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Heritability and molecular genetic studies of endometriosis

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Endometriosis is a common disease defined as the growth of endometrial tissue outside the uterine cavity that often results in a vast array of gynaecological problems including dyspareunia, dysmenorrhoea, pelvic pain and infertility. Despite the increasing evidence that supports a genetic component to this common gynaecological condition, the basic aetiology and pathogenesis of endometriosis remain unknown. It is likely that endometriosis is a common polygenic/multifactorial disease caused by an interaction between multiple genes as well as the environment. Such conditions do not have a clear Mendelian pattern of inheritance. Recent molecular cytogenetic studies on endometriotic tissue and an established endometriosis-derived cell line provide novel evidence that acquired chromosome-specific alterations may be involved in endometriosis, possibly reflecting clonal expansion of chromosomally abnormal cells. Molecular DNA studies examining the role of loss of heterozygosity in endometriotic lesions has identified candidate tumour suppressor gene loci, including 5q, 6q, 9p, 11q and 22q, that may play a role in the malignant transformation of endometriotic implants to endometrioid ovarian cancers. Evidence of mutations in the tumour suppressor *PTEN* gene in the endometrioid subtype of epithelial ovarian cancer further suggests that somatic genetic alterations represent early events in the transformation of benign endometriotic cells. Genetic factors are also likely to influence individual susceptibility to endometriosis. There is now evidence that heritable allelic differences in drug-metabolizing enzymes play an important role in the development of endometriosis. Further studies are warranted to identify major susceptibility gene(s) and the mechanism involved in endometriosis to assist in the development of better methods for early detection, diagnosis and prevention.

Key words: endometriosis/endometrioid ovarian cancers/environmental factors/heritability/somatic mutation

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Familial aggregates

Since the 1940s, published reports have recorded multiple affected relatives with endometriosis, often suggesting genetic tendencies (Gardiner, 1953; Frey and Bluefield, 1957). In 1971, Ranney reported the results of a questionnaire study of 350 women with endometriosis (Ranney, 1971). Of the 237 women who replied, 53 (22.4%) reported a first- or second-degree relative with endometriosis. In the first formal genetic study—conducted in 1980 (Simpson *et al.*, 1980)—a total of 123 probands with histologically confirmed endometriosis were identified. Nine of 153 (5.9%) female siblings aged >18 years had endometriosis; 10 of the 123 (8.1%) mothers were affected. Only 1% of the patients' husband's first-degree relatives (controls) had endometriosis. Of two sets of monozygotic (MZ) twins, one was concordant for endometriosis. In the same group of patients, the authors reported that women with an affected sibling or parent were more likely to have severe rather than mild or moderate endometriosis (Malinak *et al.*, 1980). Severe endometriosis was present in 11 of the 18 probands (61%) who had an affected first-degree relative. Among women having no

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Table I. Incidence of endometriosis among proband's first-degree relatives as compared with controls

Reference	Proband's relatives		
	Overall	Mothers and sisters	Controls
Simpson *et al.* (1980)	6.9% (19/276)	5.9% Mothers 8.1% Sisters	0.9% (2/211)[a]
Lamb *et al.* (1986)	4.9%	6.2% Mothers 3.8% Sisters	2.0%
Moen and Magnus (1993)	4.3% (45/1038)	3.9% Mothers 4.8% Sisters	0.6% (2/318)[a]
Coxhead and Thomas (1993)	5.5% (7/127)		0.8% (2/258)[a]

[a]Statistically significant

affected first-degree relative, severe endometriosis was present in only 25 of 105 (23%) cases.

Subsequent studies throughout the world have been consistent with these initial observations (Table I). In one such study, questionnaires received from 491 members of the Endometriosis Association based in the USA were used (Lamb *et al.*, 1986). A positive family history was reported by 18% of respondents. In addition, 66 women were evaluated in greater detail, with 43 returning a detailed questionnaire which both they and a friend (control) completed. Endometriosis was present in 6.2% of proband's mothers and in 3.8% of sisters; endometriosis was reported in less than 1% in first-degree relatives of friends. The frequency in second-degree relatives was 0.4% in grandmothers and 3.1% in aunts. In this study, most (93%) affected relatives were in the maternal lineage. A theoretical limitation of this study is that no attempts were made to confirm the diagnosis in relatives said to be affected; however, members of the Endometriosis Association can be assumed to be knowledgeable concerning the disorder. In the UK, a six-fold increase was reported in the incidence of endometriosis among first-degree relatives (Coxhead and Thomas, 1993). When 64 patients who had had visual diagnosis of endometriosis were compared with 128 control women, these authors found that 9.4% (6/64) of the patients and 1.6% (2/128) of controls had first-degree relatives affected with endometriosis.

