World Journal of Gastroenterology

Online Submissions: http://www.wjgnet.com/1007-9327office
wjg@wjgnet.com
doi:10.3748/wjg.v16.i27.3432

World J Gastroenterol 2010 July 21; 16(27): 3432-3436
ISSN 1007-9327 (print)

BRIEF ARTICLE

Association of *E-cadherin* (*CDH1*) gene polymorphisms and gastric cancer risk

Mansour S Al-Moundhri, Manal Al-Khanbashi, Mohammed Al-Kindi, Maryam Al-Nabhani, Ikram A Burney, Abdulaziz Al-Farsi, Bassim Al-Bahrani

Mansour S Al-Moundhri, Ikram A Burney, Medical Oncology Unit, Department of Medicine, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Sultanate of Oman
Manal Al-Khanbashi, Maryam Al-Nabhani, Department of Clinical Genetics, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Sultanate of Oman
Mohammed Al-Kindi, Department of Biochemistry, College of Medicine and Health Sciences, Sultan Qaboos University, Muscat 123, Sultanate of Oman
Abdulaziz Al-Farsi, Bassim Al-Bahrani, National Oncology Center, Royal Hospital, Muscat 111, Sultanate of Oman
Author contributions: Al-Moundhri MS designed the research, analyzed the data and wrote the manuscript; Al-Khanbashi M, Al-Nabhani M and Al-Kindi M performed the technical analysis; Burney IA, Al-Farsi A and Al-Bahrani B contributed to recruitment of patients and reviewed the manuscript.
Correspondence to: Dr. Mansour S Al-Moundhri, Associate Professor and Consulting Oncologist, Medical Oncology Unit, Department of Medicine, College of Medicine and Health Sciences, Sultan Qaboos University, PO Box 35, Muscat 123, Sultanate of Oman. mansours@squ.edu.om
Telephone: +968-99437301 Fax: +968-24415175
Received: March 24, 2010 Revised: April 24, 2010
Accepted: May 1, 2010
Published online: July 21, 2010

Abstract

AIM: To investigate the associations between *CDH1* gene polymorphisms and gastric cancer (GC) risk predisposition.

METHODS: We analyzed four *CDH1* polymorphisms (*+54 T>C*, *-160 C>A*, *-616 G>C*, *-3159 T>C*) in an Omani population, by extraction of genomic DNA from the peripheral blood of 192 patients with GC and 170 control participants and performed *CDH1* genotyping using DNA sequencing.

RESULTS: *CDH1 -160 -AA* genotype was associated with an increased risk of GC (OR = 3.6, 95% CI: 1.1-11.8) (P = 0.03). There was no significant association between the other polymorphisms and GC risk. The haplotype analysis of *+54 T>C*, *-160 C>A*, *-616 G>C*, *-3159 T>C* genotypes revealed that the OR of *CCGC* and *CAGC* haplotypes was 1.5 (95% CI: 0.7-3.5) and 1.5 (95% CI: 0.2-3.0), but did not reach statistical significance.

CONCLUSION: The current study suggests that the *-160 AA* genotype was associated with an increased risk of GC in Oman.

Key words: Gastric cancer; Polymorphism; *CDH1*

Peer reviewer: Ki-Baik Hahm, MD, PhD, Professor, Gachon Graduate School of Medicine, Department of Gastroenterology, Lee Gil Ya Cancer and Diabetes Institute, Lab of Translational Medicine, 7-45 Songdo-dong, Yeonsu-gu, Incheon 406-840, South Korea

Al-Moundhri MS, Al-Khanbashi M, Al-Kindi M, Al-Nabhani M, Burney IA, Al-Farsi A, Al-Bahrani B. Association of *E-cadherin* (*CDH1*) gene polymorphisms and gastric cancer risk. *World J Gastroenterol* 2010; 16(27): 3432-3436 Available from: URL: http://www.wjgnet.com/1007-9327/full/v16/i27/3432.htm DOI: http://dx.doi.org/10.3748/wjg.v16.i27.3432

