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KLİNİK ARAŞTIRMA *İç Hastalıkları*

Relation of chronic atrophic gastritis and intestinal metaplasia with *Helicobacter pylori* and tumor necrosis factor-α and macrophage migration inhibitory factor polymorphisms in a population of Easten Anatolia

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SUMMARY

Objective: *We aimed to determine the effects of tumour-necrosis factor-α (TNF-α) and macrophage migration inhibitory factor (MIF) gene polymorphisms, Helicobacter pylori (H. pylori) infection, on the risk of developing chronic atrophic gastritis (CAG) and intestinal metaplasia (IM).*

Material and Method: *The TNF-α-308G/A and MIF-173G/C single nucleotide polymorphisms (SNPs) were genotyped using polymerase-chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis, in 84 patients (43 CAG and 41 IM) and 40 healthy controls in a region of Eastern Anatolia.*

Results: *An increased risk of CAG was found in subjects with TNF-α-308 GA genotype, negative for H. pylori infection or TNF-α-308 AA genotype carriers and negative for H. pylori infection. An elevated risk of IM was found in subjects with TNF-α-308 GA genotype and negative for H. pylori infection or TNF-α-308 AA genotype and negative for H. pylori infection. An increased risk of CAG was found in subjects with TNF-α-308GA genotype , and positive for H. pylori infection. An increased risk of CAG was found in subjects with MIF-173GC genotype and negative for H. pylori infection or MIF-173CC genotype carriers and negative for H. pylori infection. An elevated risk of IM was found in subjects with MIF-173GC genotype and negative for H. pylori infection. An elevated risk of IM was found in subjects with MIF-173GC genotype and positive for H. pylori infection.*

Conclusion: *Therefore, the TNF-α-308G/A and MIF-173G/C genotyping may be used as biomarkers at the early stage of the gastric cancer.*

Key words: *Chronic atrophic gastritis, Intestinal metaplasia, gene polymorphisms, tumor necrosis factor- α, macrophage migration inhibitory factor, Helicobacter pylori*

ÖZET

Türkiye'de bir doğu populasyonunda kronik atrofik gastrit ve intestinal metaplazi ile tümör nekrozis faktör-α ve makrofaj migrasyon inhibitör faktör gen polimorfizmleri ve *Helikobakteri pilorinin* ilişkisi

Amaç: *Kronik atrofik gastrit (KAG) ve intestinal metaplazi (İM) gelişme riskinde tümör nekrozis faktör-α (TNF-α), makrofaj migrasyon inhibitör faktör (MIF) gen polimorfizmleri ve Helikobakteri pilori (H. pilori)'nin etkisini tespit etmek amaçlandı.*

Gereç ve Yöntem: *TNF-α-308G/A ve MIF-173G/C tek nükleotid polimorfizmi Türkiyenin doğusunda bir bölgede 84 hastada (43 KAG ve 41 IM) ve 40 sağlıklı kontrolde polimeraz zincir reaksiyon-restriksiyon fragman uzunluk polimorfizim (PZR-RRUP) analizi kullanılarak genotiplendirildi.*

Bulgular: *H. pilori infeksiyonu negatif ve TNF-α-308G/A genotipli olgularda ya da H. pilori infeksiyonu negatif ve TNF-α-308 AA taşıyıcı olgularda KAG riskinde bir artış bulundu. H. pilori infeksiyonu negatif ve TNF-α-308G/A genotipli olgularda yada H. pilori infeksiyonu negatif ve TNF-α-308AA taşıyıcı olgularda IM riskinde bir artış bulundu. H. pilori infeksiyonu pozitif ve TNF-α-308G/A genotipli olgularda KAG riskinde bir artış bulundu. H. pilori infeksiyonu negatif ve MIF-173GC genotipli olgularda ya da H. pilori infeksiyonu negatif ve MIF-173CC taşıyıcı olgularda KAG riskinde bir artış bulundu. H. pilori infeksiyonu negatif ve MIF-173GC genotipli olgularda IM riskinde bir artış bulundu. H. pilori infeksiyonu pozitif ve MIF-173GC genotipli olgularda IM riskinde bir artış bulundu.*

Sonuç: *Böylece, TNF-α-308G/A ve MIF-173G/C genotipleri erken evre gastrik kanserde biyomarkır olarak kullanılabilir.*

Anahtar kelimeler: *Kronik atrofik gastrit, intestinal metaplazi, gen polimorfizmleri, tümör nekrozis faktör-α, makrofaj migrasyon inhibitör faktör, Helikobakter pilori*

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The polymorphisms in the promoter region of TNF-α gene have been extensively studied in relation to gastric cancer. Three polymorphisms in TNF-α genes have been studied more than the other polymorphisms. TNF-α-308 GA is associated with an increased production of TNF-α [1], which is a central mediator of the immune response, and shares many biological properties with IL-1. Another important cytokine is TNF-α, the biallelic polymorphism in position -308 of the region promoting the gene codifying that cytokine, which has been associated with the development of gastric cancer (GC) [2,3]. This suggests that the TNF single-nucleotide polymorphisms (SNPs) may be used as stratification markers to predict the risk of cancer development.

