

Genetic influences on endometriosis in an Australian twin sample

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Objective: To investigate the prevalence of and twin pair concordance for endometriosis.

Design: A questionnaire survey incorporating validation.

Setting: An Australia-wide volunteer sample of female monozygotic (MZ) and dizygotic (DZ) twin pairs from the Australian National Health and Medical Research Council Twin Register.

Patient(s): Twins were selected only on the basis of previous participation in twin research.

Intervention(s): Questionnaires were sent to 3,298 individuals. Information was requested from physicians named by consenting twins.

Main Outcome Measure(s): Reported endometriosis, validated where possible by pathology or surgical report.

Result(s): Three thousand ninety-six (94%) of the twins and 145 (82%) of the physicians responded to the survey. Two hundred fifteen twins reported endometriosis, for a prevalence rate of .07 among question respondents. Tetrachoric twin pair correlations for self-reported endometriosis (MZ: n = 854 and DZ: n = 493) were $r_{MZ} = .46 \pm .09$ and $r_{DZ} = .28 \pm .13$. When available medical and pathology reports were included, they changed to $r_{MZ} = .52 \pm .08$ and $r_{DZ} = .19 \pm .16$, suggesting that 51% of the variance of the latent liability to endometriosis may be attributable to additive genetic influences.

Conclusion(s): These findings support the hypothesis that genes influence liability to endometriosis. (*Fertil Steril*® 1999;71:701–10. ©1999 by American Society for Reproductive Medicine.)

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The causes of endometriosis, and the causes of variations in its presentation and pathologic development, have remained enigmatic (1). In some affected women, the condition is a pathologic process with progression, scarring and tissue damage, severe pain, and infertility, whereas in others, it is not. Endometriosis represents a therapeutic challenge, and new treatment approaches have been urgently called for. We performed a study using the classic twin design that addresses the importance of genetic influences on endometriosis.

In the past, endometriosis was diagnosed either on symptomatic grounds or by laparotomy, but it currently is diagnosed on the basis of surgical visualization by laparoscopy. Revised criteria of the American Society for Reproductive Medicine (previously the American Fertility Society) for the diagnosis of stages I–IV endometriosis (2, 3) provide a standard

commonly recognized by gynecologists, although other classification systems have been used (4). Defining the population prevalence of endometriosis in an unbiased (and ethical) manner is impossible because diagnostic laparoscopy cannot be performed on a large, random, population-based sample of women (5).

Endometriosis can be asymptomatic (4); questions concerning the possibly self-limiting nature and the prevalence of minimal or mild disease (6) remain unanswered. Wide variations in prevalence have been reported according to the population under investigation and the criteria used (7).

The determinants of endometriosis are poorly defined, with relatively few studies performed until the last decade (8). A review of almost 100 studies, which identified only 6 with satisfactory methodologic soundness, concluded that surprisingly little is known

about risk factors for the disease (9). The risk of pelvic endometriosis is strongly associated with age, even within the reproductive period (9, 10). Increased exposure to menstruation (through shorter cycle length, longer duration of flow, and reduced parity) and retrograde menstruation have been identified as reproductive risk factors (9).

Estrogen levels appear to encourage the growth of endometriosis, so hormonal factors seem logically relevant (9), as they are to the malignant proliferation of endometrial tissue. Abnormal immune function (11, 12), possibly from the negative effects of agents such as dioxin (13, 14), has been noted, but animal findings have yet to be replicated in humans (15). In summary, clear epidemiologic factors have not been identified and the chronology of associations with the condition remains highly uncertain (10).

Evidence of genetic influences on endometriosis has been available in published studies for some time, but the robustness of the evidence has been questionable because of small sample sizes, often in "convenience" clinical samples, and the possibility of selection or ascertainment bias. An increased incidence of endometriosis in the first-degree relatives of patients with endometriosis has been reported (4, 16–22), suggesting a familial predisposition and possible genetic influences. An investigation of a series of 538 patients with endometriosis in Brisbane, Australia found a familial incidence of approximately 1 in 5, compared with a general incidence in patients in the same obstetric practice of 1 in 9 (4). Although this was a large patient series, the sample was a "convenience" rather than a community sample, and thus subject to bias.