INTRODUCTION

Gastric cancer (GC) is the fourth most common cancer and second most common cause of cancer mortality worldwide; therefore, it remains a global health burden[1,2]. GC has been associated with *Helicobacter* infection and environmental factors such as smoking, salted fish, and low intake of fruit and vegetables[3,4]. However, while these factors might affect large proportions of some populations, only subsets of these populations develop GC, and

therefore, increased genetic susceptibility has been postulated. Possible genetic risk factors have included single nucleotide polymorphisms (SNPs) in several pathways involved in chronic inflammation of gastric mucosa and subsequent carcinogenesis. The involved SNPs affect agents such as pro-inflammatory cytokines, xenobiotic metabolizing enzymes, and growth factors[5-11]. The study of these molecular pathways has helped to identify individuals at higher risk, particularly when examined with *Helicobacter pylori* (*H. pylori*) infection and other environmental exposure[7,8].

Adhesion molecules, especially the calcium-dependent intercellular adhesion molecule E-cadherin and its *CDH1* gene (located on chromosome 16), play a central role in carcinogenesis and metastasis[10,12]. The *CDH1* gene encodes a transmembrane glycoprotein that mediates intercellular adhesion and cellular polarity. The E-cadherin protein is a tumor invasion suppressor, and loss of its function results in transition to an invasive phenotype in human epithelial cancers[10,12].

Several SNPs in the *CDH1* gene are associated with GC. The most widely studied polymorphism is *CDH1 -160C>A*, where the *A* allele decreases transcriptional activity of the *CDH1* gene and E-cadherin expression, and increases susceptibility to GC in some populations[9,13-19]. Moreover, several other SNPs, including *+54 T>C*, *-3159 T>C*, *-160 C>A*, *-2076 C>T* and *-616 G>C*, were studied in Japanese and Italian populations, which resulted in the identification of haplotypes associated with increased risk of GC[12,20].

The above studies have highlighted the ethnic variation in frequency and risk predisposition of these SNPs[15,16]. Therefore, we studied in an Omani population, four *CDH1* gene polymorphisms (*+54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C*) that were previously examined in Japanese and Italian populations[12,20]. We evaluated the potential association of these SNPs and their haplotypes with GC susceptibility in a case-control design.

MATERIALS AND METHODS

Study participants

The study population consisted of a series of unrelated patients with GC who were diagnosed at two main hospitals in the Sultanate of Oman (Sultan Qaboos University Hospital and Royal Hospital). The healthy control group comprised persons of the same ethnic and geographical origin as the patients. The Medical Research and Ethics Committee of the College of Medicine of Sultan Qaboos University approved the study design. The study participants provided informed consent prior to participation, in compliance with the Declaration of Helsinki.

Genotyping method

From each participant, 10 mL blood was collected in an EDTA tube and stored frozen until the extraction of the DNA. DNA was extracted from whole blood using a commercial DNA blood kit (Gentra Puregene DNA Purification kit; Qiagen, Gaithersburg, MD, USA) and stored until processing for genotyping.

Analysis of the *CDH1* SNPs, *+54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C*, was performed using multiplex polymerase chain reaction (PCR) with an ABI premix. Genomic DNA from whole blood was used as a PCR template in a total reaction volume of 10 μL that contained 10 pmol designed primers: *+54 T>C (rs3743674)*: [5'-CCCCTGGTCTCATCATTTC-3' (forward) and 5'-AATTCCTCCAAGAATCCCCAG-3' (reverse)]; *160 C>A (rs16260)*: [5'-TGATCCCAGGTCTTAGTGAG-3' (forward) and 5'-GCTCCTCAGGACCCGAAC-3' (reverse)]; *-616 G>C (rs7203904)*: [5'-TTGACTGAGGCCACAGAGTG-3' (forward) and 5'-CTGCCTAAATCTGCTGAGCC-3' (reverse)]; *-3159 T>C* (*rs2010724*): [5'-GAGCTTCCCAGAGCCTTTCT-3' (forward) and 5'-ATTGGACTTGCCAAGGGTG-3' (reverse)]. PCR was performed as follows: one cycle at 94℃ for 10 min, 35 cycles at 94℃ for 30 s, 59℃ for 30 s, and 72℃ for 30 s, followed by 72℃ for 5 min. The final extension was at 72℃ for 10 min. PCR products were analyzed on a 2.5% agarose gel stained with ethidium bromide and photographed under UV light. The PCR product was subsequently sequenced in an ABI PRISM 3100 sequencer using BigDye Terminator v3.1 Cycle Sequencing method (Applied Biosystems,USA) as recommended by the manufacturer. Candidate SNP regions were detected and typed with the aid of DNA Star Software (DNASTAR, Madison, WI, USA).

