

The International Endogene Study: a collection of families for genetic research in endometriosis

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Objective: The aim of the International Endogene Study is to discover genes that influence susceptibility to endometriosis.

Design: The study brings together two research groups based in Australia and the United Kingdom that independently have been collecting families for linkage analysis and candidate gene studies. Both groups used similar methods to recruit families, obtain clinical notes, assign disease status based on the operative records and available histology, and collect common clinical data including age at onset of symptoms, age at diagnosis, and symptoms experienced.

Setting: Recruitment has been mainly from Australia, the United Kingdom, and the United States.

Patient(s): All affected participants have surgically confirmed disease.

Intervention(s): None.

Main Outcome Measure(s): Clinical and epidemiological data.

Result(s): To date, >1,100 families with affected sisters have been recruited, and >1,200 triads (affected women and both parents), for case-control studies.

Conclusion(s): We have created the largest resource yet assembled of clinical data and DNA for linkage and association studies in endometriosis. The increase in power to detect susceptibility genes vindicates the decision to merge the two studies and demonstrates the value of large-scale international collaboration. (*Fertil Steril* 2002;78:679–85. ©2002 by American Society for Reproductive Medicine.)

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The evidence that genes influence susceptibility to endometriosis is extensive (1, 2). Familial aggregation has been shown in both clinical (3, 4) and population-based (5) samples and in twin studies (6–8). Genetic recurrence risks to siblings (9) have been estimated at 2.3 (λ_s) for self-reported endometriosis in an Australian sample of twins and their families (8), and at 15 in the sisters of women with more severe disease based on imaging studies (10).

The search for genes predisposing individuals to common diseases such as endometriosis has traditionally been a choice between testing candidate genes for allelic association with the disease, or conducting larger-scale linkage studies to identify chromosomal regions using

the affected sibling-pair (ASP) method (11). A number of associations have been reported with candidate genes selected for their biological plausibility (12, 13). Results, however, have been inconsistent. For the *N*-acetyl transferase 2 (NAT2) gene (13, 14) and glutathione-S-transferase (GSTM1) genes (13, 15), initial positive reports were not confirmed (13). This might result from the fact that many case-control studies have been underpowered and poorly designed with inappropriate controls (16).

The linkage approach, using genomewide scanning of informative polymorphic microsatellite markers to identify regions of significant excess sharing in affected siblings (9, 17–19),

has been adopted by six groups (in Oxford, United Kingdom; Australia; Iceland; India; Puerto Rico; and Utah, United States). Specifically, the ASP method determines which chromosomal regions of affected siblings have a significant excess of shared alleles for given genetic markers that are identical by descent from the parents. Chromosomal regions that are shared between affected siblings more often than expected under random segregation will have a higher probability of containing genes that contribute to susceptibility for endometriosis. For diseases with a high genetic recurrence risk to siblings, the most informative relative pairs are distant ones, but for lower estimates of genetic risk, as for endometriosis, siblings constitute the best design (20). The ASP method is ideal for diseases in which diagnosis is problematic and defining someone as unaffected is uncertain. Our strategy to date is based on the ASP method and the assumption that there is at least one gene involved in predisposition to endometriosis. A positional candidate gene approach can then be used to identify potential susceptibility genes within that region.

This paper outlines the methods used in the recruitment of participants in the Oxford (Oxegene) and Australian (Genes Behind Endometriosis) studies, the collection and comparability of phenotypic information, and the merger of the studies into the International Endogene Study in August, 2001. The collaboration has arisen because of the need, for a complex disease such as endometriosis, to collect very large numbers of families in the search for susceptibility genes.

MATERIALS AND METHODS

Study Design

The International Endogene Study represents a collaboration between the University of Oxford and the Australian Cooperative Research Centre for Discovery of Genes for Common Human Diseases (Gene CRC) and their respective commercial partners, Oxagen Ltd., Abingdon, UK, and Cerylid Biosciences Ltd., Melbourne, Australia. The studies started independently in 1995, in each case based on previous work (4, 21, 22).

Both studies have recruited ASPs, and their parents if available. If one or both parents were unavailable, male or female siblings were collected to allow imputation of missing parental genotypes. Other affected relatives in these families were also collected as they can add power to detect linkage (23). For the second stage of determining allelic association with endometriosis in candidate genes, both studies are recruiting additional triads of cases and their parents whose nontransmitted alleles act as genetic controls in the Transmission Disequilibrium Test (TDT). We are also collecting single cases without parents for case-control studies.

