Research Article

Evaluation of CD86 rs17281995Gene Polymorphism in Gastric and Intestinal Cancer Subjects in Tehran and Khorramabad Cities

Masoumeh Aalipour [1], Asghar Aalipour [2], Mohammad Mousaei Ghasroldasht [1 *]

[1] *Department of Genetics, Faculty of Biological Sciences and Technology, Shahid Ashrafi Esfahani, Esfahan, Iran*
[2] *Department of Pathology, School of Medicine, Lorestan University of Medical Sciences, Khorramabad, Iran*

Received 26 May 2022 Accepted 16 October 2022

Abstract

Colorectal cancer (CRC) and gastric cancer (GC) are multifactorial diseases likely influenced by genetic susceptibility. Gastric cancer is also the fourth most common cancer in the world and the second leading cause of cancer-related mortality. CD86 (B7-2) is a costimulatory molecule found on antigen-presenting cells (APCs) that is important in autoimmune, transplantation, and tumor immunity. This protein is expressed in the immune system cells and is involved in the pathogenesis of various inflammatory disorders and inflammation. Rs17281995 polymorphism is located in section 3' UTR, and given the regulatory role of 3' UTR gene sequences, SNPs located in these regions can affect the expression and function of the corresponding protein. In the present study, the relationship between rs17281995 polymorphism located in the 3' UTR regulatory region of the CD86 gene sequence and the risk of colorectal and gastric cancer in Iranian patients was analyzed. Polymorphism was identified in 26 patients with colorectal cancer, 30 patients with gastric cancer, and 36 healthy controls using the high-resolution DNA melting curve analysis (HRM) technique. The Data was then analyzed using SPSS software. There was no significant relationship between rs17281995 polymorphism and colorectal ($P = 0.75$) and gastric cancers ($P = 0.97$) in the Iranian population. In addition, genotypic distribution analysis showed no significant difference between the patient and control groups ($P>0.05$). Among people with colorectal cancer, 0.577 had the G allele and 0.423 had the C allele. In the control group, 0.639 had the G allele and 0.361 had the C allele. In conclusion, our data indicate that the CD86 rs17281995 gene polymorphism does not seem to be a risk factor for colorectal and gastric cancers in the Iranian population.

***Keywords*:** Polymorphism, CD86, HRM, rs17281995, SNP

Introduction

Colorectal cancer (CRC) is globally the third most typical cancer (Shademan et al., 2022). Likewise, the incidence of colorectal cancer in the elderly Iranian population is lower than that of Western societies. Yet, the incidence of this type of cancer at a young age is high and rising (Arani & Kerachian, 2017). Gastric cancer (GC) is also the fourth most common cancer in the world and the second leading cause of cancer-related mortality (Jemal et al., 2011). Cancer is a multifactorial disease in which both environmental and genetic factors are involved (Poorolajal et al., 2020). The main components of cancer-causing genetic factors are mutations and polymorphisms that function by changing the proteins' expression or functioning(Landi et al., 2008). Molecular epidemiological research indicates that genetic changes such as single-nucleotide polymorphism can greatly influence the risk of developing diseases, specifically cancer(Di Pasqua et al., 2009). Given the effective role of the immune system in the fight against diseases and malignancies, today's studies utilize immune profiles and the involved genes to select the appropriate marker (Fang et al., 2014; Koido et al., 2013). The most important immunological genes expressed on the surface of colon epithelial cells are B7.2 costimulatory molecules (CD86).

CD86 (B7-2) is a costimulatory molecule found on antigen-presenting cells (APCs) that is important in autoimmune, transplantation, and tumor immunity. Through the CD28/CTLA-4 pathway, CD86 influences T-cell responses. Tumor cells deficient in B7-1 and B7-2 are unable to produce the costimulatory signals required for T-cell activation, resulting in T-cell anergy and immunological inactivity. As a result, alterations in CD86 may have an impact on how this disease develops(Hu et al., 1999; Ohtani, 2007). Eight exons make up the CD86

*Corresponding author's e-mail address: *Mghasroldasht@gmail.com*

gene on chromosome 3q21. The exon 8 and 3' untranslated region regulatory domain polymorphisms rs1129055 (+1057 G>A) and rs17281995 (+2379G>C), respectively, modulate the degree of protein kinase C phosphorylation of the CD86 cytoplasmic tail. The polymorphisms rs1129055 and rs17281995 in the CD86 gene have been extensively studied. Since then, the initial publication on the link between rs17281995 and the incidence of sporadic colorectal cancer in Caucasians was released in 2008 (Landi et al., 2008). Geng et al. (2014) demonstrated that rs17281995 and rs1129055 mononucleotide polymorphisms could cause changes in CD86 gene expression, ultimately raising the risk of malignancy and colorectal cancer in individuals (Geng et al., 2014).