Statistical analysis

The genotypic distributions of different polymorphic loci in the control samples were compared with those expected from the Hardy-Weinberg equilibrium using the χ^2 test. The differences in frequency distributions of the genotypes between the patient and control groups were also tested using the χ^2 test. Age- and sex-adjusted ORs and 95% CIs were calculated using logistic regression analysis. Haplotype frequencies, haplotype-survival analyses, and standardized disequilibrium coefficients (D) were calculated using Thesias software available at http://genecanvas.ecgene.net/. $P < 0.05$ was considered statistically significant. Analysis of data was performed using SPSS version 10.0 software (SPSS, Chicago, IL, USA).

RESULTS

One hundred and ninety-two GC patients and 170 unrelated controls were included. The age range for the participants included in the study was 19-80 years, and the mean ages for the patients and controls were 55.1 ± 12.5 and 32.8 ± 6.6 years, respectively. The percentages of male and female participants were 58.3% and 41.7% for GC patients respectively, and 56.5% and 43.5% for controls. *H. pylori* infection status was available in 116 GC patients and 90 control participants, with a positivity rate of 58% and 60% (Table 1). Most GC patients in this cohort presented at an advanced stage, with slight predominance of non-intestinal type according to Lauren's classification, as shown in Table 2.

Table 1 Demographic data, *Helicobacter* status, and smoking in gastric cancer patients and control subjects

Variable	GC patients	Control
No. of subjects	192	170
Age (yr), mean ± SD	32.8 ± 6.6	55.1 ± 12.5
Male, %	58.30	56.50
H. pylori status, *n*	116[1]	90[1]
Positive, *n* (%)	67 (58)	54 (60)

[1]The number of GC patients and control participants for whom *Helicobacter pylori* (*H. pylori*) serology was available. GC: Gastric cancer.

Table 2 Clinicopathological features of 192 gastric cancer patients

Variable	*n* (%)
Lauren's classification	
Intestinal	93 (48.5)
Mixed and diffuse	99 (51.5)
Histological grade	
G1	11 (5.8)
G2	80 (41.6)
G3	101 (52.6)
T stage	
T1 + T2	32 (16.7)
T3 + T4	160 (83.3)
Lymph node involvement	
Negative	26 (13.5)
Positive	166 (86.5)
TNM stage	
Ⅰ + Ⅱ	33 (17.2)
Ⅲ + Ⅳ	159 (82.8)

CDH1 genotypic frequencies and GC risk

The frequencies of the *+54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C* genotypes are shown in Table 3. The SNP analysis was successful in the majority of GC patients and control subjects, however, 15-23 samples failed for GC patients and 4-13 samples for control subjects, as shown in Table 3. The allelic distributions for control subjects did not deviate significantly from those expected from the Hardy-Weinberg equilibrium. There was a significant association between the *CDH1-160 AA* genotype, with an increased risk of GC, with OR 3.6 (95% CI: 1.1-11.8, $P = 0.03$) (Table 3). There was no significant association between the other *CDH1* polymorphisms and GC risk (Table 3).