The recruitment methods used in the Oxegene Study have previously been reported (11). The Australian study re-

cruited affected women who volunteered after media exposure and appeals (80%). A smaller number of participants were referred by collaborating gynecologists (14%) or endometriosis associations (2%) or were from the twin databases of Queensland Institute of Medical Research (QIMR; 4%). Institutional ethics committee approvals to obtain medical records, for blood collection for DNA extraction, and for all questionnaires and interview schedules were obtained from the human research ethics committees of the Queensland University of Technology and QIMR and from the Australian Twin Registry Ethics Committee. In the United Kingdom, the study received approval from the regional Multi-centre Research Ethics Committee and local research ethics committees; the appropriate approval was also obtained in collaborating centers in Leuven and Dublin.

Power Calculations for Sample Sizes

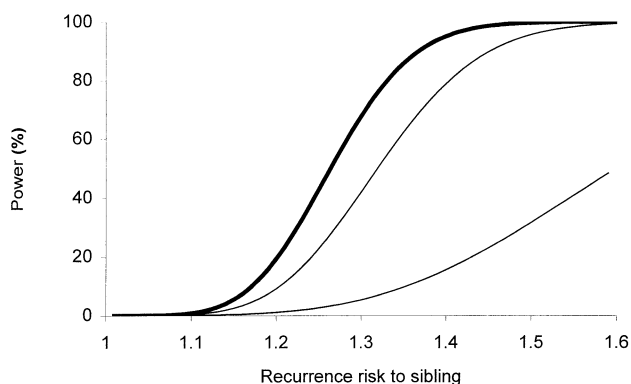
In the context of complex diseases, where multiple genes contribute to susceptibility, large sample sizes are required to detect susceptibility loci of modest effect. One measure of the genetic influence on disease is the recurrence risk to siblings, denoted λ_s , which is the ratio of the probability that an individual is affected, given that their sibling is affected, divided by the population prevalence of the disease (9). This parameter is important for estimating the power of a sample of ASPs because it determines the power to detect linkage in the case that there is a single susceptibility locus. In the case that there are multiple susceptibility loci, it determines the upper limit of the power. In an Australian sample of twins' families, the value of λ_s for endometriosis has been estimated at 2.3 (8). The value of λ_s may be ≤ 15 in the sisters of women with more severe disease (10).

In the context of multiple-susceptibility loci, each locus has its own recurrence risk to siblings (λ_{is}), and assuming multiplicative interaction and unlinked loci, the overall value of λ_s is the product of its value for each locus individually. That is, for n loci, $\lambda_s = \lambda_{1s} \times \lambda_{2s} \times \dots \times \lambda_{ns}$. Thus, in the case that λ_s equals 2.3, and assuming that there are two susceptibility loci of equal effect with multiplicative interaction, the locus-specific values are approximately equal to 1.52 (because 2.3 equals approximately 1.52^2). If there are three loci of equal effect, the locus-specific values are 1.32 (because 2.3 equals approximately 1.32^3). In the case that λ_s equals 15, and there are five susceptibility loci of equal effect, the locus-specific values are approximately equal to 1.72; if there are 10 loci, the value is approximately 1.31.

Under the assumption of no dominance variance, the power to detect linkage to a susceptibility locus depends on the number of ASPs in the sample and the locus-specific value of λ_{is} (9). Using a conservative critical LOD score of 3.6 for a genomewide significance level of 0.05 (19). Figure 1 shows the power to detect susceptibility loci with different values of λ_{is} when the sample size is approximately equal to that of the U.K. data set (300), the Australian data set (800), and the combined data set (1,100). If, for example, the value

FIGURE 1

Power for different values of the recurrence risk to siblings for different sample sizes of ASPs. *Thinnest line, 300; medium line, 800; thickest line, 1100.*



Treloar. *The International Endogene Study. Fertil Steril* 2002.

of λ_{is} for a locus is equal to 1.35, then the power is approximately 9%, 62%, and 86% for the United Kingdom, Australian, and combined data sets, respectively. Thus we see that combining the data sets provides a large improvement in the chances of finding susceptibility loci of modest effect. Also, for the U.K. data set, more severely affected patients were recruited, and so if such patients have a larger value of λ_s , then the U.K. data set may contribute proportionally more to the power of the combined data set.

