E-CADHERIN (CDH1) GENE -160C>A PROMOTER POLYMORPHISM AND RISK OF GASTRIC AND ESOPHAGEAL CANCERS

HALIT AKBAS[1], AHMET UYANIKOGLU[2], TIMUCIN AYDOGAN[2], AHMET ENGIN ATAY[3], FUAT DILMEC[1], SERKAN CERRAH[4], FERIDUN AKKAFA[1], HASIM NAR[2]

[1]Department of Genetics, Medical School of Harran University, Sanlıurfa - [2]Department of Gastroenterology, Harran University Faculty of Medicine, Sanliurfa - [3]Department of Internal Medicine, Bagcılar Education and Research Hospital, Istanbul - [4]Department of Gastroenterology, Erzurum Arial Education and Research Hospital, Erzurum,Turkey

ABSTRACT

Aim: The aim of this study was to investigate whether -160C>A single nucleotide polymorphism of the promoter region of the CDH1 gene (E-cadherin) is associated with gastric and esophageal cancers.

Methods: Ninety-eight patients with gastric and esophageal cancers and 105 gender- and age-matched controls were enrolled in the study. Genotyping of CDH1 -160C>A polymorphism (rs16260) was done by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP).

Results: The CDH1 -160C>A genotype and allele frequencies of the gastric and esophageal cancer patients did not differ significantly from those of healthy controls (p>0.05). With respect to tumour localization or histopathologic type, there was no significant association between CDH1 -160C>A genotype with gastric or esophageal carcinomas. (p>0.05).

Conclusıon: The present study indicates that the CDH1gene -160C>A polymorphism is not associated with gastric and esophageal cancers in the Turkish population.

***Key words**: Gastric cancer, esophageal cancer, E-cadherin, CDH1 gene, polymorphism.*

***Received** August 01, 2013; **Accepted** August 28, 2013*

Introduction

Gastric and esophageal cancers comprise a significant proportion of cancers affecting the upper gastrointestinal tract. Although the incidence of gastric cancers (GC) has decreased in the last 2 decades, it is still the 4th common cancer and 2nd most frequent cause of malignancy-related deaths worldwide[(1)]. On the other hand, the incidence of oesophagus cancer (OC) is relatively low however associated with poor prognosis due to advanced stage at the time of diagnosis[(2)]. The role of environmental factors on the development of GC and OC have been established. However only a small proportion of patients with risk factors may experience GC or OC that supports the role of genetical factors in carcinogenesis[(3-6)]. These two cancer types are associated with different pathologic characteristics, clinical features and prognosis. However common genetical background of two entities is an area of ongoing researches.

E-cadherin is a transmembrane glycoprotein encoded by the E-cadherin gene (CDH1) located on chromosome 16q22.1. It plays important roles in the establishment of adherent type junctions by mediating calcium-dependent cellular interactions, and is thought to be a tumour suppressor protein[(7)]. E-cadherin is also thought to be involved in intracellular signalling in normal epithelial cells. Downregulation of this molecule in epithelial cells is frequently associated with tumour formation and differentiation[(8)]. Partial or total loss of E-cadherin gene (CDH1) expression occurs in the majority of human carcinomas[(9)].

There are a number of polymorphisms clustered around the transcription start site of the E-cadherin gene. It was reported that single nucleotide polymorphisms (SNP)-160C>A

(rs16260) in the promoter region might alter the transcriptional activity of this gene[10, 11], which attracted a lot of attentions to investigate the possible effects of these polymorphism on the susceptibility of cancers including gastric and esophageal cancers (12-17). However, results of these association studies were controversial[18-22]. It is established that CDH1-160C>A gene polymorphism is an ethnicity-dependent risk factor for GC[23, 24]. To the best of our knowledge, lack of available data exist about the relation of CDH1-160C>A gene polymorphism and gastric and esophageal cancers in Turkish population. The aim of the present case-cohort study is to investigate the association of between the CDH1 gene-160C>A polymorphism with gastric and esophageal cancers in Turkish population.

