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Polymorphisms in the *E-cadherin* (*CDH1*) gene promoter and the risk of bladder cancer

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ABSTRACT

Aim: E-cadherin plays a role in carcinogenesis. For two genetic polymorphisms in the gene (*CDH1*) promoter, a reduced transcription has been reported: a C/A single nucleotide polymorphism (SNP) and a G/GA SNP at −160 bp and −347 bp, respectively, upstream of the transcriptional start site. We studied the association between both polymorphisms and the risk of bladder cancer.

Methods: One hundred and ninety-seven patients with bladder cancer and 344 population controls were genotyped and haplotyped for both SNPs.

Results: A borderline significantly increased risk for bladder cancer was found for A allele carriers (OR 1.36; 95% CI: 0.96–1.94). We did not find any association between the –347 G/GA SNP and bladder cancer. Haplotype analyses did not yield much stronger associations with bladder cancer than the –160 C/A genotype analyses.

Conclusion: This study supports earlier suggestions that the –160 C/A SNP in the *CDH1* promoter is a risk factor for bladder cancer.

1. Introduction

Bladder cancer is usually considered to be caused by exogenous carcinogens. Cigarette smoking and occupational exposure to aromatic amines and polycyclic hydrocarbons are by far the most important risk factors. Increasing evidence suggests that genetic susceptibility to bladder cancer should also be considered as an important risk factor for bladder cancer.[1] Although it is still not clear whether a Mendelian (or high-penetrance susceptibility) subtype of bladder cancer exists, evidence for lower penetrance susceptibility genes for bladder cancer such as *NAT2* and *GSTM1* is accumulating.[2]

Another gene that may play a role in bladder cancer susceptibility is *E-cadherin* (*CDH1*). The E-cadherin protein plays a major role in the establishment and maintenance of intercellular adhesion, cell polarity and tissue architecture. This function of E-cadherin is frequently lost during the development of human epithelial cancers, including carcinomas of the breast, colon, prostate, stomach, liver, and bladder. *E-cadherin* is widely recognised as an invasion-suppressor gene,

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because inactivation or downregulation of E-cadherin by mutations, allelic deletions or epigenetic changes (e.g. hypermethylation of the 5′-promoter region) is associated with tumour aggressiveness and metastasising potential.[3,4]

The accumulating experimental data indicate that E-cadherin expression is directly involved in contact-dependent inhibition of cell growth (first described by Watabe *et al.*[5]). It has been shown that E-cadherin expression results in increased levels of the cyclin-dependent kinase (cdk) inhibitor $p27^{Kip1}$, a reduction in cyclin-CDK activity and dephosphorylation of the retinoblastoma protein.[6,7] The direct mechanism responsible for the E-cadherin-mediated expression and/or activation of those genes involved in the G1 to S transition of the cell cycle is not yet clear, although it has been suggested that the ability of E-cadherin to interfere with β-catenin transcriptional activity is essential for the control of cell proliferation.[8,9] Thus, E-cadherin is not only an invasion suppressor but also a growth suppressor, suggesting that the loss of E-cadherin function may directly contribute to the development of cancer.

Polymorphisms within gene promoter regions can have profound effects on the transcriptional efficiency of genes. Two such polymorphisms with an effect on transcription have been identified in the region of the *E-cadherin* promoter.[10,11] The first is a C/A single nucleotide polymorphism (SNP), 160 bp upstream of the transcriptional start site of *CDH1*. Transcription from the A allele has been reported to be 68% less efficient than that from the C allele.[10] The second reported promoter variant is a G/GA SNP, 347 bp upstream of the transcriptional start site. In the original report by Nakamura *et al.*[11], it was shown that this polymorphism has no effect on transcriptional activity, but a more recent study in multiple cell types suggested that the GA allele decreases the transcriptional efficiency 10-fold compared with the G allele.[12]