Table 3 *CDH1* genotype frequencies and their associated risk of gastric cancer predisposition

CDH1 genotype	Patients *n* (%)[1]	Control *n* (%)[1]	OR[2] (95% CI)	*P* value
+54 T>C	*n* = 174	*n* = 157		
TT	25 (14.4)	22 (14.0)	1	
TC	70 (40.2)	75 (47.8)	0.9 (0.4-2.2)	0.9
CC	79 (45.4)	60 (38.2)	0.9 (0.4-2.4)	0.8
CC + TC	149 (85.6)	135 (86.0)	0.9 (0.4-2.1)	0.8
TT + TC	95 (54.6)	97 (61.8)	1.0 (0.5-2.0)	1.0
C allele	66.0	62.0		
-160 C>A	*n* = 174	*n* = 166		
CC	93 (53.6)	93 (56.0)	1	
CA	60 (34.5)	65 (39.2)	0.6 (0.3-1.1)	0.1
AA	21 (12.0)	8 (4.8)	3.6 (1.1-11.8)	0.03
AA + CA	81 (46.5)	73 (44.0)	0.8 (0.4-1.5)	0.8
CC + CA	153 (88.1)	158 (95.2)	3.4 (1.4-13.9)	0.01
A allele	29.0	24.0		
-616 G>C	*n* = 172	*n* = 159		
GG	84 (48.8)	71 (44.7)	1	
GC	65 (37.8)	69 (43.4)	0.9 (0.6-1.8)	0.7
CC	23 (13.4)	19 (12.0)	1.8 (0.6-5.1)	0.3
GC + CC	88 (51.2)	88 (55.4)	1.0 (0.6-1.9)	0.9
GG + GC	149 (86.6)	140 (88.1)	1.8 (0.7-5.2)	0.3
C allele	32.0	34.0		
-3159 T>C	*n* = 177	*n* = 166		
TT	52 (29.7)	47 (28.3)	1	
TC	72 (41.1)	78 (47.0)	0.9 (0.4-1.7)	0.7
CC	53 (30.3)	41 (24.7)	1.0 (0.5-2.0)	0.9
CC + TC	125 (71.4)	119 (71.7)	0.9 (0.5-1.7)	0.8
TT + TC	124 (70.8)	125 (75.3)	1.1 (0.54-2.2)	0.8
C allele	50.0	48.0		

[1]The number of patients and control indicates successful single nucleotide polymorphism analysis for each polymorphism; [2]Age and sex-adjusted.

Haplotype analysis

The common haplotypes were identified, as shown in Table 4. There were significant differences in the distribution of these haplotypes between patients and controls (Table 4). The haplotype analysis of *+54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C* genotypes revealed that the OR of *CCGC* and *CAGC* haplotypes was 1.5 (95% CI: 0.7-3.5) and 1.5 (95% CI: 0.2-3.0), respectively, but did not reach statistical significance.

Table 4 Frequencies of *CDH1* haplotypes and associated risk of gastric cancer predisposition

Haplotype	Frequency (%)		OR[1] (95% CI)	*P* value
	Patient	Control		
TCGT	20.1	22.1	1	
TACG	10.4	11.0	0.99 (0.5-1.9)	1.0
CCGT	16.5	17.7	1.0 (0.5-1.8)	1.0
CCGC	7.0	5.0	1.5 (0.7-3.5)	0.3
CCCT	11.1	9.9	1.1 (0.5-2.3)	0.8
CCCC	15.1	16.6	0.9 (0.6-1.6)	0.8
CAGC	10.7	7.1	1.5 (0.8-3.0)	0.2
CACC	5.8	5.7	1.1 (0.5-2.4)	0.8

[1]Age and sex-adjusted.

DISCUSSION

Six polymorphisms of the *CDH1* gene have been studied previously in Caucasian, East Asian, and Mexican populations and included: *-616 G>C*, *-160 C>A*, *-3159 T>C*, *+54 T>C*, *2076C>T* and *347G>GA*[12-17,20]. A recent meta-analysis has highlighted the role of ethnic differences by showing that the associations between these polymorphisms and GC among Asian and Caucasian populations are in opposite directions[15,18]. Therefore, we investigated the association between GC and the *CDH1 +54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C* polymorphisms in an Omani population, an ethnic group in which the association between GC and these polymorphisms has not been studied previously.