A recent British study by the Oxegene group identified 230 women with surgically confirmed endometriosis in 100 families, supporting a familial tendency toward endometriosis (21). Another recent report on 16 pairs of monozygotic (MZ) twins who were part of a sample of affected sister pairs recruited for a linkage study found that 14 of the 16 pairs were concordant for endometriosis (23), but this concordance rate is likely to have been subject to serious sampling bias because the respondents were recruited by advertisements for sister pairs. An analysis of age at the onset of pain symptoms in sister pairs concordant for endometriosis suggested genetic influences rather than a common environmental exposure because of the much greater similarity between sisters in age at symptom onset than in year of symptom onset (24).

An earlier, very small case study of MZ twins recruited through the Norwegian Twin Panel, and their mothers, reported that six of eight MZ twin pairs were concordant for endometriosis, which is a much higher concordance rate than that of ordinary siblings (25). Other evidence of a genetic basis for endometriosis comes from nonhuman primates (26). Molecular and cytologic research has suggested a possible path for genetic control of endometriosis (27, 28). Several candidate genes have been identified for investiga-

tion, including genes involved in galactose metabolism and dioxin detoxification (22).

The publication of twin study evidence on the heritability of other reproductive characteristics, including menstrual flow, pain, and impact of, interference of, and perceived limitation caused by periods (29, 30); age at menarche (31); premenstrual tension (32); and, in particular, liability to hysterectomy (33) initially focused our attention on this disease. We found strong evidence for genetic factors influencing liability to hysterectomy, accounting for 66% of the total variance, and these genetic influences on liability to hysterectomy were stable across birth cohorts (33). A diagnosis of endometriosis was a key significant predictor of hysterectomy in the new data, which form the basis of the present article (unpublished observations). We present the results of hypothesis testing concerning genetic influences on endometriosis using these new twin pair data.

MATERIALS AND METHODS

Sample

The sample had participated in two previous health surveys performed in 1981 and 1989. Participants were members of the cohort of 1,979 female twin pairs that were identified originally in 1980–1982 from the Australian National Health and Medical Research Council (NHMRC) Twin Register (33–36) and followed up in 1988–1990 (37). In 1993–1994, a questionnaire focused on gynecologic conditions and hysterectomy was sent to both members of 1,570 female twin pairs, plus a further 158 individual female twins in incomplete pairs (3,298 individuals) who could still be contacted and were willing to participate in twin research.

The present study therefore comprised a third wave of data collection from the original cohort of female twins. Approval to conduct the research was obtained from the Bancroft Ethics Committee (Queensland Institute of Medical Research) and from the Australian NHMRC Twin Registry.

Two items concerning similarity in appearance and being mistaken by others were included to determine zygosity. Pairs that gave inconsistent responses were recontacted for clarification. Such questionnaires have been shown to give at least 95% agreement with diagnosis based on extensive blood typing (38, 39).

More recently, members of a subsample of 198 same-sex pairs (both male and female and obtained from the wider sample) who reported themselves to be MZ were typed for 11 independent highly polymorphic markers in the course of an asthma study, and no errors in our previous zygosity diagnosis were detected. Of 131 like-sex pairs (male and female) who reported themselves to be dizygotic (DZ) and who had DNA available, 5 (3.8%) were concordant at the 11 loci, with a probability of dizygosity of $<10^{-4}$. This gave a sensitivity for self-reported monozygosity of 0.98 (exact

95% confidence interval [CI] 0.94–0.99) and a specificity of 1.00 CI (0.97–1.00) in this sample (40).

Assessment

Participants were asked to complete a four-page questionnaire (“Gynaecological Health Study”), which included questions on gynecologic problems that might predispose to hysterectomy, other surgical interventions, and medical and hormonal treatments. Twins were asked to report whether they had ever had endometriosis, their age at its onset, the nature of its investigation and/or treatment, and whether their cotwin or mother had had endometriosis.

Validation

When twins gave written consent and reported having had either a hysterectomy or endometriosis, questionnaires were sent to medical sources. Validation of self-reported hysterectomy and/or endometriosis was sought where possible from the named specialist involved. If he or she could not be contacted, the next contact attempted was the hospital or pathology laboratory or the general practitioner (family physician) involved in the treatment of the relevant condition. If no other medical source responded, the current general practitioner was contacted. Physicians were mailed a two-page questionnaire with an explanatory cover letter and a copy of the twin’s written consent, and those who had not responded after 6 weeks were telephoned by a research nurse to encourage their participation.

