Association of E-cadherin single nucleotide polymorphisms with the increased risk of endometriosis in Indian women

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ABSTRACT: The objective of the present study was to investigate the association between gene E-cadherin single nucleotide polymorphisms (SNPs) and risk of developing endometriosis in Indian women and to evaluate the role of E-cadherin expression in the pathophysiology of endometriosis. A genetic association study was conducted in 715 endometriosis cases and 500 controls of Indian origin. We genotyped −160 C/A, +54 C/T and −347 G/GA SNPs of gene E-cadherin by PCR-sequencing and PCR-restriction fragment length polymorphism techniques. Haplotype frequencies for multiple loci and the standardized disequilibrium coefficient (D') for pair-wise linkage disequilibrium (LD) were assessed by Haploview Software. In addition, to better understand genetic contributions to the pathophysiology of endometriosis, the expression pattern of E-cadherin in the endometrium of women with and without endometriosis was analyzed by western blot and immunohistochemical analysis. The frequencies of −347GA/GA (P = 0.026) and −160A/A (P = 0.0019) genotypes and −347G/−160A/+54C (P = 0.007) and −347GA/−160A/+54C (P < 0.0001) haplotypes were significantly different between patients and controls. Strong LD was observed between −347G/−160A/+54C and −347G/−160A/+54C (D' = 0.585) or −347G/−160A/+54C (D' = 0.05) loci in cases. Furthermore, increased membranous E-cadherin expression was observed in cases than in controls. The expression seems to be genotype dependent. In conclusion, the E-cadherin −347GA/GA and −160A/A genotypes and −347GA/−160A/+54C and −347G/−160A/+54C haplotypes may jointly modify the risk of endometriosis in Indian women. In addition, the differential expression of E-cadherin may play an important role in pathogenesis of endometriosis.

Key words: E-cadherin / endometriosis / Indian women / polymorphism / differential expression

Introduction

Endometriosis is a chronic gynecological disorder that occurs in 10% of women of reproductive age and in up to 50% of women with infertility (Eskenazi and Warner, 1997). It is characterized by the presence of endometrium-like tissue outside the uterine cavity. Although endometriosis is a benign lesion, it often displays characteristics that are associated with malignancy. It can invade the abdominal tissues and the viscera and has a ‘metastatic pattern’ of spread (Spuijbroek et al., 1992). The most widely accepted theory regarding the pathogenesis of endometriosis involves the implantation and invasion of the viable cells from retrograde menstruation into the peritoneum (Simpson, 1927). Lack of cell adhesion must play a major role in the migration of cells to ectopic sites but to date, the mechanisms by which cell adhesion molecules mediate the development of human endometriosis remains unclear. Previously we demonstrated the correlation between single nucleotide polymorphisms (SNPs) of various candidate genes and endometriosis in Indian population (Bhanoori et al., 2008; 2007; 2005a, b). The emerging evidence strongly suggests that the disease has a polygenic and multifactorial basis (Tempfer et al., 2009).

Cadherins constitute a gene superfamily of integral membrane glycoproteins that mediate and regulate Ca²⁺-dependent homophilic cell—cell adhesion and modulate a wide variety of processes, including cell polarization, migration and cancer metastasis (Takeichi, 1990; Wheeloock and Johnson, 2003). E-cadherin or CDH1 (16q22.1) is an epithelial cellular junction protein expressed in almost all epithelial cells. It is an invasion-suppressor molecule, plays an important role...
in the maintenance of epithelial development, organization and cell integrity (Fri xen et al., 1991). Diminished expression of CDH1 promotes malignant transformation, tumor invasion and metastasis. Reduced expression of CDH1 has been observed in a series of different cancers (Risinger et al., 1994). Normal endometrium shows intense CDH1 expression throughout the menstrual cycle (Poncelet et al., 2002), whereas the reported expression of CDH1 in the endometriotic lesions has been inconsistent.

