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1. Introduction

E-Cadherin (*CDH*1) plays a key role in cell adhesion, which is vital to the normal development and maintenance of cells. Dysfunction of the cell–cell adhesion system triggers neoplastic development. Since *CDH*1 is the prime cell adhesion mediator, the gene is thought to serve as a tumour invasion suppressor. Down regulation of *CDH*1, may lead to a loss of *CDH*1 mediated cell–cell adhesion, resulting in increased susceptibility to tumour development and subsequent tumour cell invasion and metastasis.[1] In humans, *CDH*1 underexpression has been observed in several cancers, including gastric cancer (GC)[2] where it is thought to be stronger in the diffuse than the intestinal sub-type.[3] In fact, *CDH*1 inactivating somatic mutations are detected in over 50% of sporadic GCs[4–6] and germline *CDH*1 pathogenic mutations are believed to be present in one-third of hereditary diffuse GCs.[7]

Several polymorphisms have been identified in the coding regions of the *CDH*1 gene. Of these, the best known is in the −160C/A (promoter region; rs16260), which has shown a 70% reduced level of transcriptional activity of the A allele compared to the C.[8] While two studies on Asian populations show a lower GC risk association for this polymorphism,[9,10] one New Zealand study shows an association for higher GC risk in the diffuse histological sub-type.[11] Other studies in Asian and European populations show no associations.[12–16] Very little information exists on the GC risk association of other *CDH*1 polymorphisms.

A case–control study was conducted nested within the European Prospective investigation into Cancer and Nutrition (EPIC-EurGast) to assess the GC risk association of the *CDH*1–160C/A (rs16260) polymorphism and 7 other *CDH*1 haplotype-tagging polymorphisms (htSNPs), with the consideration of potential differences by GC anatomical sub-sites, histological sub-type, *Helicobacter pylori* (Hp) infection status and smoking status.

2. Materials and methods

2.1. Subjects

The EPIC-EurGast study was established in order to elucidate the individual and joint effects of dietary/environmental factors, Hp infection and genetic polymorphisms that are putatively involved in GC aetiology in European populations. The study is part of the prospective EPIC study which is detailed elsewhere.[17,18] Cases were gastric adenocarcinomas newly diagnosed during the follow-up period. Gastric lymphomas,

gastric stump cancers, other gastric non-adenocarcinoma and unspecified cancers of the stomach were excluded. For each case (n = 245), up to four controls (n = 950) were randomly selected amongst cohort members alive and free of cancer at the time of case diagnosis, with blood samples available, and matched by gender, age (±2.5 years), centre and date of blood collection (±45 days). This study was approved by the Ethical Review Boards of IARC and all EPIC centres.

GCs were divided into three groups by anatomical sub-site: (i) tumours originating from the gastric cardia (n cases = 69, n matched controls = 257), combining tumours that reached the gastroesophageal junction, either crossing it or from below (all 16 GEJ cancers) or not, (ii) non-cardial tumours (n cases = 128, n matched controls = 508) grouping cases from other sites in the stomach, and (iii) tumours from unknown/mixed sites (n cases = 48, n matched controls = 185). GCs were also divided by histological sub-type according to the Lauren classification: (i) diffuse (n cases = 93, n matched controls = 370), (ii) intestinal (n cases = 96, n matched controls = 372), and (iii) unknown/mixed (n cases = 56, n matched controls = 208). Laboratory methods for Hp infection status are detailed elsewhere.[19]

2.2. SNP selection/genotyping

The software programme *tagSNPs*[20] was used to select a set of htSNPs in which all common SNPs had an estimated pairwise correlation coefficient (R^2_p) > 0.8 with at least one tagging SNP. For all those SNPs poorly correlated with other SNPs, but efficiently correlated with a haplotype of tagging SNP, (R^2S) > 0.8 was also used. When extensive haplotype diversity was observed, the gene was divided into haplotype blocks and the tagging SNPs were selected for each block separately. A haplotype block was defined as the graphical representation of the pattern of linkage disequilibrium (LD) based on D' and selected blocks such that the common haplotypes in each block accounted for at least 80% of all haplotypes observed using the Haploview program.[21] In total, eight SNPs tagging for three *CDH1* haplotype blocks were selected.

Genotyping was performed by Taqman® methodology in 384-well plates read with the Sequence Detection Software on an ABI-Prism7900 instrument, according to the manufacturer's instructions (Applied Biosystems). Primers and probes were supplied by Applied Biosystems (Assays-by-Design™). Each plate included a negative control (no DNA). Positive controls were duplicated on a separate plate. Failed genotypes were not repeated. Assays in which the genotypes of duplicate samples did not show >95% concordance were discarded and replaced with alternative assays with the same tagging properties.

