Evaluation of clinical parameters and estrogen receptor alpha gene polymorphisms for patients with endometriosis

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Abstract

Endometriosis is a chronic inflammatory disease, which is especially found in women with subfertility problems with an incidence of up to 30%. The disease is considered an estrogen-dependent disorder, where DNA polymorphisms of the estrogen receptor α (ERα) in connection with endometriosis are controversially discussed. From a German population of women, clinical data associated with the disease, including the American Fertility Society (AFS) I-IV classification, and non-clinical parameters were evaluated statistically in endometriosis patients (n = 98) and in control women (n = 98) without endometriosis. Using a multivariate statistical analysis, significant associations of endometriosis with dysmenorrhea (P < 0.001) and allergies against medicaments (P = 0.042) were found. A positive trend between first grade family history of endometriosis and allergies against medicaments was also observed, suggesting a genetic relationship. From both collectives, DNA from peripheral blood was analyzed for the frequency of the ERα DNA polymorphisms Xba1 (A/G) and PvuII (T/C) in intron 1 and the ERα exonic DNA polymorphism (G229A) with an amino acid exchange (Gly77Ser) in the transactivation domain. DNA samples from endometriosis lesions and control tissues from the same collectives were also analyzed for the exonic G229A polymorphism. Only homozygote wild-type alleles for the polymorphism G229A were found, making it a rare polymorphism in mid-European individuals. Allele types for the PvuII and Xba1 polymorphisms were analyzed with the observed statistically significant clinical parameters and showed no significant association with endometriosis; however a trend with AFS IV was noted, which could contribute to lesion severity. In conclusion, the analyzed polymorphisms in the ERα do not have a functional role concerning specific clinical parameters associated with endometriosis.


Introduction

Endometriosis is a chronic painful inflammatory disease representing one of the most common benign gynecological disorders. Estimates of its frequency vary from 2–10% in women of reproductive age and up to 30% in women with subfertility problems (Mahmood & Templeton 1991, Moen & Schei 1997, Witz & Burns 2002, Vigano et al. 2004). A strong familial occurrence in first degree relatives in families with endometriosis has been noted for over 20 years, supporting a genetic role (Simpson et al. 1980). Contributing factors for endometriosis-associated subfertility include impaired follicular growth, circulating aberrant hormone concentrations, reduction of oocyte fertilization and implantation rates (reviewed in Giudice & Kao 2004). Surgery can provide relief, but in 75% of patients disease symptoms recur within a period of 2 years (Kuohung et al. 2002). Other diseases associated with endometriosis include, for example, autoimmune diseases such as thyroidal autoimmunity, hypothyroidism, fibromyalgia, chronic fatigue syndrome, allergies and asthma (Sinaii et al. 2002, Poppe & Velkeniers 2003). In addition, endometriosis patients have an increased risk of ovarian cancer and possibly breast and skin cancers (Swiersz 2001). Taken together, a woman’s quality of life is impaired by endometriosis, including loss of work from chronic pain, surgery, and continued therapies.

The endometriotic lesion is defined as a steroid hormone-dependent endometrium-like tissue consisting of glands and stroma, which establishes growth outside the uterine cavity. Target tissues and organs include fallopian tubes, ovaries, peritoneum, colon, the recto-vaginal region, bladder, uterus, and more rarely kidney, lung, liver, pancreas, muscle and the central nervous system (Giudice & Kao 2004). Laparoscopy or laparotomy should...
be performed to confirm and classify the stage of endometriosis according to the American Fertility Society (AFS), where AFS I represents a minimal and AFS IV a severe occurrence. The molecular etiology of endometriosis is still unknown but two main theories exist. As first described by Sampson (1927) based upon retrograde menstruation, endometrial cells adhere, invade and neovascularize the peritoneum resulting in abnormal growth. The metaplasia theory describes an aberrant cellular differentiation process occurring at the site, which results in endometrium-like tissue (Meyer 1919, Gazvani & Templeton 2002). Even though more than 90% of women of reproductive age show at least some degree of retrograde menstruation, it remains unsolved why only some women develop endometriosis. Thus, unidentified factors must be involved that render certain women susceptible to adhering estrogen, invasion, growth and persistence of the lesion (Sharpe-Timms 2001). Some of these factors might involve an aberrant immunologic response (Paul Dmowski & Braun 2004), genetic predisposition (Lamb & Nichols 1986, Stefansson et al. 2002, Kashima et al. 2004), or an altered peritoneal cavity or altered eutopic endometrium (Sharpe-Timms et al. 2000, Mahutte et al. 2004).

