**ENDOMETRIOSIS: New Genetic Approaches and Therapy**

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**Key Words** pelvic pain, infertility, genetics, linkage analysis

**Abstract** Endometriosis is a relatively common condition in women of reproductive age and is associated with considerable morbidity. Despite an extensive literature describing its multiple clinical manifestations, their management, and many aspects of the biology of endometriotic lesions, the pathophysiological mechanisms involved remain poorly understood. A genetic component in endometriosis is now recognized, and several groups have taken up the challenge of using genetic techniques to identify the aberrant molecular and cellular mechanisms in endometriosis with the intention of providing much-needed insights that might, in turn, lead to new therapies. The techniques that have been applied include expression profiling, tumor genetic studies, functional candidate gene studies, and linkage studies that can adopt a hypothesis-free approach. This review describes the current status of these studies and explores the prospects for new therapies.

**INTRODUCTION**

Endometriosis is one of the major conditions affecting women of reproductive age, but it is encountered relatively infrequently by medical specialties other than gynaecology. Despite its high prevalence (perhaps as high as 10% of all premenopausal females) (1) and its recognition for most of the past century as an important cause of infertility and pelvic pain, its underlying mechanisms and pathophysiology are still poorly understood.

Teaching at medical school defines the disease simply in terms of the presence of tissue resembling endometrial glands and stroma at sites outside the uterine cavity, but this is an incomplete definition given what we now recognize about the complexity of endometriosis and its various manifestations. Sadly, however, more than 30 years of biomedical research have provided only limited insights into its basic biology. Recently, molecular genetic approaches to understanding the condition have promised to provide much-needed fresh insights by adopting strategies that make no assumptions about any of the aberrant mechanisms involved.
Before discussing the emerging evidence, it is worthwhile to describe the major clinical manifestations encountered in endometriosis. These might be summarized as (a) peritoneal involvement, ranging from sparse peritoneal implants to diffuse peritoneal endometriosis that affects extensive areas of the pelvis; (b) ovarian endometriosis, usually manifesting as ovarian cysts (endometriomas); (c) deeply infiltrating peritoneal implants, often producing nodules deep in the rectovaginal space or at the base of the Cul-de-Sac with tethering of bowel; (d) lesions infiltrating bowel or the urinary tract; (e) lesions in sites outside the pelvis, including the umbilicus and lung.

Currently we do not know whether these manifestations (a) are diverse facets of a single disease process, (b) are different but possibly related conditions, or more radically (c) represent forms of expression of multiple, underlying, pathophysiological processes, all of which result in the expression of the endometriosis phenotype. Examples of this latter possibility include hypertension and osteoporosis; neither of these is now regarded as a single “disease” but rather as the clinical endpoint of a number of different pathophysiological processes. Physicians would normally not attempt to manage hypertension or osteoporosis without appreciating which of the underlying disease mechanisms applies to their particular patient, but in trying to manage endometriosis, gynecologists lack this level of insight. It is an aspiration of current molecular genetic work that this situation may eventually be rectified.

No reliable, nonsurgical diagnostic methods exist to identify and assess the extent of endometriosis present. Therefore, the disease, which is found in the lower peritoneal cavity and adjacent tissues, is predominantly diagnosed at gynecological and abdominal/colorectal surgery. The lack of adequate diagnostic alternatives to surgery has proved a huge hindrance to the study of endometriosis, as did the tardiness of gynecologists to recognize that multiple appearances of the disease exist on the peritoneal surface. In addition, deeply infiltrating lesions may not be visible at surgery if located beneath the peritoneum in the pouch of Douglas, especially if the rectum has been tethered to the uterus by inflammatory processes (2). Worse still, this type of endometriosis may be mistaken for minimal disease because only the uppermost part of the lesion may be visible at laparoscopy. Consequently, a woman with significant, painful endometriosis may be incorrectly told, after laparoscopy, that her pelvis looks normal and that there is no cause for her pain.