Few studies have been conducted on multiple polymorphisms of CD86 and +2379 G / C genes with the risk of CRC development in the world and, moreover, little is known about the frequency of rs17281995 polymorphisms of CD86 gene in Iranian patients with gastric cancer. Research has established that single-nucleotide polymorphisms can lead to significant alteration of gene pathways within cancer cells. Consequently, early diagnosis of these polymorphisms can pave the way for early detection and prognosis of cancer. The role of the CD86 gene in various diseases and cancer occurrences has been investigated, yet accurate and comprehensive information on rs17281995 polymorphism in patients with colorectal cancer is not available. Thus, the present study tries to examine the relationship between CD86 rs17281995 gene polymorphism and its frequency distribution in target populations.

Materials and Methods

Participants

This study includes 30 (20 men and 10 women) and 26 (13 women and 13 men) formalin-fixed, paraffin-embedded (FFPE) blocks of tissue for gastric cancer and intestine cancer, that were selected from Imam Reza (AS) hospitals located in Tehran and Shahid Rahimi hospitals located in Khorramabad. Of all the FFPE blocks of tissue, those selected that were collected through biopsy or surgery, and their tumor stage was confirmed by the pathologist after histological and pathological examinations were performed. Control samples consisted of 18 (9 males and 9 females) FFPE blocks of tissue without cancer diagnosed. Healthy control samples were selected from those who experienced biopsy, and their test results were non-cancerous and negative.

DNA extraction and determination of its quality

After preparing tissue samples from healthy and sick people, genomic DNA was extracted by the Genomic DNA Extraction kit (Genet Bio) from paraffin tissue samples, according to the manufacturer's instructions. In order to evaluate the purity and concentration of the obtained DNA, absorption measurements were performed with a spectrophotometer (Eppendorf) at wavelengths of 260 and 280 nm.

Polymerase chain reaction (PCR method)

After DNA extraction, a PCR test was performed to amplify the preferred oligonucleotide sequence. To amplify the gene, the CD86 gene was first extracted by searching the NCBI-specific site. The sequence of this gene was entered into OLIGO Primer Analysis Software and a pair of specific primers were designed. In the subsequent step, to determine the specificity of the primers, their sequences were examined by NCBI Primer-BLAST. A desired 200 bp DNA fragment in the Beta actin gene was amplified by a polymerase chain reaction by a thermocycler. After the PCR reaction, the PCR products, which are 200 bp in length, were transferred to a 1.5% agarose gel along with a DNA marker with a molecular weight of 50 bp. After the electrophoresis technique was performed and the DNA bands were separated, the gel was photographed using Gel Doc.

Preparation of electrophoresis 1x TBE buffer

In this study, the buffer was employed both to create the gel and to establish the electric current in the electrophoresis tank. The buffer utilized for the agarose gel in the electrophoresis tank was buffer X1. The 1x TBE buffer (Aron Gene) has low ionic strength, which was first purchased as the 10x. To make 1000 ml 1x TBE buffer, about 100 ml 10x TBE buffer was dissolved in 900 ml of distilled water and diluted to make 1x TBE buffer. In this study, 1.5% gel electrophoresis was utilized. After the electrophoresis technique was performed and DNA bands were separated, the gel was photographed using Gel Doc (Compact, ATP).

Performing the melting curve analysis (HRM Method) on the sample of patients in the Study

Primer Design

In this study, CD86 gene polymorphism was assessed. In order to design a specific primer, the

nucleotide sequence of the CD86 gene was first collected from the NCBI site. Then it was checked if the two sequences were matched using the BLAST server in NCBI. The required primer design to amplify the region around the target SNP was first developed by OLIGO Primer Analysis Software. Additionally, to ensure that only the desired fragment was amplified, not attached elsewhere in the genome, their accuracy and uniqueness were confirmed using Primer 3 and Primer BLAST. Table 1 indicates the primer sequence used in the PCR reaction.