The −160C/A and −347G/GA are two common SNPs located in upstream from the transcriptional start site of CDH1, which may modulate the transcriptional efficiency of the gene (Li et al., 2002; Nakamura et al., 2002; Shin et al., 2004). In addition, a C to T transition polymorphism at position +54 after the stop codon of CDH1 was linked with the mRNA stability (Becker et al., 1995; Keirseblick et al., 1998). These polymorphisms were associated with the development and progression of various human cancers. As far as we know, little investigation has been carried out on the association between CDH1 polymorphisms and endometriosis (Hsieh et al., 2005; Shan et al., 2007). The present study was conducted to analyze the prevalence of CDH1 polymorphisms with the risk of developing endometriosis in two ethnically different Indian populations. In addition, to better understand genetic contributions to the pathophysiology of endometriosis, the expression pattern of CDH1 in the endometrium of women with and without endometriosis was also analyzed.

Materials and Methods

Study design
Two independent case–control groups were recruited from two ethnically different linguistic groups of Indian subcontinent (Reich et al., 2009). A total of 715 premenopausal unrelated Indian women with moderate–severe (III–IV) endometriosis staged using the revised American Fertility Society (rAFS) classification system (1997), Hyderabad, India (South Indian or Dravidian linguistic group, n = 370) and Institute of Reproductive Medicine (IRM), Kolkata, India (North Indian or Indo-European linguistic group, n = 345). All women had a trans-vaginal ultrasound scan (TVS) at screening followed by a laparoscopy to confirm the diagnosis (rAFS III = 259; IV = 456). All the patients had different forms of endometriosis such as peritoneal lesions, adhesions and endometrioma. Women with other ovarian cysts, adenomyosis, ovarian cancer, fibroids and Stage I and II endometriosis were excluded from the study. The aim was to focus on patients with more severe endometriosis (Stages III and IV) because the more severe forms include ovarian cystic disease, which almost certainly has a different etiology to peritoneal forms, and the diagnosis is usually unequivocal, which is not the case for Stages I and II. Their mean age ± SD was 26.5 ± 5.5 (range: 20–40) years. All the patients complained of dysmenorrhea (mild, 45%; moderate, 31% and severe, 24%) and 75% had dyspareunia. Most women (98.1%) were infertile (primary, 82% and secondary, 18%).

Five hundred women (250 of each from IRM and IIRC) were recruited from the same clinic population and had an equal opportunity to be identified as cases, thereby meeting the criteria for appropriate controls set by Zondervan et al. (2002). The controls consisted of 431 (86.2%) women with no evidence of endometriosis on TVS, laparoscopy and 69 (13.8%) women with no evidence of an ovarian endometrioma on TVS (and no clinical symptoms of endometriosis) who therefore did not subsequently have a laparoscopy. Their mean age ± SD was 27.9 ± 4.95 (range: 22–40). Seventy-six percent had primary and 24% had secondary infertility. Written informed consent was obtained from all participants. The Institutional Review Board of the Centre for Cellular and Molecular Biology, Hyderabad, approved the study.

Tissue collection
Secretory phase endometrial tissue was obtained from the uterus fundus by curettage at the time of laparoscopy. Each sample was divided into two portions: one was immediately immersed in 4% paraformaldehyde in 0.05 M of Tris–HCl buffer (pH 7.4) for at least 24 h and embedded in paraffin for histological examination. Another portion was rinsed in ice cold phosphate-buffered saline on ice to remove excess blood and then rapidly frozen in liquid nitrogen and stored at −70°C for subsequent immunoblot assay. Histological assessment of endometrial morphology was carried out using standard criteria (Noyes et al., 1950).

DNA extraction
Genomic DNA was extracted from 1 ml of EDTA anti-coagulated whole blood by the salting out method (Miller et al., 1988). The genotypes of CDH1 −160C/A and +54C/T SNPs were analyzed by PCR and sequencing analysis, while the −347G/GA genotypes (insertion mutation) were determined by PCR-restriction fragment length polymorphism method (PCR-RFLP). Both cases and controls were genotyped in a randomized, blinded fashion.