It has been hypothesised that SNPs in the *E-cadherin* (*CDH1*) gene promoter region are responsible for interindividual variation in the production of E-cadherin and in turn lead to individual susceptibility to (invasive) carcinoma.[10] Since the discovery of the –160 C/A polymorphism, several studies found a relationship between A-allele carriership and the risk of cancer (see Table 1). Two studies from a South Korean group suggested an association between the –347 G/GA polymorphism and gastric and colorectal cancer,[12,13] but the association between the –347 G/GA polymorphism and urological cancers has not been studied yet. In this case control study, germline DNA samples from patients with bladder cancer and population controls were analysed using restriction fragment length polymorphism (RFLP) to investigate the relationship between the –160 C/A SNP and the –347 G/GA SNP and the risk of bladder cancer.

2. Materials and methods

2.1. Study population

Since 1999, all new patients at the urology outpatient clinic of the Radboud University Nijmegen Medical Centre (RUNMC), the Netherlands, are requested to fill out a lifestyle questionnaire and to donate a 10 ml EDTA blood sample for research into genetic susceptibility for urological diseases. The blood samples are registered and stored at –40 °C. This routine data collection has been approved by the Institutional Review Board and the samples are only collected with informed consent. For this study, the blood samples were selected from 197 patients with a histologically confirmed diagnosis of bladder cancer between 1999 and 2003. Blood samples from controls were obtained from the Nijmegen Biomedical Study, a population-based survey conducted by the Departments of Epidemiology and Biostatistics, Clinical Chemistry, and Human Genetics of the RUNMC. In 2002, 6473 age and sex stratified randomly selected inhabitants of the municipality of Nijmegen filled out a postal questionnaire on, e.g. lifestyle and medical history, and donated a 10 ml EDTA blood sample.[14] Blood samples were used from 344 controls.

2.2. Genotyping and haplotyping

Genomic DNA was isolated from peripheral blood using salt-precipitation. Genotype and haplotype analyses of the –160C/A (SNP ID: rs16260) and the –347G/GA (SNP ID: rs5030625) polymorphisms were performed by RFLP. A fragment encompassing both SNPs was amplified using the sense primer 5′-GCCCCGACTTGTCTCTCTAC-3′ and the anti-sense primer 5′-GGCCACAGCCAATCAGCA-3′. PCR amplification was carried out in a final volume of 25 μl containing 40 ng genomic DNA, 25 pmol of each primer, 0.25 mM dNTP, and 0.5 U of *SuperTaq* DNA polymerase, using the following PCR protocol: 95 °C for 2 min for 1 cycle; 94 °C for 1 min/61 °C for 1 min/72 °C for 1 min for 35 cycles, followed by an elongation cycle of 72 °C for 10 min. PCR products were double-digested for 2 h with *Ban*II and *Hinc*II at 37 °C and separated and visualised on a 2% agarose gel. With the *Ban*II digestion, the –347 G allele will be digested and with *Hinc*II the –160 A allele will be digested (Fig. 1A), yielding specific digestion products for each genotype and haplotype (Fig. 1B).

2.3. Statistical analysis

The observed genotype frequencies among controls were tested for Hardy Weinberg Equilibrium (HWE) using a χ^2 test. Odds ratios (OR) and corresponding 95% confidence intervals (CI) were calculated in order to quantify the association between bladder cancer and all genotypes and haplotypes. Odds ratios were also calculated for subgroups of patients with tumours of different TNM-stages and WHO differentiation grade. Statistical analyses were performed using the statistical software SPSS version 12.0.1.