Environmental Similarity

The twin method relies on the assumption that the environments of MZ cotwins are no more similar than those of DZ cotwins, or if they are, that this does not influence intrapair similarity in the variable being analyzed. Our study assessed environmental similarity in two ways. In a 1989 survey, the twins were asked to rate how much they currently see and contact each other on a seven-point scale ranging from no contact to living together. They also were asked about four aspects of childhood “environmental” similarity (sharing the same room, being dressed alike, sharing the same playmates, and being in the same class), which were scored on a four-point scale.

Data Analysis

The statistical package SAS 6.11 (SAS Institute Inc., Cary, NC) (41) was used for preliminary and phenotypic data analyses. Twin pair matrices of polychoric correlations and corresponding asymptotic covariance matrices were computed separately for MZ and DZ twin pairs using the Windows interactive version of PRELIS 2.2 (Scientific Software International Inc., Chicago, IL) (42).

Phenotypic variation in human behavior and health can be decomposed into four basic components, although only three can be estimated at any one time, given the twin methods available (43, 44). These components are additive gene action (a), environmental influences specific to the individual

(e), and either environmental effects common to both cotwins (c) or nonadditive gene action (d), such as genetic dominance or epistasis. Total phenotypic variance is defined by the equation $V_p = a^2 + d^2 + c^2 + e^2 = 1$, where genotypic effects (h) comprise a and any effects of d . Additive genetic influences are those in which the total genetic effect is simply the sum of effects at individual loci, with no interaction. Nonadditive genetic effects comprise dominance, which involves allelic interaction, and epistasis, which involves nonallelic interaction between genes at different loci on one or more chromosomes (45). Genetic nonadditivity and shared environment are completely confounded in data on twin pairs reared together (43, 44, 46–48), and only one of them can be estimated.

Inferences concerning the genetic and environmental etiology of a particular trait or disease can be made from the relative concordance of MZ compared with DZ twin pairs (49). If the DZ twin pair correlation is less than half the MZ twin pair correlation, genetic nonadditivity (dominance or epistasis) is indicated, whereas shared environment increases the DZ correlation to more than half the MZ correlation. Genetic models were fitted by the method of asymptotic least squares. Univariate genetic models, estimating the contributions of additive genetic, shared, and nonshared environmental effects, were fitted using the Mx statistical modeling program (50).

In addition to the likelihood ratio χ^2 test (51), the Akaike Information Criterion (measured as $\chi^2 - 2df$) (52) was used as an indicator of fit. On the grounds of parsimony, the model with the least number of parameters that offered a fit not significantly worse than the full model was chosen. Data analysis methods are described more fully elsewhere (32, 53, 54).

RESULTS

Individual Twin Data

Response by Twins

Of the 3,298 individual twins to whom the questionnaire was mailed (both members of 1,570 pairs and 158 individuals in incomplete pairs), a response was obtained from both members of 1,431 female pairs (910 MZ and 521 DZ) plus 234 individual twins whose cotwins did not respond. There was therefore a 91% pairwise response rate. The individual response rate was 94%, with a total of 3,096 individuals, including those in incomplete pairs, responding.

Most ($n = 1,954$, 71%) respondents gave consent for the research team to request further information from their physicians if necessary. Of the twins who reported having endometriosis ($n = 215$), a higher percentage ($n = 178$, 86%) gave their consent. Twenty-nine women who reported having endometriosis (14%) did not sign the consent form. Most, but not all, of the consenting respondents provided

adequate written information about physicians who diagnosed the endometriosis or about hospitals or pathology services where records might be obtained.

Response by Physicians

One hundred forty-five (82%) physicians responded to a letter and two-page questionnaire asking for details of the endometriosis. Of these physicians, 80 (55.2%) were specialist gynecologists, 10 (6.9%) were general practitioners who had treated the twin in relation to the endometriosis or hysterectomy, 34 (23.4%) were the current general practitioners, and 21 (14.5%) were hospital medical superintendents, pathologists, or hospital gynecology clinic staff. However, 10 of these sources could provide no data on the diagnosis and a further 10 responded that they “did not know” whether endometriosis had ever been diagnosed. We use “medical” reports as a generic term to cover questionnaire responses from physicians. In the questionnaire, physicians were asked whether there had been a surgical diagnosis of the patient’s endometriosis. The response is described as a medical report (of a surgical diagnosis), although in a few cases, the physician also voluntarily provided a copy of the surgical operative report.