A study similar to that of Simpson *et al.* was conducted in Norway (Moen and Magnus, 1993). Among 522 informative cases, 3.9% of mothers and 4.8% of sisters had endometriosis; only 0.6% of sisters of women not having endometriosis (controls undergoing laparoscopy for other reasons) were affected. In this study, either endometriosis or adenomyosis were considered grounds for positive diagnosis. Interestingly, the affected mothers were far more likely to have adenomyosis than the affected sisters. Similar to the cases described in Texas (Malinak *et al.*, 1980; Simpson *et al.*, 1980), familial cases in Norway were more likely to show severe endometriosis than were non-familial cases. In another report from the same Norwegian centre, eight MZ twins were observed among 515 endometriosis cases (Moen, 1994). Six of the eight (75%) sets were concordant as compared with a rate of 3.7% (19 of 515) for non-twin sisters ($P < 0.0005$). In three of the six concordant twin sets, mothers were also affected. More recently, concordance of endometriosis was demonstrated in 14 of 16 (88%) MZ twin pairs (Hadfield *et al.*, 1997). In addition, endometriosis (detected in the context as a cause for surgical menopause) shows greater correlation in MZ than dizygotic (DZ) ($r = 0.52$ versus 0.19) twins. Similar epidemiological studies have been reported in Icelandic women with endometriosis. A kinship analysis was performed on 744 affected women, and showed the prevalence of disease to be higher in the relatives of affected women than in controls (Stefansson *et al.*, 1998).

As part of the OXGENE (OXford Endometriosis GENE) group, Kennedy and colleagues are soliciting familial cases for whole genome linkage studies using sibling-pair analysis by DNA polymorphic variants to identify endometriosis susceptibility loci. Although unavoidably subject to selection biases, the accrued cases further confirm familial tendencies (Kennedy *et al.*, 1995, 1996). In one report of 100 families with endometriosis from six different countries, 19 mother–daughter pairs and 56 sibling pairs were ascertained (Kennedy *et al.*, 1995). In 18 families, three or more relatives in more than one generation have been observed. Among the familial cases, women tended to have more severe disease (65.7%), and onset of pain symptoms occurred at an early age (mean age of 22.1 years). In a subsequent study of 83 non-twin sisters concordant for endometriosis, a 0.53 year mean difference in age was reported for the onset of pain symptoms which was not significantly different from zero (Kennedy *et al.*, 1996). The difference in age of onset supports the thesis that endometriosis may have a genetic basis. Moreover, the finding that the older sisters developed symptoms approximately five calendar years before their younger sister (difference in year of onset = 5.15 years) suggests that common exposure to an environmental agent is unlikely.

To facilitate the collection of families, magnetic resonance imaging (MRI) has been used for diagnosis of endometriosis in relatives without the requirement of a surgical procedure (Togashi et al., 1991; Kennedy *et al.*, 1997). In one recent report, unequivocal endometriosis was found on MRI in five of 14

(36%) first-degree relatives and in one of 12 (8%) other relatives (Kennedy *et al*., 1998).

Knowledge that a first-degree relative has endometriosis should have profound clinical consequences, but until genetic markers are determined it is difficult to be precise in counselling. Other than the observation (Malinak *et al.*, 1980) that familial endometriosis is more likely to be severe than moderate or mild, no characteristics distinguish familial versus non-familial endometriosis. Similarly, until a genetic marker is determined, it is impossible to determine how many individuals with endometriosis have the genetically predisposed form. This concept was explored in our earlier publications (Malinak *et al.*, 1980; Simpson *et al.*, 1980) and remarks remain extant. At a minimum, some 7–10% of all cases would be expected to be 'genetic or familial'; however, many individuals with endometriosis might simply be the first of their family to be affected. Thus, far more individuals could actually have a 'genetic' form.

