognostic predictive marker for GC.

***Terminology***

CD24 is a glycosylphosphatidylinositol-anchored cell-surface glycoprotein with functions in signal transduction and cell adhesion. CD24 over-expression is associated with a more aggressive malignant phenotype of greater proliferative and migration capability; down-expression shows a less malignant phenotype.

***Peer-review***

This study investigated the role of *CD24* genetic variantsin susceptibility and overall survival of GC and shows that P-534 of *CD24* may serve as an independent prognostic marker for GC.

**REFERENCE****S**

1 **Ferlay J**, Soerjomataram I, Ervik M, Dikshit R, Eser S, Mathers C, Rebelo M, Parkin D, Forman D, Bray F. GLOBOCAN 2012 v1.0, Cancer Incidence and Mortality Worldwide: IARC CancerBase No. 11 [Internet]. Lyon, France: International Agency for Research on Cancer 2013. Available from: URL: http: //globocan.iarc.fr, accessed on 15/01/2015

2 **IARC**. Schistosomes, liver flukes and Helicobacter pylori. IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Lyon, 7-14 June 1994. *IARC Monogr Eval Carcinog Risks Hum* 1994; **61**: 1-241 [PMID: 7715068]

3 **Cao XY**, Jia ZF, Jin MS, Cao DH, Kong F, Suo J, Jiang J. Serum pepsinogen II is a better diagnostic marker in gastric cancer. *World J Gastroenterol* 2012; **18**: 7357-7361 [PMID: 23326145 DOI: 10.3748/wjg.v18.i48.7357]

4 **Naumov I**, Zilberberg A, Shapira S, Avivi D, Kazanov D, Rosin-Arbesfeld R, Arber N, Kraus S. CD24 knockout prevents colorectal cancer in chemically induced colon carcinogenesis and in APC(Min)/CD24 double knockout transgenic mice. *Int J Cancer* 2014; **135**: 1048-1059 [PMID: 24500912 DOI: 10.1002/ijc.28762]

5 **Baumann P**, Cremers N, Kroese F, Orend G, Chiquet-Ehrismann R, Uede T, Yagita H, Sleeman JP. CD24 expression causes the acquisition of multiple cellular properties associated with tumor growth and metastasis. *Cancer Res* 2005; **65**: 10783-10793 [PMID: 16322224 DOI: 10.1158/0008-5472.can-05-0619]

6 **Sagiv E**, Starr A, Rozovski U, Khosravi R, Altevogt P, Wang T, Arber N. Targeting CD24 for treatment of colorectal and pancreatic cancer by monoclonal antibodies or small interfering RNA. *Cancer Res* 2008; **68**: 2803-2812 [PMID: 18413748 DOI: 10.1158/0008-5472.can-07-6463]

7 **Horiguchi K**, Toi M, Horiguchi S, Sugimoto M, Naito Y, Hayashi Y, Ueno T, Ohno S, Funata N, Kuroi K, Tomita M, Eishi Y. Predictive value of CD24 and CD44 for neoadjuvant chemotherapy response and prognosis in primary breast cancer patients. *J Med Dent Sci* 2010; **57**: 165-175 [PMID: 21073135]

8 **Athanassiadou P**, Grapsa D, Gonidi M, Athanassiadou AM, Tsipis A, Patsouris E. CD24 expression has a prognostic impact in breast carcinoma. *Pathol Res Pract* 2009; **205**: 524-533 [PMID: 19243896 DOI: 10.1016/j.prp.2009.01.008]

9 **Wang L**, Liu R, Ye P, Wong C, Chen GY, Zhou P, Sakabe K, Zheng X, Wu W, Zhang P, Jiang T, Bassetti MF, Jube S, Sun Y, Zhang Y, Zheng P, Liu Y. Intracellular CD24 disrupts the ARF-NPM interaction and enables mutational and viral oncogene-mediated p53 inactivation. *Nat Commun* 2015; **6**: 5909 [PMID: 25600590 DOI: 10.1038/ncomms6909]