There is increasing evidence that host inflammation related cytokines and their gene polymorphisms are related to chronic atrophic gastritis (CAG) and intestinal metaplasia (IM) [4,5]. Macrophage migration inhibitory factor (MIF), an important activator of T lymphocytes and macrophages, plays a pivotal role in the development of inflammatory and immune diseases [6,7]. *Helicobacter pylori (H. pylori)* infection is associated with an increased expression of MIF mRNA, and protein in gastric epithelial and inflammatory cells. Increased expression of the MIF protein correlates with the histological severity of GC and its precursor [8]. As these inflammatory cytokines and their gene polymorphisms may potentially influence the outcome of *H. pylori* infection, a few studies have investigated the association of gene polymorphisms in these inflammatory cytokines with the risk of atrophy and IM [9-14].

MIF is a T-cell derived lymphokine that inhibits the migration of macrophages and contributes to a delayed type hypersensitivity [15,16]. For a number of years, MIF was thought to be a T-cell product that acted on macrophages. More recently, it has been discovered that in addition to being a target, the macrophage itself is an important source of MIF. MIF is released from monocytes/macrophages upon stimulation by TNF-alpha, interferon-gamma, lipopolysaccharide and streptococcal exotoxin [17,18]. The human MIF gene situated on chromosome 22 (q11.23) has a 2-kb structure with three exons and two introns [19,20].

CAG and IM are two important precursor lesions of intestinal type GC [21]. These precursor lesions may significantly elevate the risk of intestinal type GC [22,23]. Some bacterial factors, such as the pathogenic island of *H. pylori* including cagA, sIm1 vacA, babA2, sabA, and oipA, are correlated with the severity of atrophic gastritis and occurrence of IM (24-29). However, bacterial factors alone are not sufficient to explain the diverse outcomes of *H. pylori*-related diseases. A previous study has shown that the proportion of cagA + *H. pylori* strains in children living in Linqu County, an area with a high risk of GC in China, is very high (88.5%) [30,31].

The objective of this study was to determine the frequency of polymorphisms in genes TNF-α, and MIF in patients in the east region of Turkey, with various precancerous lesions and in a control group. The relationship of these polymorphisms to *H. pylori* infection, and to the histopathological characteristics of gastric tissue were also determined.

MATERIALS and METHODS

Patient and control samples: Peripheral blood and gastric fragment samples were collected from 43 CAG and 41 IM patients living the city of Erzurum-Turkey. Gastric fragment samples were obtained by the endoscopy service of the Erzurum State Hospital. For the control, peripheral blood samples were collected from 40 asymptomatic patients without any clinical or metabolic diseases or gastrointestinal disturbances who were not referred for endoscopic examinations. All patients and controls in comparable socioeconomic level with similar cultural habits were enrolled in the study between May 2010 and June 2012. The study was approved by the Ethics Committee of Erzurum State Hospital.

Identification of *H. pylori* infection: Serum level of *H. pylori*-specific IgG and IgA in all samples was measured by enzyme-linkedimmunosorbent assay (ELISA). The presence of H. pylori was further confirmed by immunohistochemistry (IHC) assays. Polyclonal rabbit anti-H. pylori (ab7788, Abcam, Cambridge, UK) was used as a primary antibody.

DNA extraction: Genomic DNA was extracted from total blood using a leukocyte lysis solution (100 mmol/L Tris-HCl, 20 mmol/L EDTA, 200 mmol/L NaCl, 1 % dodecylsodium sulfate, 0.2 % β mercaptoethanol) and was purified using the phenol-chloroform method [32].

TNF-α genotyping: Polymorphisms of the TNF-α-308 gene were characterized using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The volume for the PCR was 25 μL, containing 0.5 mmol/L of each primer, 1 X PCR buffer, 1.5 mmol/L of MgCl2, 0.2 mmol/L of each nitrogenated base, 1.25 U of Taq DNA polymerase, 50 ng of DNA and sterile water. The primers for amplifying the TNF-α-308G/A SNP: forward primer was 5'- AGG CAA TAG GTT TTG AGG GCC AT-3' and the reverse primer was 5'-TCC TCC CTG CTC TGA TTC CG-3'. The amplification conditions were as follows:5 minutes at 94 followed by 35 cycles of 60 seconds at 94 °C, 60 seconds at 60 °C (TNF-α-308G/A), and 60 seconds at 72 °C, and with a final step at 72 °C for 5 minutes to allow for the complete extension of all PCR fragments. The PCR products were digested overnight at 37°C with NcoI restriction endonuclease, and separated by electrophoresis in 2 % agarose gel stained with ethidium bromide [33].

