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Association between the *CDH1*-472delA and -160C>A polymorphisms and diffuse and intestinal gastric cancer in a Mexican population

A.R. Bustos-Carpinteyro[1,2], N. Delgado-Figueroa[1], E. Santiago-Luna[3], M.T. Magaña-Torres[1] and J.Y. Sánchez-López[1]

[1]División de Genética, Centro de Investigación Biomédica de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México
[2]Programa de Doctorado en Genética Humana, Centro Universitario de Ciencias de la Salud, Universidad de Guadalajara, Guadalajara, Jalisco, México
[3]Gastroenterología, Hospital de Especialidades, Centro Médico Nacional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, Jalisco, México

Corresponding author: J.Y. Sánchez-López
E-mail: yosalo1795@yahoo.com

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ABSTRACT. Gastric cancer (GC), the third leading cause of cancer-related deaths in Mexico and worldwide, can be classified into diffuse (DGC) or intestinal (IGC) types based on its histological characteristics. DGC is characterized by reduced expression of the cell adhesion protein E-cadherin, which is encoded by *CDH1*. The -472delA (rs5030625) and -160C>A (rs16260) polymorphisms in *CDH1* induce a decrease in

gene transcription; in fact, these mutated alleles have been associated with GC in some populations, with conflicting results. The aim of this study was to determine the association between the *CDH1* -472delA and -160C>A polymorphisms and DGC and IGC in Mexican patients. The study was conducted in 24, 23, 48, and 93 individuals with DGC and IGC, without GC (control), and belonging to the general Mexican population (GMP), respectively. The genotypes were obtained by polymerase chain reaction - restriction fragment length polymorphism and the obtained data analyzed using Arlequin 3.1. The frequencies of the mutated allele (A) of -472delA were 0.326, 0.318, 0.284, and 0.296 in the DGC, IGC, control, and GMP groups, respectively, and those of the -160C>A polymorphism were 0.174, 0.318, 0.313, and 0.280, respectively. The genotype and allele frequencies of the two polymorphisms did not differ significantly ($P > 0.05$) among DGC, IGC, and control subjects. Therefore, we concluded that the *CDH1* -472delA and -160C>A polymorphisms are not associated with DGC or IGC in patients from western Mexico.

Key words: Gastric cancer; Polymorphisms; *CDH1*; E-cadherin

INTRODUCTION

Gastric cancer (GC) is the fifth-most frequent type of cancer and the third highest cause of cancer-related mortality worldwide, after lung and liver cancers (IARC, 2012). In Mexico, GC affects 5.5 in every 100,000 individuals, accounting for ~8.0% of all cancer-related deaths (IARC, 2012). According to Lauren's classification, GC can be histologically classified into diffuse (DGC) and intestinal (IGC) GC (Lauren, 1965). Approximately 33% of all gastric adenocarcinoma cases can be classified as DGC. This type of GC is generally undifferentiated and infiltrative and is frequently found in the stomach fundus (Espejo-Romero and Navarrete-Siancas, 2003); moreover, DGC is not associated with preneoplastic lesions and its development is rarely associated with environmental factors. Diffuse GC mainly occurs in younger patients; however, hereditary cases have also been reported. At the genetic level, DGC is mainly associated with mutations in *CDH1*, which encodes E-cadherin (Van Domselaar et al., 2007; Lochhead and El-Omar, 2008). On the other hand, IGC, which accounts for 53% of all gastric adenocarcinoma cases, is mainly associated with environmental factors. This could account for the notable differences in the geographical distribution of IGC. Other factors associated with IGC are old age, the male gender (male:female = 2:1), and infection with *Helicobacter pylori* (Espejo-Romero and Navarrete-Siancas, 2003; Lochhead and El-Omar, 2008; Fuentes-Pananá et al., 2009). Gastric injuries, such as atrophic gastritis, intestinal metaplasia, and dysplasia, can be described as precursor lesions of IGC (Correa et al., 1975). Furthermore, contrary to the trend seen in DGC, several genes (such as *K-ras*, *erbB2*, *p53*, and *p16*) are correlated with IGC incidence and development (Tamura, 2006).