10 **Sahlberg SH**, Spiegelberg D, Glimelius B, Stenerlöw B, Nestor M. Evaluation of cancer stem cell markers CD133, CD44, CD24: association with AKT isoforms and radiation resistance in colon cancer cells. *PLoS One* 2014; **9**: e94621 [PMID: 24760019 DOI: 10.1371/journal.pone.0094621]

11 **Weichert W**, Denkert C, Burkhardt M, Gansukh T, Bellach J, Altevogt P, Dietel M, Kristiansen G. Cytoplasmic CD24 expression in colorectal cancer independently correlates with shortened patient survival. *Clin Cancer Res* 2005; **11**: 6574-6581 [PMID: 16166435 DOI: 10.1158/1078-0432.ccr-05-0606]

12 **Yang XR**, Xu Y, Yu B, Zhou J, Li JC, Qiu SJ, Shi YH, Wang XY, Dai Z, Shi GM, Wu B, Wu LM, Yang GH, Zhang BH, Qin WX, Fan J. CD24 is a novel predictor for poor prognosis of hepatocellular carcinoma after surgery. *Clin Cancer Res* 2009; **15**: 5518-5527 [PMID: 19706825 DOI: 10.1158/1078-0432.ccr-09-0151]

13 **Sano A**, Kato H, Sakurai S, Sakai M, Tanaka N, Inose T, Saito K, Sohda M, Nakajima M, Nakajima T, Kuwano H. CD24 expression is a novel prognostic factor in esophageal squamous cell carcinoma. *Ann Surg Oncol* 2009; **16**: 506-514 [PMID: 19050962 DOI: 10.1245/s10434-008-0252-0]

14 **Wang YC**, Wang JL, Kong X, Sun TT, Chen HY, Hong J, Fang JY. CD24 mediates gastric carcinogenesis and promotes gastric cancer progression via STAT3 activation. *Apoptosis* 2014; **19**: 643-656 [PMID: 24327257 DOI: 10.1007/s10495-013-0949-9]

15 **Takahashi M**, Nakajima M, Ogata H, Domeki Y, Ohtsuka K, Ihara K, Kurayama E, Yamaguchi S, Sasaki K, Miyachi K, Kato H. CD24 expression is associated with progression of gastric cancer. *Hepatogastroenterology* 2013; **60**: 653-658 [PMID: 23159387 DOI: 10.5754/hge12763]

16 **Zhang C**, Li C, He F, Cai Y, Yang H. Identification of CD44+CD24+ gastric cancer stem cells. *J Cancer Res Clin Oncol* 2011; **137**: 1679-1686 [PMID: 21882047 DOI: 10.1007/s00432-011-1038-5]

17 **Ke J**, Wu X, Wu X, He X, Lian L, Zou Y, He X, Wang H, Luo Y, Wang L, Lan P. A subpopulation of CD24⁺ cells in colon cancer cell lines possess stem cell characteristics. *Neoplasma* 2012; **59**: 282-288 [PMID: 22329848 DOI: 10.4149/neo\_2012\_036]

18 **Li C**, Heidt DG, Dalerba P, Burant CF, Zhang L, Adsay V, Wicha M, Clarke MF, Simeone DM. Identification of pancreatic cancer stem cells. *Cancer Res* 2007; **67**: 1030-1037 [PMID: 17283135 DOI: 10.1158/0008-5472.can-06-2030]

19 **Duckworth CA**, Clyde D, Pritchard DM. CD24 is expressed in gastric parietal cells and regulates apoptosis and the response to Helicobacter felis infection in the murine stomach. *Am J Physiol Gastrointest Liver Physiol* 2012; **303**: G915-G926 [PMID: 22899822 DOI: 10.1152/ajpgi.00068.2012]

20 **Lisiansky V**, Kraus S, Naumov I, Kazanov D, Nabiochtchikov I, Toledano O, Leshno M, Avivi D, Dotan I, Arber N, Moshkowitz M. Role of CD24 polymorphisms in the susceptibility to inflammatory bowel disease. *Int J Biol Markers* 2014; **29**: e62-e68 [PMID: 24557789 DOI: 10.5301/jbm.5000072]

