Genetics and Molecular Biology, 28, 4, 661-664 (2005)
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Short Communication

The cuttable C-related genotype and allele for the E-cadherin 3'-UTR *Pml* I polymorphism are associated with higher susceptibility to endometriosis

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Abstract

Epithelial cadherin (E-cadherin; CDH1) may influence pericellular proteolysis and intracellular signal transduction, which plays an essential part of tumor invasion. In our study we investigated the correlation between CDH1 gene polymorphism and endometriosis in two groups of pre-menopausal Taiwanese women, group 1 (n = 150) consisting of women with severe stage IV endometriosis and group 2 (n = 159) of women with no endometriosis. The polymerase chain reaction (PCR) was used to identify the cuttable (C) and uncuttable (T) polymorphism of the CDH1-*Pml* I gene (rs1801026) located on the 3'-untranslated region (3'-UTR) of chromosome 16 and compare the genotypes and allelic frequencies of this gene in both groups. We found that the genotype and allele distributions of the CDH1-*Pml* I C/T polymorphism were significantly different in both groups. In group 1 the CDH1*C frequency was 47.7% and the T frequency 52.3%, while the CC homozygote frequency was 6.7%, the TT homozygote 11.3% and the CT heterozygote 82%. In group 2 the CDH1*C frequency was 17% and the T frequency 83%, while the CC frequency was 0.6%, the TT 66.1% and the CT 33.3%. These data indicate that the CDH1 gene polymorphism may be associated with the development of severe endometriosis and that the CDH1 gene C allele is related to higher susceptibility to endometriosis.

Key words: endometriosis, E-cadherin, polymorphism, SNP.

Received: June 25, 2004; Accepted: March 22, 2005.

Endometriosis is one of the most frequent diseases in gynecology. Despite its obscure mechanism, the implantation of endometrial cells from retrograde menstruation into the peritoneum is a widely accepted theory. It is thought that the cell adhesion molecule might play a central role in the development of endometriosis by mediating endometrial-peritoneal cell interactions (Chen *et al*., 2002). Cadherins are a family of cell adhesion molecules that mediate and regulate homophilic cell-cell adhesion and motility and functions as an invasion suppressor system. Reduced expression of cadherin is observed in numerous cancers (Risinger *et al*., 1994) and abnormal cadherin molecules may cause dysfunction of the cell-cell adhesion system and trigger cancer invasion and the formation of metastasis (Risinger *et al*., 1994).

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Epithelial E-cadherin 1 (CDH1), a member of the cadherin family of cell surface glycoproteins, is a transmembrane adhesion molecule that plays a key role in intercellular communication and the control of cell growth (Takeichi *et al*., 1991), loss of which may be associated with the evolution of malignant changes in epithelial tissue (Perl *et al*., 1998). Kim *et al*. (2000) pointed out that CDH1 is associated with invasiveness, lymph node metastasis, distant metastasis and other poor prognostic factors, and, therefore, it is logical to suspect that CDH1 expression might be involved in the development of endometriosis (Poncelet *et al*., 2002).

In the present paper we describe the results of a study to evaluate whether or not the CDH1-*Pml* I gene polymorphism is a useful marker for predicting susceptibility to severe endometriosis. The polymerase chain reaction (PCR) was used to identify the cuttable (C) and uncuttable (T) polymorphism of the CDH1-*Pml* I gene (rs1801026) lo-

cated on the 3'-untranslated region (3'-UTR) of chromosome 16.

Pre-menopausal Taiwanese women patients were divided into two groups, group 1 (n = 150) consisting of women with surgically diagnosed severe stage IV endometriosis and group 2 (n = 159) of women with no endometriosis as confirmed during cesarean sections or diagnostic laparoscopy performed by two of the authors (YYH and CCC). There were no significant differences between the members of either of the groups in terms of age, weight or height.

For genotyping, peripheral blood samples were taken from each woman and genomic DNA isolated using the Genomaker DNA extractor kit (Blossom, Taiwan). About 50 ng of genomic DNA was mixed with 20 pmole of each PCR primer in a total volume of 25 l containing 10 mM Tris-HCL, pH 8.3, 50 mM KCL, 2mM $MgCl_2$, 0.2 mM of each deoxyribonucleotide triphosphate, and 1 unit of Amplitaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The primer for the CDH1-*Pml* I C/T gene polymorphism (rs1801026) was designed as follows: forward, 5'-CAGACAAAGAC CAGG ACTAT-3'; reverse, 5'-CACCGACCACCAAAAAGTC GAGGGAA-3'. PCR amplification was performed in a programmable thermal cycler GeneAmp PCR System 2400 (Perkin Elmer Applied Biosystems, Foster City, CA, USA). The cycling condition for the CDH1 gene 3'-untranslated region (UTR) C/T polymorphism was set as follows: one cycle at 94 °C for 5 min, 35 cycles at 94 °C for 20 s, 56 °C for 20 s, and 72 °C for 20 s, and one final cycle of extension at 72 °C for 15 min.

The PCR products were separated by standard methods and the 172-bp product mixed with 2 units of *Pml* I (New England Biolabs, Beverly, USA) restriction enzyme which (mapping to the 3'-UTR of the CDH1 gene on chromosome 16) and the reaction buffer according to the manufacturer's instructions. The reaction mixture was incubated for 3 h at 37 °C, after which 10 μL of the product mixture was loaded into 3% (w/v) agarose gel containing ethidium bromide for electrophoresis. Each allele was recognized according to its fragment size, the cuttable CC homozygote producing 146+26 bp fragments, the uncuttable TT homozygote with a single 172 bp fragment and the CT heterozygote with 172+146+26 bp fragments. Genotypes and allelic frequencies in both groups were compared using the chi-squared (χ^2) test (SAS program; Version 8.1, SAS Institute Inc., Cary, North Carolina, USA) with $p < 0.05$ being considered statistically significant.