The molecular basis of morphological differences between diffuse and intestinal GC has been thought to be the expression of the E-cadherin protein (Van Domselaar et al., 2007). The cell adhesion protein E-cadherin plays a major role in maintaining the structure and function of epithelial tissues. Reduced or null E-cadherin expression has been correlated with infiltrative capacity and metastasis in GC, as well as other types of cancer, such as breast,

colorectal, and neuroepithelial tumors (Motta et al, 2008; Abascal et al, 2016).

CDH1 is down-regulated in epithelial tumors, which in turn influences the cell motility and invasiveness; therefore, it is believed to play a tumor-suppressive role. The consequent null or reduced E-cadherin expression is indicative of loss of functionality, and is correlated with increased tumor cell proliferation, invasion, and metastasis, which affects cancer progression (Berx and van Roy, 2009).

Several polymorphisms in *CDH1* are correlated with down-regulated E-cadherin such as the -472delA polymorphism (rs5030625; also known as the -347G/GA or -347-/A-polymorphism), where the mutated allele (A) binds weakly to transcription factors compared to the (delA) allele (Shin et al., 2004). Furthermore, the A allele has been associated with colorectal cancer susceptibility (Zou et al., 2009; Wang et al., 2012a), papillary thyroid carcinoma (Wang et al., 2012b), hepatocellular carcinoma (Chien et al., 2011), and GC (Shin et al., 2004) in some populations. Additionally, the -160 C>A (rs16260) polymorphism in *CDH1* resulted in a mutated allele (A) that reduced the efficacy of *CDH1* transcription *in vitro* by 68% (Li et al., 2000). The direct effect of the mutated alleles (-472A and -160A) in the transcriptional regulation of E-cadherin could increase the risk of some types of cancer, as the reduction in E-cadherin promotes the invasive capacity of tumors (Li et al., 2000; Wang et al., 2012c). The aim of this study was to analyze the association between the -472delA and -160C>A polymorphisms in *CDH1* and DGC or IGC in patients from western Mexico, as well as to report their frequency in the general population.

MATERIAL AND METHODS

Study population

Patients and controls were recruited from the Gastroenterology department of Hospital de Especialidades, Centro Médico Nacional de Occidente-Instituto Mexicano del Seguro Social (CMNO-IMSS). The general population samples were recruited from among subjects older than 18 years of age, who donated blood at the CMNO-IMSS Blood Bank. Signed informed consent forms were obtained from all included subjects. This study was approved by the National Committee on Research in Health-IMSS.

DNA was extracted from the peripheral blood leukocytes obtained from all patients and control subjects, comprising patients with DGC (N = 23) and IGC (N = 22), individuals without preneoplastic lesions such as atrophic chronic gastritis or metaplasia (control subjects, N = 44) (Table 1), and 93 unrelated blood donors [sample general Mexican population (GMP) from western Mexico].

Blood samples (5 mL) were collected in tubes containing EDTA anticoagulant. DNA was isolated from these samples using the salting-out method (Miller et al., 1988). The sample concentration and purity were quantified by spectrophotometry. Samples with the ratio of absorbance at 260 and 280 nm ($A_{260/280}$) > 1.7 were of acceptable purity. Working dilutions were prepared at 100 ng/μL and all DNA samples were stored at -20°C.