Determination of the CDH1 genotype
PCRs were carried out in a total volume of 25 μl, containing 50 ng genomic DNA, 2–6 pmole of each primer, 1 x Taq polymerase buffer (1.5 mM MgCl2) and 0.25 U of AmpliTaq DNA polymerase (Perkin Elmer, USA). The primers and PCR conditions used were given in Table I. The PCR products of 448 bp (−160C/A) and 172 bp (+54C/T) were analyzed by 1.5% agarose gel then sequenced with a Taq-Dye deoxy-terminator cycle sequencing kit (Applied BioSystems, USA) using an automated ABI 3770 DNA sequencer (Applied BioSystems). Genotype calling was performed using Chromas V.2 software (Technelysium Ltd., Australia).

<table>
<thead>
<tr>
<th>Table I</th>
<th>Primers and PCR conditions.</th>
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<tbody>
<tr>
<td>SNP</td>
<td>Primer</td>
</tr>
<tr>
<td>−347 G/GA and −160 C/A</td>
<td>5′-CGCCCCGACTTGCTCTTAC-3′ (forward) 5′-GGCCCAAGCCAATGCA-3′ (reverse)</td>
</tr>
<tr>
<td>+54 C/T</td>
<td>5′-CAGACAAAAAGACCAAGCTAT-3′ (forward) 5′-AAGGGAGCTGA AAAACCACCAGC-3′ (reverse)</td>
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</table>
values were calculated using the online Vassar Stats Calculator (http://www.faculty.vassar.edu/lowry/VassarStats.html). The sample power was analysed by Compare2 software (Abramson, 2011). The Student’s t-test was used to analyze differences between immunoblot band densities; \( P < 0.05 \) was considered statistically significant based on three or more independent experiments. Significance values were corrected by the Bonferroni method.

**Results**

All 715 cases and 500 controls were successfully genotyped. Amongst both cases and controls, the genotype distributions of individual SNPs as well as CDH1 allele system were all in HWE \( (P > 0.05) \). Sequencing analysis of 448 bp \((-160C/A)\) and 172 bp \((+54C/T)\) PCR products were shown in Fig. 2A and B. The CC, AA \((-160)\) and CC, TT \((+54)\) homozygotes manifested as a single peak, whereas heterozygotes CA \((-160)\) and CT \((+54)\) as double peaks.

**Genotyping of CDH1 polymorphisms**

The \(-347G/GA\) genotype distribution and allele frequencies amongst the 715 cases and 500 controls were shown in Table II. Although the allele frequencies were not significantly different between cases and controls \((P = 0.353)\), the genotype frequency distribution exhibited significant variation \((P = 0.026)\), which resulted from an increased proportion of homozygous GA/GA genotype among patients as compared with controls.

The genotype and allelotype distribution of the \(-160C/A\) SNP revealed significant difference between patients and controls \((P < 0.05)\). There was significant reduction in ‘CC’ genotype frequency and elevation of ‘AA’ genotype frequency in endometriosis cases as compared with controls \((P = 0.001)\). The allele frequency also showed significant difference \((P < 0.0001)\), indicating that the ‘A’ allele might confer to endometriosis risk and the ‘C’ allele offers protection against the disease.

The frequencies of genotypes \((P = 0.6)\) and allelotypes \((P = 0.47)\) of the \(+54C/T\) polymorphism were not significantly different between cases and controls (Table II). The genotype and allelotype distribution showed high prevalence of ‘C’ allele in Indian women.

The Indian population is ethnically different (Reich et al., 2009), so we performed statistical analysis independently for both North (Indo-European) and South Indian (Dravidian) women (Supplementary data, Tables SIA, B and SIIA, B). Our study provides very good evidence of ethnic differences leading to different association statuses. The analysis surprisingly revealed two different scenarios in the two groups. We found no association between the \(-347G/GA\) polymorphism and endometriosis in the Dravidian group (Supplementary data, Table SIIA); however, the Indo-European group showed strong association of this polymorphism with endometriosis (Supplementary data, Table SIA). In addition, the allele and genotype frequencies of \(+54C/T\) SNP were considerably different between two groups. The study, apart from presenting useful data for these populations of India, also brings forward important issue of variation in association levels due to ethnicity.