2.3. Statistical analyses

Hardy–Weinberg equilibrium (HWE) for each polymorphism was tested in controls. The association between each SNP

Table 1 – Baseline characteristics and description of the study population of gastric cancer cases and matched controls

	Gastric cancer	
	Cases n = 245	Matched controls n = 950
Age at recruitment[a]	59.1 ± 7.9	59.4 ± 7.8
Age at diagnosis[a]	62.4 ± 8.3	–
Mean number of years between blood donation and diagnosis[a]	3.2 ± 2.1	–
No. of Hp positive subjects[b]	203	646
No. of Hp negative subjects[b]	40	300
Body mass index[a]	26.2 ± 3.8	26.5 ± 4.2
No. of males	138	528
No. of females	107	422
Smoking status		
No. of never smokers	83	418
No. of ex-smokers	87	325
No. of smokers	73	193
No. with missing smoking status	2	14
Grouping by anatomical sub-site		
Cardia, No. of subjects	69	257
Non-cardia, No. of subjects	128	508
Unknown or mixed sub-site, No. of subjects	48	185
Grouping by histological sub-type		
Diffuse, No. of subjects	93	370
Intestinal, No. of subjects	96	372
Unknown or mixed sub-type, No. of subjects	56	208

a Values are means ± standard deviation.

b No. of subjects with missing information on Hp infection status: GC cases = 2, controls = 4. Distribution of cases/controls by EPIC country: Denmark = 22/74, France = 3/12, Germany = 30/120, Greece = 12/48, Italy = 44/173, Netherlands = 19/76, Spain = 29/113, Sweden = 58/224, United Kingdom = 28/110. Details of smoking duration in ex-smokers and smokers: No. of Ex-smokers, duration of smoking < 10 years = 10/44; No. of ex-smokers, duration of smoking ⩾10 years = 72/263; No. of ex-smokers, missing duration of smoking = 5/17; No. of smokers, <15 cigarettes per day = 28/86; No. of smokers, ⩾15–<25 cigarettes per day = 29/59; No. of smokers, ⩾25 cigarettes per day = 10/19.

Table 2 – Odds ratio (OR) and 95% confidence interval (CI) for the GC risk associations of CDH1 polymorphisms, for all GCs and GCs by anatomical sub-site and histological sub-type