Knowing that a first-degree relative has endometriosis logically should influence the timing of child bearing and the type of contraception. One might recommend early rather than delayed childbearing, but only general statements could be made. One would expect the age of onset to be similar in multiple relatives with endometriosis, so childbearing should be encouraged by the end of the third decade. It would also be logical to use oral contraceptives rather than an intrauterine device for contraception. Similarly, it would be reasonable to avoid gynaecological manipulation during menstruation to minimize the likelihood of reflux menstrual flow. However, relatives of affected individuals should not be given the impression that these recommendations guarantee avoiding the development of endometriosis.

Possible genetic mechanisms

Endometriosis is clearly heritable, but what is the mode of inheritance? The magnitude of the increased risk (5–8% for first-degree relatives) generally favours polygenic/multifactorial tendencies. However, this risk of recurrence is slightly higher than expected (2–5%) for polygenic inheritance; the frequency of affected relatives might be even higher if one could directly measure a gene product(s). Although Mendelian mechanisms cannot be excluded, polygenic inheritance still seems more likely.

The polygenic model postulates that a given disease is caused by cumulative individual effects of many different genes acting together in an additive fashion. If an individual is unfortunate enough to have a large number of these genes, there exists a threshold beyond which disease is manifested. Therefore, different genetic backgrounds will create differential susceptibility so that the aetiology of the common disease is genetically heterogeneous. Environmental factors appear to play a role in all common diseases and are also likely to be involved in endometriosis; thus, the genetic mechanism should more properly be considered to be polygenic/multifactorial.

The polygenic/multifactorial model makes several predictions. One is that the first-degree relatives will have more of the susceptibility genes and will be more frequently affected than the general population. Increased severity in familial cases is also consistent with predictions based on a polygenic model. This model is based upon predictions that the greater the severity, the greater the underlying genetic liability and, hence, the greater the proportion of affected relatives. As noted, this is the case in endometriosis. Because endometriosis was more severe in familial cases also lessens the likelihood that presence of an affected family member led to the diagnosis of another affected member merely because of a higher index of clinical suspicion. That no studies have shown human lymphocyte antigen (HLA) associations (Moen *et al.*, 1984; Simpson *et al.*, 1984; Maxwell *et al.*, 1989) is also consistent with the hypothesis of endometriosis being a single disorder characterized by polygenic/multifactorial inheritance. Common adult-onset disorders showing HLA associations (e.g. diabetes mellitus, peptic ulcer disease) usually are characterized by genetic heterogeneity with some forms of Mendelian inheritance.

Identification of a major gene(s) will not only advance the understanding of genotype–phenotype interactions, but also enhance the development of specific therapies and/or preventative measures and identification of those women at highest risk. Quantitative genetic analysis is underway by several groups all seeking to localize the various genes paramount to the aetiology of endometriosis. A collaborative project between the Oxford Group and 52 centres around the world is in performing sibling-pair analysis using polymorphic DNA markers and fluorescence-based automated analysis (Kennedy *et al.*, 1995; Kennedy, 1998). The sibling-pair method is based on the demonstration that affected relatives inherit identical copies of the allele (identity by descent; IBD) more often than expected by chance alone. Unlike linkage analysis, sibling-pair methods focus only on affected individuals and do not depend on the specification of a model of inheritance for the trait because IBD sharing at a given locus is compared with random expectation (0.5 for first-degree relatives). Because of this, excess IBD sharing is detected in affected relatives despite incomplete penetrance, phenocopy or genetic heterogeneity. This approach has been used successfully in the analysis of candidate genes in other complex traits such as hypertension. However, for this approach to be successful, large numbers of families need to be ascertained with accurate diagnosis of disease.

More recently, a linkage approach is being pursued in Icelandic women. A genome-wide scan of 15 families, with a total of 33 affected individuals and 23 relatives, was carried out at a marker density of 5 cM. A 'suggestive locus' was found on 9q, but not in the region of the *GALT* gene on the short arm of chromosome 9 (Stefansson *et al*., 1998). Clearly, there are also limits to this linkage analysis approach for the study of endometriosis, including difficulties in ascertaining sufficient numbers of extended multi-generational families and an inability to confirm the absence of disease in unaffected individuals.