21 **Li D**, Zheng L, Jin L, Zhou Y, Li H, Fu J, Shi M, Du P, Wang L, Wu H, Chen GY, Zheng P, Liu Y, Wang FS, Wang S. CD24 polymorphisms affect risk and progression of chronic hepatitis B virus infection. *Hepatology* 2009; **50**: 735-742 [PMID: 19610054 DOI: 10.1002/hep.23047]

22 **Zhou X**, Cao Y, Luo J, Zeng X. [Association between CD24 polymorphism and genetic susceptibility to breast cancer: a case-control study]. *Zhong Nan Da Xue Xue Bao Yi Xue Ban* 2013; **38**: 1122-1129 [PMID: 24316932 DOI: 10.3969/j.issn.1672-7347.2013.11.007]

23 **Jiang J**, Jia Z, Cao D, Jin MS, Kong F, Suo J, Cao X. Polymorphisms of the DNA methyltransferase 1 associated with reduced risks of Helicobacter pylori infection and increased risks of gastric atrophy. *PLoS One* 2012; **7**: e46058 [PMID: 23049933 DOI: 10.1371/journal.pone.0046058]

24 **Cao X**, Cao D, Jin M, Jia Z, Kong F, Ma H, Wang Y, Jiang J. CD44 but not CD24 expression is related to poor prognosis in non-cardia adenocarcinoma of the stomach. *BMC Gastroenterol* 2014; **14**: 157 [PMID: 25212506 DOI: 10.1186/1471-230x-14-157]

25 **Lin DY**, Zeng D, Millikan R. Maximum likelihood estimation of haplotype effects and haplotype-environment interactions in association studies. *Genet Epidemiol* 2005; **29**: 299-312 [PMID: 16240443 DOI: 10.1002/gepi.20098]

26 **Sakamoto H**, Yoshimura K, Saeki N, Katai H, Shimoda T, Matsuno Y, Saito D, Sugimura H, Tanioka F, Kato S, Matsukura N, Matsuda N, Nakamura T, Hyodo I, Nishina T, Yasui W, Hirose H, Hayashi M, Toshiro E, Ohnami S, Sekine A, Sato Y, Totsuka H, Ando M, Takemura R, Takahashi Y, Ohdaira M, Aoki K, Honmyo I, Chiku S, Aoyagi K, Sasaki H, Ohnami S, Yanagihara K, Yoon KA, Kook MC, Lee YS, Park SR, Kim CG, Choi IJ, Yoshida T, Nakamura Y, Hirohashi S. Genetic variation in PSCA is associated with susceptibility to diffuse-type gastric cancer. *Nat Genet* 2008; **40**: 730-740 [PMID: 18488030 DOI: 10.1038/ng.152]

27 **Shi Y**, Hu Z, Wu C, Dai J, Li H, Dong J, Wang M, Miao X, Zhou Y, Lu F, Zhang H, Hu L, Jiang Y, Li Z, Chu M, Ma H, Chen J, Jin G, Tan W, Wu T, Zhang Z, Lin D, Shen H. A genome-wide association study identifies new susceptibility loci for non-cardia gastric cancer at 3q13.31 and 5p13.1. *Nat Genet* 2011; **43**: 1215-1218 [PMID: 22037551 DOI: 10.1038/ng.978]

28 **Wang L**, Lin S, Rammohan KW, Liu Z, Liu JQ, Liu RH, Guinther N, Lima J, Zhou Q, Wang T, Zheng X, Birmingham DJ, Rovin BH, Hebert LA, Wu Y, Lynn DJ, Cooke G, Yu CY, Zheng P, Liu Y. A dinucleotide deletion in CD24 confers protection against autoimmune diseases. *PLoS Genet* 2007; **3**: e49 [PMID: 17411341 DOI: 10.1371/journal.pgen.0030049]