Both projects also adopted the strategy of recruiting affected women and both parents for association and linkage analysis using the TDT (24). This was done so that candidate loci could be analyzed and also so that regions showing linkage using the ASPs could be saturated with single-nucleotide polymorphisms (SNPs) to help locate the disease mutations. Power calculations to determine appropriate sample sizes for the TDT are difficult in the absence of knowledge of the genetic model. As an example, if a biallelic susceptibility locus has penetrances of 0.1, 0.15, and 0.2 for carriers of 0, 1, or 2 disease genes, respectively, and the disease gene frequency is 0.1, then the sample size required for 80% power at a significance level of 0.05 when a correction for multiple testing for 1,000 SNPs is used is equal to 1,604 triads (25). The combined study has >1,200 triads. In addition, it is possible to extract a triad from most ASP families. Thus the combining of the two studies makes it much more likely that the sample size will be large enough to detect genes of modest association.

Confirmation and Staging of Disease

Confirmation of the diagnosis from the operative records with any available histology was sought in both studies, but disease severity was staged differently. In the Australian study, the gynecologist who made the initial diagnosis was

provided with a copy of the Revised American Fertility Society (revised AFS) classification system (26) and asked to stage the disease severity. Gynecologists complied in approximately 50% of the cases. If they did not and the medical records were available and sufficiently informative, one of the authors (D.O'C.) assigned a disease stage.

In the U.K. study, another author (S.K.) or the collaborators in Guildford, Dublin, and Leuven assessed >90% of the operative records and assigned a disease stage using the revised AFS classification system (26). In the remaining cases, the assessment of the woman's own gynecologist was used. Cases were divided into three categories: stage A (revised AFS stage I); stage A+ (defined as some ovarian disease plus some adhesions), and stage B (revised AFS stage III–IV disease). This simplified system was used because of the difficulty of accurately staging disease in retrospect using the clinical records alone. In both studies, the diagnosis was in some cases only confirmed in general practitioner records or hospital discharge letters, in which case it was usually not possible to assign disease severity, and these patients were excluded.

After a meeting in September, 2001 between D.O'C. and S.K., who jointly reviewed 50 randomly selected case records as examples, it was agreed that the same general principles had been used in both studies. Thus, a woman was not accepted into the study if there was no evidence of endometriosis in the clinical records or adhesions were found with evidence of previous infection in the absence of any mention of endometriosis, or a so-called chocolate cyst was the sole finding in the absence of any adhesions or histological evidence of endometriosis. Neither assessor relied on hearsay in the clinical records (i.e., mention of a past history of endometriosis) without obtaining the relevant operation note. The same approach was also adopted for unusual cases: for example, the rare finding of histologically confirmed endometriosis in an appendix or umbilicus in an otherwise normal pelvis was interpreted as revised AFS stage I and stage A disease in the respective studies. Finding histologically confirmed bowel endometriosis was interpreted as revised AFS stage IV and stage B disease, irrespective of the pelvic findings.

There were some differences in the way in which the operation records were interpreted. If a woman had more than one operation, disease stage was assigned in the Australian study on the basis of the findings at the first operation, whereas in the U.K. study, as many of the operative records were obtained as possible to enable disease stage to be assigned on the basis of the most severe findings. If it was not possible to stage the disease severity using the clinical records, revised AFS stage I was assigned in the Australian study, whereas "stage unknown" with confirmed status was assigned in the U.K. study.

Approaches to the diagnosis of endometriosis have changed over time (27). This is an important consideration in

TABLE 1

Composition of families recruited.

Family composition	United Kingdom (N)	Australia (N)	International Endogene Study (N)
Families for ASP analysis			
At least 1 affected sibship	230	728	958
At least 1 affected sibship with other affected relatives	33	117	150
Total families with affected sibships	263	845	1,108
ASP families			
2 affected sisters	245	758	1,003
2 affected pairs in different sibships	1	11	12
3 affected sisters	15	64	79
3 affected sisters in 1 sibship and 2 in another sibship	0	3	3
4 affected sisters	1	9	10
5 affected sisters	1	0	1
Families for association analysis: triads for TDT ^a			
1 triad	358	711	1,069
1 triad and 1 other affected relative	48	128	176
1 triad and 2 other affected relatives	2	12	14
1 triad and 3 other affected relatives	1	0	1
Total families with ≥ 1 triad (some with an additional affected relative)	409	851	1,260
Individual cases for case-control study ^b	248	316	564
Total families and cases recruited	920	2,012	2,932

^a Triads consist of a case and both parents.^b Only one case per family can be used for case-control association testing.Treloar. *The International Endogene Study. Fertil Steril* 2002.

genetic linkage studies, because it suggests that some patients may be misclassified. Patient's age at study participation may also be important, because younger women classified as unaffected may later be diagnosed with the disease. Disease stage classification may also change over time. Misclassification of disease status or stage adds noise to the genetic linkage signals, and reduces the power to detect susceptibility loci. This further highlights the value of a large data set for the International Endogene Study.