3. Results

The mean age of the 197 patients at the time of diagnosis was 62 years (minimum 29, maximum 89). The controls were somewhat younger: mean age 57 years (minimum 20, maximum 88). Eighty-four percent of the patients were men, while only 65% of the controls were men. However, both age and sex appeared to be unrelated to the –160 C/A genotype and the –347 G/GA genotype so that the difference in age and sex distribution cannot confound the relation between the *CDH1* genotype and bladder cancer (this absence of confounding

Table 1 – CDH1 genotype frequencies in cases and controls and genotype specific risks (odds ratios and 95% confidence limits) by study

Type of cancer	Authors (reference)	Cases			Controls			Odds ratio	Selection/characteristics of cases	Selection/characteristics of controls	Country
		No. of cases	CDH1 genotype (%)		No. of controls	CDH1 genotype (%)					
Gastric cancer	Shin *et al.*[12]	28	C/C	75	142	C/C	77.5	1	Familial gastric cancer patients, >one 1st or 2nd degree relatives affected, at least one <50 years, without germline mutations in *CDH1*	Randomly selected healthy individuals	Korea
			C/A	21.4		C/A	21.8	1.0 (0.3–2.7)			
			A/A	3.6		A/A	0.7	5.2 (0.3–87.1)			
			A allele	25.0		A allele	22.5				
	Lu *et al.*[18]	206	C/C	57.8	261	C/C	58.2	1	Histologically confirmed primary gastric adenocarcinoma patients in 2 high cancer mortality counties	Randomly selected cancer-free individuals from neighbouring counties	China
			C/A	36.4		C/A	34.9	1.2 (0.80–1.7)			
			A/A	5.8		A/A	6.9	0.9 (0.4–2.0)			
			A allele	42.2		A allele	41.8				
	Kuraoka *et al.*[19]	106	C/C	58	90	C/C	36	1	Microscopically confirmed gastric adenocarcinoma patients, excluding patients with tumour-cell invasion or significant inflammation	Cancer-free individuals, with endoscopical and histological confirmed no gastric cancer	Japan
			C/A	32		C/A	58				
			A/A	10		A/A	6				
			A allele	44		A allele	64	2.7 (1.5–4.8)			
	Wu *et al.*[20]	201	C/C	47.3	196	C/C	42.3	1	Histologically confirmed gastric adenocarcinoma patients, free from diseases with genetic disposition	Randomly selected healthy subjects matched on age, gender and ethnicity, with normal mucosa or minimal gastritis	Taiwan
			C/A	50.7		C/A	48.0	not sign.			
			A/A	2.0		A/A	9.7	0.2 (0.1–0.6)			
			A allele	52.7		A allele	57.7				
	Park *et al.*[21]	292	C/C	63.7	146	C/C	58.2	1	Gastric cancer patients, with no family history of gastric cancer	Healthy individuals	Korea
			C/A	31.5		C/A	37.7	0.8 (0.5–1.2)			
			A/A	4.8		A/A	4.1	1.1 (0.4–2.9)			
			A allele	36.3		A allele	41.8	0.9 (0.6–1.2)			
	Pharoah *et al.*[22]	148	C/C	39.2	93	C/C	46.2	1	Pathological confirmed gastric cancer patients, with either lymph nodes or mucosal margins negative for malignancy	Normal controls obtained from a pediatric molecular diagnostics laboratory	Canada
			C/A	51.4		C/A	47.3	1.3 (0.8–2.2)			
			A/A	9.4		A/A	6.5	1.7 (0.6–4.9)			
			A allele	60.8		A allele	53.8	1.3 (0.8–2.3)			
		132	C/C	46.2	42	C/C	52.4	1	Operated gastric cancer patients	Healthy individuals	Germany
			C/A	43.9		C/A	35.7	1.4 (0.7–2.2)			
			A/A	9.9		A/A	11.9	0.9 (0.3–2.9)			
			A allele	53.8		A allele	47.6	1.3 (0.6–2.6)			
		153	C/C	40.5	331	C/C	46.2	1	Gastric cancer patients	Healthy blood donors	Portugal
			C/A	52.3		C/A	43.6	1.3 (0.9–2.0)			
			A/A	7.2		A/A	8.2	1.0 (0.5–2.2)			
			A allele	59.5		A allele	51.8	1.3 (1.0–1.7)			

(*continued on next page*)

