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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