Additional Phenotypic Data

In the U.K. study, additional phenotypic data were obtained by questionnaire only, whereas in the Australian study, a telephone interview was also conducted to obtain details of diagnosis and family structure, and verbal consent for study documentation to be forwarded. The two studies used different questionnaires, but common questions included age at diagnosis, experience of symptoms such as pelvic pain, age of onset of pelvic pain, other gynecological diagnoses, fertility problems, and pregnancy history.

Data Stratification

Disease staging of patients allows for stratification of pedigrees based on disease severity. Thus, for the ongoing genotypic analyses, families are being stratified as follows: 1) all affected family members have stage A disease; 2) at least one affected family member has stage B disease, and 3)

all affected family members have stage B disease. We are also investigating other ways in which pedigrees may be stratified, for example on the basis of age at onset, pelvic pain, or infertility. Stratifying the data in this way can be useful in identifying subsets of families in which genetic effects are stronger. However, power can be lost in analyzing subsets unless they have considerably higher values of λ_s (see Power Calculations for Sample Sizes and Fig. 1). Stratifying the data can also reduce power because it necessitates correction for multiple testing.

RESULTS

As of April 2002, the combined data set consists of >2,500 families (see Table 1). In the Australian study, 4,207 women with self-reported endometriosis have volunteered to be contacted regarding participation in the study; 3,744 have reached a definite endpoint, but 18 of these women were uncontactable or overseas. Of these 3,726 women, 3,521 (94%) have been fully recruited. One hundred thirty-eight (4%) were ineligible for various reasons, 35 (1%) actively and 17 (<1%) passively decided not to participate after reconsidering, and 15 failed to complete their participation (<1%). Confirmation of disease has not yet been obtained for 199 (6%) of the women recruited, and for now they are classified with "unknown" affected status. Fourteen women

could not be assigned disease stage because of insufficient information, although their records clearly indicated a diagnosis.

In the U.K. study, probands were identified through endometriosis, infertility, and general gynecology clinics (5%); referrals from a large network of gynecologists (33%) as well as through self-help groups (1%); information published in national newspapers and magazines (11%); and the Oxegene Web site (50%). The database comprises 2,380 families containing 5,106 individuals, of whom 3,649 reported being affected and 1,561 are relatives. To date, 58% of affected individuals have consented to participate in the study; 4% decided not to participate after receiving the study information pack. Recruitment of 100 further families is still in progress. Disease has been confirmed by operation reports in 2,218 individuals; in 11 women, endometriosis was confirmed by operation records, but insufficient information was given to assess accurately the stage of disease. Questionnaires have been sent to 1,252 women with endometriosis and have been completed and returned by 840 women, giving a response rate of 67%.

Country of Residence and Ancestry

In the Australian study, the majority of affected participants resided in Australia itself (98%); in the U.K. study, the majority resided in the United Kingdom (57%) or North America (37%). Most (>95%) participants were Caucasian. In the Australian study, 67% of the participants' parents were both born in Australia: 59% of women reported their father's ancestry as British, followed by Irish (15%), German (6%), Italian, or Greek (4%), and a range of other countries (16%), with a similar pattern in their mothers.

Diagnosis and Stage of Disease

The mean ages (\pm SD) at diagnosis were 29.6 ± 7.6 years; range = 10–71 years (Australian study) and 30.5 ± 7.4 years; range = 15–70 years (U.K. study).

In the Australian study, doctors or hospitals provided operative reports confirming the diagnosis in 45% of the cases. Twenty-eight percent of doctors provided operative notes, 21%, a diagram of the patient's disease; and 27%, a histology report. Twenty-six percent of women reported having had a hysterectomy, at ages ranging from 19 to 71 years, with a mean of 37.2 ± 6.4 years. Stage of disease was assigned for a total of 3,318 women: stage I = 45%; II = 21%; III = 19%; and IV = 15%. Using the simplified system, 2,220 (67%) had stage A and 1,098 (33%) had stage B disease.