Analysis of *CDH1* polymorphisms

A 448-base pair fragment including the polymorphic regions of interest was amplified by PCR using the following primers: 5'-CGCCCCGACTTGTCTCTCTAC-3' and

5'-GGCCACAGCCAATCAGCA-3' (Shin et al., 2004). PCR was performed in a 12.5-μL reaction mixture comprising 200 ng genomic DNA, 5 pM each primer, 0.5 U Taq polymerase (Invitrogen, Carlsbad, CA, USA), 1X PCR buffer, 1.0 mM $MgCl_2$, and 2.0 mM deoxynucleotide triphosphate mix (dNTP Set; Vivantis, Subang, Jaya, Malaysia). The PCR conditions were set as follows: initial denaturation at 96°C for 5 min; 30 cycles at 96°C for 40 s, 60°C for 25 s, and 72°C for 20 s; and a final extension at 72°C for 3 min, in a 2720 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). The amplified fragment (5 μL) comprising the -472delA SNP was digested with 5 U *Ban*II restriction enzyme (New England Biolabs, Ipswich, MA, USA) in 1X NEBuffer 4 (at a total volume of 10 μL) at 37°C for 1 h. The fragment containing the -160C>A polymorphism was digested with 1 U *Hin*cII (New England Biolabs) in 1X NEBuffer 3 at 37°C for 1 h. Restriction digestion of the -472delA region yielded several fragments based on the genotype (delA/delA: 263, 117, and 68 bp; delA/A: 331, 263, 117, and 68 bp; and A/A: 331 and 117 bp). Similarly, digestion of the -160C>A region yielded fragments of the following sizes (according to the genotype): 448 bp (C/C), 448, 369, and 79 bp (C/A), and 369 and 79 bp (C/A). The digested fragments were visualized on 6% polyacrylamide gels stained with silver nitrate (Figure 1).

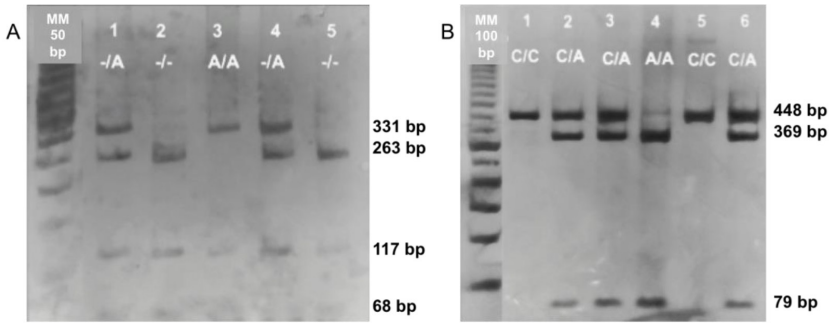

Figure 1. Visualization of the -472delA and -160C>A polymorphisms in *CDH1* observed in 6% polyacrylamide gels. **A.** SNP -472delA: the wild genotype -/- is seen in *lanes 2* and *5*, whereas the heterozygotes are observed in *lanes 1* and *4* and the mutated genotype A/A in *lane 3*. The sizes of all fragments were determined relative to a 50-bp molecular marker. **B.** SNP -160C>A: *lanes 1* and *5* show the wild genotype C/C, *lanes 2*, *3*, and *6* are indicative of the heterozygous genotype C/A, and *lane 4* displays the mutated genotype A/A. The fragment sizes were determined using a 100-bp molecular marker.

Statistical analysis

The differences in genotypic and allelic frequencies, obtained by direct counting, between the patients and controls were analyzed by a chi-square test. Haplotypes were directly established at the two study sites (-472delA and -160C>A) in homozygous or single-site heterozygous individuals; in individuals who were heterozygous for the two polymorphisms, the gamete phase was assigned using the Bayesian algorithm. The conformance of the GMP group with the Hardy-Weinberg equilibrium was determined, and the genotype frequency of

the GMP group was compared against that seen in the world populations. Statistical analysis was performed using the Arlequin v.3.11 software (Excoffier et al., 2005). Differences were considered to be statistically significant at P values < 0.05.

RESULTS

No significant differences were observed in the average age, male-to-female ratio, and familial antecedents of GC in first-degree relatives between the study groups (Table 1).

Table 1. Demographic characteristics of the study groups.