Table 1 – *continued*

Type of cancer	Authors (reference)	Cases			Controls			Odds ratio	Selection/characteristics of cases	Selection/characteristics of controls	Country
		No. of cases	CDH1 genotype (%)		No. of controls	CDH1 genotype (%)					
Breast cancer	Lei *et al.*[23]	576	C/C C/A A/A A allele	52.1 39.2 8.7 47.9	348	C/C C/A A/A A allele	53.4 38.5 8.0 46.5	1 1.1 (0.9–1.3)	Swedish sporadic and familial breast cancer patients Czech breast cancer patients	Mothers of patients not expected to have a statistically different prevalence of breast cancer than the general population and female blood donors	Sweden & Czech Republic
Colorectal cancer	Porter *et al.*[24]	128	C/C C/A A/A A allele	55.5 41.4 3.1 44.5	171	C/C C/A A/A A allele	59.1 32.1 8.8 40.9	1 1.3 (0.8–2.2) 0.4 (0.1–1.1)	Sporadic colorectal cancer patients, randomly ascertained through population-based surveys	Cancer-free individuals undergoing genetic testing for unrelated disorders	UK
		162	C/C C/A A/A A allele	58.0 48.4 4.7 53.1	171	C/C C/A A/A A allele	59.1 32.1 8.8 40.9	1 1.2 (0.8–1.9) 0.4 (0.2–1.2)	Familial colorectal cancer patients, ascertained from genetics centres	Cancer-free individuals undergoing genetic testing for unrelated disorders	UK
	Shin *et al.*[13]	260	C/C C/A A/A A allele	71.9 25.4 2.7 28.1	147	C/C C/A A/A A allele	77.6 21.8 0.6 22.4	1 1.8 (0.9–3.6) 1.9 (0.1–26.8)	Colorectal cancer patients, with no family history of colorectal cancer	Blood samples from healthy cancer-free individuals	Korea
Bladder cancer	Tsukino *et al.*[25]	314	C/C C/A A/A A allele	63.4 29.9 6.7 36.6	314	C/C C/A A/A A allele	66.2 30.9 2.9 33.8	1 1.0 (0.7–1.4) 2.3 (1.0–5.2)	Histologically confirmed urothelial transitional cell carcinoma patients	Individuals getting a general health check-up, frequency-matched for age and gender	Japan
	Zhang *et al.*[26]	50	C/C C/A A/A A allele	22 34 44 78	50	C/C C/A A/A A allele	54 24 22 46	1 3.5 (1.3–9.6) 4.9 (1.8–13.5) 4.2 (1.7–9.9)	Cytoscopically and pathologically confirmed urothelial transitional cell carcinoma patients, excluding heavy smokers	Urological patients with various diagnoses of benign disease, matched for age and gender, excluding heavy smokers	China
Prostate cancer	Jonsson *et al.*[27]	1036	C/C C/A A/A A allele	50.8 40.6 8.7 49.3	669	C/C C/A A/A A allele	53.1 37.2 9.7 46.9	1 1.1 (0.9–1.4) 0.9 (0.7–1.3) 0.9 (0.9–1.3)	Sporadic, hereditary and familial prostate cancer patients	Men, frequency matched for age and residence, and from randomly selected cancer-free controls, frequency matched for age, gender, and residence	Sweden
	Verhage *et al.*[15]	82	C/C C/A A/A A allele	25.6 70.7 3.7 74.4	188	C/C C/A A/A A allele	55.3 40.0 4.8 44.8	1 3.8 (2.1–6.8) 1.7 (0.4–6.6) 3.6 (2.0–6.4)	Sporadic and hereditary prostate cancer patients	Subjects with benign prostatic hyperplasia, or visiting the urology ward, or requesting vasectomy	Holland