Pathology reports were provided for 77 (35.8%) of the women who reported having endometriosis. Neither surgical operative reports nor pathology reports were necessary for patient classification, but they were used where available to maximize the correct assignment of the ultimate diagnosis. Medical and/or pathology information also was available for 27 twins who had had a hysterectomy but had not reported having endometriosis.

Endometriosis Self-Report

Two hundred fifteen individual twins reported having endometriosis, for an overall self-reported prevalence rate of 7.23% among the 2,973 respondents to the question.

Validity of Self-reported Data

We used information from medical, surgical, and/or pathology reports where available. There was histologic confirmation of endometriosis for 38 (49.4%) women of the 77 for whom pathologic findings were provided. In all, 66 self-reports of endometriosis (21 negative and 45 positive) were contradicted by the reports received from physicians, whereas pathology reports confirmed endometriosis in a further 6 cases where the physician either had reported negatively ($n = 3$) or had provided no information ($n = 3$). In the former case, we allowed the pathology report to override the physician’s report.

Medical and pathology reports were accepted as prima facie evidence of false-positive or false-negative twin reporting, although there were circumstances that may have resulted in the medical report received not being a true indication of a surgical diagnosis ever having been made. New variables were computed to allow for medical, surgical, or

TABLE 1

Agreement between self-reported endometriosis, medical report, and self-reported endometriosis adjusted by available medical and pathology data.

	Self-report cross-tabulated with indicated value		
	Kappa (κ)	95% CI	No. of respondents
Medical/pathology report			
Adjusted for false-negative reports only	0.52	0.42–0.61	306
Adjusted for false-positive reports only	0.94	0.92–0.96	2,973*
Adjusted for both positive and negative medical report data	0.88	0.84–0.91	2,973*
	0.82	0.78–0.86	2,973*

Note: CI = confidence interval.

*N is limited to respondents who answered the question regarding endometriosis.

pathology reports, when obtained, to override twin self-reports in specified data analyses. When other reports were not obtained, self-reported data were retained to maximize sample size and power. Self-reports therefore could be modified by medical reports, which in turn could be modified by contradictory pathology reports. Simple κ coefficients for agreement between self-reported endometriosis, medically reported endometriosis, and different combinations of data incorporating positive medical data, negative medical data, or both are shown in Table 1.

In the case of 21 women for whom histology reports at hysterectomy indicated the presence of endometriosis but the twin gave a negative self-report, possible explanations include that the endometriosis was asymptomatic and that an earlier laparoscopy had shown no evidence of disease. Other asymptomatic cases may have existed, but we were unable to detect them because validation was only actively sought for positive self-reports, and the 21 “false-negative” self-reports were picked up from medical reports sought to confirm twin-reported hysterectomy. In the case of the 45 false-positive twin reports, a number of explanations were possible, but again, the medical report was accepted at face value. See Discussion for further detail.

Age at Diagnosis

Age at the time of diagnosis of endometriosis was provided by less than half ($n = 93$, 43.3%) the women who reported having the condition. This probably was because of poor positioning of the question on the questionnaire. The mean age at the time of diagnosis reported by the twins was >4 years earlier than that reported by their physicians (Table 2). The wording of the question on the twins’ questionnaire was ambiguous, and it is likely that the twins responded with the age at which symptoms and investigations started rather than the age at which a diagnosis was made. These problems have been rectified for subsequent work.

TABLE 2

Age at diagnosis from twins' reports, medical reports, and combined data.

Type of report	Age at diagnosis (y)			
	Mean \pm SD	Range	Median	Mode
Twin's report (n = 93)	31.4 \pm 7.3	14–49	30	28
Medical report (n = 99)	35.6 \pm 7.3	18–53	35	30
Combined data* (n = 150)	34.4 \pm 7.6	18–53	34	30

* Where available, physician's data on age at diagnosis overrode twin's data.