For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system. For 10 % of samples, the PCR reaction was repeated once for quality control. For the TNF-α-308G/A SNP, the GG genotype was represented by two DNA bands with sizes of 87 bp and 20 bp; the GA genotype (lacking the NcoI site) was represented by the original 107 bp fragment, whereas the heterozygote displayed three DNA bands with sizes of 107 bp, 87 bp and 20 bp. For a negative control, each PCR reaction used distilled water instead of DNA in the reaction system. For 10% of the samples, the PCR reaction was repeated once for quality control.

MIF genotyping: This was carried out as previously described by Donn et al. (20) PCR was used to amplify a 366 bp fragment. Forward primer was 5'-ACT-AAGAAA-GAC CCG-AGG-C-3', and the reverse primer was 5'-GGG-GCA-CGT-TGG-TGTTTA-C-3'. The annealing temperature used was 59°C. The resulting fragment was digested with AluI restriction endonuclease overnight at 37°C and the digest was resolved on 2.5 % agarose gel stained with 10 % ethidium bromide, and visualised using UV light. The 366 bp PCR product had a consistent restriction site resulting in a 98 bp and a 268 bp fragment. The GG genotype did not have a second cutting site for Alu I. The CC genotype had a second cutting site resulting in 3 fragments: 205 bp, 98 bp, 63 bp size. The heterozygous GC genotype was characterised by 4 bands: 268 bp, 205 bp, 98 bp, and 63 bp.

Histological evaluation: Biopsy specimens from the lesion and the adjacent area in each patient were obtained. The specimens were fixed in 10% buffered formalin solution, embedded in paraffin, cut into sequential 0.4-μm sections, and stained with hematoxylin and eosin (HE). The histopathological parameters were graded (0-3) using the criteria described in the updated Sydney classification system [34], to be used for the analysis of chronic inflammation, polymorphonuclear activity, and intestinal metaplasia.

Statistical analysis

Statistical analysis was performed using SPSS10.0 software package (SPSS Company, Chicago, Illinois, USA). Comparison of the distribution of TNF-α-308 and MIF-173 genotypes in CAG, two-sided contingency tables using chi-square tests were employed on IM patients and healthy controls. The odds ratio (OR) and 95% confidence interval (CI)

were calculated using a logistic regression model adjusted to the age and gender of the study population. The association was determined as an OR at a confidence interval of 95%. Differences were considered statistically significant if p values were less than 0.05.

RESULTS

Demographic data, and characteristics of *H. pylori* infection in CAG, IM and control groups are listed in Table 1. The mean (± standard deviation) age of CAG patients (43.2±3.8 years; range, 34-65 years), and IM patients (45.8.3±4.3 years; range, 37-66 years) was similar to that of the healthy controls (42.5±7.5 years; range, 31-58 years). The gender distribution male, and female patients in CAG and IM groups (60.5 vs and 39.5 %, and 60.1 vs 39.9 %, respectively) was comparable to that in healthy controls (62.5 vs 37.5 %). The percentage of the cases with H. pylori infection was significantly higher in CAG (58.1 %), IM (73.1 %) groups than in the control group (35%) (p=0.002). The patients expressed alleles at the individual loci were expressed in patients showing control group, with no significant p values (Table 2).

TNF-α-308 polymorphism in CAG and IM: Multivariate analysis showed that the frequencies of TNF-α-308 and MIF-173 in groups CAG and IM were significantly different from those in the control group (Table 2). Compared with TNF-α-308 GG genotype, TNF-α-308 AA genotype and TNF-α-308 A allele carriers exhibited a significantly increased risk for progression of lesions to CAG (Table 3). The patients with TNF-α-308 GA genotype or TNF-α-308 A allele carriers had an increased risk for progression of lesions to IM.

MIF-173 polymorphism in CAG and IM: Multivariate analysis showed that the MIF-173 GC genotype or MIF-173 C allele carriers were significantly associated with an increased risk for progression of lesions to CAG and IM (Table 4). The risk for CAG in association with TNF-α-308 and MIF-173 genotypes was further examined with stratification by H. pylori infection. The ORs for the development of CAG in subjects carrying TNF-α-308 A allele, and those with H. pylori infection alone were 2.18 (95% CI: 0.54-8.82), and 1.5 (95 % CI:0.378-5.95), respectively (Table 3). However, the OR was not significantly increased in subjects with *H. pylori* infection carrying the AA genotype. There was an interaction between TNF-α-308 A allele carriers, and *H. pylori* infection, with a relative risk for the development of CAG due to the interaction of 1,50. Contrarily, a similar trend to develop CAG was not observed between the MIF-173 C allele carriers and *H. pylori* infection (OR = 0.75, 95 % CI: 0.19-2.97) (Table 4).

