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

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**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.

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**Key words:** Gastric cancer; Polymorphism; CDH1

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**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[12].

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 -160 C>A, where the A allele decreases transcriptional activity of the CDH1 gene and E-cadherin expression, and increases susceptibility to GC in some populations[5,12-19]. 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[10,12].

The above studies have highlighted the ethnic variation in frequency and risk predisposition of these SNPs[5,12,14]. 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[10,12,18]. 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 (Genta 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'-CCCTCTGTTCTCATCATTTT-3' (forward) and 5'-AACCTCTTCAAGAATCCCG-3' (reverse)]; 160 C>A (rs16260); [5'-TGATCCAG-GTCTTAGTGAG-3' (forward) and 5'-GCTCTCAG-GACCCGAAC-3' (reverse)]; -616 G>C (rs7203904); [5'-TTGACTGAGGCCACAGATG-3' (forward) and 5'-CTGCCCTAAATCGTCTAGCC-3' (reverse)]; -3159 T>C (rs2010724); [5'-GAGCTTCCACAGCCTTCT-3' (forward) and 5'-ATTGGACTCCAGGGTG-3' (reverse)]. PCR was performed as follows: one cycle at 94°C for 10 min, 35 cycles at 94°C for 30 s, 95°C for 30 s, and 72°C for 30 s, followed by 72°C for 5 min. The final extension was at 72°C 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 PRIS 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 χ2 test. The differences in frequency distributions of the genotypes between the patient and control groups were also tested using the χ2 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.
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### 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<sup>1</sup> | 90<sup>1</sup> |
| Positive, n (%)                 | 67 (58)     | 54 (60) |

<sup>1</sup>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) |

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

| *CDH1* genotype | Patients n (%)<sup>1</sup> | Control n (%)<sup>1</sup> | OR<sup>2</sup> (95% CI) | P value |
|-----------------|--------------------------|---------------------------|---------------------|--------|
| +54 T>C         | n = 174                  | n = 157                   | 1                   |        |
| 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                   | 1                   |        |
| 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                   | 1                   |        |
| 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                   | 1                   |        |
| 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 (0.5-2.2)       | 0.8    |
| C allele        | 50.0                     | 48.0                      |                     |        |

<sup>1</sup>The number of patients and control indicates successful single nucleotide polymorphism analysis for each polymorphism;<sup>2</sup>Age and sex-adjusted.

### 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).

### 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.

### 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>C.<sup>[12-17,20]</sup> 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.<sup>[15,18]</sup> 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.
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The most widely studied CDH1 polymorphism in various cancers is CDH1 -160 C>A. 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. 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. The altered expression of 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 has found an association between +54 T>C and esophageal and gastric cancer, other studies were negative.

It has been suggested that haplotype analysis might be more useful than single SNP analysis in identifying cancer risk. In particular, the combined analysis of CDH1 -160 C>A, -2076 C>T and +54 T>C has suggested that a haplotype ATT increases susceptibility to GC, whereas the CTT haplotype has a protective effect. 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. 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 environmental 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.

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