Clonal origins and gene over-expression

The pattern of aberrations, combined with the invasive nature of the disorder, suggests parallels between endometriosis and neoplasia. In tumours, the pathogenesis of neoplasia is now accepted to involve a clonal origin of a progenitor cell developing selective advantage. Two 'hits' (mutational events) are necessary for this clone; both events may be somatic mutations, or one may be germline and the other somatic. To this end, monoclonal cell expansion in endometriosis has been observed (Nibert *et al*., 1995; Jimbo *et al*., 1997). Over-expression of certain oncogenes (c-*myc*, c-*erbB*-1 and -2) has also been observed, as has DNA amplification on 6p (Gogusev *et al*., 1998). However, none of several potential candidate genes on 6p was over-expressed.

Immunocytochemical studies have demonstrated high protein levels of various proto-oncogenes in endometriotic tissue as compared with normal endometrium, including c-*myc*, c-*fms*, c-*erbB*-1/-2 and *ras* (Bergqvist *et al.*, 1991; Schenken *et al.*, 1991). These results suggest that altered proto-oncogene expression maybe involved in disregulated growth and differentiation of endometriotic cells. The *bcl-2* gene is a member of the Bcl-2 gene family, and over-expression of this gene leads to a decreased rate of cell death (Yang and Korsmeyer, 1996). This gene is considered to play a role in the normal endometrium cycle by regulating cellular homeostasis and apoptosis; increased expression is detected in the proliferative endometrial phase but not in the secretory phase (Lu *et al.*, 1993). Although several studies have examined the expression of *bcl-2* in endometrial carcinomas and hyperplasia, the results have been controversial (Henderson *et al*., 1996). In a recent study, *bcl-2* over-expression in ectopic endometrial lesions by immunohistochemical staining was reported, indicating that endometriotic cells fail to undergo apoptosis (Watanabe *et al*., 1997). Using a cell death detection enzyme-linked immunosorbent assay (ELISA) kit, it was further demonstrated that apoptosis is significantly decreased in eutopic endometrium of women with endometriosis as compared with fertile controls ($P < 0.0001$) (Dmowski *et al.*, 1998).

Molecular alterations

Where are the genes responsible for endometriosis localized? One approach, as described earlier, is to search for gene over-expression. However, the over-expressed genes are not necessarily the same ones as are involved in initiated pathogenesis. One vexing problem has been difficulty in obtaining and studying pure endometriosis tissue, in distinction to tissue samples admixed with contiguous (normal) endometrium or connective tissues. Genetic analysis of mixed tissue specimens may reveal normal results, and possibly explain the failure to find non-random cytogenetic abnormalities in any of 42 endometrial implants (Dangel *et al*., 1994). Similarly, another group failed to find *ras* oncogene- and *p53* tumour suppressor gene-specific mutations in ectopic and eutopic endometrial tissue specimens from 10 women with severe endometriosis (Vercellini *et al*., 1994).

More recently, techniques have been developed that can provide greater confidence that information will be derived from endometriosis tissue, and not other tissue. Our group has used fresh tissue touch preparations of endometriotic tissues to permit direct placement of cells from select tissue areas onto slides, thus reducing the number of potentially contaminating cells. Using chromosome-specific probes, we employed a multi-colour fluorescence in-situ hybridization (FISH) approach to examine single cells, and reported non-random chromosome alterations including trisomy 11 and monosomy 16 and 17 in late-stage disease (Shin *et al*., 1997).The undisrupted localization of cells from endometriotic tissue onto the surface of a slide is illustrated in Figure 1A, in which 80% of the nuclei analysed were monosomy 16 (Figure 1B). These studies provided novel evidence that acquired chromosome-specific alterations may be involved in endometriosis, possibly reflecting clonal expansion of chromosomally abnormal cells. That candidate tumour suppressor genes and oncogenes have been mapped to these chromosomes suggests that chromosomal loss or gain plays a role in the development and/or progression of endometriosis.