Thus, endometriosis presents enormous challenges to biomedical research and clinical practice, which are difficult to meet because of the following factors:

■ The cell biology and pathophysiology remain poorly defined.
■ The natural history and progression are difficult to assess.
■ Noninvasive assessment is currently inadequate, which confounds accurate assessment of the community prevalence.
■ Adequate assessment of the pelvis requires a high degree of skill and knowledge about the numerous types and appearances of the disease.
■ Although laparoscopic surgery for visible peritoneal deposits is widely available and relatively risk-free, surgery for deeply infiltrating disease, which
frequently involves the rectum, demands highly specialized skills and is hazardous.

- Medical options suitable for long-term management are currently unsatisfactory.
- Multidisciplinary chronic pain support facilities are often underdeveloped and given low priority by health care funders.

As a result, the challenge in endometriosis is at every level in the health care enterprise. It is a common disease with a marked effect on quality of life (3), but the kind of progress made during the past 30 years in developing new diagnostic and therapeutic strategies in so many other conditions has not occurred in endometriosis. A major reason for this relative lack of progress is undoubtedly the difficulty in obtaining research funding for a condition that does not appear on any list of priorities because it is not life-threatening, even though it is associated with considerable morbidity and suffering.

Further hampering research is the traditional view of a single disease spectrum with minimal (Stage I) endometriosis at one end and severe (Stage IV) at the other, as assessed by the revised American Fertility Society (rAFS) classification system (4), which defines disease severity in terms of the amount of ectopic endometrial tissue present, its location, and the damage it has caused. The various components—ovarian cysts with or without adhesions, and peritoneal surface and deeply infiltrating lesions—may involve completely different biological mechanisms. They may therefore represent entirely distinct disease entities with their own etiologies rather than different points on a single disease spectrum. Decades of research into the immunology and cell biology of endometriosis have so far failed to clarify this issue.

HERITABILITY OF ENDOMETRIOSIS

A novel approach to understanding the etiology of endometriosis is based on the realization that it appears to be highly heritable. The evidence was confined initially to small, case-control studies conducted in North America, Norway, and the United Kingdom. These reports showed that surgically confirmed disease in general occurs 6–9 times more commonly in the first-degree relatives of affected women than in controls (5–7), and radiological studies suggested that this effect may be more pronounced in the relatives of women with severe disease (8).

Subsequently, compelling evidence of the genetic basis of endometriosis has emerged from the analysis of large, clinical databases in Australia, Iceland, and Utah. Utilizing the Australian National Health and Medical Research Council Twin Register, Treloar et al. sent questionnaires to 3298 monozygotic (MZ) and dizygotic (DZ) twin pairs and obtained confirmatory information regarding disease status where possible (9). Of the twins surveyed, 3096 (94%) responded, among whom 215 self-reported that they were affected—a 0.07 prevalence rate among
respondents. Including only those women with surgically confirmed disease, the MZ and DZ twin pair correlations were $0.52 \pm 0.08$ and $0.19 \pm 0.16$, respectively, which suggests that 51% of the variance of the latent liability to the disease may be attributable to additive genetic influences.

In Iceland, Stefansson et al. have conducted the first population-based study examining the heritability of endometriosis (10). Having identified all patients in the country who were diagnosed surgically with the disease over a defined time period, they then determined to what extent the women were related, using a national genealogy database dating back 10 generations (11). The 750 women identified were significantly more related to each other than to population controls, with risk ratios for sisters and cousins of 5.20 and 1.56, respectively. The mean kinship coefficient (KC) for the affected women was significantly higher than that for 1000 sets of 750 matched controls, and this remained significant even when the contribution from first-degree relatives was excluded.

In a similar way, Ward et al. have recently investigated the degree of heritability using a large genealogy database that contains relationship data on 17 million ancestors and 3 million descendants of the ~10,000 founders who settled the Utah region in the mid-1800s (12). The names of the 448 grandparents of 112 sister pairs with surgically confirmed disease were identified in the database. Although there appeared to be no founder effect, their KC showed a high degree of relatedness ($F = 7.1 \times 10^{-4}$)—significantly higher than the mean KC ($F = 1.5 \times 10^{-4}$) obtained after sampling 100 controls randomly from the database 100 times. The authors concluded that endometriosis is highly heritable because the KC value for the grandparents of the affected sisters was more than 8 standard deviations higher than that of the control population.