**Haplotype analysis**

To analyze the combined effect of CDH1 SNPs on endometriosis development, the haplotype frequencies were estimated (Table III). There was strong linkage disequilibrium between \(-347G/GA\) and

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**Figure 1** Genotyping of the E-cadherin \(-347G/GA\) polymorphism by PCR–RFLP analysis. Lane M, 100 bp λ DNA marker; lanes 1 and 2, heterozygous mutant genotype (G/GA); lanes 3 and 4, wild genotype (G/G); lanes 5 and 6, homozygous mutant genotype (GA/GA).
immunostaining of CDH1 was more intense in the surface and glandular epithelial cells of endometrium, but undetectable in the stromal cells. CDH1 was present in almost all cases and controls. In some sections, fluorescence was seen in red blood cells, but they did not pick up DAPI as they are anucleated. We observed increased membranous CDH1 expression in cases than in controls (Fig. 4).

Western blot analysis
Western blot analysis confirmed the immunohistochemical data. The case endometrial samples exhibited higher levels of CDH1 expression than the controls (Fig. 5).

Discussion
The present study showed significant association between CDH1 \(-160\) C/A, \(-347\) G/GA polymorphisms and endometriosis risk in Indian women. Shan et al. (2007) reported no association between \(-160\) C/A, \(-347\) G/GA polymorphisms and endometriosis in north Chinese women, which is not in agreement with the present result. Possible reasons for this discrepancy could be: first, ethnic variation observed in the SNPs analysed. Indeed drastic difference in the ‘A’ allele distribution was observed in the two populations studied. The frequencies of ‘A’ allele in their controls and cases were 19.6 and 16.8%, respectively, compared with 22 and 29.44% in the present study. Second, the sample size in their study is much smaller than that of the present study.

The \(-160\) C/A and \(-347\) G/GA SNPs are at the upstream of the transcriptional start site of CDH1 gene. Several cis-acting elements such as E boxes, CAAT box, SPI-binding site, etc. are present within the proximity of above sites (Giroldi et al., 1997). Hence, they have significant effects on the transcriptional activity of CDH1 gene. The \(-160\) A allele decreases the transcriptional efficiency by 68% when compared with the ‘C’ allele in vitro (Li et al., 2002), whereas the \(-347\) GA allele reduces the transcriptional efficiency by 10-fold and has a weak transcription factor binding strength when compared with the ‘G’ allele (Shin et al., 2004). Thus, the above allelic variations may act as potential genetic markers for diseases with high invasive or metastatic nature.

There are very few reports regarding the effect of the \(+54\) C/T SNP on the development of tumors. It is suspected that the \(+54\) C/T SNP
Table II  Genotype and allele frequencies of CDH1 polymorphisms in endometriosis patients and controls of Indian women.