At present, molecular research has focused on steroid hormone receptors and hormone metabolism and their role in endometriosis. For example, both estrogen and progesterone receptors are present in endometriotic lesions; however conflicting results have shown that the expression levels of estrogen receptor (ER) α and progesterone receptor (PR) are either increased or decreased compared with eutopic endometrium (Lessey et al. 1989, Jones et al. 1995). Importantly, it has been demonstrated in endometrium and endometriotic cysts that estrogens bind preferentially to ERα rather than to ERβ (Matsuzaki et al. 2000). Estrogen modulating enzymes in lesions and cell lines showed that, in contrast to normal endometrium of women without endometriosis, expression of CYP19 and 17β-hydroxysteroid dehydrogenase type 2 (17-HSD2) was present in the lesions and uterine endometrium from patients (Noble et al. 1996, Kitawaki et al. 1999) but, in contrast, expression of 17β-hydroxysteroid dehydrogenase type 1 (17-HSD1) was deficient (Zeitoun et al. 1998). Both of these enzymatic pathways result in a local cellular accumulation of 17β estradiol; thus, together with circulating estrogen, a stimulation of growth occurs in endometriotic tissue mediated by the ER, further supporting the view that endometriosis is an estrogen hormone-dependent disease.

A single nucleotide polymorphism (SNP) results from a base substitution mutation. SNPs in protein-coding regions (cSNPs) can be classified as synonymous and non-synonymous, where the latter can result in a missense mutation, with a change of amino acids or a nonsense mutation occurring in a termination codon. In addition, SNPs in promoter regions can result in reduced or increased gene expression, whereas SNPs in introns can result in defective splicing or a change in transcription rate if a regulatory element is mutated. A restriction fragment length polymorphism (RFLP) occurs where cleavage of DNA by a restriction enzyme is due to a nucleotide polymorphism in the restriction recognition site. SNPs occur on average every 1.9 kb in the genome where 1.42 million SNPs have been mapped with over 60 000 being represented within exons and untranslated regions (Marth et al. 2001). Thus, determining DNA sequence variations between individuals could contribute to differences in for example drug sensitivities, disease risk and the clinical course of the disease. There have been a variety of DNA polymorphisms in genes, but only a few have shown an association with endometriosis including genes in the immune system, galactose metabolism, cancer susceptibility genes and in nuclear receptors (Cramer et al. 1996, Baranova et al. 1999, Georgiou et al. 1999, Hsieh et al. 2001, Kitawaki et al. 2001, Chang et al. 2002, Kado et al. 2002, Wieser et al. 2003). Recent studies have postulated that ERα SNPs may influence their action as a modulator of estrogen. Two SNPs, a Pvull restriction site (CAG/CTG) with a T → C base change and an Xba1 restriction site (T/CTAGA) with an A → G base change, are both located in intron 1, S’ of exon 2 (Yaich et al. 1992, Yamada et al. 2002). Interestingly, the Pvull polymorphic site abolishes an activator-protein 4 (AP-4) transcription factor binding site as determined by homology with the DNA consensus sequence (Hu et al. 1990). A TA-dinucleotide repeat polymorphism has also been identified in S’ of exon 1 in the ERα gene (Sano et al. 1995, Becherini et al. 2000). The ERα Pvull polymorphism has been analyzed and reported in over 140 publications involving specific diseases, for example Alzheimer’s, mineral bone density, breast cancer and endometriosis; however, the Xba1 polymorphism of ERα has been analyzed more rarely (Hill et al. 1989, Parli et al. 1989, Yaich et al. 1992, Georgiou et al. 1999, Becherini et al. 2000, Kitawaki et al. 2001, Brodowska 2003, Kurabayashi et al. 2004, Seko et al. 2004, Wang et al. 2004, Zhang et al. 2004). Both ERα polymorphisms Pvull and Xba1 have demonstrated contradictory results in the literature. Therefore, more analysis is needed in the general population to ultimately determine their involvement with diseases, especially with endometriosis.