Prostate cancer	Tsukino *et al.*[28]	219	C/C C/A A/A A allele	60.7 35.2 4.1 39.3	219	C/C C/A A/A A allele	67.1 30.1 2.7 32.8	1 1.3 (0.9–1.9) 1.7 (0.6–4.8)	Histologically confirmed prostate cancer patients	Individuals getting a general health check-up, individually matched for age	Japan
	Kamoto *et al.*[30]	236	C/C C/A A/A A allele	65.3 30.0 4.7 34.7	139	C/C C/A A/A A allele	75.5 20.9 3.6 24.5	 1.61 (1.0–2.6)	Histologically confirmed sporadic prostate cancer patients	Hospital based volunteers without voiding complaints, negative PSA and negative DRE	Japan
	Lindström *et al.*[31a]	211	C/C C/A A/A A allele	44 44 12 56	540	C/C C/A A/A A allele	53 41 6 47	1 1.4 (0.9–2.0) 2.8 (1.4–5.3)	Histologically confirmed familial prostate cancer patients (at least 2 cases in a nuclear family)	Men, frequency matched for age and residence, and from randomly selected cancer-free controls, frequency matched for age, gender, and residence	Sweden
	Bonilla *et al.*[32]	427	C/C C/A A/A A allele	62.2 33.4 4.4 37.8	337	C/C C/A A/A A allele	66.3 31.9 1.8 33.7	1 0.9 (0.6–1.3) [b]	Sporadic prostate cancer African Americans (*N* = 119), Jamaicans (*N* = 89), and European Americans (*N* = 219)	Unaffected African American (*N* = 112), Jamaican (*N* = 123), and European American (*N* = 102) volunteers	U.S.A. Jamaica
	Hajdinjak *et al.*[29]	183	C/C C/A A/A A allele	49.2 39.3 11.5 50.8	198	C/C C/A A/A A allele	53 40.9 6.1 47.0	1 1.1 (0.7–1.9) 2.9 (1.3–6.8) 1.4 (0.9–2.4)	Histologically confirmed sporadic prostate cancer patients	168 women blood donors and 30 patients with histologically verified benign prostatic hyperplasia	Slovenia
Gastric cancer	Shin *et al.*[12]	28	G/G G/GA GA/GA GA allele	57.1 39.3 3.6 42.9	142	G/G G/GA GA/GA GA allele	72.5 27.5 0 27.5	1 1.8 (0.7–4.2)	Familial gastric cancer patients, >one 1st or 2nd degree relatives affected, at least one <50 years, without germline mutations in *CDH1*	Healthy individuals	Korea
Colorectal cancer	Shin *et al.*[13]	260	G/G G/GA GA/GA GA allele	59.6 35.8 4.6 40.4	147	G/G G/GA GA/GA GA allele	72.1 27.9 0 27.9	1 1.7 (0.9–3.2)	Colorectal cancer patients, with no family history of colorectal cancer	Healthy cancer-free individuals collected from the hospital	Korea

a Lindström *et al.*[31] (which is a replication study of Jonsson *et al.*[27]): An association was not found between the control group and a group of 612 sporadic prostate cancer patients. However, two of six tagging SNPs (rs2010724 in intron 2 and rs1801026 in exon 16 of the gene) were significantly associated with sporadic prostate cancer. Furthermore, among 123 informative PC families, both the –160 bp promoter SNP as well as the only haplotype with a frequency over 1% containing the A allele of this SNP were transmitted to affected offspring in a greater extent than expected.
b Bonilla *et al.*[32]: the odds ratios (95% CI) for the different ethnic groups were: 1.1 (0.6–2.0) for African Americans, 0.6 (0.3–1.1) for Jamaicans, and 1.2 (0.6–2.6) for European American.