CDH1 polymorphism	Genotype	All gastric cancers		GC anatomical site				GC histological type			
				Cardia		Non-cardia		Intestinal		Diffuse	
		Case/ control (*n*)	OR (95% CI)	Case/ control (*n*)	OR (95% CI)	Case/ control (*n*)	OR (95% CI)	Case/ control (*n*)	OR (95% CI)	Case/ control (*n*)	OR (95% CI)
			Age adjusted		Age adjusted		Age adjusted		Age adjusted		Age adjusted
CDH1 –160C/A	CC	119/451	1.0	30/125	1.0	64/243	1.0	46/175	1.0	49/173	1.0
rs16260	CA	101/408	0.9 (0.7–2.2)	31/107	1.2 (0.7–2.0)	51/222	0.9 (0.6–1.3)	39/167	0.8 (0.5–1.5)	37/158	0.8 (0.5–1.3)
	AA	25/90	1.00 (0.6–1.6)	8/25	1.3 (0.5–3.3)	13/41	1.2 (0.6–2.3)	11/30	1.3 (0.6–2.8)	7/37	0.6 (0.3–1.5)
P trend			0.7		0.5		0.9		0.9		0.2
rs1078621	CC	63/281	1.0	15/78	1.0	34/145	1.0	26/108	1.0	24/108	1.0
	CT	125/465	1.2 (0.9–1.7)	37/133	1.4 (0.7–2.7)	3/247	1.1 (0.7–1.8)	49/189	1.0 (0.6–1.8)	47/175	1.2 (0.7–2.1)
	TT	53/188	1.2 (0.8–1.8)	17/46	1.9 (0.8–4.2)	27/99	1.1 (0.6–2.0)	21/74	1.1 (0.6–2.1)	19/75	1.1 (0.6–2.2)
P trend			0.3		0.1		0.7		0.8		0.7
rs4076177	TT	82/350	1.0	23/106	1.0	44/184	1.0	30/138	1.0	34/133	1.0
	TC	122/444	1.2 (0.9–1.6)	32/109	1.4 (0.7–2.5)	63/247	1.1 (0.7–1.6)	48/188	1.1 (0.7–1.9)	47/162	1.1 (0.7–1.9)
	CC	40/145	1.1 (0.7–1.8)	14/39	1.7 (0.8–3.7)	21/74	1.1 (0.6–2.0)	18/45	1.8 (0.9–3.5)	11/66	0.6 (0.3–1.4)
P trend			0.4		0.2		0.7		0.1		0.4
rs7188750	GG	181/666	1.0	51/167	1.0	93/372	1.0	73/260	1.0	69/251	1.0
	GA	55/263	0.8 (0.6–1.1)	15/84	0.6 (0.3–1.2)	30/127	0.9 (0.6–1.5)	19/106	0.6 (0.4–1.1)	20/109	0.7 (0.4–1.2)
	AA	9/21	1.6 (0.7–3.5)	3/6	1.7 (0.4–6.9)	5/9	2.3 (0.7–7.2)	4/6	2.2 (0.6–7.8)	4/10	1.5 (0.4–4.9)
P trend			0.6		0.4		0.6		0.5		0.5
rs3785076	AA	233/866	1.0	64/231	1.0	123/464	1.0	93/336	1.0	89/331	1.0
	AG	10/71	0.5 (0.3–1.0)	4/21	0.7 (0.2–2.3)	4/37	0.4 (0.1–1.1)	3/33	0.3 (0.1–1.1)	2/29	0.3 (0.1–1.1)
	GG	0/1	–	0/0	–	0/1	–	0/0	–	0/1	–
P trend			–		–		–		–		–
rs2276330	AA	185/709	1.0	51/180	1.0	95/394	1.0	74/282	1.0	69/270	1.0
	AG	52/219	0.9 (0.7–1.3)	15/65	0.9 (0.5–1.6)	29/107	1.1 (0.7–1.9)	20/83	0.9 (0.5–1.7)	18/89	0.8 (0.5–1.4)
	GG	7/12	2.3 (0.9–5.8)	2/4	1.7 (0.3–9.5)	4/5	3.8 (0.9–14.4)	2/3	2.2 (0.4–13.5)	5/5	3.8 (1.1–13.5)
P trend			0.7		0.9		0.2		0.8		0.6
rs7203904	GG	149/560	1.0	41/143	1.0	78/314	1.0	62/220	1.0	53/209	1.0
	GC	79/339	0.9 (0.7–1.2)	24/103	0.9 (0.5–1.5)	40/164	1.00 (0.7–1.5)	28/133	0.8 (0.5–1.2)	33/141	0.9 (0.6–1.5)
	CC	17/47	1.4 (0.8–2.5)	4/11	1.4 (0.4–4.4)	10/26	1.6 (0.7–3.4)	6/15	1.5 (0.5–3.9)	7/20	1.4 (0.5–3.5)
P trend			0.8		0.9		0.5		0.7		0.8
rs2276329	AA	221/834	1.0	61/223	1.0	119/447	1.0	90/322	1.0	83/327	1.0
	AG	23/109	0.8 (0.5–1.3)	8/31	1.0 (0.4–2.3)	9/56	0.6 (0.3–1.2)	6/44	0.5 (0.2–1.2)	9/41	0.8 (0.4–1.8)
	GG	1/6	0.6 (0.1–5.7)	0/1	–	0/5	–	0/4	–	1/2	1.9 (0.2–21.5)
P trend			0.3		–		–		–		0.9

Values are odds ratios with 95% confidence intervals.

and GC risk was assessed by odds ratio (OR) and corresponding 95% confidence interval (95% CI) estimated by logistic regression models conditioned on the matching factors plus additional adjustment for age of subject at blood collection (age-adjusted model), plus further adjustments for smoking status/duration/intensity and Hp infection status (mulitvariate-adjusted model). Effect modification by Hp infection status, gender and smoking status/duration/intensity was assessed by the likelihood ratio test. To assess whether Hp infection status, gender or smoking status (never smoker, former smoker, smoker) modify the association of GC risk with *CDH1* polymorphisms, unconditional logistic regression models were used adjusted for the matching factors plus additional adjustment for age of subject at blood collection.

3. Results

3.1. *CDH1 individual SNP analyses*

Baseline characteristics and description of the study population are shown in Table 1.

The *CDH1*–160C/A (rs16260) polymorphism appears to be in linkage disequilibrium with rs1078621 and rs4076177. Although mutual adjustment of these SNPs for each other was attempted in order to determine independent effects, this did not materially alter the findings and so results for these SNPs are presented without any mutual adjustments. All polymorphisms were in HWE. No statistically significant GC risk associations were noted for any of the *CDH1* polymorphisms (Table 2). In the multivariate adjusted model, further adjustments for smoking status/duration/intensity and Hp infection status made no meaningful differences to any of the findings (results not shown).

For *CDH1*–160C/A (rs16260) and most of the other *CDH1* polymorphisms, no differences of effect were observed by GC anatomical site or histological type (Table 2). For rs2276330, the GG versus the AA genotype was associated with higher GC risk in the non-cardia anatomical site (OR = 3.75, 95% CI = 0.98–14.40) and in the diffuse histological type (OR = 3.82, 95% CI = 1.08–13.50).