Materials and methods

Sixty eight gastric and 30 esophageal cancer patients and 105 age and sex matched controls from same region and ethnic origin were enrolled to the study. Patients with gastric or oesophageal cancer were recruited from those admitted to the Endoscopy unit of Erzurum Education and Research Hospital, Erzurum, Turkey, between June 2011 and November 2012. Patients without overt cancer and family history of cancer were enrolled as healthy controls. Family history of cancer was defined as any cancer in at least first or second degree relatives and information was obtained by interview with the participants. Written informed consent was obtained from all patients and Ethics committee of Medical School of Harran University approved the study.

Diagnosis of GC was confirmed by endoscopic biopsy and histopathologic examination. A written informed consent was obtained from all patients before endoscopic intervention. Intravenous midazolam (0.05-0.1 mg/kg) was used for conscious sedation. Upper gastrointestinal system endoscopy was performed by Olympus GIFQ 160Z, Exera II (Olympus, America Corp., Melville, NY, USA). Endoscopy procedure and endoscopic examination was performed by the same gastroenterologist. Similarly, single pathologist carried out the histopathologic examination of biopsy samples.

OC and GC where macroscopically differentiated by their position about the cardioesophageal junction (respectively 1 cm above or 2 cm below). Differentiation of cancers located on cardioeosophageal junction was done by standard criteria which is introduced by Siewert and Stein[25]. Staging procedure was performed in accordance with Union Internationale Contre le Cancer system.

DNA extraction

Two ml of peripheral venous blood was collected from each subject and put in Vacutainer tubes containing EDTA and stored at-20°C until the extraction of the DNA. Genomic DNA extraction was performed by commercially available kits "Thermo Scientific GeneJET™ Whole Blood Genomic DNA Purification Kit" according to manufacturer's instructions.

Genotyping analysis

Polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assays were used to determine the-160 C>A single nucleotide polymorphism (rs16260) of the CDH1 gene as previously described[26]. PCR amplification was generated using the following oligonucleotide primers: forward 5'- GCCCCGACTTGTCTCTCTAC-3' and reverse 5'- GGCCACAGCCAATCAGCA-3' (product of 447 bp).

To amplify the region containing the-160 C>A polymorphism of the CDH1 gene, a PCR reaction was carried out in a 10 µl reaction volume containing 1 x PCR buffer, 2 mM MgCl2, 0.2 mM each deoxynucleotide triphosphate (dNTPs, Fermentas, St. Leon-Rot, Germany), 40 ng of DNA, 0.2 μM of each primer (Bio Basic Inc., Ontario, Canada), and 0.5 unit of Taq DNA Polymerase (Fermentas). The PCR conditions were: 3 min of initial denaturation at 94 °C, followed by 30 cycles at 95 °C for 30 s, 30 s at 60 °C for annealing, and 30 s at 72 °C for extension, followed by 5 min at 72 °C for final extension. The PCR products were 447 bp.

For the RFLP analysis, PCR-amplified products were digested with HincII for 2-5 h at 37 °C (New England Biolabs, Hertfordshire, UK). The amplicon with the homozygous AA allele of CDH1 was cleaved by HincII, yielding 367 and 80 bp fragments, whereas the amplicon with the homozygous CC allele remained uncut, yielding a 447 bp band. The amplicon with the heterozygous CT allele yielded three fragments of 447, 367, and 80 bp length. The digested products were separated on 3% agarose gel along with a 100-1500 bp DNA ladder (Bio Basic Inc.).

Ethidium bromide-stained gels were visualized under UV light using the Alpha Imager System (AlphaInnotech, San Leandro, CA, U.S.A.)(Fig. 1).