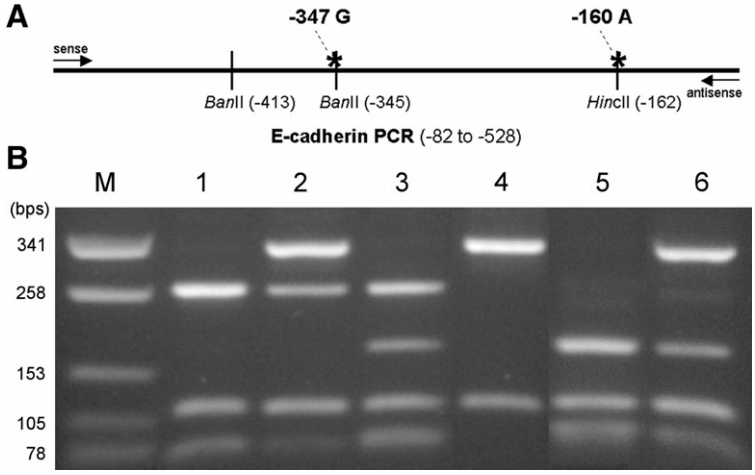

Fig. 1 – Genotype and haplotype analysis of *E-cadherin* (*CDH1*) gene promoter single-nucleotide polymorphisms by PCR-RFLP. (A) Schematic overview of the *E-cadherin* gene promoter PCR fragment and the location of the *Ban*II and *Hinc*II restriction sites with respect to the SNP sites (G–A haplotype). (B) RFLPs of the –347G/GA and –160C/A SNPs using *Ban*II and *Hinc*II double-digestion. M, DNA size marker; 1, G–C (263, 116, 68 bps)/G–C haplotypes; 2, G–C/GA–C (332, 116 bps); 3, G–C/G–A (183, 116, 80, 68 bps); 4, GA–C/GA–C; 5, G–A/G–A; 6, G–A/GA–C; (the GA–A haplotype was not observed in these studies).

was confirmed by multivariable logistic regression analysis; data not shown). Of all patients, 67.8% had superficial bladder cancer (pTa, pT1, or pTis), the remaining 32.2% had invasive disease (pT2-4).

Genotype and allele frequencies for cases and controls are presented in Table 2. There was no evidence that the genotype frequencies among the controls deviated from those expected under Hardy Weinberg Equilibrium: –160 C/A: $p = 0.94$, and –347 G/GA: $p = 0.15$.

A borderline significantly increased risk for bladder cancer was found for A allele carriers compared to the homozygous C allele carriers (OR 1.36; 95% CI: 0.96–1.94). The risk for the heterozygous and homozygous A allele carriers was increased approximately 1.3- and 1.9-fold, respectively (both odds ratios borderline significant). There was no association between carrying the GA allele and the risk of bladder cancer (OR = 1.01; 95% CI: 0.66–1.54). The odds ratio for GA homozygotes was 1.77, but its confidence interval was very wide because of

Table 2 – Genotype and haplotype frequencies of *E-cadherin* promoter polymorphisms

		Bladder cancer ($N = 197$)[a]	Controls ($N = 344$)	OR (95% CI)
–160 bp	C/C (%)	99 (51.0)	200 (58.7)	1
	C/A (%)	77 (39.7)	122 (35.8)	1.28 (0.88–1.85)
	A/A (%)	18 (9.3)	19 (5.6)	1.91 (0.96–3.80)
	C/A or A/A (%)	95 (49.0)	141 (41.4)	1.36 (0.96–1.94)
–347 bp	G/G (%)	141 (76.2)	249 (76.4)	1
	G/GA (%)	42 (22.7)	75 (23.0)	0.99 (0.64–1.52)
	GA/GA (%)	2 (1.1)	2 (0.6)	1.77 (0.25–12.66)
	G/GA or GA/GA (%)	44 (23.8)	77 (23.9)	1.01 (0.66–1.54)
Haplotype	G-C/G-C	64 (35.0)	137 (42.4)	1
	G-C/GA-C	28 (15.3)	54 (16.7)	1.11 (0.64–1.91)
	G-C/GA-A	–	–	–
	GA-C/GA-C	2 (1.1)	2 (0.6)	2.14 (0.29–15.62)
	G-C/G-A	58 (31.7)	94 (29.1)	1.32 (0.85–2.05)
	GA-C/G-A	13 (7.1)	20 (6.2)	1.39 (0.65–2.96)
	GA-C/GA-A	–	–	–
	G-A/G-A	18 (9.8)	16 (5.0)	2.40 (1.15–5.03)
	G-A/GA-A	–	–	–
	GA-A/GA-A	–	–	–

a The –160 C/A SNP was assessed in 194 patients and 341 controls; the –347 G/GA SNP was assessed in 185 patients and 326 controls; the haplotypes of both SNPs were assessed in 183 patients and 323 controls. We did not find any GA-A haplotype in our study.