In the present investigation, following clinical data evaluation of a German population of women with endometriosis and of control individuals, we first determined statistically significant associations with disease, clinical history and lifestyle characteristics. Secondly, the incidence of different genetic polymorphisms of ERα in 98 endometriosis patients and 98 control individuals was determined: (1) the RFLPs or SNPs Pvull and Xba1 in intron 1 of ERα (401 bp and 354 bp 5’ of exon 2 respectively) and (2) the cSNP G229A in exon 1 of ERα with a missense mutation of Gly77Ser. These SNPs were then analyzed for an association with the statistically significant determined parameters concerning endometriosis.
Materials and Methods

Patient and control groups

All women who participated in this study were of German nationality, with middle European descent and were recruited between 2002 and 2003. All individuals gave written informed consent and all handling of patients and their blood and tissue samples were in accordance with the Ethics Committee review and approval at the University-Clinics Erlangen. The 98 endometriosis patients ranged in age from 16 to 54 years (mean age: 35.3 years) and the 98 control individuals ranged in age from 15 to 54 years (mean age: 36.2 years). They were seen at the Department of Gynecology and Obstetrics at the University-Clinics Erlangen or at the Buergers-Hospital Frankfurt a.M., where control individuals had their annual pelvic examination with sonography. Clinical examination of internal organs was determined either by laparoscopy or laparotomy and endometriotic lesions were classified according to the AFS classification. Endometriosis lesions were isolated surgically in the operation theatre at the Department of Gynecology and Obstetrics, University-Clinics Erlangen and were flash frozen in liquid nitrogen. At the time of this study, some of the endometriosis patients were treated with gonadotropin-releasing hormone (GnRH) agonists or with contraceptives. For all endometriosis and control individuals, data regarding clinical history including any data concerning endometriosis, and lifestyle characteristics were collected personally using a questionnaire designed by the Department of Gynecology and Obstetrics at the University-Clinics Erlangen. The control study requirements were: age-matched individuals, premenopausal with a regular menstruation cycle, no subfertility problems or any abnormal or severe pelvic pain during the menstrual cycle or intercourse and no clinical signs of endometriosis. In 2005 more than 75% of the control women were contacted again for an update of the questionnaire: None had any clinical signs of endometriosis or other symptoms relating to endometriosis. In addition, by that time 37% of all controls had undergone lower abdominal surgery, such as sterilization, and no sign of endometriosis was noted. Statistical analyses were performed using the Student’s t-test, Fisher’s exact test and a multivariate analysis using SPSS 13.0 software (SPSS, Chicago, IL, USA).

Blood and tissue DNA isolation

From the 98 endometriosis and 98 control individuals genomic DNA isolation from peripheral blood was performed as previously described (Oppelt et al. 2005). Tissue samples were also analyzed from a portion of the endometriosis and control cohort. Genomic DNA was isolated from 42 of the 98 endometriosis lesions (AFS I (n = 2), AFS II (n = 4), AFS III (n = 8), AFS IV (n = 28)) and from 13 of the 98 normal patient-matched control tissues including endometrium (n = 5), peritoneum (n = 5), ovarian (n = 1), sacro-uterine ligament (n = 2). For tissue DNA isolation, 20–50 mg frozen tissue were demembranated (Mikro-Dismembrator, B.Braun, Germany), then incubated overnight at 37 °C in a cell lysis buffer containing 1% SDS and proteinase K (Oppelt et al. 2005) and after a brief incubation with RNase A, the DNA was purified with phenol/chloroform, ethanol precipitated and quantitated. For all blood and tissue DNA samples an average of 70–100 μg DNA per patient were isolated and stored at −80 °C.

Standard and real-time PCR, restriction enzyme digestion

A standard PCR reaction was performed using oligonucleotides and reaction specificities as described previously (Becherini et al. 2000). The PCR fragment was purified (PCR clean up kit; Qiagen, Hilden, Germany), digested to completion with Pvull or Xba1 (Roche, Mannheim, Germany) and then electrophoresed in 1% (w/v) agarose, ethidium bromide stained, photographed and analyzed. Genotypic allelic determination using RealTime PCR for the exonic DNA polymorphism G229A, which detects a G→A base change resulting in a non-synonymous amino acid change (Gly77Ser) in the transactivation domain at codon 77 of the ERα (refSNP ID: rs9340773) was designed using the PCR primers: 5′ GCCAACCGCGAGGTCTA 3′ and 5′ CCCAGGCCGTGGA 3′, and the allelic specific primers 5′ TCAGACCCGCTCC (VIC) and 5′ TCAGACCA-GCCTCC (FAM), then SNP analysis was performed using the ABI7000 according to the manufacturer’s instructions on the same patient DNA analyzed for Pvull and Xba1, as well as endometriotic lesions and control tissues. The ERα G229A SNP was quality control tested by Applied Biosystems (Applera, Darmstadt, Germany). Quality control was established in the laboratory by routine tests of DNA from the control collective with different SNP assays for homozygosity and heterozygosity.

