Possible involvement of the E-cadherin gene in genetic susceptibility to endometriosis

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BACKGROUND: Endometriotic cells display invasive characteristics, despite their benign histological appearance. Recently, the epithelial–mesenchymal transition, in which epithelial cells acquire mesenchymal and migratory properties, has attracted attention as a mechanism of tumor invasion. We aimed to investigate the association between endometriosis and polymorphisms of the E-cadherin gene, a central player in the epithelial–mesenchymal transition, in Japanese women.

METHODS: Twelve single-nucleotide polymorphisms (SNPs) in the E-cadherin gene were identified by real-time polymerase chain reaction using a TaqMan assay in 511 women with endometriosis (the majority in Stages III and IV) and 498 healthy controls.

RESULTS: Allele frequency analysis indicated that there was a marginally higher frequency of the rs4783689 C allele in women with endometriosis compared with controls (corrected \( P = 0.007 \); odds ratio = 1.37; 95% confidence interval, 1.14–1.64). No significant associations with endometriosis were found for the other 11 SNPs.

CONCLUSIONS: Although this study was limited by sample size, the E-cadherin gene polymorphism rs4783689 was marginally associated with endometriosis in the Japanese population, suggesting that E-cadherin might be involved in genetic susceptibility to endometriosis.

Key words: E-cadherin / polymorphism / endometriosis / epithelial–mesenchymal transition

Introduction

Endometriosis is an estrogen-dependent disorder observed in 5–10% of women of reproductive age (Giudice and Kao, 2004) and in 20–50% of women with infertility (Gao et al., 2006). The disorder is characterized by the presence of endometrial glands and stroma outside the uterine cavity, primarily on the pelvic peritoneum and ovaries (Bulun, 2009). The most widely accepted theory is that retrograde menstruation through the Fallopian tube leads to the transfer of endometrial cells into the peritoneal cavity, where they implant on the pelvic structures (Giudice and Kao, 2004). Adhesion, invasion and proliferation of the ectopic endometriotic cells are all necessary for the establishment of endometriotic lesions. It is noteworthy that endometriotic cells are histologically benign but display invasive characteristics.

Recently, the epithelial–mesenchymal transition has been attracting attention as one of the molecular mechanisms of invasion in malignant tumors (Peinado et al., 2007; Kalluri and Weinberg, 2009). The epithelial–mesenchymal transition refers to the cellular and molecular processes by which epithelial cells lose their cell–cell interactions and apico-basal polarity while acquiring mesenchymal and migratory properties. In particular, decreased expression of E-cadherin (CDH1)
is a hallmark of the epithelial-mesenchymal transition. E-cadherin is a transmembrane glycoprotein that mediates calcium-dependent homophilic cell–cell adhesion and plays a major role in the establishment and maintenance of intercellular adhesion, cell polarity and tissue architecture (Takeichi, 1991). In various human malignancies, a reduction in E-cadherin is thought to result in dysfunction of the cell–cell junction system and to trigger malignant tumor invasion (Peinado et al., 2007; Kalluri and Weinberg, 2009, Yoshihara et al., 2009). Endometriotic epithelial cells also show decreased E-cadherin expression and an increase in the expression of N-cadherin, which is a representative mesenchymal marker. This finding suggests that endometriotic cells might acquire invasive ability via the epithelial–mesenchymal transition (Gaetje et al., 1997; May et al., 2011).

There is a body of evidence indicating that genetic factors are involved in the risk of developing endometriosis (Kennedy et al., 1995; Stefansson et al., 2002; Treharre et al., 2002). The association between endometriosis and genetic polymorphisms has been well established (Tremper et al., 2009). Previously, two reports described a relationship between single-nucleotide polymorphisms (SNPs) of the E-cadherin gene and endometriosis (Hsieh et al., 2005; Shan et al., 2007). In these two studies, the rs1801026 SNP was associated with endometriosis in both Taiwanese and Chinese women. We aimed to clarify the association between polymorphisms of the E-cadherin gene and endometriosis in the Japanese population.

