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Short Communication

CDH1 gene polymorphisms, smoking, *Helicobacter pylori* infection and the risk of gastric cancer in the European Prospective Investigation into Cancer and Nutrition (EPIC-EURGAST)

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ABSTRACT

Despite declining incidence rates, gastric cancer (GC) is a major cause of death worldwide. E-Cadherin is an adhesion molecule that is thought to be involved in GC. Germline mutations in the E-Cadherin gene (CDH1) have been identified in hereditary diffuse GC. Also, a promoter polymorphism at position -160 C/A has been suggested to lead to transcriptional down regulation and has been shown to affect GC risk in some studies. However, very little information exists on the GC risk association of other CDH1 polymorphisms and it is unclear whether any associations may be different by GC anatomical sites or histological types. Thus, a case-control study (cases = 245/controls = 950) nested within the European Prospective Investigation into Cancer and Nutrition (EPIC) cohort was conducted to assess the GC risk association of eight CDH1 gene polymorphisms. None of the CDH1 polymorphisms or haplotypes analysed were associated with GC risk and no differences of effect were observed by *Helicobacter pylori* infection status. However, three CDH1 polymorphisms in the same haplotype block, including the CDH1-160C/A, interacted with smoking to increase GC risk in smokers but not in never smokers. These findings should be confirmed in larger independent studies.

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1. Introduction

E-Cadherin (CDH1) 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 CDH1 is the prime cell adhesion mediator, the gene is thought to serve as a tumour invasion suppressor. Down regulation of CDH1, may lead to a loss of CDH1 mediated cell-cell adhesion, resulting in increased susceptibility to tumour development and subsequent tumour cell invasion and metastasis.¹ In humans, CDH1 under-expression 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, CDH1 inactivating somatic mutations are detected in over 50% of sporadic GCs^{4–6} and germline CDH1 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 CDH1 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 CDH1 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 CDH1-160C/A (rs16260) polymorphism and 7 other CDH1 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_p^2) > 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^2_S) > 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 $\pm$ 7.9	59.4 $\pm$ 7.8
Age at diagnosis ^a	62.4 $\pm$ 8.3	–
Mean number of years between blood donation and diagnosis ^a	3.2 $\pm$ 2.1	–
No. of Hp positive subjects ^b	203	646
No. of Hp negative subjects ^b	40	300
Body mass index ^a	26.2 $\pm$ 3.8	26.5 $\pm$ 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 $\pm$ 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		All gastric cancers		GC anatomical site				GC histological type			
Genotype		Case/ control (n)	OR (95% CI)	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)
		Age adjusted		Age adjusted		Age adjusted		Age adjusted		Age adjusted	
CDH1 –160C/A rs16260	CC	119/451	1.0	30/125	1.0	64/243	1.0	46/175	1.0	49/173	1.0
	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 (multivariate-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 rs16260	CC	44/186	1.0	44/161	1.0	29/99	1.0	0.02
	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 *CDH1*-160C/A (rs16260), rs1078621 and rs4076177 polymorphisms, all in the same haplotype block. Previously, Lu and colleagues¹² reported that the *CDH1*-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 *CDH1* gene, but there are indications from animal models that it may interfere with *CDH1* 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 *CDH1* polymorphisms chosen.

In summary, this study shows no association of any of the *CDH1* polymorphisms tested with GC risk, particularly the *CDH1*-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 *CDH1* polymorphisms and their functionality.

Conflict of interest statement

None declared.