Phenotypic Associations With Endometriosis

No statistically significant association was found between parity and either self-reported endometriosis (polychoric $r = .11 \pm .10$) or endometriosis incorporating all medical reports ($r = .08 \pm .11$). The latter translates to an odds ratio of 1.12 for endometriosis diagnosis according to (nulli)parity (nulliparous vs. parous) (95% CI 0.78–1.62). However, if the relation is clinically meaningful, assuming a nulliparous proportion in the population (as in our sample) of 0.18, detecting a relative risk associated with nulliparity of 1.12 with a chance of a type II error of 20% would require a sample of 4,349 women with endometriosis and 60,973 without the disease, given the 1:14.02 ratio of cases to noncases in our sample. We would need larger samples that included 991 and 302 cases of endometriosis to detect odds ratios of 1.2 and 1.5, respectively, under the same assumptions.

Our power to detect increased risk associated with nulliparity indicated by an odds ratio of 1.12, given the number of cases of diagnosed endometriosis (n = 198) and the number of noncases (n = 2776) in the sample (55), is only 12%, and the chance of a type II error is very high. Hence, we would need a much larger sample to detect a true clinically significant association between nulliparity and endometriosis.

Polyserial correlations between age at the time of response to the questionnaire and the respective endometriosis variables were negligible ($r = .01$ and $r = .02$). One might expect that risk would increase with age, but in these data, changing patterns of diagnosis over time may have influenced the likelihood of a diagnosis being made. No statistically significant associations were found between endometriosis (self-reported or adjusted) and highest level of education reached, occupational category using the Australian Statistical Classification of Occupations major grouping (56), or current employment status. We again note the reduced power of our study to detect true clinical associations of low magnitude.

Twins were asked to report whether their mother and/or their twin sister had had endometriosis. Of the 2,483 total question respondents, 39 (1.3%) said their mother had had endometriosis, 544 (18%) did not know, and 2,444 (80.7%) said their mother had not had endometriosis. Of the women with endometriosis (using the best available data), the proportion who reported that their mother also had had endometriosis (using the best available data), the proportion who reported that their mother also had had endometriosis increased to 9 (4.6%), with 54 (27.8%) unknown and 131 (67.5%) negative responses. There was a significantly increased likelihood of women with endometriosis themselves reporting that their mother had had endometriosis ($\chi^2_1 = 21.82, P = 0$).

Respondents who reported that their cotwin had endometriosis numbered 135 (4.5%) of all respondents (n = 2,998), whereas 303 (10.1%) said they did not know and 2,560 (85.4%) said their twin did not have endometriosis. However, women who had endometriosis themselves were significantly more likely to report having a twin sister with endometriosis than were women who did not have endometriosis ($\chi^2_1 = 144.66, P = 0$). The possibility of response bias could not be excluded. There was no statistically significant difference between the two zygosity groups for the likelihood of a twin with endometriosis reporting a mother who had had endometriosis.

Twin Pair Data

Concordance

Twin pair concordances for the range of self-reported and computed variables, including data adjusted for false-positive data only, false-negative data only, and both false-positive and false-negative data, show a clear suggestion of genetic influences operating on endometriosis in twins aged ≥ 29 years in 1993 (Table 3).

We investigated the possibility that twin pair concordance for endometriosis might be affected by the environmental similarities of twins using two indicators: contact between cotwins as adults and childhood similarity based on four recalled criteria (dressed alike, shared same room, shared same playmates, in same class at school). These were reported by these same twins in 1988–1990.

The issue of environmental similarity for MZ compared with DZ twins is important. Zygosity is significantly associated with the amount of contact between cotwins and the extent to which they share environments as children. Spearman correlation coefficients ranged from $r = .10$ ($P = .007$) for seeing each other as adults to $r = .42$ ($P = .0001$) for sharing the same playmates as children. The essential issue is whether twin pair concordance for endometriosis is related to these indicators of shared environment after controlling for zygosity. On the measures we had, we found that it was not.

TABLE 3

Twin pair concordances for self-reported endometriosis and data adjusted by different categories of medical and pathology reports.