### Materials and Methods

#### Subjects

All women in the study with endometriosis were registered in Japan at the Niigata University Hospital, the Nagasaki University Hospital, the Kumamoto University Hospital, the Takarazuka City Hospital and the National Hospital Organization Kyoto Medical Center. Case samples were collected from 520 Japanese women who underwent a laparotomy or laparoscopic surgery and had a pathological confirmation of endometriosis. Controls were recruited at the Niigata University Hospital and the Nagasaki University Hospital. All control samples were collected from 520 Japanese women who underwent laparotomy or laparoscopic surgery and had no pathological confirmation of endometriosis. Patents with endometriosis were diagnosed according to the revised American Society for Reproductive Medicine classification (1997).

The control group was recruited at the Niigata University Hospital. All control samples for the association study were collected from 520 Japanese women as follows: (i) 293 fertile women with no uterine or ovarian tumors as diagnosed by ultrasonography, and (ii) 227 fertile women who underwent laparoscopic surgery for benign ovarian tumors and had no endometriosis lesions. In the control group, none of the women had any history of endometriosis or any of the following endometriosis-related symptoms: infertility, dysmenorrhea, hypermenorrhea or irregular menstruation.

Genomic DNA was extracted from peripheral blood lymphocytes using a QIAamp DNA Blood Maxi Kit (QIAGEN, Tokyo, Japan) according to the manufacturer’s protocol. The ethics committees of the participating institutions approved the study protocol, and each participant gave written informed consent.

#### SNP selection and genotyping

We extracted seven tag SNPs (rs11642413, rs12931189, rs2961, rs7186053, rs4783689, rs2276329 and rs13689) in the E-cadherin gene from the JSNP database (http://snp.ims.u-tokyo.ac.jp/index.html), a repository of Japanese SNP data. SNP rs1801026, which has been reported to be associated with endometriosis in both Taiwanese and Chinese women (Hsieh et al., 2005; Shan et al., 2007), was genotyped in this study. We also selected a functional SNP, rs16260, which is associated with E-cadherin gene regulation (Li et al., 2000). SNP rs2010724, which was reported to be associated with prostate cancer in Swedish populations, was added (Lindström et al., 2005). Based on HapMap Japanese data in the National Center for Biotechnology Information SNP database (http://www.ncbi.nlm.nih.gov/snp), we confirmed that the minor allele frequencies (MAFs) of these 10 SNPs were >0.05 (Supplementary data, Fig. S1). The average distance between any two adjacent SNPs was 10.8 kb (SD = 5.9 kb) (the positions of SNPs are presented in Supplementary data, Fig. S1). Additionally, we selected two non-synonymous SNPs (rs2276311 and rs34507583), of which the minor allele was available in the HapMap Japanese data: these two SNPs were genotyped for the rare variant analysis because the MAFs were <0.01, according to the HapMap Japanese data.

To analyze these SNPs, a TaqMan SNP genotyping assay was performed on an ABI PRISM 7900HT Sequence Detection System (Applied Biosystems, Foster City, CA, USA). In brief, a polymerase chain reaction (PCR) was conducted by using TaqMan Universal Master Mix (Applied Biosystems). PCR was conducted in a 5 µl final reaction volume using 20 ng of genomic DNA. Thermal cycling conditions were 95 °C for 10 min, followed by 40 cycles of 92 °C for 15 s and 60 °C for 1 min. The genotypes were automatically attributed in each sample by measuring the allele-specific fluorescence with the ABI Prism 7900 HT Sequence Detection System, and we used the SDS 2.1 software for allele discrimination (Applied Biosystems). TaqMan probes used for identification of each allele are listed in Supplementary data, Fig. S1.

#### Statistical analysis

The difference in the age of the women with endometriosis and controls was analyzed using an unpaired t-test. As quality controls for this association study, the call rate and Hardy–Weinberg equilibrium (HWE) were calculated. The HWE was tested using the χ² test by comparing the expected and actual allele frequencies. The relative proportions of alleles were compared using the χ² test. All analyses, except for the Bonferroni correction and haplotype analysis, were performed using GraphPad PRISM version 4.0 (GraphPad Software, San Diego, CA, USA). The Bonferroni correction was performed using the R statistical environment version 2.13.0 (2011). P-values of <0.005 were considered to be statistically significant. Power calculations were performed using CaTS (Power Calculator for Genetic Studies) (Skol et al., 2006), assuming a significance level of 0.005, prevalence of endometriosis at 10% and genotype relative risk (GRR) of 1.1, 1.3 or 1.5.