Table 3 – Genotype frequencies of *E-cadherin* promoter polymorphisms by stage and grade

	–160 bp C → A polymorphism			–347 bp G → GA polymorphism		
	C/C (%)	C/A or A/A (%)	OR (95% CI)	G/G (%)	G/GA or GA/GA (%)	OR (95% CI)
T-stage						
Controls	200 (59)	141 (41)	1	249 (76)	77 (24)	1
Ta/CIS	49 (57)	37 (43)	1.07 (0.66–1.73)	64 (76)	20 (24)	1.01 (0.57–1.78)
T1	20 (46)	24 (54)	1.70 (0.90–3.21)	32 (78)	9 (22)	0.91 (0.42–1.99)
T2-T4	29 (47)	33 (53)	1.61 (0.94–2.78)	44 (76)	14 (24)	1.03 (0.53–1.98)
Differentiation grade						
Good (G1/G2a)	18 (51)	17 (49)	1.34 (0.67–2.69)	27 (79)	7 (21)	0.84 (0.35–2.00)
Moderate (G2/G2b)	30 (53)	27 (47)	1.28 (0.73–2.24)	40 (76)	13 (24)	1.05 (0.53–2.07)
Poor (G3)	50 (50)	50 (50)	1.42 (0.91–2.22)	73 (76)	23 (24)	1.02 (0.60–1.76)

small numbers (only four study participants were homozygous for the GA allele).

The haplotype analyses did not lead to much stronger associations with bladder cancer, but resembled the genotype analyses (Table 2). A homozygous haplotype GA–C gave some suggestion for a 2-fold increased risk but was found among only four study participants. A homozygous G–A haplotype resulted in a statistically significant 2.4-fold increased risk. None of the study participants appeared to have a GA–A haplotype.

The association between the –160 C allele and bladder cancer seems to be stronger for pT1 and pT2-4 disease than for pTa/pTis (Table 3) but approximately similar for all tumour grades. The absence of an association between the –347 GA allele and bladder cancer was seen for all tumour stages and grades (Table 3).

4. Discussion

It has been hypothesised that SNPs in the *E-cadherin* (*CDH1*) gene promoter region are responsible for interindividual variation in the production of E-cadherin and in turn lead to individual susceptibility to cancer.[10] In this study, we found a borderline significant association between the *CDH1* –160 C/A polymorphism and bladder cancer. The risk for heterozygous and homozygous A allele carriers was increased approximately 1.3- and 1.9-fold, respectively. We did not find any significant association between the *CDH1* –347 G/GA polymorphism and the risk of bladder cancer.

The latter finding is surprising because it has been reported previously that the GA-allele decreases transcriptional efficiency even more than the A-allele does.[12] Several major *cis*-acting elements, such as two E-boxes and a CAAT box, have been identified within the proximal *E-cadherin* gene promoter.[16] Deletion of those elements is detrimental for the *E-cadherin* promoter to function at an adequate level. However, deletion of upstream sequences had no effect on promoter activity *in vitro*.[16] Paradoxically, it was found that the two SNPs upstream of the proximal *E-cadherin* promoter diminish its transcriptional efficacy, which might be explained by a difference in the affinity of, yet unidentified, DNA-binding proteins to those allelic variants.[10,12] In a comparative study by Nakamura *et al.*[11], however, only a minor effect of the –160 A-allele on *E-cadherin* promoter activity was reported, whereas the –347 GA-allele did not affect its activity at all. Although in all three SNP promoter studies the same pGL3-E reporter system was used, different *E-cadherin* promoter fragments were applied, extending to different degrees at the 5′ and 3′ ends. Binding of transcription factors to the regions encompassing the SNPs may depend on the binding of other factors further upstream or downstream. Therefore, subtle variations in the length of promoter fragments used in reporter assays may explain the discrepancies found for the relative promoter activities of the different allelic variants *in vitro*. Hence, further evidence for a decreased transcriptional efficiency of the *E-cadherin* promoter variants is indispensable.