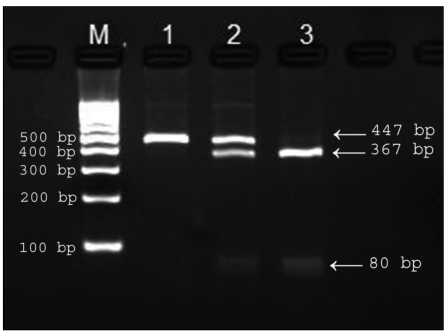

Figure 1: PCR-RFLP products of CDH1 gene -160C>A polymorphism obtained by 2.5% agarose gel electrophoresis. Lane M shows 100bp DNA ladder. Lane 1 shows homozygous alleles (C/C), lane 2 shows heterozygous alleles (C/A) and lane 3 shows homozygous polymorphic alleles (A/A).

Statistical analysis

Statistical analysis were performed using the SPSS Statistical package, version 17.0 for Windows (SPSS. Inc., U.S.A.). The normality of distribution was evaluated by the Kolmogorov-Smirnov test, and the normally distributed data were analyzed by the Student's t-test. Genotype distributions and allele frequencies of the CDH1 gene-160 C>A polymorphism were analysed by the chi-square test. The observed and expected genotype frequencies of control and patient groups were compared using chi-square test to determine if they were in Hardy-Weinberg equilibrium. All the statistical tests were two-sided, and a P value <0.05 was considered to be statistically significant.

Results

The demographic characteristics of gastric and esophageal cancer cases and controls are summarized in Table 1. There was no statistically significant difference in the distribution of sex and age between case and control group (P>0.05).

	Patient group	Control group	P
Age (years) (mean ± SD)	64.28 ± 11.6	61.44 ± 14.24	>0.05
Sex (female/male)	40/60	45/60	>0.05

Table 1: Demographic characteristics of the study group.

Genotyping of CDH1 -160 C>A polymorphism was entirely achieved in patients and control subjects. Genotype distributions of both patients and controls were consistent with Hardy-Weinberg equilibrium.

CDH1-160C>A	Patients n=98 (%)	Controls n=105 (%)	OR	95% CI	P-value
Genotype					
CC	31 (32%)	44 (42%)	Reference		
CA	56 (57%)	54 (51%)	1.47	0.81-2.66	0.2
AA	11 (11%)	7 (7%)	2.23	0.77-6.39	0.12
Allele					
C	118 (60%)	142 (68%)	Reference		
A	78 (40%)	68 (32%)	1.38	0.91-2.07	0.11

Table 2: Genotype distributions and allele frequencies of CDH1-160C>A polymorphism in gastric and esophageal cancer patients and healthy controls.

Genotype distributions and allele frequencies of CDH1-160C>A polymorphism in gastric and esophageal cancer patients and healthy controls were shown in table 2. When homozygote CC genotype (wild type) was considered as reference, there was no significant difference between genotype distribution of patients and controls (P>0.05). Also the difference between allele frequencies of patients and controls were nonsignificant (P>0.05). Genotype distribution and allele frequency of CDH1 -160 C>A in 68 patients with GC and 30 patients with esophageal cancer were similar to that of control subjects (Table 3).

CDH1-160C>A	Genotype					Allele frequency			
	CC	CA	AA	OR (95% CI)	P	C	A	OR (95% CI)	P
Tumor localization									
Controls	44	54	7	Reference		142	68	Reference	
Stomach	22	36	10	1.5 (0.7-2.8)	>0.05	80	56	1.4(0.9-2.2)	>0.05
Esophagus	9	20	1	1.6 (0.7-4.0)	>0.05	38	22	1.2(0.6-2.2)	>0.05
Tumor Pathology									
Controls	44	54	7	Reference		142	68	Reference	
Adenocarcinoma	14	26	6	1.6 (0.7-3.4)	>0.05	46	34	1.4(0.8-2.4)	>0.05
Squamous cell carcinoma	10	17	3	1.4 (0.6-3.3)	>0.05	35	21	1.2(0.7-2.3)	>0.05

Table 3: Comparison of genotype distribution and allele frequency of CDH1-160 C>A polymorphism in patients with gastric and oesophageal cancer and control subjects in terms of tumour localization and Tumour Pathology.