Pairwise linkage disequilibrium (LD) between 10 SNPs (excluding the two non-synonymous SNPs at the E-cadherin gene region) was estimated using r-squared statistics, and the results were displayed graphically using Haplovew software v4.2 (http://www.broadinstitute.org/scientific-community/science/programs/medical-and-population-genetics/haplovew/haplovew). In the haplotype analysis, permutation tests were conducted to exclude false positives related to multiple tests using Haplovew.

#### Results

The overall genotype call rate using the TaqMan genotyping SNP assay was more than 95%; nine samples (1.7%) from a total of 520 endometriosis cases and 22 samples (4.2%) from a total of 520 controls were discarded from the analysis because genotyping was not successful in one or more of the SNPs analyzed. Therefore, a total of 1001 women (511 cases and 498 controls) were included in the analysis. The clinical characteristics of the cases and controls are listed in Table I. The mean age and SD were 33.6 ± 7.3 years for the cases
and $33.7 \pm 7.5$ years for controls. There was no significant difference in age between the two groups ($P = 0.85$).

The observed frequencies of each SNP met the assumptions of HWE in both the case and control subjects. The results of the allele frequency analysis are presented in Table II. Our results from controls are in line with HapMap Japanese data (Table II and Supplementary data, Fig. S1). There were differences in the allele frequency of rs4783689, rs2276329, rs1801026 and rs13689 between endometriosis cases and controls (nominal $P = 0.0007, 0.015, 0.032$ and $0.045$).

After the Bonferroni correction, there was a marginally higher frequency of the rs4783689 C allele in women with endometriosis compared with the controls (corrected $P = 0.007$; odds ratio $= 1.37$; 95% confidence interval, 1.14–1.64). There were no significant differences in the allelic frequencies of the remaining nine SNPs between the cases and the controls after the Bonferroni correction. This association analysis had 47–68% power to detect a risk allele with a GRR of 1.3 when the risk allele frequency was between 0.25 and 0.70 (Supplementary data, Fig. S2).

The comparison of allele frequencies between women with advanced-stage endometriosis and controls showed similar findings (Supplementary data, Table SI). In the rare variant analysis, no association was found between the two non-synonymous SNPs (rs2276331 and rs34507583) and endometriosis (Supplementary data, Table SII).

To perform the haplotype analysis, we selected two SNPs (rs2276329 and rs1801026) in LD block 4 (Supplementary data, Fig. S3). The haplotype distribution was significantly different between the cases and controls (Table III). After 1000 permutation tests were performed, the association remained significant for haplotype G-T (permutation $P < 0.05$).

### Table I Clinical characteristics of the population in a study of the association of polymorphisms in the E-cadherin gene (CDH1) with susceptibility to endometriosis.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Endometriosis</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No.</td>
<td>511</td>
<td>498</td>
</tr>
<tr>
<td>Ethnicity</td>
<td>Japanese</td>
<td>Japanese</td>
</tr>
<tr>
<td>Age (mean ± SD)</td>
<td>33.6 ± 7.3</td>
<td>33.7 ± 7.5</td>
</tr>
<tr>
<td>BMI (kg/m², mean ± SD)</td>
<td>20.8 ± 2.9</td>
<td>NA</td>
</tr>
<tr>
<td>Stage of endometriosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>14</td>
<td>NA</td>
</tr>
<tr>
<td>II</td>
<td>2</td>
<td>NA</td>
</tr>
<tr>
<td>III</td>
<td>225</td>
<td>NA</td>
</tr>
<tr>
<td>IV</td>
<td>215</td>
<td>NA</td>
</tr>
<tr>
<td>Unknown</td>
<td>55</td>
<td>NA</td>
</tr>
</tbody>
</table>