Results

Association of clinical disease, history and lifestyle characteristics with endometriosis compared with controls

In order to analyze our population group for an association with endometriosis, clinical data was collected from each patient, representing a wide spectrum of clinical history and personal data for analyses. Table 1 shows the clinical characteristics of all 196 patients in our study and the statistical associations between endometriosis patients and controls. Results showed no significant differences regarding mean age, age at menarche, body mass index (BMI) and the use of oral contraceptives. The AFS classification of the endometriosis collective resulted in 14 patients with AFS I, 12 patients with AFS II, 34 patients with AFS III and 38 patients with AFS IV. Table 2 shows specific categories, including clinical and additional lifestyle characteristics, which were compared statistically between the two population groups. For example, an
analysis using the Fisher’s exact test showed that only benign breast tumors (P = 0.003) were observed in the control individuals. No significant difference was found between the smoking habits or the living place of patients with endometriosis and the controls. Regarding fertility problems, it was found that the control population had 1.5-fold and 1.7-fold more pregnancies and positive live births, respectively, than endometriosis patients. In addition, only endometriosis patients (17%) had subfertility problems compared with controls (data not shown).

Using both the Fisher’s exact test and a more stringent statistical multivariate analysis, controls had significantly more myomas than endometriosis patients (P = 0.04 (Fisher’s), P = 0.024 (multivariate)); however, endometriosis patients had significantly more dysmenorrhea (P < 0.001 (Fisher’s), P < 0.001 (multivariate)) and more allergies against medications (P = 0.005 (Fisher’s), P = 0.042 (multivariate)) than controls. For 10 endometriosis patients a positive first grade family history existed where 6 of these 10 also had a first grade family history of allergies to medications, suggesting a trend for a genetic predisposition.