Primer Preparation

The primers were collected by Pishgam Company and received as lyophilized. According to the manufacturer's protocol, adding distilled water increased the primer's concentration to 100 pmd / ml. Stock and working solutions were both kept at -20 ° C.

HRM Test

This method is a homogeneous and new method after PCR amplification that is performed in a closed tube. The HRM technique enables the analysis of genetic changes (SNPs, mutations, and methylations) in PCR products. HRM differentiates nucleic acid samples based on GC sequence, length, and volume. HRM analysis includes the amplification of the desired gene in 80-250 bp fragments in a reaction that contains a dye that binds to fluorescent double-stranded DNA.

After distributing the mixture to the strip, the required amount of DNA was added to each strip from different samples. Each time an HRM was performed, a strip was considered as a negative control, implying that the DNA sample was not contaminated. The final volume of the reaction mixture in each strip was considered to be 25µl. After mixing the ingredients, including Eva Green (5 µl), DNA (18/57µl), Primer Forward, and Reverse (1/67 pmol/µlit) in the microtube, the microtube was placed in the Rotor-Gene Q device. According to the following program, firstly the samples were exposed to 95 °C for 15 min and then 45 cycles with 95 °C for 15 seconds and 60 °C for 20 seconds. Finally, the graphs were illustrated in temperatures ranging from 65 to 95 °C with a decreased temperature of 0.1 °C.

Statistical Analysis

SPSS software was employed to perform statistical analysis on the data when p-values of less than 0.05 were considered significant. It should be noted that the tests performed for this project were conducted in two independent experiments.

Results

Characteristics of Colorectal Cancer Specimens

In this study, a total of 44 people were studied, of whom 26 (13 men and 13 women) had colorectal cancer and 18 (nine men and nine women) were healthy individuals who were considered as controls. The subjects' ages ranged from 31 to 93 years. Most people with colorectal cancer were over 62 years old, and most of the subjects had moderate differentiation with PT between 3 and 4.

Characteristics of Gastric Cancer Specimens

In this study, a total of 48 people were studied, of which 30 (20 men and 10 women) had gastric cancer and 18 (nine men and nine women) were healthy individuals who were considered controls. The subjects' ages ranged from 44 to 85 years. Most people with gastric cancer were over 58 years old. Most patients had moderate differentiation and showed PT between 3 and 4. The results of the genotypic CD86 gene (rs17281995 polymorphism) in colorectal cancer patients and the control group are shown in Table 2.

After performing electrophoresis and separating the DNA bands from each other, the gel was photographed using the Gel Doc device. According to Figure 1, it is clear that the bright single bands of DNA, with a length of 200 bp, indicate the quality of the PCR product.

Statistical analysis of genotypic data revealed that the p-value was equal to 0.75. Thus, no significant difference was observed in the genotypic distribution of the patient and control groups (P>0.05). After the genotypic analysis was performed, allelic abundance was also discussed. Among people with colorectal cancer, 0.577 had the G allele and 0.423 had the C allele. In the control group, 0.639 had the G allele and 0.361 had the C allele. The bar graph of rs17281995 allelic frequency distribution in patients with colorectal and a control cancer is shown in Figure 2.

The p-value was equal to 0.55; hence, there was no significant difference in the allelic distribution of the patient and control groups (P>0.05). The results of genotypic polymorphism rs17281995 of CD86 gene in gastric cancer patients and the control group are examined in Table 3.

As shown by the table above, the p-value was equal to 0.97. Thus, no significant difference was observed in the genotypic distribution of the patient and control groups (P>0.05). After genotypic analysis, allelic distribution was also discussed. Among patients with gastric cancer, 0.533 had the G allele and 0.467 had the C allele. In the control group,

0.528 had the G allele and 0.472 had the C allele. The bar graph of rs17281995 allelic distribution in patients with gastric and control cancers is shown in Figure 3.

The p-value was equal to 0.95, so there was no significant difference in the allelic distribution of patients and controls (P>0.05).

Table 1. Sequence and characteristics of the primer used to amplify the studied gene polymorphism.

Product Length (PCR)	Primer Sequence	Annealing Temperature	SNP ID
162	F:CCTACAGATGTCCTACGGGAA R:TAGTGATCCCACCTTAGAGCC	60 ℃	rs17281995

Table 2. Genotype and allele frequency of the rs17281995 polymorphism in patients with colorectal cancer and the control group.