### Table II Allele frequencies of the CDH1 polymorphisms in Japanese patients with endometriosis and controls.

<table>
<thead>
<tr>
<th>SNP</th>
<th>Localization</th>
<th>Variation</th>
<th>MAF cases ($n = 511$)</th>
<th>MAF controls ($n = 498$)</th>
<th>OR*</th>
<th>95% CI*</th>
<th>Nominal P-value</th>
<th>Corrected P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>rs16260</td>
<td>5′-upstream</td>
<td>C/A</td>
<td>0.18</td>
<td>0.18</td>
<td>0.97</td>
<td>0.77–1.22</td>
<td>0.80</td>
<td>1</td>
</tr>
<tr>
<td>rs11642413</td>
<td>Intron 2</td>
<td>A/G</td>
<td>0.41</td>
<td>0.45</td>
<td>1.13</td>
<td>0.95–1.35</td>
<td>0.16</td>
<td>0.96</td>
</tr>
<tr>
<td>rs12931189</td>
<td>Intron 2</td>
<td>A/G</td>
<td>0.25</td>
<td>0.27</td>
<td>1.14</td>
<td>0.93–1.39</td>
<td>0.23</td>
<td>1</td>
</tr>
<tr>
<td>rs2961</td>
<td>Intron 2</td>
<td>A/G</td>
<td>0.16</td>
<td>0.16</td>
<td>1.00</td>
<td>0.79–1.26</td>
<td>0.99</td>
<td>1</td>
</tr>
<tr>
<td>rs2010724</td>
<td>Intron 2</td>
<td>A/G</td>
<td>0.20</td>
<td>0.19</td>
<td>0.94</td>
<td>0.76–1.18</td>
<td>0.65</td>
<td>1</td>
</tr>
<tr>
<td>rs7186053</td>
<td>Intron 3</td>
<td>A/G</td>
<td>0.38</td>
<td>0.39</td>
<td>1.05</td>
<td>0.88–1.25</td>
<td>0.61</td>
<td>1</td>
</tr>
<tr>
<td>rs4783689</td>
<td>Intron 11</td>
<td>C/T</td>
<td>0.34</td>
<td>0.41</td>
<td>1.37</td>
<td>1.14–1.64</td>
<td>0.0007</td>
<td>0.007</td>
</tr>
<tr>
<td>rs2276329</td>
<td>Intron 14</td>
<td>A/G</td>
<td>0.068</td>
<td>0.098</td>
<td>1.48</td>
<td>1.08–2.04</td>
<td>0.015</td>
<td>0.14</td>
</tr>
<tr>
<td>rs1801026</td>
<td>3′ UTR</td>
<td>C/T</td>
<td>0.11</td>
<td>0.15</td>
<td>1.34</td>
<td>1.03–1.75</td>
<td>0.032</td>
<td>0.26</td>
</tr>
<tr>
<td>rs13689</td>
<td>3′ UTR</td>
<td>T/C</td>
<td>0.12</td>
<td>0.15</td>
<td>1.31</td>
<td>1.01–1.69</td>
<td>0.045</td>
<td>0.32</td>
</tr>
</tbody>
</table>

*OR and CI denote odds ratio and confidence interval, respectively. MAF, minor allele frequency.

*P-values were calculated by the χ² test.

*The Bonferroni correction was applied for multiple comparisons.

### Table III Haplotype distribution of polymorphisms in the E-cadherin gene in patients with endometriosis (cases) and controls.

<table>
<thead>
<tr>
<th>Haplotype</th>
<th>Case</th>
<th>Control</th>
<th>χ²</th>
<th>Nominal P-value</th>
<th>Permutation P-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-C</td>
<td>0.89</td>
<td>0.85</td>
<td>4.99</td>
<td>0.026</td>
<td>0.062</td>
</tr>
<tr>
<td>G-T</td>
<td>0.068</td>
<td>0.098</td>
<td>5.61</td>
<td>0.018</td>
<td>0.042</td>
</tr>
<tr>
<td>A-T</td>
<td>0.044</td>
<td>0.048</td>
<td>0.21</td>
<td>0.21</td>
<td>0.88</td>
</tr>
</tbody>
</table>

*P-value was adjusted after 1000 permutation test.
Discussion

In this study, we focused on the E-cadherin gene, which plays an important role in the epithelial–mesenchymal transition. We also demonstrated that the rs4783689 SNP of the E-cadherin gene was marginally associated with endometriosis among the 12 analyzed SNPs in the E-cadherin gene.