	DGC	IGC	Control group
Age	60.5	61.1	61.2
Male-to-female ratio	1.7	1.8	0.9
Familial antecedents of GC (first degree relatives)	13% (3/23)	13.6% (3/22)	2.3% (1/44)

GC, gastric cancer; DGC, diffuse gastric cancer; IGC, intestinal gastric cancer.

The genotypic and allelic frequencies of the -472delA and -160C>A polymorphisms are summarized in Table 2. The genotype and allele frequencies were similar among the DGC, IGC, and control groups (P > 0.05).

Haplotype analysis indicated the presence of only three of the four possible combinations: the delA-C, delA-A, and A-C; the combination of the mutated alleles A-A was not observed. Haplotype combinations were observed at frequencies of 52.3% (N = 23), 18.2% (N = 8), and 29.5% (N = 13), respectively, in the DGC group; 42.5% (N = 17), 25.0% (N = 10), and 32.5% (N = 13), respectively, in the IGC group; and 39.8% (N = 35), 31.8% (N = 28), and 28.4% (N = 25), respectively, in the controls. The distribution of haplotype frequencies was similar between patients and controls (diffuse GC *vs* control, P = 0.06; intestinal GC *vs* control P = 0.19).

The genotype and allele frequencies of -472delA and -160C>A polymorphisms in the general Mexican population were in Hardy-Weinberg equilibrium (-472delA, P = 0.569; -160C>A, P = 0.837) (Table 2).

The genotype and allele frequencies of the -472delA SNP were compared to those reported in nine other ethnic populations (dbSNP, 2015); the frequencies observed in this study were similar to those observed in Caucasian (HISP and HISP1), Pacific (PAC1), African (YORUB, AD, AFR1), and Asian (ASIAN) populations (P > 0.05); however, these frequencies were different from those reported in two other Caucasian populations (CEPH and CAUC1) (P < 0.01) (dbSNP, 2015).

The genotype and allele frequencies of SNP -160C>A were compared to those reported in two other populations of Mexican origin (Medina-Franco et al., 2007; dbSNP, 2015) and 11 worldwide populations (dbSNP, 20015). These frequencies were similar to those seen in the populations of Mexican origin [(C/C: 0.620, C/A: 0.300, A/A: 0.080, P = 0.337) (dbSNP, 2015) and (C/C: 0.564, C/A: 0.385, A/A: 0.051, P = 0.732) (Medina-Franco et al., 2007)], as well as Amerindian (GIH), Caucasian (CEU and TSI), and Asian (CHB, CHD, JPT, HCB) populations (P > 0.05); however, differences were seen between our results and those seen in African populations (ASW, LWK, MKK, YRI) (dbSNP, 2015) (P < 0.001).

Table 2. Genotype and allele frequencies of the E-cadherin-encoding *CDH1* -472delA and -160C>A polymorphisms in the study groups.

	-472delA>A						-160 C>A					
	Genotypes			P value	Alleles		Genotypes			P value	Alleles	
	delA/delA	delA/A	A/A		delA	A	C/C	C/A	A/A		C	A
Diffuse GC group (N = 23)	0.44 (10)	0.48 (11)	0.09 (2)	0.960	0.67 (31)	0.33 (15)	0.70 (16)	0.26 (6)	0.04 (1)	0.207	0.83 (38)	0.17 (8)
Intestinal GC group (N = 22)	0.46 (10)	0.46 (10)	0.09 (2)	0.983	0.68 (30)	0.32 (14)	0.50 (11)	0.36 (8)	0.14 (3)	0.715	0.68 (30)	0.32 (14)
Control group (N = 44)	0.50 (22)	0.43 (19)	0.07 (3)		0.72 (63)	0.28 25)	0.44 (21)	0.50 (24)	0.06 (3)		0.69 (66)	0.31 (30)
General Mexican population (N = 93)	0.47 (44)	0.46 (43)	0.07 (6)		0.70 (131)	0.30 (55)	0.51 (46)	0.43 (39)	0.07 (6)		0.72 (131)	0.28 (51)

DISCUSSION

The main aim of this study was to explore the association between the -472delA and -160C>A polymorphisms in *CDH1* and DGC or IGC in Mexican patients. This investigation has not been previously performed simultaneously in this population subset. We also determined the frequency of these polymorphisms in a general population sample from western Mexico.