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REFERENCES

1. Perl AK, Wilgenbus P, Dahl U, Semb H, Christofori G. A causal role for E-cadherin in the transition from adenoma to carcinoma. *Nature* 1998;**392**(6672):190–3.
2. Chan AO. E-cadherin in gastric cancer. *World J Gastroenterol* 2006;**12**(2):199–203.
3. Tamura G. Alterations of tumor suppressor and tumor-related genes in the development and progression of gastric cancer. *World J Gastroenterol* 2006;**12**(2):192–8.
4. Becker KF, Atkinson MJ, Reich U, et al. E-cadherin gene mutations provide clues to diffuse type gastric carcinomas. *Cancer Res* 1994;**54**(14):3845–52.
5. Chan AO, Lam SK, Wong BC, et al. Promoter methylation of E-cadherin gene in gastric mucosa associated with *Helicobacter pylori* infection and in gastric cancer. *Gut* 2003;**52**(4):502–6.
6. Machado JC, Oliveira C, Carvalho R, et al. E-cadherin gene (*CDH1*) promoter methylation as the second hit in sporadic diffuse gastric carcinoma. *Oncogene* 2001;**20**(12):1525–8.
7. Oliveira C, Ferreira P, Nabais S, et al. E-Cadherin (*CDH1*) and p53 rather than SMAD4 and Caspase-10 germline mutations contribute to genetic predisposition in Portuguese gastric cancer patients. *Eur J Cancer* 2004;**40**(12):1897–903.
8. 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**(4):873–6.
9. Kuraoka K, Oue N, Yokozaki H, et al. Correlation of a single nucleotide polymorphism in the E-cadherin gene promoter with tumorigenesis and progression of gastric carcinoma in Japan. *Int J Oncol* 2003;**23**(2):421–7.
10. Wu MS, Huang SP, Chang YT, et al. Association of the -160 C → a promoter polymorphism of E-cadherin gene with gastric carcinoma risk. *Cancer* 2002;**94**(5):1443–8.
11. Humar B, Graziano F, Cascinu S, et al. Association of *CDH1* haplotypes with susceptibility to sporadic diffuse gastric cancer. *Oncogene* 2002;**21**(53):8192–5.
12. Lu Y, Xu YC, Shen J, et al. E-cadherin gene C-160A promoter polymorphism and risk of non-cardia gastric cancer in a Chinese population. *World J Gastroenterol* 2005;**11**(1):56–60.
13. Park WS, Cho YG, Park JY, 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**(4):501–4.
14. Pharoah PD, Oliveira C, Machado JC, et al. *CDH1* c-160a promoter polymorphism is not associated with risk of stomach cancer. *Int J Cancer* 2002;**101**(2):196–7.
15. Shin Y, Kim IJ, Kang HC, et al. The E-cadherin -347G → GA promoter polymorphism and its effect on transcriptional regulation. *Carcinogenesis* 2004;**25**(6):895–9.
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 Yi Xue Yi Chuan Xue Za Zhi* 2005;22(5):557–9.
17. Bingham S, Riboli E. Diet and cancer—the European Prospective Investigation into Cancer and Nutrition. *Nat Rev Cancer* 2004;4(3):206–15.
 18. Riboli E, Hunt KJ, Slimani N, et al. European Prospective Investigation into Cancer and Nutrition (EPIC): study populations and data collection. *Public Health Nutr* 2002;5(6B):1113–24.
 19. Palli D, Masala G, Del GG, et al. CagA+ *Helicobacter pylori* infection and gastric cancer risk in the EPIC-EURGAST study. *Int J Cancer* 2007;120(4):859–67.
 20. Stram DO, Haiman CA, Hirschhorn JN, et al. Choosing haplotype-tagging SNPS based on unphased genotype data using a preliminary sample of unrelated subjects with an example from the Multiethnic Cohort Study. *Hum Hered* 2003;55(1):27–36.
 21. Barrett JC, Fry B, Maller J, Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. *Bioinformatics* 2005;21(2):263–5.
 22. Liu YC, Shen CY, Wu HS, et al. *Helicobacter pylori* infection in relation to E-cadherin gene promoter polymorphism and hypermethylation in sporadic gastric carcinomas. *World J Gastroenterol* 2005;11(33):5174–9.
 23. Wang X, Hao T, Wu R. Effect of cigarette smoke extract on E-cadherin expression of airway epithelial cells. *Zhonghua Jie He He Hu Xi Za Zhi* 1999;22(7):414–6.
 24. Xu S, Wu R, Chen C. The study of E-cadherin expression on injury and repair of epithelium of respiratory tract in smoking mice. *Zhonghua Jie He He Hu Xi Za Zhi* 1999;22(7):417–9.