Table 1. Epidemiyological characteristics of the patients and control group n (%).

Demographic data	CAG (n=43)	IM (n=41)	Control (n=40)
Age (yr)			
<50	14 (32.6)	16 (39.0)	15 (35,5)
>50	29 (67.4)	25 (60.1)	25 (64,5)
		p=0.812	
Sex			
Male	26 (60,5)	25 (60.1)	25 (62,5)
Female	17 (39,5)	16 (39.9)	15 (37,5)
		p=0.911	
H. pylori infection			
Positive	25 (58.1)	30 (73.1)	14 (35)
Negative	18 (41.9)	11 (26.9)	26 (65)
		p=0.002	

CAG: Chronic atrophic gastritis; IM: Intestinal metaplasia

Table 2. Distribution of genotypes for TNF-α-308 and MIF-173 in the patients and in control group n (%).

Genotypes	CAG (n=43)	IM (n=41)	Control (n=40)
TNF-α-308			
G/G	23 (53.5)	25 (61.0)	29 (72.5)
G/A	16 (37.2)	14 (34.1)	10 (25.0)
A/A	4 (9.3)	2 (4.9)	1 (2.5)
		p=0.405	
MIF-173			
G/G	22 (51.2)	23 (56.1)	27 (67.5)
G/C	18 (41.9)	17 (41.4)	11 (27.5)
C/C	3 (6.98)	1 (2.44)	2 (5.0)
		p=0.500	

TNF: Tumor necrosis factor; MIF: Macrophage migration inhibitory factor

Table 3. Combined risk of polymorphism in the TNF-α-308 gene and *H. pylori* infection for development of chronic atrophic gastritis (CAG) and intestinal metaplasia (IM).

TNF-α	*H. pylori* infection	Control	CAG n (%)	OR (95%CI)	p	IM n (%)	OR (95%CI)	p
GG	-	20 (76.9)	11 (61.1)	Ref	0.273	5 (45,5)	Ref	0.086
GA	-	5 (19.2)	6 (33.3)	2.18 (0.54-8.82)	0.623	5 (45,5)	4.00 (0.824-19.423)	0.355
AA	-	1 (3.8)	1 (5.6)	1.82 (0.103-31.99)		1 (9)	4.00 (0.21-25.66)	
GG	+	9 (64.3)	12 (48)	Ref	0.564	20 (66.7)	Ref	0.759
GA	+	5 (35.7)	10 (40)	1.50 (0.378-5.95)	1.00	9 (30)	0.81 (0.21-3.12)	
AA	+	0 (0)	3 (12)	0		1 (3.3)	0	
Total		40	43			41		

Table 4. Combined risk of polymorphism in the MIF-173 gene and *H. pylori* infection for development of chronic atrophic gastritis (CAG) and intestinal metaplasia (IM).

MIF	*H. pylori* infection	Control	CAG n (%)	OR (95%CI)	p	IM n (%)	OR (95%CI)	p
GG	-	20 (76.9)	8 (44.4)	Ref	0.031	8 (72.7)	Ref	0.630
GC	-	5 (19.2)	9 (50)	4.50 (1.15-17.65)	0.534	3 (27.3)	1.50 (0.29-7.81)	
CC	-	1 (3.8)	1 (5.6)	2.50 (0.139-49.01)		0 (0)	0	
GG	+	7 (50)	14 (56)	Ref	0.682	15 (50)	Ref	0.899
GC	+	6 (42.9)	9 (36)	0.75(0.19-2.97)	1.00	14 (46.7)	1.089 (0.293-.4.042)	0.608
CC	+	1 (7.1)	2 (8)	1.00(0.077-13.02)		1 (3.3)	0.467 (0.025-8.596)	
Total		40	43			41		

The OR of developing CAG significantly increased in healthy subjects carrying at least one MIF-173 C allele (OR = 4.50, 95 % CI: 1.15-17.65) (Table 4). However, an interaction between the MIF-173 C allele carriers and H. pylori infection was not observed (OR = 0.75, 95 % CI: 0.19-2.97).

The association of IM and TNF-α-308 with MIF-173 genotypes was further examined using stratification by *H. pylori* infection. However, the OR for IM in subjects with *H. pylori* infection carrying TNF-α-308 A allele did not increase significantly (OR = 0.81, 95 % CI:0.21-3.12) (Table 3). However, there was an interaction between the MIF-173 C allele carriers and *H. pylori* infection (OR = 1.089, 95% CI:0.293-4.042).

DISCUSSION

Gastric cancer is the second most common cause of cancer mortality in the world [35,36]. Because inflammation is one of the initial phases of gastric carcinogenesis [37], inflammation-related polymorphisms, including single-nucleotide polymorphisms (SNPs) in TNF-α gene, have been extensively studied in relation to gastric cancer [38].