### Table 2 Specific clinical and life style categories compared between endometriosis patients (EM) and controls.

<table>
<thead>
<tr>
<th>Category</th>
<th>Parameter</th>
<th>Total number</th>
<th>EM (n=98)</th>
<th>Control (n=98)</th>
<th>P-value (Fisher)</th>
<th>OR</th>
<th>95% CI</th>
<th>P-value (multivariate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diseases</td>
<td>Uterine myoma</td>
<td>22</td>
<td>6 (27.3)</td>
<td>16 (72.7)</td>
<td>0.04*</td>
<td>0.334</td>
<td>0.125; 0.894</td>
<td>0.024*</td>
</tr>
<tr>
<td></td>
<td>Ovarian cyst</td>
<td>2</td>
<td>0 (0)</td>
<td>2 (100)</td>
<td>0.497</td>
<td></td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Benign breast tumor</td>
<td>9</td>
<td>2 (22.2)</td>
<td>7 (77.8)</td>
<td>0.170</td>
<td>0.271</td>
<td>0.055; 1.338</td>
<td>0.128</td>
</tr>
<tr>
<td></td>
<td>Autoimmune</td>
<td>2</td>
<td>0 (0)</td>
<td>0 (0)</td>
<td>0.497</td>
<td>0.497</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Sexual transmitted disease</td>
<td>2</td>
<td>1 (50)</td>
<td>0 (0)</td>
<td>0.497</td>
<td>0.497</td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Chronic infections</td>
<td>8</td>
<td>3 (37.5)</td>
<td>5 (62.5)</td>
<td>0.721</td>
<td>0.587</td>
<td>0.136; 2.528</td>
<td>0.253</td>
</tr>
<tr>
<td></td>
<td>Circulatory</td>
<td>5</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td>0.059</td>
<td></td>
<td></td>
<td>0.999</td>
</tr>
<tr>
<td></td>
<td>Thyroid problems</td>
<td>21</td>
<td>8 (38.1)</td>
<td>13 (61.9)</td>
<td>0.356</td>
<td>0.581</td>
<td>0.229; 1.472</td>
<td>0.409</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>179</td>
<td>89 (49.7)</td>
<td>90 (50.3)</td>
<td>0.879</td>
<td>0.587</td>
<td>0.229; 1.472</td>
<td>0.409</td>
</tr>
<tr>
<td>Food</td>
<td>House dust</td>
<td>9</td>
<td>4 (44.4)</td>
<td>5 (55.6)</td>
<td>0.767</td>
<td>0.791</td>
<td>0.206; 3.080</td>
<td>0.700</td>
</tr>
<tr>
<td>Food</td>
<td>Latex</td>
<td>2</td>
<td>1 (50)</td>
<td>1 (50)</td>
<td>1</td>
<td>1</td>
<td>0.062; 16.217</td>
<td>0.832</td>
</tr>
<tr>
<td>Food</td>
<td>Pollen</td>
<td>24</td>
<td>10 (50)</td>
<td>10 (50)</td>
<td>1</td>
<td>1</td>
<td>0.397; 2.522</td>
<td>0.723</td>
</tr>
<tr>
<td>Food</td>
<td>Medicaments</td>
<td>26</td>
<td>20 (76.9)</td>
<td>6 (23.1)</td>
<td>0.005*</td>
<td>3.932</td>
<td>1.504; 10.277</td>
<td>0.042*</td>
</tr>
<tr>
<td>Food</td>
<td>Others</td>
<td>36</td>
<td>22 (61.1)</td>
<td>14 (38.9)</td>
<td>0.196</td>
<td>1.737</td>
<td>0.830; 3.635</td>
<td>0.152</td>
</tr>
<tr>
<td>Smoking</td>
<td>Smoking now</td>
<td>61</td>
<td>24 (39.3)</td>
<td>37 (60.7)</td>
<td>0.064</td>
<td>0.535</td>
<td>0.289; 0.988</td>
<td>0.999</td>
</tr>
<tr>
<td>Smoking</td>
<td>Ex-smoker</td>
<td>19</td>
<td>13 (68.4)</td>
<td>6 (31.6)</td>
<td>0.146</td>
<td>2.435</td>
<td>0.835; 3.447</td>
<td>0.999</td>
</tr>
<tr>
<td>Smoking</td>
<td>Nons smoker</td>
<td>112</td>
<td>57 (50.9)</td>
<td>55 (49.1)</td>
<td>0.136</td>
<td>1.087</td>
<td>0.617; 1.914</td>
<td>0.999</td>
</tr>
<tr>
<td>Pain</td>
<td>Dysmenorrhea</td>
<td>114</td>
<td>90 (78.9)</td>
<td>24 (21.1)</td>
<td>&lt;0.001*</td>
<td>39.643</td>
<td>16.177; 97.15</td>
<td>&lt;0.001*</td>
</tr>
<tr>
<td>Menstrual cycle</td>
<td>Regular</td>
<td>122</td>
<td>59 (48.4)</td>
<td>63 (51.6)</td>
<td>0.624</td>
<td>0.807</td>
<td>0.425; 1.534</td>
<td>0.171</td>
</tr>
<tr>
<td>Living</td>
<td>Rural</td>
<td>74</td>
<td>34 (45.9)</td>
<td>40 (54.1)</td>
<td>0.461</td>
<td>0.770</td>
<td>0.432; 1.374</td>
<td>0.363</td>
</tr>
<tr>
<td>Living</td>
<td>Others</td>
<td>88</td>
<td>45 (51)</td>
<td>43 (48.9)</td>
<td>0.086</td>
<td>1.086</td>
<td>0.618; 1.907</td>
<td>0.234</td>
</tr>
<tr>
<td>Living</td>
<td>Others</td>
<td>33</td>
<td>18 (54.2)</td>
<td>15 (45.5)</td>
<td>0.073</td>
<td>1.245</td>
<td>0.588; 2.638</td>
<td>0.730</td>
</tr>
</tbody>
</table>

OR, odds ratio; CI, confidence interval.
Significant P-values are indicated (*).
analyzed in the literature; therefore, we tested for any allelic segregation differences in blood DNA (98 controls + 98 patients) and tissue DNA from the same patients (47 lesions, 14 control tissues) in our collective groups. Our results showed that the SNP G229A was homozygote for GG at both alleles in all cases; therefore, at position 77 in the transactivation domain only glycine was present.