Adenocarcinoma (n=46) and squamous cell carcinoma (n=30) were the most frequent histologic

type in the present study. Less frequent histologic types were lymphoma (n=3), adenosquamous carcinoma (n=3), mucinous adenocarcinoma (n=2) and ringed cell carcinoma (n=1). The histologic type was not determined in 13 patients. Due to limited number of patients with cancers other than adenocarcinoma and squamous cell carcinoma, statistical analysis was performed only in patients with this last ones. The difference between genotype distribution and allele frequency of CDH1 -160 C>A of patients with adenocarcinoma or squamous cell carcinoma and controls were nonsignificant (Table 3).

Discussion

The present study indicated that a significant association does not exist between CDH1gene-160C>A polymorphism and gastric or esophageal cancers in the Turkish population. Our study population was composed of residents of eastern Anatolia which is known as the highest incidence area for GC and EC OC in Turkey[27]. High incidence of upper gastrointestinal system malignancies in eastern region of Turkey is related to, at least in part, genetical factors as well as environmental ones. Environmental factors including nutritional inhabits and helicobacter pylori serum positivity are well established risk factors that are frequent in Turkey[28]. However, lack of available data exist in genetical background of gastric and esophageal cancers from Turkey.

Despite well known risk factors, GC or OC develops only in a small group of patients having majority of these predisposing conditions that suggests the central role of genetical factors[29]. A group of epidemiological studies indicated that OC and GC may share similar multifactorial background resulted from complex interaction of environmental and genetic factors[30, 31].

E-cadherin acts as a calcium-dependent intercellular adhesion molecule and plays a major role in the maintenance of intercellular adhesion[24, 26]. Watabe et al first described the involvement of E-cadherin in contact-dependent inhibition of cell growth. Although the idea that E-cadherin is involved in a group tumours has been determined, the exact molecular role of of E-cadherin in tumour formation is still unclear. Increased accumulation of β-catenin as a consequence of E-cadherin loss during tumour formation is a possible mechanism[32]. In some human cancer studies it was established that E-cadherin expression is suppressed and considered as critical step in tumour development and progression[10].

Polymorphisms of the E-cadherin gene are responsible for interindividual variation in the production of E-cadherin and accused of increasing individual susceptibility to cancer[26]. Transcriptional activity of E-cadherin is influenced by-160C>A single nucleotide polymorphism at promoter region of the gene. When compared to the wild-type C allele, the A allele has diminished transcriptional efficiency by 68%, possibly depending on stronger transcriptional factor binding activity of the C allele[10]. Therefore, several cancers including gastric malignancies were found to associated with-160C>A polymorphism of CDH1 gene[12-17]. However, contrary results including gastric and esophageal cancers have also been reported (18-22). It is suggested that the-160C/A polymorphism in the CDH1 promoter may play different roles in different cancer types. Also in the present study we did not observe any association between-160C>A SNP and the risk of gastric and esophageal cancers.

Recent two meta analysis revealed that the frequency of CDH1 -160C<A polymorphism of E-cadherin gene vary significantly and ıt was determined as an ethnicity dependent risk factor for GC[23, 24]. Among them, Wang et al. reported that the variant genotypes of the_160C>A polymorphism were associated with significantly decreased GC risk among Asians, but not among Europeans (23). On the other hand, meta-analysis by Chen et al suggests that CDH1 -160C>A polymorphism may be associated with risk of GC among Caucasians, but not among Asians[24]. Due to controversial results from different ethnic populations, we aimed to examine the association of CDH1 -160C>A polymorphism and upper gastrointestinal cancers in Turkish population.