In the U.K. study, an operative report was obtained in >97% of cases, and a histology report was available in 26% of cases. The diagnosis was made at laparoscopy (68%), laparotomy (19%), or hysterectomy (13%). Stage of disease was assigned for a total of 2,218 women, of whom 737 (33%) had stage A and 1,470 (66%) had stage A+, stage B, or deeply infiltrating endometriosis; 11 (<1%) women were

assigned "stage unknown" with endometriosis-confirmed status.

Prevalence of Dysmenorrhea and Pelvic Pain

In the Australian study, 90% of women reported experiencing "severe" dysmenorrhea, with onset at mean age of 17.1 ± 5.8 years, range = 8–45 years; and 79% reported having experienced "severe" pelvic pain at some stage, with a mean age at onset of 20.4 ± 7.3 years, range = 9–50 years. Seventy-five percent of women who had had sexual intercourse reported experiencing pain during intercourse.

In the U.K. study, 91% of participants reported having at some stage experienced pelvic pain (including dysmenorrhea and also pain on opening bowels and passing urine); women reported that their symptoms started between the ages of 7 and 54 years, with a mean of 22.0 ± 8.4 years. Forty-four percent of women who had had sexual intercourse reported experiencing pain during sexual intercourse.

Problems Conceiving

In the Australian study, 23% of the women had never tried to conceive, but of those who had tried to conceive, 60% reported experiencing difficulties. Fifty-five percent of the women, who had tried for 12 months or more to conceive, had done so without success.

In the U.K. study, 43% of the women had been infertile at some stage. Of the women who became pregnant, 44% had tried to conceive for 12 or more months. Forty-five percent of the women who had tried for 12 months or more to conceive had done so without success.

DISCUSSION

This is the largest clinical resource for linkage and association studies in endometriosis ever assembled. Such a large collection of families is necessary because there are likely to be several genes of relatively small effect, but with major clinical ramifications in terms of disease burden. The length of time that it has taken to reach these recruitment levels (>8 years) is an issue itself in that knowledge about the endometriosis phenotype has increased and that candidate gene work has proceeded apace while we have been collecting families. Such studies require a long recruitment period, enormous resources, and huge efforts on the parts of clinicians, recruitment teams, and laboratory staff. Without doubt, we could not have achieved such large numbers of participants without the considerable goodwill of clinicians, secretaries, health information officers, and numerous endometriosis self-help groups.

Both groups have relied on a retrospective review of clinical records to diagnose endometriosis and stage the disease. Every effort was made to minimize mistakes that may be made because some clinicians will have misdiagnosed endometriosis and some notes contained too little detail. However, in general, we feel that the criteria used by

D.O'C. and S.K. to interpret the records have led to accurate phenotyping for future genotypic analyses. The only alternative to our approach would have been to collect families prospectively, which would have been prohibitively time-consuming and expensive.

Outstanding clinical issues relate to the small differences in interpretation noted earlier, and whether the respective databases should be altered to take these differences into account. Although these are not major issues, we are still grappling with how best to deal with them. There are many issues in deciding which clinical information is important for stratification of the samples to best identify linkage regions. Other population stratification questions arise, for example when identifying genetic (allelic) associations with the disease, as ancestry may be important in terms of differing allele frequencies in population groups.

The resource will clearly be a source of interesting epidemiological data in the future. For example, preliminary analysis of the Australian database shows an average 8-year gap reported by women between symptom onset and diagnosis of endometriosis. The gap is comparable to that previously reported for a sample of patients from the U.K. study (28). Although potential reasons for delay are numerous, it is inevitable that women's well-being must be greatly compromised. Pathology and progression of endometriosis and/or endometriotic disease may occur unnecessarily. Although retrospective, the very high prevalence of reported lifetime experience of severe menstrual and pelvic pain in this group of patients is also noteworthy and of serious clinical concern.

The increased power to detect genes predisposing women to endometriosis vindicates the decision to merge the two studies and demonstrates the value of large-scale international collaboration. By the end of 2002 we will have completed genotyping all the ASP families and better characterized linkage regions previously reported in preliminary findings (29). We will be testing candidate genes in those regions for association with endometriosis, and we expect that this strategic approach will bypass the inconclusive route taken by sometimes ad hoc selection and association testing of candidate genes.

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