<table>
<thead>
<tr>
<th>Genotypes/alleles</th>
<th>Cases Genotypes/alleles</th>
<th>Stage III, n = 259 (%)</th>
<th>Stage IV, n = 456 (%)</th>
<th>Total, n = 5</th>
<th>Controls, n = 500</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>–347G/GA G/G</td>
<td>157 (60.6)</td>
<td>271 (59.4)</td>
<td>428 (59.8)</td>
<td>298 (59.6)</td>
<td>0.026a</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>G/GA</td>
<td>86 (33.2)</td>
<td>149 (32.7)</td>
<td>235 (32.9)</td>
<td>183 (36.6)</td>
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<td></td>
<td></td>
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</tr>
<tr>
<td>GA/GA</td>
<td>16 (06.2)</td>
<td>36 (07.9)</td>
<td>52 (07.3)</td>
<td>19 (03.8)</td>
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<td></td>
<td></td>
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<tr>
<td>Alleles</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G</td>
<td>400 (77.2)</td>
<td>691 (75.8)</td>
<td>1091 (76.3)</td>
<td>779 (77.9)</td>
<td>0.353b</td>
<td>0.913</td>
<td>0.752–1.107</td>
<td></td>
</tr>
<tr>
<td>GA</td>
<td>118 (22.8)</td>
<td>221 (24.2)</td>
<td>339 (23.7)</td>
<td>221 (22.1)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>–160 C/A C/C</td>
<td>163 (62.9)</td>
<td>283 (62.1)</td>
<td>446 (62.4)</td>
<td>347 (69.4)</td>
<td>0.001a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/A</td>
<td>44 (17.0)</td>
<td>73 (16.0)</td>
<td>117 (16.4)</td>
<td>86 (17.2)</td>
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</tr>
<tr>
<td>A/A</td>
<td>52 (20.1)</td>
<td>100 (21.9)</td>
<td>152 (21.2)</td>
<td>67 (13.4)</td>
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<td></td>
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<tr>
<td>Alleles</td>
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</tr>
<tr>
<td>C</td>
<td>370 (71.4)</td>
<td>639 (70.1)</td>
<td>1099 (70.6)</td>
<td>780 (78.0)</td>
<td>&lt;0.0001b</td>
<td>0.676</td>
<td>0.56–0.815</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>148 (28.6)</td>
<td>273 (29.9)</td>
<td>421 (29.4)</td>
<td>220 (22.0)</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>+54 C/T C/C</td>
<td>216 (83.4)</td>
<td>371 (81.4)</td>
<td>587 (82.1)</td>
<td>420 (84.0)</td>
<td>0.6a</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C/T</td>
<td>32 (12.4)</td>
<td>65 (14.2)</td>
<td>97 (13.6)</td>
<td>58 (11.6)</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>T/T</td>
<td>11 (04.2)</td>
<td>20 (04.2)</td>
<td>31 (04.3)</td>
<td>22 (04.4)</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Alleles</td>
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</tr>
<tr>
<td>C</td>
<td>464 (89.6)</td>
<td>807 (88.5)</td>
<td>1271 (88.9)</td>
<td>898 (89.8)</td>
<td>0.47b</td>
<td>0.908</td>
<td>0.698–1.181</td>
<td></td>
</tr>
<tr>
<td>T</td>
<td>54 (10.4)</td>
<td>105 (11.5)</td>
<td>159 (11.1)</td>
<td>102 (10.2)</td>
<td></td>
<td></td>
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</tbody>
</table>

CI, confidence interval.

aFisher’s exact test (3 × 2 table at 2 df); P < 0.05.
bFisher’s exact test (2 × 2 table at 1 df); P < 0.05.

Table III  Haplotype frequencies of CDH1 polymorphisms in endometriosis patients and controls of Indian women.

<table>
<thead>
<tr>
<th>Haplotypes</th>
<th>–347</th>
<th>–160</th>
<th>54</th>
<th>Controls</th>
<th>Cases</th>
<th>P-value</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>G C C</td>
<td>508 (50.8)</td>
<td>644 (45.0)</td>
<td>0.577</td>
<td>1.106</td>
<td>0.774–1.580</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G C T</td>
<td>57 (05.7)</td>
<td>80 (05.6)</td>
<td>0.007</td>
<td>1.334</td>
<td>1.081–1.646</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G A C</td>
<td>192 (19.2)</td>
<td>326 (22.8)</td>
<td>0.152</td>
<td>1.451</td>
<td>0.870–2.420</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>G A T</td>
<td>22 (02.2)</td>
<td>41 (02.9)</td>
<td>0.764</td>
<td>1.034</td>
<td>0.829–1.288</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA C C</td>
<td>193 (19.3)</td>
<td>253 (17.7)</td>
<td>0.624</td>
<td>1.145</td>
<td>0.661–1.985</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA C T</td>
<td>22 (02.2)</td>
<td>32 (02.2)</td>
<td>&lt;0.0001</td>
<td>4.123</td>
<td>2.363–7.194</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA A C</td>
<td>5 (0.5)</td>
<td>48 (03.4)</td>
<td>0.113</td>
<td>3.351</td>
<td>0.750–14.968</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GA A T</td>
<td>1 (0.1)</td>
<td>6 (0.4)</td>
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</table>