With respect to the relation of GC that constitute vast majority of our patients group, and CDH1 -160C>A polymorphism, there are controversial reports from different ethnic populations. Reports from Oman and Mexico determined that CDH1 -160C>A polymorphism is associated with an increased risk of GC[12, 13]. In another study from Brazil, CDH1 -160C>A polymorphism may increase the risk of developing GC[14]. Cattaneo et al. conducted a study in an Italian population and stated that the A allele of E-cadherin gene may act as a low penetrance cancer susceptibility gene[33]. Studies from Caucasian population have apparently pointed out a significant association between CDH1

-160C>A polymorphism and GC. In contrast, no significant association was observed in Asian population such as Korea, China and Japan[20, 34, 35]. However some reports of Caucasian populations from Italy and England have failed to determine the same association[36, 37]. Also in this first report from Turkish population that examine the association of CDH1 -160C>A polymorphism with GC, we failed to demonstrate a significant relation.

Two study from China have shown that there is no significant association between CDH1 -160C>A polymorphism and OC[21, 34]. Although the sample size of patients with esophageal cancer was small in our study, similarly we could not observe a significant association between CDH1 -160C>A polymorphism and OC.

The present study have some limitations. First, the sample size was relatively low. Second, analysing additional single nucleotide polymorphisms (SNPs) of CDH1 gene may augment correctness of the study. The study population of our study was composed of Turkish patients with GC or OC and control subjects that inhibits to generalize our results to our populations. The final limitation in our study was lack of information about smoking status, dietary inhabits and helicobacter pylori positivity of our participants.

Conclusion

Our study provided evidences that the CDH1gene -160C>A polymorphism does not raised the risk of SCC and GCA in the population of Western Anatolia. Our results along with the interpretation of previous reports indicates that the160A allele of E-cadherin is an ethnicity-dependent risk factor for GC. it is safe to conclude that the negative association of CDH1gene -160C>A polymorphism with GC and EC OC should be regarded as preliminary until results are confirmed by large scaled prospective studies.