Two prior reports indicated that the rs1801026 SNP in the 3′ untranslated region (UTR) region was associated with endometriosis in both Taiwanese and Chinese women (Hsieh et al., 2005; Shan et al., 2007). It is known that SNPs in the 3′ UTR region can affect protein expression by altering the stability of the mRNA (Nackley et al., 2006; Sauna et al., 2007). Intriguingly, E-cadherin protein level is lower in ovarian cancer tissue of carriers of the major allele at rs1801026 SNP than in carriers of the minor allele (Li et al., 2008). Therefore, women carrying the major allele of rs1801026 might exhibit decreased E-cadherin expression in their endometrial cells. We generated a hypothesis that the E-cadherin gene might be a candidate gene involved in endometriosis in East Asian populations. However, we were unable to show that two SNPs (rs1801026 and rs13689) in the 3′ UTR region were significantly related to endometriosis after the Bonferroni correction. Two previous studies (Hsieh et al., 2005; Shan et al., 2007) analyzed only patients with Stages III and IV as endometriosis cases. Because genetic differences between Stages I/II and III/IV have been previously indicated (Montgomery et al., 2008), we re-analyzed whether the E-cadherin gene SNPs were associated with advanced-stage endometriosis. However, there were no significant differences in allele frequencies of the two SNPs between women with advanced-stage endometriosis and controls (Supplementary data, Table S1). This discordancy between our result and two previous reports might be related to differences in sample size and ethnicity of the study populations. Indeed, the allele frequency of rs1801026 in control women shows a major difference between our result and theirs and ours.

The development of microarray technology permits the genotyping analysis of hundreds of thousands of SNPs. In endometriosis, three genome-wide association studies have been conducted (Adachi et al., 2010; Uno et al., 2010; Painter et al., 2011). However, there are no genes associated with the development of endometriosis in common among these three publications. This discrepancy might be related to differences in array platforms, analysis methods and the ethnicity of the study populations. Unfortunately, SNPs related to the E-cadherin gene surpassed a genome-wide significance threshold of 5 × 10−7 in three genome-wide association studies (Wellcome Trust Case Control Consortium, 2007).

There have been many studies of the molecular mechanisms that regulate the invasiveness of endometriotic cells (Gaetje et al., 1997; Grund et al., 2008; Liu et al., 2009; Sotnikova et al., 2010). Gaetje et al. (1997) have reported that primary cells from human endometriotic biopsies are invasive in an in vitro collagen invasion assay and that invasive endometriotic cells display a lack of E-cadherin expression. Publicly available microarray data sets (GSE5108 and GSE7305 submitted on Gene Expression Omnibus) show that E-cadherin mRNA expression was significantly reduced in endometriotic tissue compared with normal endometrium (Eyster et al., 2007; Hever et al., 2007). In addition, it is well known that matrix metalloproteinases are increased in an endometriosis lesion (Sotnikova et al., 2010). Alteration of cell adhesion, as typified by the epithelial–mesenchymal transition, is one of the most important processes in endometriosis. Intriguingly, Chen et al. (2010) have reported that in the epithelial component of adenomyotic lesions, vimentin expression was up-regulated and E-cadherin expression was down-regulated compared with the eutopic endometrium. This finding suggests that the epithelial–mesenchymal transition occurs in adenomyosis. By contrast, Gorbacheva et al. (2008) reported increased expression of E-cadherin and β-catenin in the eutopic and ectopic endometrium in adenomyosis. One recent report showed that N-cadherin but not E-cadherin expression was altered in gastrointestinal tract endometriosis (Van Patten et al., 2010).

In conclusion, our data demonstrated a possible association between the rs4783689 SNP in the E-cadherin gene and endometriosis in a Japanese population, although this study was limited by sample size. Further studies of protein function in endometriotic cells are necessary to clarify the roles of E-cadherin in endometriosis.

Supplementary data

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

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Authors’ roles

Ku.Y., Ko.Y. and K.T. designed the experiments, and K.Y. and S.A. conducted the experiments. K.H., K.N., M.Y., N.N., K.K., H.M., H.K., K.I. and H.S. coordinated clinical sample acquisition. Ku.Y., Ko.Y., T.Y. and K.T. interpreted the experiments. Ku.Y., Ko.Y. and K.T. wrote the manuscript. K.T. supervised this project. All authors critically reviewed the manuscript and approved the final version.

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Conflict of interest

None declared.

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