The heritability of endometriosis is also apparent in nonhuman primates, which develop the disease spontaneously. Over a 20-year period, at the Wisconsin National Primate Research Center, 142 rhesus macaques with endometriosis have been identified principally from necropsy records, giving a prevalence of 31.4% (95% CI: 26.9–35.9%) (13). All the cases have been used to construct an extended, multigenerational pedigree and 9 nuclear families consisting of 1602 females in total. A high degree of heritability is suggested, as in the human data, by a significantly higher mean KC among affected compared with unaffected animals, and a higher recurrence risk for full sibs (0.75, 95% CI: 0.45–1.0) compared with paternal (0.47, 95% CI: 0.42–0.52) and maternal (0.26, 95% CI: 0.10–0.41) half-sibs.

The evidence from these epidemiological studies strongly suggests that endometriosis is inherited as a complex genetic trait; the phenotype results from interactions among allelic variations in several susceptibility genes, and from interactions between those genes and environmental factors. Moreover, the best available data indicate that the value of $\lambda_S$ (the risk of the sister of an affected woman having endometriosis compared with the risk in the general population) probably lies between 2 and 9 for all disease severities, although $\lambda_S$ may be as high as 15 in women with severe endometriosis. Clearly, there remain many unanswered questions as to the relative influence of genetic versus environmental factors and possible gene-environment interaction. Given the large number of different
environmental factors women may be exposed to up to and during their reproductive years, studying such interactions is problematic and may never be achieved without collecting long-term data prospectively.

Trying to identify the genes implicated in the etiology, pathophysiology, and progression of endometriosis currently involves a number of different tissue-based approaches, including expression profiling, comparative genomic hybridization, and loss of heterozygosity (LOH) studies. In addition, there are strategies based on the analysis of genomic DNA, which aim to identify genes that increase disease susceptibility (described below). Such approaches may be able not only to illuminate the elusive question of disease mechanisms but also to provide new insights into the management of the disease.

**EXPRESSION PROFILING**

Expression profiling has largely focused on comparing ectopic and eutopic endometrium from affected individuals, and endometria from women with and without endometriosis. For example, using a cDNA microarray consisting of 23,040 genes, Arimoto et al. identified genes that were upregulated in ovarian endometriotic cysts compared with eutopic endometrium at various stages of the menstrual cycle, including genes that encode some human leukocyte antigens, complement factors, and ribosomal proteins (14). Downregulated elements included the tumor suppressor TP53, genes related to apoptosis, and the gene encoding OVGP1, a protein involved in maintenance of early pregnancy.

Kao et al. investigated differentially expressed genes in endometrium obtained during the window of implantation from women with and without endometriosis using Affymetrix microarrays (15). Of the 12,686 genes analyzed, 91 were significantly increased more than twofold in their expression, and 115 were decreased more than twofold. The data implicate some genes (encoding aromatase and the progesterone receptor) in disease pathogenesis, and the selective dysregulation of other genes (involved in embryonic attachment and toxicity, immune function, and apoptotic responses) in a reduced likelihood of implantation. A similar set of experiments showed that the Cyr61 gene, which encodes a secreted, cysteine-rich, heparin-binding protein that promotes cell adhesion, migration, and neovascularization, was expressed at significantly higher levels in endometriotic tissue than in eutopic endometrium. In its secretory phase, Cyr61 was expressed at significantly higher levels in eutopic endometrium from women with endometriosis compared to those without the disease (16).

**TUMOR GENETIC STUDIES**

Although generally acknowledged as a benign disease, endometriosis has many features in common with neoplasia, such as clonal proliferation (17–21) and a tendency to metastasis and tissue invasion (22). The disease has also been associated
clinically with subtypes of ovarian malignancy, in particular endometrioid and clear cell carcinoma (22). Genetic alterations in endometriotic tissue have been described in LOH studies (23–26) and in studies using comparative genomic hybridization (27, 28) and fluorescence in situ hybridization (29–31). The alterations involve chromosomal regions that contain known or putative tumor suppressor genes previously implicated in ovarian cancer, such as 1q21, 9p21, and 17p13.1. These data led a number of groups to suggest that ovarian and probably deeply infiltrating endometriosis both develop like a tumor and that identifying the genes responsible might be important in understanding the initiation of endometrioid and clear cell ovarian carcinomas. More recently, however, other groups—using more reliable methodology, and using laser capture microdissection to isolate endometriotic from normal tissue—have questioned whether endometriotic cysts are truly monoclonal (18) and whether they demonstrate LOH at tumor suppressor gene loci (28a).