29 **Sánchez E**, Abelson AK, Sabio JM, González-Gay MA, Ortego-Centeno N, Jiménez-Alonso J, de Ramón E, Sánchez-Román J, López-Nevot MA, Gunnarsson I, Svenungsson E, Sturfelt G, Truedsson L, Jönsen A, González-Escribano MF, Witte T, Alarcón-Riquelme ME, Martín J. Association of a CD24 gene polymorphism with susceptibility to systemic lupus erythematosus. *Arthritis Rheum* 2007; **56**: 3080-3086 [PMID: 17763438 DOI: 10.1002/art.22871]

30 **Piotrowski P**, Lianeri M, Wudarski M, Łacki JK, Jagodziński PP. CD24 Ala57Val gene polymorphism and the risk of systemic lupus erythematosus. *Tissue Antigens* 2010; **75**: 696-700 [PMID: 20230526 DOI: 10.1111/j.1399-0039.2010.01447.x]

31 **Dawidowicz K**, Dieudé P, Avouac J, Wipff J, Hachulla E, Diot E, Tiev K, Cracowski JL, Mouthon L, Amoura Z, Frances C, Carpentier P, Meyer O, Kahan A, Boileau C, Allanore Y. Association study of B-cell marker gene polymorphisms in European Caucasian patients with systemic sclerosis. *Clin Exp Rheumatol* 2011; **29**: 839-842 [PMID: 21961844]

32 **Zhou Q**, Rammohan K, Lin S, Robinson N, Li O, Liu X, Bai XF, Yin L, Scarberry B, Du P, You M, Guan K, Zheng P, Liu Y. CD24 is a genetic modifier for risk and progression of multiple sclerosis. *Proc Natl Acad Sci U S A* 2003; **100**: 15041-15046 [PMID: 14657362 DOI: 10.1073/pnas.2533866100]

33 **Goris A**, Maranian M, Walton A, Yeo TW, Ban M, Gray J, Dubois B, Compston A, Sawcer S. CD24 Ala/Val polymorphism and multiple sclerosis. *J Neuroimmunol* 2006; **175**: 200-202 [PMID: 16631259 DOI: 10.1016/j.jneuroim.2006.03.009]

34 **Otaegui D**, Sáenz A, Camaño P, Blázquez L, Goicoechea M, Ruíz-Martínez J, Olaskoaga J, Emparanza JA, López de Munain A. CD24 V/V is an allele associated with the risk of developing multiple sclerosis in the Spanish population. *Mult Scler* 2006; **12**: 511-514 [PMID: 16900767]

35 **Ronaghi M**, Vallian S, Etemadifar M. CD24 gene polymorphism is associated with the disease progression and susceptibility to multiple sclerosis in the Iranian population. *Psychiatry Res* 2009; **170**: 271-272 [PMID: 19896210 DOI: 10.1016/j.psychres.2009.01.002]

36 **Diaz-Gallo LM**, Medrano LM, Gómez-García M, Cardeña C, Rodrigo L, Mendoza JL, Taxonera C, Nieto A, Alcain G, Cueto I, López-Nevot MA, Urcelay E, Martin J. Analysis of the influence of two CD24 genetic variants in Crohn's disease and ulcerative colitis. *Hum Immunol* 2011; **72**: 969-972 [PMID: 21684315 DOI: 10.1016/j.humimm.2011.05.028]

37 **Sheng L**, Shui Y. Clusters of differentiation 24 polymorphism and hepatocellular carcinoma. *Hepatology* 2011; **54**: 2273; author reply 2273-2274 [PMID: 21932403 DOI: 10.1002/hep.24676]

38 **Matysiak-Budnik T**, Mégraud F. Helicobacter pylori infection and gastric cancer. *Eur J Cancer* 2006; **42**: 708-716 [PMID: 16556496 DOI: 10.1016/j.ejca.2006.01.020]

39 **Kabir S**. Effect of Helicobacter pylori eradication on incidence of gastric cancer in human and animal models: underlying biochemical and molecular events. *Helicobacter* 2009; **14**: 159-171 [PMID: 19702845 DOI: 10.1111/j.1523-5378.2009.00677.x]