References

1) Kandaz M, Ertekin MV, Bilici M. *Retrospective analysis of patients with esophageal cancer treated with radiotherapy and/or chemoradiotherapy*. Tumori. 2012 Jul-Aug; 98(4): *445-50*.
2) Hussein N. *Helicobacter pylori and gastric cancer in the Middle East: A new enigma?* World J Gastroenterol. 2010 July 14; 16(26): *3226-3234*.
3) Zagari, R.M., Bazzoli, F. *Gastric cancer: who is at risk?* Dig. Dis. 2004, 22: *302-305*.
4) Roberts-Thomson, I.C., Butler, W. J. *Polymorphism and gastric cancer*. J. Gastroenterol. Hepatol. 2005, 20: *793-794*.
5) Hamilton JP, Meltzer SJ *A review of the genomics of gastric cancer*. Clin. Gastroenterol. Hepatol. 2006, 4: *416-425*.
6) Boccia S, Gianfagna F, La Torre G, Persiani R, D'Ugo D, van Duijn, CM, Ricciardi G *Genetic Susceptibility to Gastric Cancer: A review of the Published Meta-Analyses*. In Cardinni, D.C. (ed.), Research Focus on Gastric Cancer. Hauppauge, NY: Nova Science Publishers 2008, *137-163*.
7) Gumbiner BM. *Regulation of cadherin-mediated adhesion in morphogenesis*. Nat Rev Mol Cell Biol 2005; 6: *622-634*.
8) Cavallaro U, Christofori G. *Cell adhesion and signalling by cadherins and Ig-CAMs in cancer*. Nat Rev Cancer 2004; 4: *118-132*.
9) Chen HC, Chu RY, Hsu PN, Hsu PI, Lu JY, Lai KH, Tseng HH, Chou NH, Huang MS, Tseng CJ, Hsiao M. *Loss of E-cadherin expression correlates with poor differentiation and invasion into adjacent organs in gastric adenocarcinomas*. Cancer Lett 2003; 201: *97-106*.
10) Li LC, Chui RM, Sasaki M, et al. *A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities*. Cancer Res 2000; 60: *873-6*.
11) Shin Y, Kim IJ, Kang HC, et al. The *E-cadherin -347G!GA promoter polymorphism and its effect on transcriptional regulation*. Carcinogenesis 2004; 25: *895-9*.
12) Al-Moundhri MS, Al-Khanbashi M, Al-Kindi M, Al-Nabhani M, Burney IA, Al- Farsi A, et al. *Association of E-cadherin (CDH1) gene polymorphisms and gastric cancer risk*. World J Gastroenterol 2010; 16: *3432-6*.
13) Medina-Franco H, Ramos-De la Medina A, Vizcaino G, Medina-Franco JL. *Single nucleotide polymorphisms in the promoter region of the E-cadherin gene in gastric cancer: case-control study in a young Mexican population*. Ann Surg Oncol 2007; 14: *2246-9*.
14) Borges Bdo N, Santos Eda S, Bastos CE, Pinto LC, Anselmo NP, Quaresma JA, et al. *Promoter polymorphisms and methylation of E-cadherin (CDH1) and KIT in gastric cancer patients from northern Brazil*. Anticancer Res 2010; 30: *2225-33*.
15) Verhage B A, van Houwelingen K, Ruijter T E, Kiemeney L A, Schalken J A. *Single-nucleotide polymorphism in the Ecadherin gene promoter modifies the risk of prostate cancer*. Int J Cancer 2002; 100: *683-5*.
16) Zhang X, Ma X, Zhu Q G, Li L C, Chen Z, Ye Z Q. *Association between a C/A single nucleotide polymorphism of the E-cadherin gene promoter and transitional cell carcinoma of the bladder*. J Urol 2003; 170: *1379-82*.
17) Tipirisetti NR, Govatati S, Govatati S, Kandukuri LR, Cingeetham A, Singh L, Digumarti RR, Bhanoori M, Satti V. *Association of E-cadherin single-nucleotide polymorphisms with the increased risk of breast cancer: a study in South Indian women*. Genet Test Mol Biomarkers. 2013 Jun; 17(6): *494-500*.
18) Tsukino H, Kuroda Y, Imai H, et al. *Lack of evidence for the association of E-cadherin gene polymorphism with increased risk of progression of prostate cancer.* Urol Int 2004; 72: *203-7*.