The most widely studied *CDH1* polymorphism in various cancers is *CDH1 -160 C>A*[13-19]. In the present study, we found that this polymorphism affected the risk of developing GC. The carriage of the *CDH1 -160 AA* genotype increased the risk of GC (OR: 3.6, 95% CI: 1.1-11.8) (P = 0.03). Two meta-analyses have suggested that the association of *CDH1 -160 AA* with GC risk is ethnicity-dependent, whereby the OR estimates for *CDH1 -160 AA* carriers are less than 1.0 for Asians but significantly greater than 1.0 for Caucasians[15,18]. Thus, our results are consistent with the findings in Caucasian populations. The explanation for this observation remains unclear, however, the A variant decreases transcription efficiency by 68% compared with the C allele *in vitro*[9]. The altered expression of adhesion molecule E-cadherin results in tumor development and carcinogenesis. Possible explanations for the discrepancy between ethnic groups include the frequency of the polymorphism in the population studied or linkage disequilibrium with other, perhaps undiscovered, functional SNPs in the *CDH1* gene. The present study shows that there is no association between the *CDH1 +54 T>C* and *-616 G>C* SNPs and GC development. Although a study by Zhang *et al*[13] has found an association between *+54 T>C* and esophageal and gastric cancer, other studies were negative[15].

It has been suggested that haplotype analysis might be more useful than single SNP analysis in identifying cancer risk[12,20]. In particular, the combined analysis of *CDH1 -160 C>A*, *-2076C>T* and *+54 T>C* has suggested that a haplotype ATT increases susceptibility to GC, whereas the CTT haplotype has a protective effect[12,20]. Yamada *et al* have studied the *+54 T>C*, *-160 C>A*, *-616 G>C*, *-2076 T>C* and *3159 T>C* polymorphisms and have found that the TCGTT haplotype is the most common haplotype and has a protective effect, whereas the TAGTC haplotype increases susceptibility to GC[12,20]. The haplotype analysis of *+54 T>C*, *-160 C>A*, *-616 G>C* and *-3159 T>C* genotypes revealed that the OR of *CCGC* and *CAGC* haplotypes was 1.5 (95% CI: 0.7-3.5) and 1.5 (95% CI: 0.2-3.0), respectively, but did not reach statistical significance. The reason for the difference can be attributed to differences in polymorphisms studied, genetic background and local environmental factors, and highlights the need for comparative studies between different ethnic groups.

In conclusion, the current study confirms the ethnic variations in the association between *CDH1 -160 C>A* polymorphisms and GC susceptibility. We demonstrated that the *-160 AA* genotype was associated with an increased risk of GC. This finding could allow the identification of higher-risk groups who might benefit from intensive prevention strategies (aimed at infections or environmental factors). A better understanding of the functional aspects of these polymorphisms in tumor tissue could lead to a better understanding of tumor biology and behavior, and elucidate the discrepancies observed between and within studies.

COMMENTS

Background

E-cadherin plays a central role in carcinogenesis and metastasis. *E-cadherin* (*CDH1*) gene polymorphisms at various loci and their significance for predisposition to gastric cancer (GC) risk have been studied previously with different results that have suggested ethnic variation. The authors investigated the associations between *CDH1* gene polymorphisms and GC risk predisposition.

Research frontiers

A better understanding of *CDH1* gene polymorphisms in GC could lead to a better understanding of tumor biology and behavior.

Innovations and breakthroughs

The current study confirms the ethnic variations in the association between *CDH1 -160 C>A* polymorphisms and GC susceptibility. The authors demonstrated that the *-160 AA* genotype was associated with an increased risk of GC.

Applications

These findings could allow the identification of higher-risk groups who might benefit from intensive prevention strategies (aimed at infections or environment factors).

Terminology

CDH1 gene encodes E-cadherin protein, which is an important adhesion molecule. Single nucleotide polymorphisms are DNA sequence variations that occur when a single nucleotide is altered.

Peer review

This study provides some useful epidemiological information about genetic predisposition and the risk of GC.