			SNP			P-value
			CC	GC	GG	0.75
Group	Control	**Count**	1	11	6	
		% Within group	5.6%	61.1%	33.3%	
	Case	**Count**	3	16	7	
		% Within group	11.5%	61.5%	29.6%	

Discussion

Nowadays, gastrointestinal cancers, particularly gastric cancer and colorectal cancer are the leading causes of cancer-related death globally (Forat-Yazdi et al., 2015; Namazi et al., 2018; Namazi et al., 2017). The CRC and GC can be developed and progress in complex ways through the interaction of multiple genetic and environmental factors. The aim of this study was to determine the CD86 rs17281995 gene polymorphism in gastric and intestinal cancer subjects in the Iranian population. The results indicated that the frequency of G and C alleles did not differ between patients with colorectal cancer and that it is not a risk factor. Regarding the genotypic frequency of CD86, the results showed that the frequency of GG, GC, and CC genotypes differed between people with colorectal cancer and the control group, but it was not significant (p=0.75). This could be due to the small population of our study. Inconsistent with the results of the present study is a study performed in Iran, showing that the CC genotype and C allele in rs17281995 polymorphism are associated with the risk of intestinal cancer. This study revealed that CC genotype and C allele occur more often in female patients based on gender status (Azimzadeh et al., 2013). The reason for the difference between the results in this study and those of the present study could be due to the small population of our study. A meta-analysis by War et al. showed that the rs17281995 polymorphism was significantly associated with an increased risk of cancer, particularly colorectal cancer, in the Caucasus population, yet no significant association was observed in other Asian populations. A valid reason for this discrepancy could be the significantly different sizes of the studies (2075 vs. 781) because one of the conditions needed for the results of the sample size to be real is that the larger the sample size is, the more generalizable it is to the whole population(Geng et al., 2014). The most important genes expressed on the surface of the epithelial cells of the gastrointestinal tract are CD80 and CD86, co-stimulatory molecules of the B7 family, i.e., membrane-bound molecules that play an important role in activating the immune system, especially T lymphocytes. Lack of B7 molecules leads to decreased stimulation of T lymphocytes, especially $CD4^+$. It is important when cancer cells can escape the attacks by the immune system and T lymphocytes, which are responsible for destroying cancer cells, fail to be stimulated by them (Lenschow et al., 1996; van der Merwe et al., 1997). The results of the rs17281995 polymorphism in patients with gastric cancer are as follows: the frequency of G and C alleles is not different between patients with gastric cancer and those in the control group, thus it

is not a risk factor. As regards the genotypic frequency of CD86, the results indicated that the frequency of GG, GC, and CC genotypes did not differ between gastric cancer and control subjects. A meta-analysis by Zhuang et al. suggests that polymorphism of 592C> A in the interleukin 10 (IL-10) promoter may be associated with an increased risk of GC among Asians(Zhuang et al., 2010). Yet in another study, an association was seen between the IL-17F A7488G polymorphism and gastric cancer (Wu et al., 2010). IL-17F 7488 polymorphism has previously been indicated to be associated with increased inflammation in Helicobacter pylori infection(Arisawa et al., 2007). Saeki et al. explained that the rs4072037 polymorphism of mucin 1 (MUC1) increases the risk of GC, primarily the diffuse type (Saeki et al., 2011). The authors established that rs4072037 plays an important role in regulating transcription as well as selecting the MUC1 split site. However, no study has been conducted thus far to investigate the association of the rs17281995 polymorphism with gastric cancer. Hence, this study was the first to do so. Molecular epidemiological studies have described some relatively common genetic variations, namely single nucleotide polymorphism (SNP), as biomarkers for genetic susceptibility to GC progression (Canedo et al., 2008; Milne et al., 2009; Yin et al., 2009). These genetic variations may modulate the effects of exposure to environmental factors by regulating several biological pathways during gastric cancer. Genetic variations in inflammation-related genes, particularly cytokines and their receptors, are thought to play an important role in tumor development and progression (Canedo et al., 2008; González et al., 2002; Machado et al., 2003). Therefore, the role of genetic polymorphisms in the risk of GC has led to an increase in studies in recent years.