19) Porter TR, Richards FM, Houlston RS, et al. *Contribution of cyclin d1 (CCND1) and E-cadherin (CDH1) polymorphisms to familial and sporadic colorectal cancer*. Oncogene 2002; 21: *1928-33*.
20) Park WS, Cho YG, Park JY, Kim CJ, Lee JH, Kim HS, et al. *A single nucleotide polymorphism in the E-cadherin gene promoter-160 is not associated with risk of Korean gastric cancer*. J Korean Med Sci 2003; 18: *501-4*.
21) Zhang XF, Wang YM, Wang R, et al. *Correlation of E-cadherin polymorphisms to esophageal squamous cell carcinoma and gastric cardiac adenocarcinoma*. Chin J Cancer 2005; 24: *513-19*.
22) Song CG, Huang CM, Liu X, et al. *Association of _160C>A polymorphism in CDH1 gene with gastric cancer risk in Fujian Chinese population*. Zhonghua Yi Xue Yi Chuan Xue Za Zhi 2005; 22: *557-9*.
23) Wang GY, Lu CQ, Zhang RM, Hu XH, Luo ZW. *The E-cadherin gene polymorphism 160C->A and cancer risk: a HuGE review and meta-analysis of 26 case-control studies*. Am J Epidemiol 2008; 167: *7-14*.
24) Chen B, Zhou Y, Yang P, Liu L, Qin XP, Wu XT. *CDH1 -160C>A gene polymorphism is an ethnicity-dependent risk factor for gastric cancer. Cytokine*. 2011 Aug; 55(2): *266-73*.
25) Siewert JR, Stein H. *Classification of adenocarcinoma of the esophago-gastric junction*. Br J Cancer 1998; 85: *1457-9*.
26) Kiemeney LA, van Houwelingen KP, Bogaerts M, Witjes JA, Swinkels DW, den Heijer M, Franke B, Schalken JA, Verhaegh GW. *Polymorphisms in the E-cadherin (CDH1) gene promoter and the risk of bladder cancer*. Eur J Cancer. 2006 Dec; 42(18): *3219-27*.
27) Huang CH, Chiou SH. *Proteomic analysis of upregulated proteins in Helicobacter pylori under oxidative stress induced by hydrogen peroxide*. Kaohsiung Journal of Medical Sciences. 2011; 27: *544-53*.
28) Wang HM, Chuang SM, SuYC, Li YH, Chueh PJ. *Down-Regulation of Tumor Associated NADH Oxidase, tNOX (ENOX2), Enhances Capsaicin-Induced Inhibition of Gastric Cancer Cell Growth*. Cell Biochem Biophys. 2011; 61: *355-66*.
29) Geddert H, Kiel S, Zotz RB, Zhang J, Willers R, Gabbert HE, Sarbia M. *Polymorphism of p16 INK4A and cyclin D1 in adenocarcinomas of the upper gastrointestinal tract*. J Cancer Res Clin Oncol. 2005 Dec; 131(12): *803-8*.
30) Zhang J, Cui Y, Kuang G, Li Y, Wang N, Wang R, Guo W, Wen D, Wei L, Yu F, Wang S. *Association of the thymidylate synthase polymorphisms with esophageal squamous cell carcinoma and gastric cardiac adenocarcinom*a. Carcinogenesis. 2004 Dec; 25(12): *2479-85*.
31) Guo W, Blot WJ, Li JY, Taylor PR, Liu BQ, Wang W, Wu YP, Zheng W, Dawsey SM and Li B. (1994) *A nested case-control study of oesophageal and stomach cancers in the Linzhou nutrition intervention trial*. Int. J. Epidemiol., 23, *444-450*.
32) Nelson WJ, Nusse R. *Convergence of Wnt, beta-catenin, and cadherin pathways*. Science 2004; 303: *1483-1487*.
33) Cattaneo F, Venesio T, Molatore S, Russo A, Fiocca R, Frattini M, et al. *Functional analysis and case–control study of _160C/A polymorphism in the E-cadherin gene promoter: association with cancer risk*. Anticancer Res 2006; 26: *4627-32*.
34) Zhang XF, Wang YM, Ge H, Cao YY, Chen ZF, Wen DG, et al. *Association of CDH1 single nucleotide polymorphisms with susceptibility to esophageal squamous cell carcinomas and gastric cardia carcinomas*. Dis Esophagus 2008; 21: *21-9*.
35) Yamada H, Shinmura K, Ikeda S, Tao H, Otani T, Hanaoka T, et al. *Association between CDH1 haplotypes and gastric cancer risk in a Japanese population*. Scand J Gastroenterol 2007; 42: *1479-85*.
36) Corso G, Berardi A, Marrelli D, Pedrazzani C, Garosi L, Pinto E, et al. *CDH1 C -160A promoter polymorphism and gastric cancer risk*. Eur J Cancer Prev 2009; 18: *46-9*.
37) Pharoah PD, Oliveira C, Machado JC, Keller G, Vogelsang H, Laux H, et al. *CDH1 c-160a promotor polymorphism is not associated with risk of stomach cancer*. Int J Cancer 2002; 101: *196-7*.

Request reprints from:
HALIT AKBAS
Department of Medical Biology, Medical Faculty
University of Harran, Sanliurfa
(Turkey)