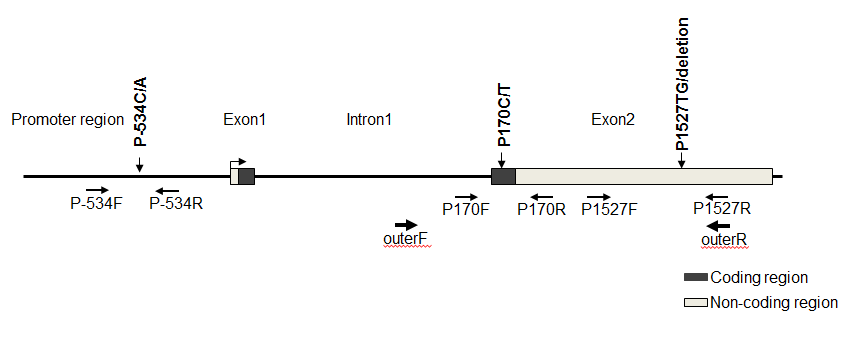
40 **Marmé F**, Werft W, Walter A, Keller S, Wang X, Benner A, Burwinkel B, Sinn P, Hug S, Sohn C, Bretz N, Moldenhauer G, Rupp C, Rupp AK, Biakhov MY, Bottini A, Friedrichs K, Khailenko VA, Manikhas GM, Ruiz A, Sánchez-Rovira P, Santoro A, Segui MA, Villena C, Lichter P, Kristiansen G, Altevogt P, Schneeweiss A. CD24 Ala57Val polymorphism predicts pathologic complete response to sequential anthracycline- and taxane-based neoadjuvant chemotherapy for primary breast cancer. *Breast Cancer Res Treat* 2012; **132**: 819-831 [PMID: 21960110 DOI: 10.1007/s10549-011-1759-9]

41 **Zhou X**. CD24 polymorphisms cannot predict pathologic complete response to anthracycline- and taxane-based neoadjuvant chemotherapy in breast cancer. *Clin Breast Cancer* 2014; **14**: e33-e40 [PMID: 24393851 DOI: 10.1016/j.clbc.2013.11.001]

42 **Buck K**, Hug S, Seibold P, Ferschke I, Altevogt P, Sohn C, Schneeweiss A, Burwinkel B, Jäger D, Flesch-Janys D, Chang-Claude J, Marmé F. CD24 polymorphisms in breast cancer: impact on prognosis and risk. *Breast Cancer Res Treat* 2013; **137**: 927-937 [PMID: 23314606 DOI: 10.1007/s10549-012-2325-9]

43 **Sadot E**, Kraus S, Stein M, Naboishchikov I, Toledano O, Kazanov D, Arber N, Kashtan H. CD24 gene polymorphism-a novel prognostic factor in esophageal cancer. *Int J Biol Markers* 2014; **29**: e49-e54 [PMID: 24474454 DOI: 10.5301/jbm.5000071]

**P-Reviewer:** Zhang J **S-Editor:** Yu J **L-Editor:** **E-Editor:**



**Figure 1 Human *CD24* gene.** Primers used were demonstrated by arrows.

**C:\Users\baishideng-2014\Desktop\revised-jyu\3-25\19515\19515-Figures\19515-Figure2.tif**

**Figure 2 Survival plots of gastric cancer patients.** A: Plot for P-534 using the recessive model (A/A *vs* C/C + C/A). B: Plot for P170 using the recessive model (T/T *vs* C/C + C/T).

C:\Users\baishideng-2014\Desktop\revised-jyu\3-25\19515\19515-Figures\19515-Figure3.tif

**Figure 3 Expression of CD24 by immunohistochemistry.** A: expression of CD24 from a gastric cancer case with P-534 AA, P170 CC and P1527 TG/TG genotypes; B: expression of CD24 from a gastric cancer case with P-534 CC, P170 TT and P1527 TG/TG genotypes.