CI, confidence interval.

aFisher’s exact test (2 × 2 table at 1 df); P < 0.05.
down-regulate CDH1 expression by modulating the mRNA stability (Keirsebilck et al., 1998). Our results showed no association between +54C/T SNP and risk of developing endometriosis in Indian women. The data are inconsistent with the previously reported findings (Hsieh et al., 2005; Shan et al., 2007).

Moreover, our results indicate higher risk of endometriosis for \(-347GA/-160A/+54C\) haplotype. As per our results it is one of the rarest haplotypes in Indian women. It has two mutated alleles (GA and A) out of three. This may be one of the reasons for its low frequency in both cases (48/715) and controls (5/500) in comparison with other haplotypes. However, its relative frequency in the cases was 6.7–times higher than that in the controls. It indicates the significance of this haplotype in pathogenesis of endometriosis. Furthermore, the \(-347GA/-160A/+54C\) haplotype exhibits two broad categories of alleles: the first category alleles (\(-347GA; -160A\)) are mutated version that decrease the transcriptional efficiency, while the second category allele is a wild type (+54C). In this context, we are hypothesizing that the decreased expression of CDH1 by first category alleles may play a crucial role in the shedding of viable cells from endometrium and in its metastatic pattern of spread. Whereas the increased CDH1 expression by the removal of the negative transcriptional effect of first category alleles by an unknown mechanism may lead to the implantation of cells on ectopic sites. Here the presence of second category wild-type allele (+54C) at third loci is also crucial as it does not alter the CDH1 expression at post-transcriptional level unlike to its relative mutated allele (+54T). However, further functional studies are necessary to confirm our findings.

The loss of CDH1 expression may be a crucial mechanism in the pathogenesis of endometriosis. Previous reports showed that E-cadherin is predominantly expressed in the glandular and surface epithelial cells of endometrium, but not in the stroma (Van der Linden et al., 1995; Poncelet et al., 2002). Our results are in agreement with this finding. According to Van der Linden et al. (1995), differential expression of CDH1 might be involved in the shedding of endometrial tissue during menstruation and the attachment of endometrial tissue fragments to the peritoneal lining during retrograde menstruation. Persistence of fibronectin expression around endometriotic glands but not in endometrium despite menstruation in corresponding eutopic endometrium indicates that CDH1 could play a role in the persistence of endometriotic lesions (Beliard et al., 1997). Endometriotic cells have been found to be non-malignant epithelial cells lacking CDH1 which were invasive in an in vitro collagen invasion assay (Gaetje et al., 1997). Uncertain results have been reported regarding the expression of CDH1 in endometriosis. Some studies reported reduced

**Figure 3** LD analysis of cases and controls are shown separately. Haploview plots are presented along the single nucleotide polymorphisms studied. The pair-wise linkage disequilibrium values ($D' = 0–100$) of all single nucleotide polymorphisms are given in each diamond. A value of 100 represents maximum possible linkage disequilibrium. (A) LD analysis of controls and (B) LD analysis of patients.