Of the three polymorphisms reviewed in a report, TNF-α-308 GA is studied more extensively and a biologic role for it has been identified. Previous studies have suggested that frequencies of genetic markers often demonstrate high variations among various ethnic and racial groups [39,40], whereas differences in terms of genetic effects (expressed in ORs) are much less common [40]. This meta-analysis found that the median prevalence of TNF-α-308 A carrier genotypes was almost twice as high in Western world as in East Asian populations (23.5 vs 13.4%). However, contrary to what has been shown for most of the previous associations [40], the OR associated with AA genotype showed a difference between Western and East Asian studies. No SNPs have been consistently associated with gastric cancer risk [41]. Indeed, because several initially promising gene–disease associations gravitated towards null over time [42,43], it has been suggested that medical journals should take a cautious approach in publishing such associations [44]. This review has found that TNF-α -308 AA genotype is moderately associated with an increased risk of gastric cancer. How-

ever, TNF-α-308 GA and GG were not statistically significantly associated with gastric cancer risk [45].

Our study showed that the TNF-α -308G/A polymorphism may be dependently associated with susceptibility to CAG and IM in the population of Eastern Anatolia. Although the moderate sample size in this study may detect minor effects of the TNF-α SNP in the development of CAG and IM, the diverse outcomes of various studies indicated that the TNF-α-308 A/G polymorphism may play different roles in susceptibility to different tumor types. In our study, the TNF-α-308 A allele was found to be associated with a high risk of CAG and IM. Moreover, an interaction was detected between TNF-α -308 A allele carriers, and *H. pylori* infection which promoted progression from baseline lesions to CAG and IM. Some studies have demonstrated that polymorphisms in gene TNF-α, together with *H. pylori*-infection are associated with an increased risk of developing stomach cancer [2,3] which requires more detailed comprehension of the factors related to the high prevalence of stomach cancer in this region. They analyzed the frequency of the genotypes of polymorphisms in gene TNF-α and the presence of infection by CagA+ strains in patients with various gastrointestinal diseases and in a control group [45], and Asiatic populations [46,47], whereas the frequency of polymorphisms in gene TNF-α-308 was significantly different from that reported in Asiatic [43], and Caucasian [44], populations, respectively.

The genetic composition of the Brazilian population is made up of a genetic mix of various ethnic groups, including Portuguese, Africans and Amerindians [48]. The differences and similarities between the allelic frequencies of the polymorphisms studied in our population with those of other ethnic groups are products of the genetic mix that has occurred in Brazil. In Brazil, Rocha et al. [49] obtained similar results in relation to the association of allele TNF-α-308 and an increased risk of developing stomach cancer.

Significantly augments T helper 1(Th1) immune response by inducing proinflammatory cytokines such as TNF-α, interferon-γ, and IL-1β secretion. It has been shown that Th1 predominant immune responses inhibit acid secretion from gastric glands, and cause gastric atrophy and metaplasia in a H. pylori infected mouse model [50]. Natural genetic variations in cancer-related genes may affect the individual susceptibility to cancers via modulating transcription and expression. The functional SNPs in TNF genes have been associated with the risk of some tumors. Individuals carrying TNF-α-308 A allele has an increased risk for several cancers, such as breast cancer [51], and gastric cancer [52].

MIF promotes the recognition of Gram-negative bacteria by the innate immune system [26]. The MIF gene appears to be a strong candidate susceptibility gene for *H. pylori* -related diseases. Xia et al. [7] reported that both mRNA and protein levels of MIF are up-regulated in *H. pylori* –infected patients and parallel to the severity of gastritis. Moreover, the expression level of MIF protein is markedly different in patients with gastritis, IM, dysplasia (Dys), and GC [7,8]. In vivo and in vitro functional studies have demonstrated that the mutant allele MIF-173 C is associated with an increased MIF protein production [53]. The presence of MIF-173 C allele stimulates protein 4 (AP-4) transcription factor binding site that may up-regulate MIF expression [53]. In our study, the MIF-173 C allele was found to be associated with a high risk of CAG and IM. Moreover, an interaction occurred between MIF-173 C allele carriers, and H. pylori infection promoting progression of baseline lesions to CAG and IM. Other studies found that MIF not only modulates the expression of proinflammatory mediators such as TNF-α, but also regulates the activation of T cells [54]. Therefore, we have hypothesized that variants of MIF gene polymorphism may contribute to the different outcomes of *H. pylori*-related gastritis. Moreover, MIF gene polymorphisms may be another important candidate prognostic gene marker for the patients infected with *H. pylori*.

In summary, *H. pylori* infection and variants in TNF-α-308 or MIF-173 polymorphisms influence

the occurrence of CAG and IM. The findings in this study indicate that the TNF-α-308 G/A and MIF-173 G/C genotyping can be used as biomarkers at the early stage of GC.