**Table 1 Primer sequences used for polymerase chain reaction**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Primer** | **Sequence** | **Length of product** | **Endonucleases** | **Bands** |
| P-534F | 5’-AGAGATAACCCTGCCCGAG-3’ | 209 bp | BsrFI | C: 126 bp + 83 bp |
| P-534R | 5’-CCAAGTTTCCTTTGTTTCCC-3’ |
| outerF | 5’-CCACTTGGCATTTTTGAGGCATCT-3’ | 1882 bp | - | - |
| outerR | 5’-TGTGTCGAGGCAGTTGTAAAAG-3’ |
| P170F | 5’-ctaaagagaatgaccttggtgggttgag-3’ | 404 bp | BstXI | T: 275 bp + 129 bp |
| P170R | 5’-GGATTGGGTTTAGAAGATGGGGAAA-3’ |
| P1527F | 5’-GCCAGGGCAATGATGAATGAG-3’ | 847 bp | BsrI | TG: 645 bp + 202 bp |
| P1527R | 5’-TGTGTCGAGGCAGTTGTAAAAGAT-3’ |

**Table 2 Characteristics of subjects included *n* (%)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | **Cancer** | **Atrophy** | **Controls** | ***P* value** |
| *n* | 679 | 111 | 976 |  |
| Gender |  |  |  |  |
| Male | 487 (71.7) | 66 (59.5) | 578 (59.2) | < 0.0001 |
| Female | 192 (28.3) | 45 (40.5) | 398 (40.8) |  |
| Age (yr) | 61.0 (54.0-70.0) | 50.0 (47.0-57.0) | 48.5 (44.0-55.0) | < 0.0001 |
| ≤ 55 | 204 (30.0) | 76 (68.5) | 756 (77.5) | < 0.0001 |
| 55-70 | 310 (45.7) | 28 (25.2) | 181 (18.5) |  |
| > 70 | 165 (24.3) | 7 (6.3) | 39 (4.0) |  |
| *H. pylori* |  |  |  |  |
| Positive | 453 (67.8) | 84 (75.7) | 478 (49.7) | < 0.0001 |
| Negative | 215 (32.2) | 27 (24.3) | 483 (50.3) |  |
| Differentiation |  |  |  |  |
| Poor | 437 (68.4) |  |  |  |
| Moderate to well | 202 (31.6) |  |  |  |
| Pathologic type |  |  |  |  |
| Tubular adenocarcinoma | 545 (82.2) |  |  |  |
| Signet ring cell | 50 (7.5) |  |  |  |
| other | 68 (10.3) |  |  |  |
| TNM stage |  |  |  |  |
| I | 97 (15.0) |  |  |  |
| II | 227 (35.1) |  |  |  |
| III | 224 (34.6) |  |  |  |
| IV | 99 (15.3) |  |  |  |
| Distant metastasis |  |  |  |  |
| Positive | 573 (85.3) |  |  |  |
| Negative | 99 (14.7) |  |  |  |
| Post-operational Chemotherapy |  |  |  |  |
| No | 456 (67.2) |  |  |  |
| FOLFOX-4 | 100 (14.7) |  |  |  |
| XELOX | 47 (6.9) |  |  |  |
| Other | 76 (11.2) |  |  |  |

Data are presented as frequency counts (percentage of total) or median (25th to 75th percentiles).