In group 1 the CDH1*C frequency was 47.7% and the T frequency 52.3%, while the CC homozygote frequency was 6.7%, the TT homozygote 11.3% and the CT heterozygote 82%. In group 2 the CDH1*C frequency was 17% and the T frequency 83%, while the CC frequency was 0.6%, the TT 66.1% and the CT 33.3% (Table 1). The C and T allele frequencies and genotype proportions of the CDH1-*Pml* I C/T polymorphisms of the groups were significantly different at p = 0.0001. We also observed the cuttable C-related genotype (CC, CT) and C allele are related to higher susceptibility to endometriosis while the uncuttable T allele is not.

The mechanisms by which cell adhesion molecules mediate the development of endometriosis remain unclear. However, it is known that CDH1 serves as a tumor suppressor and is a calcium-dependent molecule which is found in most epithelial tissue (Chesire *et al*., 2000) where it is known to play an important role in the development of epithelial structures. Furthermore, CDH1 is a useful tumor marker because altered cadherin expression correlates with increased tumor aggressiveness and un-differentiation in prostate cancer (Chesire *et al*., 2000) and to uncontrolled proliferation, un-differentiation, invasion and metastasis in gastric and colorectal cancer (Kim *et al*., 2000). Various authors have also reported that CDH1 is also associated with embryogenesis, polarization, differentiation and to cell migration in inflamed tissue (Gumbiner 1996; Takeichi *et al*., 1991).

Table 1 - Genotype and allele frequencies of the CDH1 gene 3'-UTR cuttable (C) and uncuttable (T) polymorphism in women with and without endometriosis. The differences in homozygote genotype and allele frequencies between the two groups were significant at p = 0.0001 by the chi-squared test.

Genotype frequency	Group 1 (endometriosis)		Group 2 (non-endometriosis)	
	Number of women	%	Number of women	%
CC homozygote	10	6.7	1	0.6
TT homozygote	17	11.3	105	66.1
CT heterozygote	123	82	53	33.3
Total (n)	150		159	
Allele frequencies	Number of Alleles	%	Number of Alleles	%
C	143	47.7	54	17
T	157	52.3	264	83
Total number of alleles (2n)	300	-	318	-

The loss of CDH1 expression is thought to constitute a crucial mechanism in the pathogenesis of endometriosis, endometriotic cells having been found to be nonmalignant epithelial cells lacking CDH1 which acts as an invasion suppressor molecule in carcinomas (Gaetje *et al*., 1997). Beliard *et al*. (1997) reported that fibronectin expression persisted around endometriotic glands but not in endometrium despite menstruation in corresponding eutopic endometrium, suggesting that CDH1 could play a role in the persistence of endometriotic lesions. CDH1 was detected in late luteal phase endometrium by van der Linden *et al*. (1995) who thought that this cadherin may be involved in the attachment of endometrial fragments to the peritoneal lining during retrograde menstruation and that these cell adhesion molecules could be involved in the shedding of endometrial tissue during menstruation and the attachment of endometrial tissue fragments to the peritoneum (van der Linden *et al*.,1995). In their study of human endometrium and peritoneal endometriosis, Poncelet *et al*., (2002) found that ectopic endometriosis (but not eutopic endometrial cells) are invasive in an *in vitro* collagen assay.

Genetic alterations in any component of the cadherin complex might induce the loss of adhesion function (Oyama *et al*., 1994) and polymorphisms generated by mutations in the genes might cause decreased, increased or absent gene expression or enzyme activity by multiple molecular mechanisms. Down-regulation of CDH1 protein expression might be correlated with various tumors, including those of the breast (Berx *et al*., 1996), stomach (Grady *et al*., 2000), colorectum (Kim *et al*., 2000), pancreas (Gerdes *et al*., 1999), lung (Liu *et al*., 2001) as well as prostate cancer (Li *et al*., 2001). Kim *et al*. (2000) have stated that CDH1 mutations are an important step in the carcinogenesis sequence of gastric and colorectal cancer, while Giroldi *et al*. (2000) found that decreased CDH1 expression was correlated with poor survival of bladder and prostate cancer patients and Perl *et al*. (1998) reported that CDH1 mediated cell adhesion is one of the rate limiting steps in the progression from adenoma to carcinoma. In contrast, however, Kusano *et al*. (2001) have suggested that disorders in the CDH1 system may not involved in the carcinogenesis of gastric cancer, while Murant *et al*. (2000) demonstrated that allele imbalance within the CDH1 gene is an infrequent event in prostate carcinogenesis. These controversies may be due to the multiple genetic contributions, different disease classification schemes and staging or to variations due to ethnic or environmental factors or in the disease itself. Presumably, the distinct biological condition caused by the CDH1 genotype will be among various genetic, dietary, and environmental factors regulating hormonal and non-hormonal conditions in the development of endometriosis. This polymorphism may also be in linkage disequilibrium with other unidentified functional polymorphisms in cell adhesion molecule genes which also the influence pathogenesis of endometriosis.

In summary, the cuttable C-related genotypes (CC homozygote and CT heterozygote) of the CDH1-*Pml* I gene polymorphism are related to higher risk of endometriosis whereas the uncuttable TT homozygote is related to lower risk. It therefore seems likely that the CDH1 gene polymorphism contributes to the pathogenesis of endometriosis and, after further clarification of its role in endometriosis, more detailed knowledge of this polymorphism might be useful in understanding related complex pathogenesis. Further surveys also seem to be needed to clarify the role of other cell adhesion molecule genes in the development of endometriosis.

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Associate Editor: Emmanuel Dias Neto