REFERENCES

1. **Jang WH, Yang YI, Yea SS, et al.** The 238 tumor necrosis factor-alpha promoter polymorphism is associated with decreased susceptibility to cancers. *Cancer Lett* 2001;166(1): 41-46. http://dx.doi.org/10.1016/S0304-3835(01)00438-4
2. **Shibata J, Goto H, Arisawa T, et al.** Regulation of tumour necrosis factor (TNF) induced apoptosis by soluble TNF receptors in Helicobacter pylori infection. 1999;45(1):24-31. http://dx.doi.org/10.1136/gut.45.1.24
3. **Yea SS, Yang YI, Jang WH, et al.** Association between TNF-alpha promoter polymorphism and Helicobacter pylori cagA subtype infection. *J Clin Pathol* 2001;54(9):703-706. http://dx.doi.org/10.1136/jcp.54.9.703
4. **Gao L, Weck MN, Nieters A, Brenner H.** Association between a pro-inflammatory genetic profile and the risk of chronic atrophic gastritis among older adults from Germany. *Eur J Cancer* 2009;45(3):428-434. http://dx.doi.org/10.1016/j.ejca.2008.09.019
5. **Tahara T, Arisawa T, Shibata T, et al.** Effect of RANTES promoter genotype on the severity of intestinal metaplasia in Helicobacter pylori-infected Japanese subjects. *Dig Dis Sci* 2009;54(6):1247-1252. http://dx.doi.org/10.1007/s10620-008-0497-2
6. **Calandra T, Spiegel LA, Metz CN, Bucala R.** Macrophage migration inhibitory factor is a critical mediator of the activation of immune cells by exotoxins of Gram-positive bacteria. *Proc Natl Acad Sci* 1998;95(19):11383-11388. http://dx.doi.org/10.1073/pnas.95.19.11383
7. **Xia HH, Lam SK, Huang XR, et al.** Helicobacter pylori infection is associated with increased expression of macrophage migratoryinhibitory factor--by epithelial cells, T cells, and macrophages--in gastric mucosa. *J Infect Dis* 2004;190(2):293-302. http://dx.doi.org/10.1086/421915
8. **He XX, Yang J, Ding YW, et al.** Increased epithelial and serum expression of macrophage migration inhibitory factor (MIF) in gastric cancer: potential role of MIF in gastric carcinogenesis. *Gut* 2006;55(6):797-802. http://dx.doi.org/10.1136/gut.2005.078113
9. **Taguchi A, Ohmiya N, Shirai K, et al.** Interleukin-8 promoter polymorphism increases the risk of atrophic gastritis and gastric cancer in Japan. *Cancer Epidemiol Biomarkers Prev* 2005;14(11): 2487-2493. http://dx.doi.org/10.1158/1055-9965.EPI-05-0326
10. **Ohyauchi M, Imatani A, Yonechi M, et al.** The polymorphism interleukin 8 -251 A/T influences the susceptibility of Helicobacter pylori related gastric diseases in the Japanese population. *Gut* 2005;54(3):330-335. http://dx.doi.org/10.1136/gut.2003.033050
11. **Arisawa T, Tahara T, Shibata T, et al.** Functional polymorphisms in the promoter region of macrophage migration inhibitory factor and chronic gastritis. *Int J Mol Med* 2007;20(4):539-544.
12. **Uygun A.** Peptic ulcer and Helicobacter pylori Associate and Helicobacter pylori treatment. *Turkiye Klinikleri J Gastroenterohepatol-Special Topics* 2009;2(3):19-28.
13. **Nazlıgül Y.** Helicobacter Pylori and Upper Gastrointestinal Diseases: Review. *Turkiye Klinikleri J Gastroenterohepatol* 2009;16(2):69-75.
14. **Boran Ç, Balaban YH, Yılmaz F.** Do Gene Expressions Differ Between Gastritis with and without Helicobacter pylori? *Turkiye Klinikleri J Med Sci* 2010;30(6):1806-1813. http://dx.doi.org/10.5336/medsci.2009-14585
15. **David JR.** Delayed Hypersensitivity in vitro: its mediation by cell-free substances formed by lymphoid cell-antigen interaction. *Proc Natl Acad Sci USA* 1966;56(1):72-77. http://dx.doi.org/10.1073/pnas.56.1.72
16. **Bloom BR, Bennett B.** Mechanism of a reaction in-vitro associated with delayed-type hypersensitivity. *Science* 1966;153(731):80-82. http://dx.doi.org/10.1126/science.153.3731.80
17. **Calandra T, Bernhagen J, Mitchell RA, Bucala R.** The macrophage is an important and previously unrecognized source of macrophage migration inhibitory factor. *J Exp Med* 1994;179(6):1895-1902. http://dx.doi.org/10.1084/jem.179.6.1895
18. **Bacher M, Metz CN, Calandra T, et al.** An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci USA* 1996;93(15):7849-7854. http://dx.doi.org/10.1073/pnas.93.15.7849
19. **Paralkar V, Wistow G.** Cloning the human gene for macrophage migration inhibitory factor (MIF). *Genomics* 1994;19(1):48-51. http://dx.doi.org/10.1006/geno.1994.1011
20. **Donn RP, Shelley E, Ollier WE, Thomson W.** British Paediatric Rheumatology Study Group. A novel 5'-flanking region polymorphism of macrophage migration inhibitory factor is associated with systemiconset juvenile idiopathic arthritis. *Arthritis Rheum* 2001;44(8):1782-1785. http://dx.doi.org/10.1002/1529-0131(200108)44:8<1782::AID-ART314>3.0.CO;2-#
21. **Correa P.** A human model of gastric carcinogenesis. *Cancer Res* 1988;48(13):3554-3560.
22. **Inoue M, Tajima K, Matsuura A, K, et al.** Severity of chronic atrophic gastritis and subsequent gastric cancer occurrence: a 10-year prospective cohort study in Japan. *Cancer Lett* 2000;161(1):105-112. http://dx.doi.org/10.1016/S0304-3835(00)00603-0
23. **Uemura N, Okamoto S, Yamamoto S, et al.** Helicobacter pylori infection and the development of gastric cancer. *N Engl J Med* 2001;345(11):784-789. http://dx.doi.org/10.1056/NEJMoa001999
24. **Furuta T, El-Omar EM, Xiao F, et al.** Interleukin 1beta polymorphisms increase risk of hypochlorhydria and atrophic gastritis and reduce risk of duodenal ulcer recurrence in Japan. *Gastroenterology* 2002;123(1):92-105. http://dx.doi.org/10.1053/gast.2002.34156
25. **Hansson LE, Nyrén O, Hsing AW et al.** The risk of stomach cancer in patients with gastric or duodenal ulcer disease. *N Engl J Med* 1996;335(4):242-249. http://dx.doi.org/10.1056/NEJM199607253350404
26. **Parkin DM.** The global burden of cancer. *Semin Cancer Biol* 1998;8(4):219-235. http://dx.doi.org/10.1006/scbi.1998.0080
27. **Yu J, Leung WK, Go MY, et al.** Relationship between Helicobacter pylori babA2 status with gastric epithelial cell turnover and premalignant gastric lesions. *Gut* 2002;51(4):