REFERENCES

1 **Murray CJ**, Lopez AD. Alternative projections of mortality and disability by cause 1990-2020: Global Burden of Disease Study. *Lancet* 1997; **349**: 1498-1504
2 **Shibuya K**, Mathers CD, Boschi-Pinto C, Lopez AD, Murray CJ. Global and regional estimates of cancer mortality and incidence by site: II. Results for the global burden of disease 2000. *BMC Cancer* 2002; **2**: 37
3 **González CA**. Vegetable, fruit and cereal consumption and gastric cancer risk. *IARC Sci Publ* 2002; **156**: 79-83
4 **Kelley JR**, Duggan JM. Gastric cancer epidemiology and risk factors. *J Clin Epidemiol* 2003; **56**: 1-9
5 **Correa P**. The biological model of gastric carcinogenesis. *IARC Sci Publ* 2004; 301-310
6 **Correa P**, Schneider BG. Etiology of gastric cancer: what is new? *Cancer Epidemiol Biomarkers Prev* 2005; **14**: 1865-1868
7 **El-Omar EM**, Chow WH, Rabkin CS. Gastric cancer and H. pylori: Host genetics open the way. *Gastroenterology* 2001; **121**: 1002-1004
8 **González CA**, Sala N, Capellá G. Genetic susceptibility and gastric cancer risk. *Int J Cancer* 2002; **100**: 249-260
9 **Li LC**, Chui RM, Sasaki M, Nakajima K, Perinchery G, Au HC, Nojima D, Carroll P, Dahiya R. A single nucleotide polymorphism in the E-cadherin gene promoter alters transcriptional activities. *Cancer Res* 2000; **60**: 873-876
10 **Lynch HT**, Grady W, Suriano G, Huntsman D. Gastric cancer: new genetic developments. *J Surg Oncol* 2005; **90**: 114-133; discussion 133
11 **Correa P**. New strategies for the prevention of gastric cancer: Helicobacter pylori and genetic susceptibility. *J Surg Oncol* 2005; **90**: 134-138; discussion 138
12 **Humar B**, Graziano F, Cascinu S, Catalano V, Ruzzo AM, Magnani M, Toro T, Burchill T, Futschik ME, Merriman T, Guilford P. Association of CDH1 haplotypes with susceptibility to sporadic diffuse gastric cancer. *Oncogene* 2002; **21**: 8192-8195
13 **Zhang XF**, Wang YM, Ge H, Cao YY, Chen ZF, Wen DG, Guo W, Wang N, Li Y, Zhang JH. Association of CDH1 single nucleotide polymorphisms with susceptibility to

esophageal squamous cell carcinomas and gastric cardia carcinomas. *Dis Esophagus* 2008; **21**: 21-29

14 **Corso G**, Berardi A, Marrelli D, Pedrazzani C, Garosi L, Pinto E, Roviello F. CDH1 C-160A promoter polymorphism and gastric cancer risk. *Eur J Cancer Prev* 2009; **18**: 46-49

15 **Gao L**, Nieters A, Brenner H. Meta-analysis: tumour invasion-related genetic polymorphisms and gastric cancer susceptibility. *Aliment Pharmacol Ther* 2008; **28**: 565-573

16 **Song CG**, Huang CM, Liu X, Lu HS, Zhang XF, Huang W. [Association of -160(C-->A) polymorphism in CDH1 gene with gastric cancer risk in Fujian Chinese population] *Zhonghua Yixue Yichuanxue Zazhi* 2005; **22**: 557-559

17 **Pharoah PD**, Oliveira C, Machado JC, Keller G, Vogelsang H, Laux H, Becker KF, Hahn H, Paproski SM, Brown LA, Caldas C, Huntsman D. CDH1 c-160a promotor polymorphism is not associated with risk of stomach cancer. *Int J Cancer* 2002; **101**: 196-197

18 **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

19 **Cattaneo F**, Venesio T, Molatore S, Russo A, Fiocca R, Frattini M, Scovassi AI, Ottini L, Bertario L, Ranzani GN. Functional analysis and case-control study of -160C/A polymorphism in the E-cadherin gene promoter: association with cancer risk. *Anticancer Res* 2006; **26**: 4627-4632

20 **Yamada H**, Shinmura K, Ikeda S, Tao H, Otani T, Hanaoka T, Tsuneyoshi T, Tsugane S, Sugimura H. Association between CDH1 haplotypes and gastric cancer risk in a Japanese population. *Scand J Gastroenterol* 2007; **42**: 1479-1485

S- Editor Tian L **L- Editor** Kerr C **E- Editor** Lin YP