In addition, we have developed a modified microwave-enhanced FISH method to examine individual cells localized in endometriotic lesions when using fixed archival tissue in the absence of fresh surgical specimens (Kosugi *et al*., 1999). This is a powerful tool that enables analysis of specimens whose histology can be confirmed in a mirror-image serial tissue section (Figure 2). Using a two-colour FISH approach, we observed a significantly greater frequency of chromosome 17 aneuploidy in the endometriotic specimens ($n = 8$, mean of 65%) as compared with matched normal endometrial cells (mean of 25%). Moreover, we found significant ($P < 0.0001$) differences between the distribution of FISH signals among the endometriosis samples, implying a high degree of heterogeneity involving chromosome 17 aneuploidy. These findings support a multi-step pathway involving somatic genetic alterations in the development and progression of endometriosis.

A human endometriosis-derived permanent cell line (FbEM-1) has been established and characterized (Bouquet de Jolinière *et al*., 1997). Cytogenetic R-banding showed numerous chromosomal aberrations, including monosomy X, 4q+, 5q+, trisomy 7, 8 and 10 and tetrasomy of chromosomes 17, 18, 19 and 20. The authors recently used comparative genomic hybridization (CGH) as a method for identifying somatic chromosomal alterations in DNA from the same established cell-line as well as a series of endometriotic lesions ($n = 9$) (Gogusev *et al*., 1998). Although CGH revealed various chromosome abnormalities in FbEM-1, including 1q+, 4q–, 11p–, 13q–; loss of 9, 12 and 18; and amplification of 6p, the changes were not consistent with previous cytogenetic results, suggesting that the cultured cells may be unstable and therefore genetically

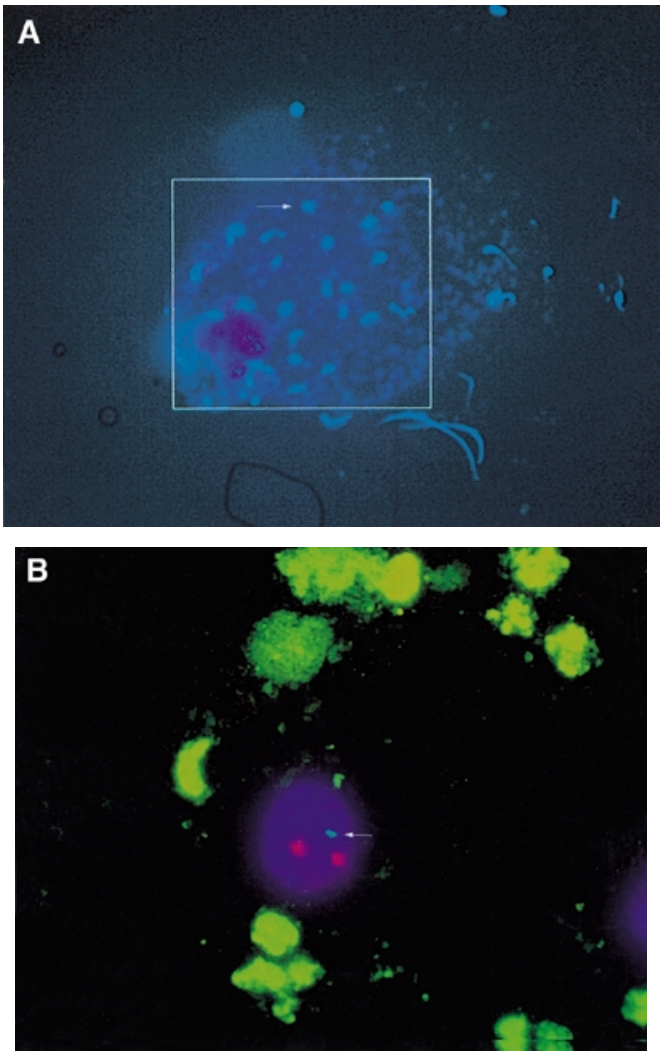

Figure 1. Multi-colour interphase FISH analysis on fresh tissue touch preparations of endometriotic tissues. (**A**) The undisrupted localization of cells placed on the surface of a glass slide. The box identifies an area of DAPI-positive (blue fluorescence) nuclei (arrow). (**B**) Detection of monosomy 16 in one of the DAPI-positive cells. The green and red fluorescent signals identify chromosomes 16 (only one signal detected) and 11 (two signals detected) respectively.

heterogeneous. Interestingly, chromosomal alterations were observed in only four of the nine endometriotic tissue DNA samples, with overall unbalanced aberrations involving gain on 1q, 4q, 11p, 17 and 20. Failure to find genetic imbalance among the remaining five cases may have been due to presence of contaminating normal DNA.