Variable	Monozygotic pairs (841–854 pairs)	Dizygotic pairs (485–493 pairs)
	No. (%)	No. (%)
Self-report		
Concordant E+ E+	16 (1.9)	6 (1.2)
Discordant E+ E–	92 (10.9)	59 (12.2)
Concordant E– E–	733 (87.2)	420 (86.6)
Total	841 (100.0)	485 (100.0)
Data adjusted for false-negative reports only*		
Concordant E+ E+	17 (2.0)	6 (1.2)
Discordant E+ E–	105 (12.3)	62 (12.6)
Concordant E– E–	732 (85.7)	426 (86.4)
Total	854 (100.0)	494 (100.0)
Data adjusted for false-positive self-reports only†		
Concordant E+ E+	16 (1.9)	3 (0.6)
Discordant E+ E–	74 (8.6)	45 (9.1)
Concordant E– E–	764 (89.5)	445 (90.3)
Total	854 (100.0)	493 (100.0)
Data adjusted for both false-positive self-reports only ‡		
Concordant E+ E+	18 (2.1)	3 (0.6)
Discordant E+ E–	87 (10.3)	49 (9.9)
Concordant E– E–	749 (88.6)	441 (89.5)
Total	854 (100.0)	493 (100.0)

* The effect is to change false-negative self-reports of endometriosis to true-positive.

† The effect is to change false-positive self-reports of endometriosis to true-negative.

‡ Using medical data where available, adjusting false-positives and false-negatives, and including medical data where twins did not respond to the question; the effect is to change false-positive twin reports of endometriosis to negative, and false-negative twin reports of endometriosis to positive. The latter was possible only for women who reported having had a hysterectomy.

After adjusting for zygosity in analyses of covariance, we found no difference between the pairs that were concordant for endometriosis, the pairs that were discordant for endometriosis, and the concordant pairs that were negative for endometriosis on any of the four reported childhood similarity indicators or on the amount of recent face-to-face or other contact between cotwins. There was no heterogeneity of regression slopes, and we found no difference between the concordance groups in the extent to which they had shared a room ($F = 1.18, P = .31$), shared the same playmates ($F = .76, P = .47$), been dressed alike ($F = 1.16, P = .32$), been in the same class ($F = .01, P = .99$), or currently saw ($F = 1.80, P = .17$) or contacted ($F = 1.79, P = .17$) each other. This suggested that the likeli-

hood of both cotwins reporting endometriosis was not related either to these measures of childhood environmental similarity or to the adult personal contact aspect of the twins' shared environment.

Members of positive-endometriosis-concordant twin pairs were coded according to whether the same physician and hospital had treated both twins for endometriosis. For available data, only one pair of seven reported the same hospital, and one of seven also named the same general practitioner. In two pairs of seven, the same specialist was named by each twin in the pair. Six of the seven pairs were MZ and one was DZ. The six MZ pairs all named different current physicians; members of the one DZ pair went to the same physician. Although the numbers were very small, members of MZ pairs were significantly less likely than members of DZ pairs to attend the same general practitioner at the time of the survey ($P = .008$). There was no significant association between zygosity and having been treated for endometriosis by the same specialist or at the same hospital.

Age at Onset and Age at Diagnosis

The number of concordant twin pairs in which both twins reported their age at the time of diagnosis was small (MZ pairs: $n = 5$, DZ pairs: $n = 1$). To maximize numbers, the physician's report of the patient's age at the time of diagnosis was calculated from the year of diagnosis in relation to the patient's year of birth and was added to the twin's self-report. This raised the numbers to nine MZ pairs and four DZ pairs. Twin pair Pearson correlations for age at onset/diagnosis data, treated as continuous, were $r_{MZ} = .84$ and $r_{DZ} = .81$, but SEs were very high.

Correlations and Genetic Risk Ratios

Tetrachoric correlations for the MZ and DZ twin pair data considered under four different conditions (Table 4) also suggest the influence of genes on endometriosis. In this survey, medical data were not obtained for all twins, and power is unacceptably reduced if only validated cases are included in analyses (Table 4). The "best possible" data are those in which medical reports are used to redistribute "false"-positive and "false"-negative twin reports to "true" reports wherever possible. By so doing, maximum possible power based on large twin pair numbers was achieved for analyses.

These results affirmed and even strengthened the results based on self-reported data only, because MZ twin pair correlations increased and DZ twin pair correlations decreased. When only cases where twins answered the endometriosis question were included and medical validation was therefore incorporated for self-reported cases only, the ratio of MZ to DZ twin pair correlations was even further in excess of 2:1 ($r_{MZ} = .50 \pm .11, N = 841$; $r_{DZ} = .16 \pm .23, n = 485$).