**Table 3 Distributions of genotypes in three groups *n* (%)**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
|  | **Controls** | **Cancer** | ***P* value** | **OR1 (95%CI)** | **Atrophy** | ***P* value** | **OR1 (95%CI)** |
| P-534 |  |  |  |  |  |  |  |
| C/C | 271 (27.8) | 181 (26.7) | - | Reference | 29 (26.1) | - | Reference |
| C/A | 488 (50.0) | 358 (52.7) | 0.099 | 1.1 (0.86-1.48) | 62 (55.9) | 0.204 | 1.2 (0.74-1.91) |
| A/A | 217 (22.2) | 140 (20.6) | 0.145 | 0.9 (0.61-1.20) | 20 (18.0) | 0.328 | 0.8 (0.46-1.54) |
| C | 1030 (52.8) | 720 (53.0) | 0.886 | - | 130 (54.1) | 0.716 | - |
| A | 922 (47.2) | 638 (47.0) | 1.0 (0.86-1.14) | 102 (45.9) | 0.9 (0.72-1.25) |
| P170 |  |  |  |  |  |  |  |
| C/C | 419 (42.9) | 295 (43.4) | - | Reference | 60 (54.1) | - | Reference |
| C/T | 439 (45.0) | 308 (45.4) | 0.277 | 1.1 (0.82-1.38) | 37 (33.3) | 0.082 | 0.6 (0.40-0.96) |
| T/T | 118 (12.1) | 76 (11.2) | 0.369 | 0.9 (0.60-1.29) | 14 (12.6) | 0.724 | 0.9 (0.47-1.65) |
| C | 1277 (65.4) | 898 (66.1) | 0.673 | - | 157 (70.7) | 0.114 | - |
| T | 675 (34.6) | 460 (33.9) | 1.0 (0.83-1.12) | 65 (29.3) | 0.8 (0.58-1.06) |
| P1527 |  |  |  |  |  |  |  |
| TG/TG | 826 (84.6) | 559 (82.3) | - | Reference | 95 (85.6) | - | Reference |
| TG/del | 142 (14.6) | 117 (17.2) | 0.069 | 1.3 (0.97-1.82) | 15 (13.5) | 0.940 | 0.9 (0.50-1.59) |
| del/del | 8 (0.8) | 3 (0.4) | 0.162 | 0.4 (0.09-1.77) | 1 (0.9) | 0.935 | 0.9 (0.10-7.30) |
| TG | 1794 (91.9) | 1235 (90.9) | 0.956 | - | 205 (92.3) | 0.821 | - |
| del | 158 (8.1) | 123 (9.1) | 1.1 (0.88-1.45) | 17 (7.7) | 0.9 (0.56-1.58) |
| Haplotype2 |  |  |  |  |  |  |  |
| ACTG | 45.6 | 47.0 | - | Reference | 45.4 | - | Reference |
| CTTG | 33.6 | 33.7 | 0.946 | 1.0 (0.85-1.16) | 28.7 | 0.407 | 0.9 (0.63-1.21) |
| CCTG | 11.4 | 10.2 | 0.382 | 0.9 (0.71-1.14) | 17.7 | 0.072 | 1.6 (0.99-2.37) |
| CCdel | 8.0 | 9.1 | 0.370 | 1.1 (0.87-1.46) | 7.7 | 0.919 | 1.0 (0.56-1.67) |

Data are presented as frequency counts (percentage of total). 1ORs of the genotypes were calculated adjusting for age, sex and *H. pylori* infection in logistic regression model; 2The haplotype was lined with P-534, P170 and P1527 and displayed as percentage.