Investigators now speculate that endometrioid ovarian cancers probably arise by the malignant transformation of endometriotic implants (Sainz de la Cueata *et al*., 1996). In the study of cancer, early non-random somatic changes in DNA are frequently observed, reflecting clonal expansion of genetically abnormal cells. Loss of heterozygosity (LoH) studies, which assay the extent and variation of allelic loss, have been used successfully to identify and localize tumour suppressor gene loci among

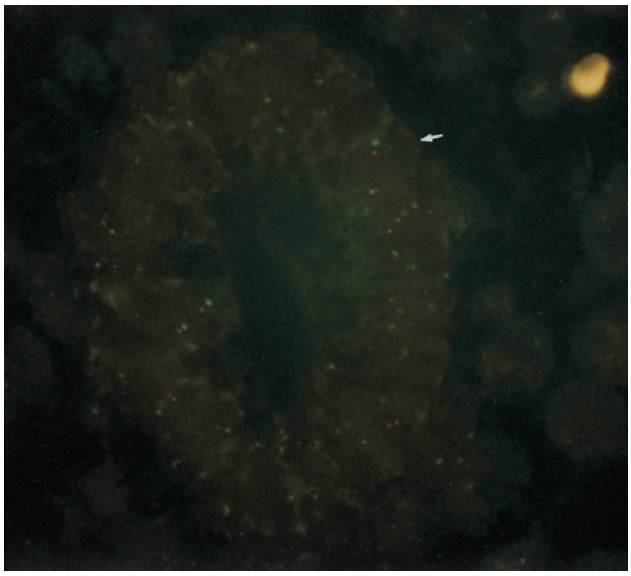

Figure 2. Two-colour FISH analysis of fixed archival endometrial tissue. A serial section of a normal endometrial gland is shown following hybridization with probes specific to the centromere of chromosome 17 (green fluorescent signals) and the p53 tumour suppressor locus on 17p13 (red fluorescent signals). Individual cells localized specifically to the gland can be identified and analysed for evidence of aneuploidy. The arrow identifies a cell that is monosomy for chromosome 17 based on the presence of only one red and one green signal.

various tumour types. Although the frequency of malignant transformation is less than 1%, there is clear association of endometriosis with ovarian endometrioid and endometrial cancer (Heaps *et al.*, 1990). LoH studies of endometrial and ovarian endometrioid tumours have reported significant loss of certain chromosomal regions. The chromosomal regions and genes commonly associated with ovarian endometrioid and endometrial cancers are summarized in Table II.

To determine whether LoH occurred in late-stage endometriosis, and whether the specific chromosomal regions were those associated with ovarian carcinomas, microdissection was used as a means of separating endometriotic glands and stroma from fixed archival tissue (Jiang *et al*., 1996). These workers first demonstrated monoclonal origin in endometriotic cysts by evaluating the pattern of allelic loss observed in multiple individual lesions within patients. Concomitantly, they found evidence of LoH involving chromosomes 9p, 11q and 22q as compared with matched normal genomic DNA. In a subsequent study, the same authors demonstrated common genetic alterations in nine of 11 cases in which ovarian carcinoma had arisen within, or adjacent to, endometriosis (Jiang *et al*., 1998). Alterations in chromosome arms 5q, 6q, 9p, 11q and 22q were observed in 25–30% of cases involving endometriosis with associated carcinoma. In one endometriosis sample, mutation in the *p53* gene at codon 220 (Tyr to Cys) was detected. Therefore, genetic alterations associated with ovarian endometrioid cancers may be involved in the development of endometriosis.