The genetic risk ratios shown in Table 4 were calculated

TABLE 4

Tetrachoric monozygotic and dizygotic twin pair correlations for endometriosis.

Variable	Monozygotic pairs (n = 854)				Dizygotic pairs (n = 493)			
	r	SE	λ_M	Prevalence	r	SE	λ_D	Prevalence
Self-report*	0.46	0.09	3.58	0.072	0.28	0.13	2.32	0.073
Data adjusted for false-negative self-reports only	0.43	0.09	3.19	0.080	0.24	0.13	1.95	0.081
Data adjusted for false-positive self-reports only	0.56	0.08	5.03	0.060	0.23	0.17	2.26	0.052
Data adjusted for false-negative and false-positive reports†	0.52	0.08	4.18	0.070	0.19	0.16	1.82	0.060
Pairs where medical reports were available for both twins‡	0.34	0.24	—	—	-0.31	0.44	—	—

* Monozygotic pairs (n = 841); dizygotic pairs (n = 485).

† Both true-positives and true-negatives are maximized.

‡ Monozygotic pairs (n = 38); dizygotic pairs (n = 13).

on the basis of the prevalence of the computed variable in the individual twin sample of each zygoty group separately. On the basis of concordance figures, λ_M and λ_D genetic risk ratios to cotwins were calculated (57). On the basis of self-reporting alone and the prevalence of self-reported endometriosis in the whole sample of individual twins, the risk ratio of affected vs. population prevalence as a result of genetic influence was 3.58 for MZ cotwins (λ_M) and 2.32 for DZ cotwins (λ_D) (Table 4). The ratio of these risks ($(\lambda_M - 1)/(\lambda_D - 1)$) (i.e., $[3.58 - 1]/[2.32 - 1]$) of 1.95 is compatible with additive genetic contributions to the risk of endometriosis.

Exploration of sibships in a preliminary pilot sample of 71 of these twin pair families gave a genetic risk ratio for siblings (λ_S) of 2.34 for self-reported endometriosis. Genetic risk ratios for endometriosis when full medical and pathologic information was incorporated were even higher for MZ pairs than for DZ pairs, supporting the possibility of additive genetic influences and even suggesting the possibility of nonadditive genetic influences. The ratio of λ_M to λ_D risks ($(\lambda_M - 1)/(\lambda_D - 1)$) for the best available data was 2.36, also suggesting additive genetic influences. When data adjusted only for false-positive reports were considered (and there is an argument that negative pathology reports may not have related to a period when endometriosis was active), the ratio of genetic risks increased to 2.77.

Genetic Model-Fitting

The "best available" data for endometriosis, using all medical reports to maximize numbers of true-positives and true-negatives in the sample, are explained best by a parsimonious model containing only additive genetic and individual environmental influences (AE) (Table 5). The lowest Akaike Information Criterion estimate suggests the best-fitting, most parsimonious model. However, the fact that model-fit does not worsen significantly when genetic influences are dropped from the model (likelihood ratio $\chi^2_1 = 3.09$), means that we cannot exclude a model that contains

only shared environment (C) and specific environmental influences (E).

Although there is some suggestion of nonadditivity in the ADE model estimate of d^2 , the 95% confidence limits suggest that it ought not to be included, and the fit is not significantly worsened by dropping it from the model. Power calculations indicate that we would require a larger sample of 3,233 twin pairs to be 80% certain of detecting an additive genetic effect accounting for 51% of the total phenotypic variation if shared environmental influences account for none of the variance.

DISCUSSION

A ratio of 2:1 between MZ and DZ twin pair correlations suggests the additive influence of genes and a negligible influence of shared environmental factors (53). A ratio in excess of 2:1 suggests the possibility of genetic nonadditivity, although the power to detect nonadditivity in the classic twin study is low, even when large amounts are estimated (43). Our twin pair correlation ratios and the Akaike Information Criterion, which was lowest for the AE model, suggest additive genetic influences on endometriosis. We note that the likelihood ratio test did not distinguish between models that contained shared environment and additive genetic influences, but we have been guided by the Akaike Information Criteria and correlation ratios in drawing our conclusions.