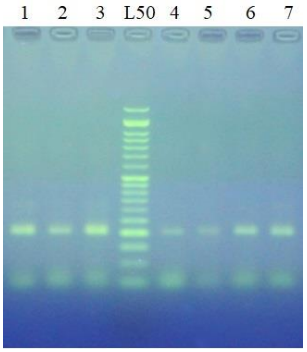

Figure 1. The electrophoresis PCR products of CD86 (200 pb) on 1.5% agarose gel. Column L50: marker 50bp, columns 1-7: PCR product.

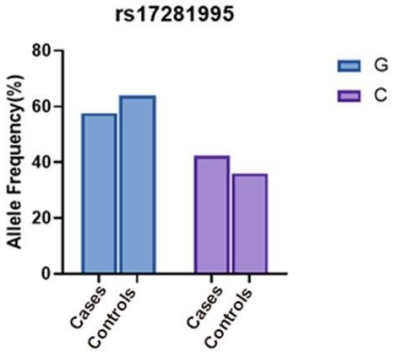

Figure2. Bar graph for allelic distribution of rs17281995 in colorectal cancer patients and controls.

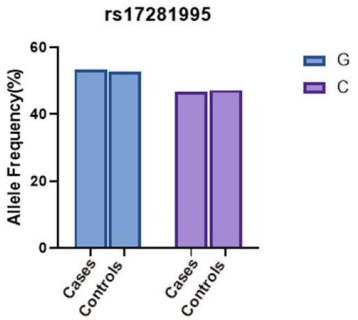

Figure 3. Bar graph for allelic distribution of rs17281995 in patients with gastric cancer and controls.

Table 3. Genotype and allele frequency of rs17281995 polymorphism in patients with gastric cancer and the control group.

			SNP			P-value
			CC	GC	GG	0.97
Group	Control	Count	4	9	5	
		% Within group	22%	50%	27%	
	Case	Count	7	14	9	
		% Within group	23.3%	46.7%	30%	

Conclusion

In the present study, it was discovered that there is no association between the frequency of alleles and genotypes of rs17281995 polymorphism in terms of colorectal and gastric cancer.

Conflict of Interests

The authors declare that there is no conflict of interest in this study.

References

Arani, S.H. and Kerachian, M.A. (2017) Rising rates of colorectal cancer among younger Iranians: is diet to blame?. Current Oncololgy 24(2):131-137.

Arisawa, T., Tahara, T., Shibata, T., Nagasaka, M., Nakamura, M., Kamiya, Y., et al. (2007) Genetic polymorphisms of molecules associated with inflammation and immune response in Japanese subjects with functional dyspepsia. International journal of molecular medicine 20(5): 717-723.

Azimzadeh, P., Romani, S., Mirtalebi, H., Fatemi, S.R., Kazemian, S., Khanyaghma, M., et al. (2013) Association of co-stimulatory human B-lymphocyte antigen B7-2 (CD86) gene polymorphism with colorectal cancer risk. Gastroenterology and Hepatology from Bed to Bench 6(2): 86.

Canedo, P., Durães, C., Pereira, F., Regalo, G., Lunet, N., Barros, H., et al. (2008) Tumor necrosis factor alpha extended haplotypes and risk of gastric carcinoma. Cancer Epidemiology, Biomarkers & Prevvention 17(9): 2416-2420.

Di Pasqua, A.J., Wallner, S., Kerwood, D.J. and Dabrowiak, J.C. (2009) Adsorption of the PtII anticancer drug carboplatin by mesoporous silica. Chemistry & Biodiversity 6(9): 1343-1349.

Fang, S.H., Efron, J.E., Berho, M.E. and Wexner, S.D., (2014) Dilemma of stage II colon cancer and decision making for adjuvant chemotherapy. Journal of the American College of Surgeons 219(5): 1056-1069.

Forat-Yazdi, M., Gholi-Nataj, M., Neamatzadeh, H., Nourbakhsh, P. and Shaker-Ardakani, H. (2015) Association of XRCC1 Arg399Gln polymorphism with colorectal cancer risk: a HuGE meta analysis of 35 studies. Asian Pacific Journal of Cancer Prevention 16(8): 3285-3291.

Geng, P., Zhao, X., Xiang, L., Liao, Y., Wang, N., Ou, J., et al. (2014) Distinct role of CD86 polymorphisms (rs1129055, rs17281995) in risk of cancer: evidence from a meta-analysis. Plos one 9(11): e109131.

González, C.A., Sala, N. and Capellá, G. (2002) Genetic susceptibility and gastric cancer risk. International Journal of Cancer 100(3): 249-260.