Table II. Common chromosomal regions and genes associated with ovarian endometrioid and endometrial cancers

Chromosomal region	Genes mapped	Gene function
2p16	*MSH2*	DNA mismatch repair protein
2p16	*MSH6 (GTBP)*	DNA mismatch (G/T) binding protein
2q31–q33	*PMS1*	DNA mismatch repair protein
3p21	*MLH1*	DNA mismatch repair protein
5q11–q12	*MSH3*	DNA mismatch repair protein
7p22	*PMS2*	DNA mismatch repair
9p21	*p16/CDKN2*	Negative regulator of cell cycle progression; cyclin-dependent kinase inhibitor
10q23.3	*PTEN*	Cell adhesion; signal transduction
16q22.1	*E-Cadherin*	Cell adhesion molecule
17p13.1	*TP53*	Transcription factor; Cell-death/-cycle regulator at G_1checkpoint of cell cycle
17q11.2–q12	*HER-2/neu*	Oncogene; transmembrane tyrosine kinase receptor; member of EGFR family
17q21	*BRCA1*	Tumour suppressor; may act directly or indirectly in transcription-coupled repair of oxidative DNA damage
18q22.1	*DCC*	Putative tumour suppressor with homology to cell adhesion molecules
18q21	*bcl-2*	Inhibitor of apoptosis (expression inhibits cell death)
Xq	Unknown	Ovarian development/function

Further support of the hypothesis that ovarian endometrioid cancers arise through malignant transformation of endometriotic lesions stem from recent evaluation of DNA alterations in the subtypes of epithelial ovarian cancers. Mutations in the tumour suppressor *PTEN* gene on chromosome 10q23 in endometrioid but not serous or mucinous epithelial ovarian tumours have been reported (Obata *et al*., 1998). These authors analysed over 81 ovarian tumours, including 34 endometrioid, 29 serous, 10 mucinous and eight clear cell tumours for LoH on 10q23 and mutations in *PTEN*. Although LoH was common among the endometrioid (43%) and serous (28%) tumours, somatic mutations involving *PTEN* in the remaining allele was observed only in the endometrioid (21%) tumours. These results demonstrate that the developmental pathways of the three epithelial ovarian cancer subtypes (serous, mucinous and endometrioid) appear to be different, and that somatic mutations in *PTEN* may represent early events in the transformation of benign endometriotic cells to malignancy. The PTEN protein is believed to function as a tyrosine phosphatase and play a role in signal transduction. Interestingly, other studies have shown *PTEN* mutations to be common in endometrial cancers when microsatellite instability is detected (Peiffer *et al*., 1995; Tashiro *et al*., 1997). Clearly, the role of *PTEN* in the pathogenesis of endometriosis is likely to be pursued in the near future.

Our findings of chromosome 11, 16 and 17 aneuploidy are consistent with genetic alterations observed in endometrial cancers (Table II). In addition, our own results of LoH involving the long arm of chromosome 17 (Bischoff *et al*., 1997) and increased heterogeneity involving chromosome 17 aneuploidy in endometriosis further suggest that genomic instability and chromosome 17 alterations may be involved in late-stage disease (Kosugi *et al*., 1999).

Environmental component

Because only some women develop endometriosis implies that there is increased susceptibility to development of disease in certain cases. Individual susceptibility is influenced not only by genetic background but also by the interaction of genes with environmental factors. Dioxin (2,3,7,8-tetrachlorodibenzo-*p*-dioxin; TCDD) and dioxin-like compounds (DLC) (e.g. polychlorinated biphenyls; PCB) have been implicated as factors involved in the development of endometriosis (Zeyneloglu *et al.*, 1997). The major route of human exposure to dioxin and DLC is through the diet. These compounds are hydrophobic and resistant to metabolic degradation, with an estimated half-life of seven years in humans. Consequently, they accumulate to high concentrations in lipids and membranes, being released slowly into the blood. In addition to well-described toxic effects, dioxin has adverse effects upon the female reproductive system. Dioxin administration severely reduced the reproductive success and oestrous cycling in rodents and non-human primates (Tryphonas *et al*., 1991; Cummings and Metcalf, 1995) and antagonized oestrogen-induced increases in uterine weights and endometrial development in mice (Johnson *et al.*, 1997). The direct relationship between dioxin and endometriosis was first shown using rhesus monkeys (Rier *et al*., 1993), with autopsy evaluation of animals exposed daily for 5 years to either 5 or 25 parts per trillion (ppt) of dioxin in their food demonstrating widespread peritoneal endometriosis in 71% and 86% of animals respectively.