The molecular alterations in *CDH1* are rarely studied in Mexico (Gamboa-Domínguez et al., 2005; Ramos-de la Medina et al., 2006; Medina-Franco et al., 2007). Of the three studies performed in Mexican populations, only one investigated the association between the -160C>A SNP in *CDH1* and diffuse gastric cancer (Medina-Franco et al., 2007).

The demographic characteristics of the patients in the DGC and IGC groups and the control group, including the average age and male-to-female ratio, were in agreement with the data reported in previous publications (Garcia et al., 2007).

The antecedents of GC in first-degree relatives observed in the DGC group (13%) were similar to those previously reported in other Mexican patients with DGC (15.3%) (Medina-Franco et al., 2007). To our knowledge, there are no reported antecedents regarding intestinal GC.

The *CDH1* -472delA polymorphism was not associated with either histological type of GC studied; this result was similar to that previously reported in Brazilian (46 cases and 53 controls) (Borges et al., 2010) and Chinese (239 cases and 343 controls) populations (Zhang et al., 2008b).

Alternately, the *CDH1* -160C>A polymorphism was not associated with diffuse or intestinal GC in our patients from the western region of Mexico. This has also been observed in several other populations, as reported in previous meta-analyses (Chen et al., 2011, Cui et al., 2011; Wang et al., 2011; Li et al, 2012; Deng et al., 2014; Jiang et al., 2015) including several studies and a large number of samples. However, the mutated genotype A/A of the -160C>A SNP was associated with increased DGC risk in a Mexican population (N = 39 diffuse GC) (20.5%, OR = 6.5, 95%CI = 2.1-19.6) (Medina-Franco et al., 2007). Our results differed significantly from the results of this study, which could be attributed to the reduced frequency of the wild genotype observed in the previous study, compared to the frequency observed in this study (CC 38.5 *vs* 69%, P = 0.017). This data suggests that alleles or polymorphisms can play different roles in diseases, depending of the genetic structure, lifestyle, and environmental conditions of the sample population, as seen in multifactorial diseases such as cancer.

None of the haplotypes identified in this study (haplotype analysis) were associated with DGC or IGC in the study population. The haplotypes at these polymorphic sites have been analyzed previously in two Chinese studies (Zhang et al., 2008a; Jiang et al, 2015). We performed an analysis with these data (exclusively for the -472delA and -160C>A polymorphic sites), which revealed no significant differences between our results and those reported in the two Chinese populations, suggesting that the -472delA and -160C>A polymorphisms are not related to risk or protection for gastric cancer in both Chinese and Mexican populations.

The genotype and allele frequencies of the -472delA polymorphism observed in the GMP were similar to those reported in other populations worldwide, except for two Caucasian populations (CEPH and CAUC1) that presented low frequencies of the genotypes containing the mutated allele (delA/A = 0.111 and 0.069, respectively; and A/A = 0.056 and 0.000, respectively) (dbSNP, 2015). The genotype frequencies of the -160C>A SNP were also similar to those reported in other populations, such as Mestizo-Mexican populations (dbSNP, 2015); however, these frequencies differed from those seen in four African populations (dbSNP, 2015). The low frequency of the A allele in the latter population could explain this difference.

The results show the considerable genetic contribution of the Asian, Caucasian, and African populations into the Mexican population, as indicated by other authors (Price et al., 2007; Silva-Zolezzi et al., 2009).

Conclusion

The -472delA and -160C>A polymorphisms in *CDH1* were not associated with GC in patients from western Mexico. However, further studies must be conducted to increase our understanding of the role of these polymorphisms in the development of GC.

Conflicts of interest

The authors declare no conflict of interest.

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