**Table 4 Distributions of genotypes according to clinical parameters in gastric cancer cases**

|  |  |  |  |  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- | --- |
|  | **P534** | | | ***P* value** | **P170** | | | ***P*** | **P1527** | | ***P* value** |
| **C/C** | **C/A** | **A/A** | **C/C** | **C/T** | **T/T** | **TG/TG** | **TG/del** |
| *n* | 181 | 358 | 140 |  | 295 | 308 | 76 |  | 559 | 120 |  |
| Age | 60 (53-70) | 61 (54-71) | 61 (55-70) | 0.715 | 61 (53-71) | 61 (54-70) | 63 (56-70) | 0.658 | 61 (54-70) | 60 (51-71) | 0.307 |
| Sex |  |  |  |  |  |  |  |  |  |  |  |
| Male | 27.3 | 50.3 | 22.4 | 0.092 | 43.9 | 44.2 | 11.9 | 0.485 | 83.6 | 16.4 | 0.172 |
| Female | 25.0 | 58.9 | 16.1 | 42.2 | 48.4 | 9.4 | 79.2 | 20.8 |
| *H. pylori* |  |  |  |  |  |  |  |  |  |  |  |
| Positive | 26.3 | 53.4 | 20.3 | 0.905 | 42.6 | 46.1 | 11.3 | 0.769 | 82.3 | 17.7 | 0.996 |
| Negative | 27.0 | 51.6 | 21.4 | 45.6 | 43.7 | 10.7 | 82.3 | 17.7 |
| Differentiation |  |  |  |  |  |  |  |  |  |  |  |
| Poor | 24.7 | 55.8 | 19.5 | 0.070 | 43.5 | 47.4 | 9.2 | 0.055 | 81.5 | 18.5 | 0.500 |
| Moderate to well | 30.2 | 46.0 | 23.8 | 45.0 | 40.1 | 14.8 | 83.7 | 16.3 |
| TNM stage |  |  |  |  |  |  |  |  |  |  |  |
| Ⅰ-Ⅱ | 26.2 | 52.8 | 21.0 | 0.999 | 45.1 | 44.7 | 10.2 | 0.695 | 82.4 | 17.6 | 0.986 |
| Ⅲ-Ⅳ | 26.3 | 52.6 | 21.1 | 42.7 | 45.2 | 12.1 | 82.4 | 17.6 |
| Pathologic type |  |  |  |  |  |  |  |  |  |  |  |
| Tubular adenocarcinoma | 26.4 | 52.3 | 21.3 | 0.754 | 45.0 | 43.9 | 11.2 | 0.173 | 82.8 | 17.2 | 0.751 |
| Signet ring cell | 26.0 | 60.0 | 14.0 | 34.0 | 60.0 | 6.0 | 80.0 | 20.0 |
| Other | 27.9 | 50.0 | 22.1 | 38.2 | 47.1 | 14.7 | 85.3 | 14.7 |
| Distant metastasis |  |  |  |  |  |  |  |  |  |  |  |
| Positive | 32.3 | 48.5 | 19.2 | 0.323 | 40.4 | 44.4 | 15.1 | 0.374 | 79.8 | 20.2 | 0.454 |
| Negative | 25.1 | 53.9 | 20.9 | 44.3 | 45.2 | 10.5 | 82.9 | 17.1 |
| CD24 staining | 60 (0-120) | 60 (0-120) | 100 (20-140) | 0.215 | 60 (0-120) | 60 (20-120) | 40 (0-100) | 0.482 | 60 (0-120) | 60 (40-100) | 0.922 |

Data are presented as percentage of total or median (25th to 75th percentiles).

**Table 5 Results of multivariate Cox regression analysis**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  |  | ***P* value** | **HR** | **95%CI** |
| P534 | C/C+C/A | - | 1.00 | - |
|  | A/A | 0.0416 | 1.38 | 1.01-1.88 |
| Age |  | 0.1875 | 1.01 | 1.00-1.02 |
| Sex | Male | - | 1.00 | - |
|  | Female | 0.2315 | 0.83 | 0.61-1.13 |
| Differentiation | Moderate to well | - | 1.00 | - |
|  | Poor | 0.0089 | 1.50 | 1.11-2.04 |
| Pathologic type | Tubular adenocarcinoma | - | 1.00 | - |
|  | Signet ring cell | 0.4750 | 0.83 | 0.50-1.39 |
|  | Other | 0.9469 | 1.01 | 0.67-1.53 |
| TNM stage | I | - | 1.00 | - |
|  | II | 0.0003 | 5.50 | 2.18-13.86 |
|  | III | < 0.0001 | 22.29 | 9.05-54.89 |
|  | IV | < 0.0001 | 32.12 | 12.63-81.70 |
| Chemotherapy | No | - | 1.00 | - |
|  | FOLFOX-4 | 0.0195 | 0.64 | 0.45-0.93 |
|  | XELOX | 0.0021 | 0.43 | 0.25-0.74 |
|  | Other | 0.0004 | 0.45 | 0.29-0.70 |