FUNCTIONAL CANDIDATE GENE STUDIES

Several “functional” candidate genes have been investigated in case-control studies comparing the frequency of single-nucleotide polymorphisms or mutations in genes that were chosen based on knowledge of their putative or actual role in mechanisms involved in the development of endometriosis. The four broad systems studied have been retrograde menstruation, the growth and differentiation of endometriotic cells, hormonal pathways, and detoxification mechanisms. It has been argued that findings have been inconsistent because most studies have used inappropriate controls (32). However, confusion is also created by underpowered studies and/or studies investigating genes without sufficient evidence to support their biological plausibility. Two examples will suffice because the subject has been well reviewed (33), and up-to-date information about all relevant studies is maintained on a genetic epidemiology Web site (34).

First, Cramer et al. reported that 10/33 (30%) affected women compared to 15/111 (14%) controls carried at least one allele with the N314D mutation in the \textit{GALT} gene, which had been previously associated with Müllerian anomalies (35). The polymorphism causes reduced activity of the enzyme galactose-1-phosphate uridyl transferase, which is involved in galactose metabolism. Three studies involving more patients have, however, since failed to replicate the association (10, 36, 37).

Second, genes that encode enzymes involved in detoxification, such as the glutathione S-transferase (GST) family, have been investigated based on the finding that the environmental pollutant 2,3,7,8-tetrachlorodibenzo-p-dioxin (dioxin) induces endometriosis in the rhesus monkey (38). Homozygotes for a null mutation in one of the GST family genes, \textit{GSTM1}, were more common in endometriosis cases than in controls (86% versus 46%), although if only women with rAFS Stage III–IV disease were included then >90% had the mutation (39). Subsequently, studies
in UK (40), Japanese (41), and Indian populations (S. Shivagi, S. Kennedy, personal communication) have failed to replicate the association, and no association has been found for a mutation in a similar gene, \textit{GSTT1} (40, 41). Moreover, in a critical appraisal of all the human and nonhuman primate evidence implicating dioxin exposure as a risk factor for endometriosis, Guo recently concluded that there was insufficient evidence to support the theory, about 10 years after it was first proposed (42). It is possible, therefore, that research groups around the world have investigated genes involved in detoxification as functional candidates based on an entirely false premise.

\section*{LINKAGE STUDIES}

Given the uncertainties regarding the etiology of the disease despite many years of hypothesis-driven research, it can easily be argued that a hypothesis-free approach is more likely to be successful. That is certainly the view held by at least six research groups, which have adopted linkage analysis as a strategy to identify susceptibility genes in endometriosis. This entails a genome-wide screen with \(\sim 400\) evenly spaced microsatellite markers followed by further fine mapping and association studies with “positional” candidates in linked regions. Two of the genome-wide scans (in India and Puerto Rico) are sponsored by public funds and four (in Australia, Iceland, UK/US, and Utah) by biotechnology companies, which inevitably influences the amount of information entering the public domain. What is known about some of these projects is summarized below.

Researchers in Iceland have collaborated with DeCode Genetics, which has a unique genealogical database that includes all individuals who have lived in the country in the past 10 generations. The investigators collected DNA from 205 women with surgically confirmed endometriosis in 64 families that include 2 to 13 affected members. To date, no genome-wide scan results have been reported, but 47 microsatellite markers have been genotyped across chromosome 9 (where the \textit{GALT} gene is located) without finding evidence of linkage (10). In Utah, researchers have collected 284 sister pairs and are planning a genome-wide screen (12). In principle, families in Iceland and Utah represent unique resources for genetic studies, and chromosomal regions linked to the disease may be identified rapidly in one or both of these populations. However, there are certain limitations to researching complex genetic traits in Iceland and Utah. Both places have small, homogeneous populations and there may be too few affected individuals to power the studies adequately, resulting in an inability to detect small genetic effects.