Hu, J.Y., Wang, S., Zhu, J.G., Zhou, G.H. and Sun, Q.B. (1999) Expression of B7 costimulation molecules by colorectal cancer cells reducestumorigenicity and induces anti-tumor immunity. World Journal of Gastroenterology 5(2): 147.

Jemal, A., Bray, F., Center, M.M., Ferlay, J., Ward, E. and Forman, D. (2011) Global cancer statistics. A Cancer Journal for Clinicians 61(2):69-90.

Koido, S., Ohkusa, T., Homma, S., Namiki, Y., Takakura, K., Saito, K., et al. (2013) Immunotherapy for colorectal cancer. World journal of gastroenterology: World Journal of Gastroenterology 19(46): 8531.

Landi, D., Gemignani, F., Naccarati, A., Pardini, B., Vodicka, P., Vodickova, L., et al. (2008) Polymorphisms within micro-RNA-binding sites and risk of sporadic colorectal cancer. Carcinogenesis 29(3): 579-584.

Lenschow, D.J., Walunas, T.L. and Bluestone, J.A. (1996) CD28/B7 system of T cell costimulation. Annul Review of Immunology 14(1): 233-258.

Machado, J.C., Figueiredo, C., Canedo, P., Pharoah, P., Carvalho, R., Nabais, S., et al. (2003) A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. Gastroenterology 125(2): 364-371.

Milne, A.N., Carneiro, F., O'morain, C. and Offerhaus, G.J.A. (2009) Nature meets nurture: molecular genetics of gastric cancer. Human Genetic 126(5): 615-628.

Namazi, A., Forat-Yazdi, M., Jafari, M.A., Foroughi, E., Farahnak, S., Nasiri, R., et al. (2017) Association between polymorphisms of ERCC5 gene and susceptibility to gastric cancer: A systematic review and meta-analysis. Asian Pacific Journal of Cancer Prevention 18(10): 2611.

Namazi, A., Forat-Yazdi, M., Jafari, M., Farahnak, S., Nasiri, R., Foroughi, E., et al. (2018) Association of interleukin-10-1082 A/G (rs1800896) polymorphism with susceptibility to gastric cancer: meta-analysis of 6,101 cases and 8,557 controls. Arq Gastroenterol 55: 33-40.

Ohtani, H. (2007) Focus on TILs: prognostic significance of tumor infiltrating lymphocytes in human colorectal cancer. Cancer immunology 7(1).

Poorolajal, J., Moradi, L., Mohammadi, Y., Cheraghi, Z. and Gohari-Ensaf, F. (2020) Risk factors for stomach cancer: a systematic review and meta-analysis. Epidemiology and health 42.

Saeki, N., Saito, A., Choi, I.J., Matsuo, K., Ohnami, S., Totsuka, H., et al. (2011) A functional single nucleotide polymorphism in mucin 1, at chromosome 1q22, determines susceptibility to diffuse-type gastric cancer. Gastroenterology 140(3): 892-902.

Shademan, B., Masjedi, S., Karamad, V., Isazadeh, A., Sogutlu, F. and Nourazarian, A. (2022) CRISPR Technology in Cancer Diagnosis and Treatment: Opportunities and Challenges. Biochemical Genetics 1-25.

van der Merwe, P.A., Bodian, D.L., Daenke, S., Linsley, P. and Davis, S.J. (1997) CD80 (B7-1) binds both CD28 and CTLA-4 with a low affinity and very fast kinetics. Journal of Experimental Medicine 185(3) : 393-404.

Wu, X., Zeng, Z., Chen, B., Yu, J., Xue, L., Hao, Y., et al. (2010) Association between polymorphisms in interleukin-17A and interleukin-17F genes and risks of gastric cancer. International Journal of Cancer 127(1): 86-92.

Yin, M., Hu, Z., Tan, D., Ajani, J.A. and Wei, Q. (2009) Molecular epidemiology of genetic susceptibility to gastric cancer: focus on single nucleotide polymorphisms in gastric carcinogenesis. American Journal of Translational Research 1(1): 44.

Zhuang, W., Wu, X.T., Zhou, Y., Liu, L., Liu, G.J., Wu, T.X., et al. (2010) Interleukin10-592 promoter polymorphism associated with gastric cancer among Asians: a meta-analysis of epidemiologic studies. Digestive Diseases and Sciences 55(6): 1525-1532.