480-484.
http://dx.doi.org/10.1136/gut.51.4.480

28. **Parsonnet J, Harris RA, Hack HM, Owens DK.** Modelling cost-effectiveness of Helicobacter pylori screening to prevent gastric cancer: a mandate for clinical trials. *Lancet* 1996;348(9021):150-154. http://dx.doi.org/10.1016/S0140-6736(96)01501-2
29. **Broutet N, Moran A, Hynes S, et al.** Lewis antigen expression and other pathogenic factors in the presence of atrophic chronic gastritis in a European population. *J Infect Dis* 2002;185(4):503-512. http://dx.doi.org/10.1086/339016
30. **You WC, Zhang L, Pan KF, et al.** Helicobacter pylori prevalence and CagA status among children in two counties of China with high and low risks of gastric cancer. *Ann Epidemiol* 2001;11(8):543-546. http://dx.doi.org/10.1016/S1047-2797(01)00227-7
31. **Zhou J, Zhang J, Xu C, He L.** cagA genotype and variants in Chinese Helicobacter pylori strains and relationship to gastroduodenal diseases. *J Med Microbiol* 2004;53(3):231-235. http://dx.doi.org/10.1099/jmm.0.05366-0
32. **Miller SA, Dykes DD, Polesky HF.** A simple salting out procedure for extracting DNA from human nucleated cells. *Nucleic Acids Res* 1998;16(3):1215. http://dx.doi.org/10.1093/nar/16.3.1215
33. **Wilson AG, Symons JA, McDowell TL, et al.** Effects of a polymorphism in the human tumor necrosis factor alpha promoter on transcriptional activation. *Proc Natl Acad Sci USA* 1997;94(7):3195-3199. http://dx.doi.org/10.1073/pnas.94.7.3195
34. **Dixon MF, Genta RM, Yardley JH, Correa P.** Histological classification of gastritis and Helicobacter pylori infection: an agreement at last? The International Workshop on the Histopathology of Gastritis. *Helicobacter* 1997;2(supp.1): 17-24. http://dx.doi.org/10.1111/j.1523-5378.1997.06b09.x
35. **Kamangar F, Cheng C, Abnet CC, Rabkin CS.** Interleukin-1B polymorphisms and gastric cancer risk- a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006;15(10): 1920-1928. http://dx.doi.org/10.1158/1055-9965.EPI-06-0267
36. **Parkin DM, Bray F, Ferlay J, Pisani P.** Global cancer statistics, 2002. CA: *A Cancer J Clin* 2005;55(2):74-108. http://dx.doi.org/10.3322/canjclin.55.2.74
37. **Correa P.** Human gastric carcinogenesis: a multistep and multifactorial process - First American Cancer Society Award Lecture on Cancer Epidemiology and Prevention. *Cancer Res* 1992;52(24):6735-6740.
38. **Camargo MC, Mera R, Correa P, et al.** Interleukin-1beta and interleukin-1 receptor antagonist gene polymorphisms and gastric cancer: a meta-analysis. *Cancer Epidemiol Biomarkers Prev* 2006;15(9):1674-1687. http://dx.doi.org/10.1158/1055-9965.EPI-06-0189
39. **Garte S, Gaspari L, Alexandrie AK, et al.** Metabolic gene polymorphism frequencies in control populations. *Cancer Epidemiol Biomarkers Prev* 2001;10(12):1239-1248.
40. **Ioannidis JP, Ntzani EE, Trikalinos TA.** 'Racial' differences in genetic effects for complex diseases. *Nat Genet* 2004;36(12):1312-1318. http://dx.doi.org/10.1038/ng1474