For all SNPs, no significant interactions were observed between GC risk and gender or Hp infection status. Sub-group analyses by these variables were not remarkable (results not shown). However, consideration of smoking status showed a significant or borderline interaction for the *CDH1*–160C/A (rs16260) polymorphism ($p = 0.02$). Table 3 shows results for sub-group analyses by smoking status. A significantly higher GC risk was observed in smokers for 3 SNPs in the same haplotype block: *CDH1*–160C/A (rs16260), rs1078621 and rs4076177. No meaningful findings were obtained for any of the other *CDH1* SNPs tested (results not shown).

3.2. *CDH1 haplotype analyses*

Haplotypes were also assessed in the context of GC risk. However, no further significant findings were noted when considering haplotypes apart from those manifesting in the SNP analysis.

4. Discussion

Polymorphic variation in the *CDH1* gene promoter region may modulate E-cadherin expression and hence GC risk. However, to date, the findings for polymorphisms in this gene have been inconsistent.[9–16] In the present study, the *CDH1*–160C/A (rs16260) polymorphism was not associated with GC risk, even in sub-group analyses by GC anatomical site or histological type. These results are in line with some of the other studies that have also considered such sub-group analyses showing overall null associations,[12–14] but in contrast with previous findings.[10]

One reason for this inconsistency may be that Hp infection is thought to be required to promote the inactivation of *CDH1* in individuals with the –160CC genotype.[22] Nevertheless, two studies that considered Hp infection status when looking at *CDH1* polymorphisms in association with GC risk have shown that it did not modulate GC risk associated with the *CDH1*–160C/A (rs16260) polymorphism.[12] In the present study, there was also no interaction between Hp infection status and the *CDH1*–160C/A (rs16260) polymorphism.

Table 3 – OR and 95% CI for GC risk associations of selected CDH1 polymorphisms by smoking status

CDH1 polymorphism		All gastric cancers						
	Genotype	Case/ control (*n*)	Never smoker OR (95% CI)	Case/ control (*n*)	Former smoker OR (95% CI)	Case/ control (*n*)	Smoker OR (95% CI)	*P*-value for interaction
CDH1–160C/A	CC	44/186	1.0	44/161	1.0	29/99	1.0	0.02
rs16260	CA	34/188	0.8 (0.5–1.3)	36/135	1.0 (0.6–1.7)	31/78	1.6 (0.8–3.0)	
	AA	5/44	0.4 (0.2–1.2)	7/29	1.0 (0.4–2.4)	13/16	3.9 (1.6–10.1)	
P trend			0.1		1.0		0.01	
rs1078621	CC	24/129	1.0	26/93	1.0	11/59	1.0	0.25
	CT	42/204	1.1 (0.6–2.00)	43/174	1.0 (0.6–1.7)	40/87	2.9 (1.3–6.4)	
	TT	16/84	1.0 (0.5–2.1)	16/56	1.1 (0.5–2.3)	21/47	2.9 (1.2–6.9)	
P trend			0.9		0.9		0.02	
rs4076177	TT	31/138	1.0	29/129	1.0	20/79	1.0	0.05
	TC	43/206	0.9 (0.5–1.6)	45/145	1.5 (0.9–2.6)	34/83	1.9 (1.0–3.8)	
	CC	9/70	0.5 (0.2–1.2)	12/46	1.3 (0.6–3.0)	19/30	3.1 (1.4–7.0)	
P trend			0.2		0.3		0.01	

Values are odds ratios with 95% confidence intervals.

Interaction with smoking status showed a statistically significant increase in GC risk in smokers for the *CDH*1–160C/A (rs16260), rs1078621 and rs4076177 polymorphisms, all in the same haplotype block. Previously, Lu and colleagues[12] reported that the *CDH*1–160C/A (rs16260) polymorphism is associated with a non-significant increase in non-cardia GC risk in smokers for the CA + AA genotypes versus the CC. It is difficult to speculate exactly how smoking may interact with the *CDH*1 gene, but there are indications from animal models that it may interfere with *CDH*1 expression and function.[23,24] It may even be speculated whether smoking status may explain some of the inconsistencies in results from previous studies. Given that chance is also a possibility for the present observations, these findings should be replicated in other populations using better powered studies.

Haplotype analysis did not show results any different than those presented for the individual SNPs. In general, analysis of haplotypes tests for a potential poly-allelic effect where several linked polymorphisms are thought to modulate cancer risk – but this did not appear to be the case here with the *CDH*1 polymorphisms chosen.

In summary, this study shows no association of any of the *CDH*1 polymorphisms tested with GC risk, particularly the *CDH*1–160C/A (rs16260) polymorphism. No interaction was observed for Hp infection status and no differences of effect were observed in sub-group analyses by GC anatomical site or histological type. Further studies are necessary to replicate these findings and to identify the causal *CDH*1 polymorphisms and their functionality.

Conflict of interest statement

None declared.

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