Among the control group, endometriosis was observed in only two (33%) animals; this is similar to the spontaneous rate of endometriosis in colonies of rhesus monkeys. In humans, there is increasing epidemiological evidence of the effects of dioxin. Higher concentrations of PCBs in 28 patients with endometriosis compared with 441 unaffected women in a population of infertile women were reported (Gerhard and Runnebaum, 1992), while other authors (Koninckx *et al.*, 1994) showed the incidence of endometriosis in Belgium to be 60–80% in infertile women—one of the highest reported incidences in the world. In 1989, the World Health Organization reported dioxin concentrations in breast milk of Belgium women to be among the highest in the world (WHO, 1989). In women, increased concentrations of dioxin in breast milk may reflect the high dioxin accumulation, and possibly explain the high incidence of endometriosis among Belgian women in particular. More recently, the concentration of dioxin in the peripheral blood of 44 women with endometriosis as compared with 35 controls was measured (Mayani *et al.*, 1997). Dioxin concentrations of 0.6 to 1.2 ppt were detected in 18% of affected cases as compared with 0.4 ppt in one (3%) control case. Together, these studies suggest that dioxin and DLC may play a role in the development of endometriosis in humans.

The phase II conjugating enzymes usually function to inactivate carcinogenic and procarcinogenic compounds. Among the phase II enzymes, two—glutathione S-transferase (GST) and N-acetyltransferase (NAT2)—have been studied extensively, and these are considered important cancer-susceptibility genes. Both the *GSTM1* and *GSTT1* genes are polymorphic with null alleles that can be detected by polymerase chain reaction (PCR)-based methods (Hand *et al*., 1996; Lear *et al*., 1996). In a study of 84 cases of epithelial ovarian cancer, an influence of the *GSTM1* and *GSTT1* null alleles on increased *p53* expression was reported (Sarhanis *et al*., 1996). The authors proposed that the *GSTM1* and *GSTT1* are critical in the detoxification of the products of oxidative stress produced during the repair of the ovarian epithelium. Thus, homozygous null alleles in both genes may function synergistically, causing inefficient detoxification of intermediates produced during stress that increase damage to various genes in the host cell, including *p53*, and resulting in persistent expression of mutant protein. A recent study has shown a possible role of the homozygous *GSTM1* null allele with the development of endometriosis in 86% ($n = 50$) cases as compared with 45.8% ($n = 72$) of control, unaffected women ($P < 0.0001$) (Baranova *et al*., 1997). Our preliminary results of an association of M1/M2 NAT2 slow-acetylator genotype provide further evidence that heritable allelic differences in drug-metabolizing enzymes may play an important role in the development of endometriosis (Bischoff *et al*., 1998).

There is also growing evidence that the micro-environment of the peritoneal fluid and ovary play a role in the pathogenesis of endometriosis. In addition to genetic and immunological factors, the type of endometriotic lesion (i.e. superficial implants versus cystic ovarian endometriosis) appears to be regulated by the local concentration of hormones, cytokines, growth factors and angiogenic factors (Healy *et al.*, 1998; Koninckx *et al.*, 1998). The role of the cytochrome P450 aromatase system is also considered to be important since it has been associated with increased local levels of oestrogen in the extra-ovarian environment (Leyendecker *et al*., 1998).

Conclusions

Genetic predisposition to endometriosis is established, and is likely to involve one or more causative genes. Severity of disease tends to vary, and progression from mild to a more severe form occurs in some cases. These findings are consistent with endometriosis being inherited as a polygenic/multifactorial trait. Despite evidence of genetic and environmental factors contributing to this disease, the gene(s) and mechanism involved remain unknown. Further studies are warranted to identify major susceptibility genes and to test whether functional allelic differences in genes that mediate cellular responses to external drugs and/or chemicals influence susceptibility to endometriosis. Identification of the gene(s) and mechanism involved in endometriosis will ultimately assist in the development of better methods for early detection, diagnosis and prevention.

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