This is not a problem faced by the large, well-phenotyped DNA bank assembled by the International Endogene Study, which is a collaboration between the University of Oxford and the Australian Cooperative Research Center for Discovery of Genes for Common Human Diseases (Gene CRC), and their respective commercial partners. Their two projects—the OXEGENE (Oxford Endometriosis Gene) Study and the Genes Behind Endometriosis Study—started independently in 1995
based on previous research (43–45). Both groups have mainly collected affected sister pairs using recruitment methods similar to those described elsewhere (46). The combined data set consists of >2500 families (46, 47). Of those women with disease that could be staged using the rAFS system, 2220 (67%) in the Australian study have Stage A (the equivalent of rAFS Stage I–II) and 1098 (33%) have Stage B (the equivalent of rAFS Stage III–IV) disease; in the UK study, 737 (33%) have Stage A and 1470 (66%) have more severe or deeply infiltrating disease. Suggestive linkage has been reported for one chromosomal locus based on the analysis of marker data (400 markers at ∼10 cM) generated for a total of 289 families from the complete data set, containing 374 sister pairs plus other affected relatives (48).

However, the results of the full genome-wide scan of all the collected families have not yet been published.

NEW THERAPIES BASED ON GENOMIC DATA

A major stimulus to the use of molecular genetic tools has been the need to develop new therapeutic options. Current drug therapies fall short of what might be considered ideal because (a) they tend to be effective during administration but do not provide consistent, long-term relief, (b) they tend to suppress disease rather than ablate it, and (c) they are associated with significant side effects in most women (49). It may also be possible to target therapy better by defining responsive patient subsets.

To date, the development of aromatase inhibitors for use in endometriosis is the best example of a potential new therapy based on translational research in this field. The application builds on the evidence that aromatase is aberrantly expressed in endometriotic tissue (50). Aromatase catalyses the rate-limiting step in estrogen biosynthesis and is expressed in many tissues, including ovarian follicular granulosa cells and adipose tissue, but not in endometrium. Endometriotic tissue has high levels of aromatase mRNA. It has been shown that PGE2 stimulates aromatase activity in endometriotic cell lines and the resulting local estrogen production induces PGE2, thereby establishing a positive feedback loop (51). Conversely, expression of 17β-hydroxysteroid dehydrogenase (17β-HSD) type 2, which inactivates conversion of estradiol to estrone, is reduced in endometriotic tissue (52). This combination of mechanisms favors the local accumulation of estradiol and PGE2 and encourages the growth of endometriotic tissue—an observation that suggests aromatase inhibition might be a useful strategy in managing endometriosis. Supportive evidence from animal models includes an effect on experimental implants with YM511 (53) and fadrozole (54). Clinical information is limited at present, but there is a case report of symptom relief and regression of a 3-cm vaginal endometriotic lesion in an overweight, postmenopausal woman treated for 9 months with anastrazole (55). The effect may have been due to general suppression of aromatase activity in adipose tissue, but equally it may have resulted from inhibition of local aromatase expression in the lesion itself. However, the
usefulness of this approach may be limited because there was a 6.2% fall in bone mineral density despite the use of alendronate and calcium. More recently, in a randomized, controlled trial, anastrazole plus a gonadotrophin-releasing hormone agonist (GnRHa) was compared to a GnRHa alone during six months of treatment. Anastrazole plus GnRHa increased the pain-free interval and decreased symptom recurrence rates following surgery for severe endometriosis (56).

GENERATING NEW INSIGHTS

The genetic research described should lead to a clearer understanding of the cellular and molecular basis of endometriosis. The discovery of genes that predispose some women to develop endometriosis and the identification of the interacting environmental factors that cause expression of the endometriosis phenotype should transform the clinical management of this debilitating disease. It might also be expected that women found to test positive for genetic susceptibility would be advised to avoid environmental risk factors and to consider having children sooner rather than later in life in view of the association between endometriosis and infertility.

A long-term goal is to be able to identify the genetic determinants that contribute to the expression of the different phenotypes seen in endometriosis. Drug design would be directed at treating disease subsets defined on the basis of genotyping. New classification systems based on genetic information about individual patients may also lead to the development of targeted drugs, which in turn will enable more effective therapy and fewer side effects.

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LITERATURE CITED

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