Discussion

In this investigation we searched for statistically significant clinical and lifestyle parameters of mid-European women with endometriosis compared with matched control individuals. In addition, we analyzed all identified statistically significant clinical parameters for an association with the PvuII and Xba1 gene polymorphisms in intron 1 of the ERα gene. Stringent statistically significant associations compared with control individuals were observed with specific allergic responses and pain. Although no association of these parameters was observed with the ERα PvuII and/or Xba1 polymorphisms, a trend was noted with the endometriosis AFS IV classification (Table 5), implicating a possible role in the pathological state of the lesion, perhaps in growth and invasion.

Many SNPs also could represent spontaneous somatic mutations, which occur at higher rates in tumors (Jackson & Loeb 1998) or possibly in benign lesions. However, in both collective groups when DNA analysis was performed between blood, normal tissue and endometriosis lesions only the homozygote GG genotype for the exonic SNP G229A was detected. Although polymorphic in a North American population with an allele frequency of G/G (0.987) and A/G (0.013) (χ²: 0.003) this exonic ERα SNP resulting in a non-synonymous Gly77Ser exchange, was not detected in our endometriosis and control collectives. Our total analyzed cohort of 196 individuals was homozygous wildtype for this SNP; therefore this SNP must be rare in the European population and irrelevant for the etiology of endometriosis.

Although endometriosis is a common gynecological disorder, the etiology is still poorly understood. Chronic pelvic inflammation with elevated cytokines is associated with endometriosis and the disease shares some similarities with autoimmune diseases (Nothnick 2001, Matarasse et al. 2003). Earlier studies found a correlation between atopic diseases in 88 patients with endometriosis compared with 88 controls (Nichols et al. 1987). Using a more recent survey from the USA, Sinaii et al. (2002) investigated the relationship of concomitant diseases in 3680 endometriosis patients. Compared with published rates in the average USA female population, they found a higher rate of hypothyroidism, fibromyalgia, chronic fatigue syndrome, autoimmune diseases, allergies and asthma. Although this survey included a large patient cohort, the presence of a defined control collective,
and XbaI restriction.
PvuII and XbaI restriction.

(52x115) their association with endometriosis, including interleukin
immune genes have been examined in lymphocytes for
immunological problems. Several DNA polymorphisms of
hormones, e.g. contraceptives or GnRH agonists, or (2)
the higher rate of allergies against medicaments: (1) an
ing a genetic relationship. Two possibilities could explain
of allergies and endometriosis was also observed, support-
2
et al.
(reviewed in Wenzl
2003). It will be important to examine other gene polymorphisms which could be involved in allergies, for
example the T-cell receptor gene. Interestingly, the presence of the ER together with estradiol was recently
found to be essential for T cell lymphopoiesis and T
cell-dependent inflammation (Islander et al. 2005). In this
present study, no statistical association was noted between
the SNPs of ERα gene and allergies in the patient and con-
trol groups, thus supporting the view that there is no role
for the tested ERα polymorphism in a T-cell-dependent
inflammatory response.

Although dysmenorrhea and subfertility problems are
clearly associated with endometriosis, the molecular basis
for subfertility is still unclear. Several molecular markers
have been associated with the pathology of endometriosis
and/or specifically with subfertility in these patients,
including aromatase, endometrial bleeding associated-
factor, hepatocyte growth factor, 17-HSD, HoxA10, A11,
alpha(v), beta-3 integrin, leukemia inhibitory factor, matrix
metalloproteases, and the estrogen and progesterone
receptors (reviewed in Berube et al. 1998, Giudice & Kao
2004). In the present investigation, it was found that with
both the Fisher’s exact and multivariate analyses dysme-
enorhea was statistically significant (P = <0.001). In
addition, the control group showed higher levels of posi-
tive pregnancies and positive live births compared with
endometriosis patients, whereas endometriosis patients
had more fertility problems. However, no positive associ-
ation was found when compared with the PvuII and Xba1
SNPs in the ERα gene, supporting the view that there is no
involvement with the above clinical phenotypes.

Endometriosis is an estrogen-dependent disease. It is
possible that genetic variations in the estrogen-mediated
pathway within the lesion could contribute to a more
aberrant or increased estrogen effect promoting endome-
triosis initiation and invasion. When both PvuII and Xba1
polymorphisms were taken together, a trend for a higher
risk for the more severe endometriosis (AFS IV) was found
in the rarer genotypic (3 patients with endometriosis
compared with 0 controls) subgroup, Xba1 (X/X) and PvuII
(P/p) when compared with the non-severe endometriosis
lesions (AFS I-III). The association of both the PvuII
and Xba1 SNPs with diseases is extremely controversial:
e.g. a positive correlation of PvuII was found in 188 tumor

Table 4 Association of endometriosis patients with AFS I-IV staging and genotype.