Apart from the confusion about the transcriptional efficiency of the –347 G/GA SNP, one may also wonder why only one of both SNPs is associated with bladder cancer even though they physically lie so close together. Only 187 bases separate the two SNPs from each other which makes strong linkage disequilibrium (LD) between both SNPs likely. Indeed, we found a clear association between the two SNPs: none of the 541 study participants had a GA-A haplotype. If we assume that the –160 A-allele is a causal factor for bladder cancer and the –347 G/GA SNP is only a marker, then one might expect a reduced risk of bladder cancer for the GA-allele just because it is in LD with the risk increasing A allele. However, the strength of an association between a marker and a disease is not only dependent on the LD with the causal SNP but also on the difference between the causal SNP and marker allele frequencies. As an illustration, we used the Genetic Power Calculator[17] to calculate the expected odds ratio for GA carriers under the null hypothesis that the –347 G/GA SNP is only a marker for a risk increasing A allele at the –160 position. Using the observed allele frequencies among controls and the genotype specific odds ratios for the –160 C/A SNP, and further assuming the population prevalence of bladder cancer to be 0.1% and the coefficient of LD (D') 0.95, the expected odds ratios under the null hypothesis are 0.93 and 0.86 for GA heterozygotes and homozygotes, respectively. The fact that these odds ratios are only marginally different from 1.00 illustrate that the different results we observed for both SNPs are not in contradiction with strong LD between the SNPs.

Several studies from Asia and Europe have examined the association between the C/A polymorphism in the *E-cadherin*

gene promoter and the risk of cancers, including urothelial and prostate cancers (see Table 1). Although the results are inconsistent, there is some suggestion that A allele carriers run a slightly increased risk for gastric, prostate, and bladder cancer. The evidence for the association with prostate cancer is strong since Lindström and colleagues[31] replicated an earlier study by the same group.[27] In a large independent series of prostate cancer patients and controls, they again found a strong association for A allele carriers among patients with a positive family history but not among sporadic patients (although two other SNPs in the gene were weakly associated with sporadic prostate cancer). This finding was further supported by haplotype analyses of six tagging SNPs plus the functional –160 bp promoter SNP. Also, family based transmission tests in 123 informative families showed that both the A allele and the haplotype containing the A allele were transmitted to affected offspring in a greater extent than expected.[31]

The available evidence for bladder cancer is less strong. Tsukino *et al*.[25] studied 314 bladder cancer patients and 314 controls in Japan. An increased risk was not found for A allele heterozygotes but homozygotes had a 2-fold increased risk. In a small study from China among 50 patients and 50 controls (heavy smokers excluded), Zhang *et al*.[26] found a very strong association between carrying the A-allele and bladder cancer (OR 4.2, 95% CI 1.7–9.9), also for heterozygotes. It is not clear what caused this discrepancy but it may be related to ethnicity, study size and differences in characteristics (including environment) of the cases and controls.

Considering the two Asian studies by Tsuniko *et al*.[25] and Zhang *et al*.[26] in combination with our results, we conclude that there is evidence that the –160 bp C/A SNP in the *CDH1* promoter region is associated with bladder cancer. The probability that this association is caused by population stratification ('genetic confounding') or other confounding by non-genetic factors is small. Whether the association is causal or due to linkage disequilibrium with yet another variant in the gene promoter is not entirely clear yet. The findings by Lindström and colleagues suggest that the association is causal but that other variants in the gene may contribute to the increased risk of cancer. Further evidence for a decreased transcriptional efficiency of *E-cadherin* with the –160 A allele will be necessary.

Conflict of interest statement

The authors declare not to have any financial or personal relationships with other people or organisations that may have biased the present study.

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