41. **Gonzalez CA, Sala N, Capella G.** Genetic susceptibility and gastric cancer risk. *Int J Cancer* 2002;100(3):249-260. http://dx.doi.org/10.1002/ijc.10466
42. **Ioannidis JP, Ntzani EE, Trikalinos TA, Contopoulos-Ioannidis DG.** Replication validity of genetic association studies. *Nat Genet* 2001;29(3):306-309. http://dx.doi.org/10.1038/ng749
43. **Kamangar F, Dores GM, Anderson WF.** Patterns of cancer incidence, mortality, and prevalence across five continents: defining priorities to reduce cancer disparities in different geographic regions of the world. *J Clin Oncol* 2006;24(14):2137-2150. http://dx.doi.org/10.1200/JCO.2005.05.2308
44. **Ioannidis JP.** Journals should publish all 'null' results and should sparingly publish 'positive' results. *Cancer Epidemiol Biomarkers Prev* 2006;15(1):186. http://dx.doi.org/10.1158/1055-9965.EPI-05-0921
45. **Rothman N, Skibola CF, Wang SS, et al.** Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. *Lancet Oncol* 2006;7(1):27-38. http://dx.doi.org/10.1016/S1470-2045(05)70434-4
46. **El-Omar EM, Rabkin CS, Gammon MD, et al.** Increased risk of noncardia gastric cancer associated with proinflammatory cytokine gene polymorphisms. *Gastroenterology* 2003;124(5):193-1201. http://dx.doi.org/10.1016/S0016-5085(03)00157-4
47. **Zeng ZR, Hu PJ, Hu S, et al.** Association of interleukin 1B gene polymorphism and gastric cancers in high and low prevalence regions in China. *Gut* 2003;52(12):1684-1689. http://dx.doi.org/10.1136/gut.52.12.1684
48. **Guo W, Wang N, Li Y, Zhang JH.** Polymorphisms in tumor necrosis factor genes and susceptibility to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma in a population of high incidence region of North China. *Chin Med J* 2005;118(22):1870-1878.
49. **Rocha GA, Guerra JB, Rocha AM, et al.** IL1RN polymorphic gene and cagA-positive status independently increase the risk of noncardia gastric carcinoma. *Int J Cancer* 2005;115(5):678-683. http://dx.doi.org/10.1002/ijc.20935
50. **Zhang JH, Li Y, Wang R, et al.** NQO1 C609T polymorphism associated with esophageal cancer and gastric cardiac carcinoma in North China. *World J Gastroenterol* 2003;9(7):1390-1393.
51. **Mestiri S, Bouaouina N, Ahmed SB, et al.** Genetic variation in the tumor necrosis factor-alpha promoter region and in the stress protein hsp70-2: susceptibility and prognostic implications in breast carcinoma. *Cancer* 2001;91(4):672-678. http://dx.doi.org/10.1002/1097-0142(20010215)91:4<672::AID-CNCR1050>3.0.CO;2-J
52. **Machado JC, Figueiredo C, Canedo P, et al.** A proinflammatory genetic profile increases the risk for chronic atrophic gastritis and gastric carcinoma. *Gastroenterology* 2003;125(2):364-371. http://dx.doi.org/10.1016/S0016-5085(03)00899-0
53. **Bacher M, Metz CN, Calandra T, et al.** An essential regulatory role for macrophage migration inhibitory factor in T-cell activation. *Proc Natl Acad Sci USA* 1996;93(15): 7849-7854. http://dx.doi.org/10.1073/pnas.93.15.7849
54. **Abe R, Peng T, Sailors J, et al.** Regulation of the CTL response by macrophage migration inhibitory factor. *J Immunol* 2001;66(2):747-753.