<table>
<thead>
<tr>
<th></th>
<th>PvuII genotype</th>
<th>PvuII allele</th>
<th>XbaI genotype</th>
<th>XbaI allele</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>pp (n = 59)</td>
<td>pp (n = 59)</td>
<td>pp (n = 59)</td>
<td>pp (n = 59)</td>
</tr>
<tr>
<td>AFS &lt; IV (n = 39)</td>
<td>37 (62.7)</td>
<td>12 (20.3)</td>
<td>10 (16.9)</td>
<td>32 (27.1)</td>
</tr>
<tr>
<td>AFS IV (n = 39)</td>
<td>21 (53.8)</td>
<td>8 (20.5)</td>
<td>10 (25.6)</td>
<td>28 (35.9)</td>
</tr>
<tr>
<td></td>
<td>χ² = 1.181, P = 0.554</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pp, xx = wild type; p or PvuII and x for XbaI enzyme restriction; Pp, Xx = heterozygote for PvuII and Xba1; PP, XX = homozygote for no PvuII
and Xba1 restriction.

Table 5 Association of AFS 1-3 and AFS 4 classification with combination of genotype.

<table>
<thead>
<tr>
<th></th>
<th>AFS &lt;4 (n = 59)</th>
<th>AFS 4 (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERα genotype</td>
<td>P-value</td>
<td></td>
</tr>
<tr>
<td>PvuII Xba1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PP XX</td>
<td>1 (1.7)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>PP Xx</td>
<td>3 (5.1)</td>
<td>3 (7.7)</td>
</tr>
<tr>
<td>PP Xx</td>
<td>5 (8.5)</td>
<td>1 (2.6)</td>
</tr>
<tr>
<td>PP xx</td>
<td>6 (10.2)</td>
<td>7 (17.9)</td>
</tr>
<tr>
<td>pp XX</td>
<td>5 (8.5)</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>pp xx</td>
<td>7 (11.9)</td>
<td>4 (10.3)</td>
</tr>
<tr>
<td>pp Xx</td>
<td>11 (18.6)</td>
<td>2 (5.1)</td>
</tr>
<tr>
<td>pp xx</td>
<td>21 (35.6)</td>
<td>17 (43.6)</td>
</tr>
<tr>
<td>χ² = 3.496, P = 0.174</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

pp, xx = wild type; p or PvuII and x for Xba1 enzyme restriction; Pp, Xx = heterozygote for PvuII and Xba1; PP, XX = homozygote for no
PvuII and Xba1 restriction.

matched for age, race, as well as for demographic and
epidemiological characteristics was lacking (Sinai et al.
2002). In the present study, endometriosis patients had
autoimmune diseases (n = 2), thyroid problems (n = 8)
and chronic inflammations (n = 3), but none of these
reached statistical significance when compared with the
control group (Table 2). No significant general difference
between endometriosis patients and controls relating to
allergies was found; however, endometriosis patients did
show significantly more allergies against medicaments
(P = 0.005) and pollen (P = 0.048) using the Student’s t-
test, but only allergies against medicaments were statisti-
cally significant using the multivariate analysis (P = 0.042)
(Table 2). A positive link between first grade family history
of allergies and endometriosis was also observed, support-
ing a genetic relationship. Two possibilities could explain
the higher rate of allergies against medicaments: (1) an
increased consumption of pain relieving medicaments and
hormones, e.g. contraceptives or GnRH agonists, or (2)
immunological problems. Several DNA polymorphisms of
immune genes have been examined in lymphocytes for
their association with endometriosis, including interleukin
(IL)-1, -4, -6, -10 and tumor necrosis factor (TNF) alpha
(reviewed in Wenzl et al. 2003). Only the IL-6 promoter
polymorphism –174 G/C found in women of middle
European origin could be involved in a predisposition to
ovarian endometriosis with chocolate cysts (Wieser et al.
2003).
Table 6  Summary of PvuII and Xba1 polymorphisms in ERα of endometriosis (EM) and control patients in various published analyses.