**Figure 4** Immunohistochemical localization analysis of E-cadherin expression in phase-matched (early secretory) endometrium from patients (A–C) and controls (D–F). DAPI (A and D), FITC (B and E) and DAPI + FITC merged (C and F).
expression of CDH1 in the glandular epithelia of endometriosis patients (Scotti et al., 2000; Poncelet et al., 2002), while others observed no difference between cases and controls (Chen et al., 2002; Levy et al., 2008; Katy et al., 2010). Interestingly, some other groups demonstrated increased levels of CDH1 in cases than in controls (Pannala et al., 2007; Matsuzaki et al., 2010). In the present study we observed increased levels of CDH1 expression in cases than in controls. These findings strongly suggest that differential expression of CDH1 may have an important role in the shedding of viable endometrial cells during the menstrual cycle, peritoneal implantation, infiltrative growth and the development of malignancy. Further elucidation of the role of CDH1 and other cell adhesion molecules is necessary to define the pathogenesis of endometriosis.

The CDH1 expression seems to be genotype dependent (Fig. 5). In overall, the −160A, −347GA and +54T alleles appear to decrease the CDH1 expression. The effect is more when the individuals carry homozygous mutated alleles (e.g. samples 5 and 7; 6 and 8 and 2 and 3). The effect also appears to be cooperative/additive (e.g. samples 7 and 8). Furthermore, case tissues showed high incidence of significant haplotypes (−347GA/−160A/+54C and 347G/−160A/+54C) than controls tissues (Fig. 5). Interestingly, all the case tissues showed at least one significant haplotype, but the controls were not (except sample 4). Although, the case tissues are associated with significant genotypes, their expression levels are higher than in control tissues. Haplotype effect on expression may be one of the reasons for this discrepancy. Actually both associated SNPs (−347GA, −160A) decrease the CDH1 expression by lowering the transcriptional efficiency (Li et al., 2002; Shin et al., 2004). But here we cannot ignore the role of alleles at third loci (+54C/T) of the haplotypes. When we compare the significant haplotypes with their related haplotypes which differ only at the third locus [-347G/−160A/+54C (P = 0.007) versus −347G/−160A/+54T (P = 0.152) and −347GA/−160A/+54C (P < 0.0001) versus 347GA/−160A/+54T (P = 0.113)], we can perceive the protective role of +54T allele. This allele (+54T) may decrease the CDH1 expression at post-transcriptional level by altering the m-RNA stability (Keirsebilck et al., 1998). Our expression results are in agreement with this finding (Fig. 5, samples 2 and 3). Interestingly our case tissues carry at least one wild allele (C) at +54 loci. The negative post-transcriptional effect of +54T allele may be one of the reasons for the lower expression of CDH1 in controls than in cases even though the cases have associated polymorphisms that lowers the expression. In addition, the haplotype effect on CDH1 expression showed two different scenarios in cases and controls as the expression is more in cases than in controls for the same haplotype (e.g. samples 4 and 5). It indicates the involvement of some other unknown factors/mechanisms in CDH1 expression between cases and controls. Although the observations are interesting, we are not going to conclude the above CDH1 genotypes and haplotypes as direct cause of endometriosis because with this very small sample size, it is very difficult to decipher the impact of genotype/haplotype on expression as it (expression) is regulated by several cis and trans acting factors.

In conclusion, CDH1 −347GA/GA and −160A/A genotypes and −347GA/−160A/+54C and −347G/−160A/+54C haplotypes may jointly modify the endometriosis risk in Indian women, whereas the remaining genotypes and haplotypes have comparatively lower risk. In addition, the differential expression of CDH1 may play an important role in pathogenesis of endometriosis. Additional, larger population-based studies as well as functional evaluation of the variants are necessary to confirm our findings.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/.

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**Authors’ roles**

S.G.: substantial contributions to the execution of experiments, analysis and interpretation of data, statistical analysis and drafting of manuscript. N.K.T.: substantial contributions to the execution of experiments, data analysis and statistical analysis. M.D.: substantial contributions to the acquisition of data. B.C.: substantial contributions to the acquisition of data. S.S.: substantial contributions to conception and design of study, analysis and interpretation of data, statistical analysis, drafting of manuscript and approval of final version. M.B.: substantial contributions to conception and design of study, analysis and interpretation of data, statistical analysis, drafting of manuscript and approval of final version.

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**Conflict of interest**

The authors of this manuscript have nothing to declare.

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