<table>
<thead>
<tr>
<th></th>
<th>Total number</th>
<th>PP (n%)</th>
<th>Pp (n%)</th>
<th>pp (n%)</th>
<th>P (n%)</th>
<th>x (n%)</th>
<th>X (n%)</th>
<th>xx (n%)</th>
<th>xX (n%)</th>
<th>XX (n%)</th>
<th>Nationality</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>EM</td>
<td>109</td>
<td>14 (12.8)</td>
<td>59 (54)</td>
<td>36 (33)</td>
<td>87 (40)</td>
<td>131 (60)</td>
<td>Japanese</td>
<td>Kitawaki et al. (2001)</td>
<td>122</td>
<td>6 (4.9)</td>
<td>38 (31.2)</td>
<td>78 (63.9)</td>
</tr>
<tr>
<td>Control</td>
<td>179</td>
<td>43 (24)</td>
<td>71 (39.6)</td>
<td>66 (36.4)</td>
<td>157 (45.3)</td>
<td>201 (56.3)</td>
<td>Japanese</td>
<td>Kitawaki et al. (2001)</td>
<td>171</td>
<td>12 (7.0)</td>
<td>56 (32.7)</td>
<td>103 (60.3)</td>
</tr>
<tr>
<td>EM</td>
<td>121</td>
<td>24 (19.8)</td>
<td>49 (40.5)</td>
<td>48 (39.7)</td>
<td>97 (40.1)</td>
<td>145 (59.9)</td>
<td>Japanese</td>
<td>Wang et al. (2004)</td>
<td>98</td>
<td>11 (11.2)</td>
<td>25 (25.5)</td>
<td>62 (63.3)</td>
</tr>
<tr>
<td>Control</td>
<td>172</td>
<td>37 (21.5)</td>
<td>88 (51.2)</td>
<td>47 (27.3)</td>
<td>162 (47.1)</td>
<td>182 (52.9)</td>
<td>Japanese</td>
<td>Wang et al. (2004)</td>
<td>98</td>
<td>12 (12.2)</td>
<td>26 (26.6)</td>
<td>60 (61.2)</td>
</tr>
<tr>
<td>EM</td>
<td>57</td>
<td>2 (3.5)</td>
<td>28 (49.1)</td>
<td>27 (47.4)</td>
<td>32 (58.1)</td>
<td>82 (71.9)</td>
<td>Greek</td>
<td>Georgiou et al. (1999)</td>
<td>220</td>
<td>17 (7.7)</td>
<td>63 (28.6)</td>
<td>140 (63.7)</td>
</tr>
<tr>
<td>Control</td>
<td>57</td>
<td>16 (28.1)</td>
<td>26 (45.6)</td>
<td>15 (26.3)</td>
<td>58 (50.9)</td>
<td>56 (49.1)</td>
<td>Greek</td>
<td>Georgiou et al. (1999)</td>
<td>269</td>
<td>24 (8.9)</td>
<td>82 (30.5)</td>
<td>163 (60.6)</td>
</tr>
</tbody>
</table>

1Total number for PvuII polymorphisms; 2total number for Xba1 polymorphism.

PP, XX = homozygote for no PvuII and Xba1 restriction; Pp, Xx = heterozygote for PvuII and Xba1; pp, xx = wild type, p for PvuII and x for Xba1 enzyme restriction.
population size. It will be important to analyze other DNA polymorphisms associated with endometriosis in candidate genes involved in estrogen pathways. For example, the Rsal (exon 5) and AluI (exon 8) SNPs of the ERβ gene in patients with endometriosis compared with controls showed no general association (Wang et al. 2004). However, a slight but statistically significant difference was observed between the frequency of the A allele in the AluI polymorphism and the AF5 IV classification. In the future it will be important to determine genotyping and the functional roles of specific SNPs in molecular pathways involved in endometriosis.

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References


Brodowska A 2003 The influence of hormonal replacement therapy on bone density in postmenopausal women depending on polymorphism of vitamin D receptor (VDR) and estrogen receptor (ER) genes. Annales d’Academie Medicaii Steimtinsen 49 111–130.

Chang CC, Hsieh YY, Tsai FJ, Tsai CH, Tsai HD & Lin CC 2002 The proline form of p53 codon 72 polymorphism is associated with endometriosis. Fertility and Sterility 77 43–45.


Malhute NG, Mataliotsakis IM, Goumenou AG, Koumantakis GE, Vassilaidis S & Arici A 2004 Elevations in peritoneal fluid macrophage migration inhibitory factor are independent of the depth of invasion or stage of endometriosis. Fertility and Sterility 82 97–101.


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