The implications of our findings depend on the extent to which the twin sample is representative of the general female population. The female twins have been shown to be representative of the Australian population on a variety of indicators, including age, general level of education, and marital status (58). There is limited ethnic diversity in the volunteer twin sample. The twins volunteered to participate in medical research in general and were unselected for endometriosis or any other characteristic.

Diagnostic difficulties introduce substantial problems for

TABLE 5

Univariate genetic model-fitting (asymptotic least squares) to tetrachoric correlations and asymptotic covariance matrices for endometriosis incorporating all available medical and pathology report data.

Model	Parameter estimates (squared)			χ^2	df	AIC
	a^2	c^2/d^2	e^2			
ACE	0.51	0.00	0.49	0.178	0	0.178
CE	—	0.45	0.55	3.270	1	1.270
AE	0.51*	—	0.49*	0.178	1	-1.822
ADE	0.23	0.28†	0.48	0.000	0	0.000
E	—	—	1.00	42.303	2	38.303

Note: ACE = estimating additive genetic, shared environmental, and individual environmental influences; ADE = estimating additive and nonadditive genetic, and individual environmental influences; AE = estimating additive genetic and individual environmental influences only; AIC = Akaike Information Criterion; CE = estimating shared and individual environmental influences only; E = estimating individual environmental influences only.

* The 95% confidence interval for the additive genetic estimate (a^2) is 0.36–0.66 and that for the specific environmental influence (e^2) is 0.33–0.65.

† The 95% confidence interval for the nonadditive genetic estimate (d^2) is 0–0.68.

epidemiologic research: [1] women can have histologic evidence of endometriosis without clinical or laparoscopic evidence of the disorder; [2] endometriosis can be asymptomatic (59), and the presence of endometrial tissue in the pelvic cavity of asymptomatic women does not necessarily constitute a pathologic condition (60), [3] clinicians may not be consistent in their definition or clinical diagnosis of endometriosis (visually on laparoscopy) or in their investigations of infertility, recurrent pain, or menorrhagia, which might lead to a diagnosis of endometriosis; [4] histologic evidence can result in a false-negative diagnosis depending on the site from which tissue is removed for biopsy, and [5] when histologic evidence is used as the “gold standard,” bias may be introduced by virtue of the indications for obtaining the tissue specimen.

Validation of self-reported data required evaluation in the light of these five factors. Validation depended on which physician responded, which pathology results were made available, and disproportionately on whether a hysterectomy had been performed. A number of negative reports were included as prima facie evidence of the absence of endometriosis, even though they were provided by physicians who might not reasonably be expected to know the patient's complete history. Moreover, identification of false-positive and false-negative reporting was not possible for all twins whose data were included in the analyses. This problem has been rectified for our subsequent and current genetic study of endometriosis.

In the case of false-positive reports, it was possible that the physician or pathology service from whom the report was obtained was not the best source of data regarding the history of the endometriosis diagnosis. Possible reasons for this may be that the physician who responded may not have been the one who performed the laparoscopy; he or she may have been a current general practitioner if no response could be obtained from the specialist or hospital named by the twin. In addition, endometriosis may have regressed spontaneously or, more probably, in response to treatment by a previous physician and may not have been seen subsequently.

No national data from health insurance or linked records relating to diagnoses were available in Australia at the time of the study, and transfer or sharing of medical records between medical practitioners or services has been discretionary rather than mandatory. Because twin reporting was retrospective, the time since diagnosis was substantial in many cases and many physicians had died or changed practices and many hospitals had been closed or records had been destroyed. We went to considerable lengths to obtain records, but obtaining specialist records was impossible in some cases and current general practitioners were contacted for information.

The results suggested a much smaller number of false-positive self-reports ($n = 3$) when data were provided by the specialists named by the twins as having treated them for endometriosis. Nevertheless, the inferences required to make distinctions between the reports of different medical practitioners were impossible to draw accurately and acceptably, so all medical reports were treated as correct.

Over and above the difficulties involved in obtaining the most pertinent medical reports, potential diagnostic problems are relevant to any research on endometriosis. They include variations in diagnostic procedures and practices between practitioners and (possibly asymptomatic) histologic endometriosis found inadvertently at surgery. Our understanding of the morphology of endometrial implants has advanced in the past decade. The timing of the clinical diagnosis of endometriosis in our sample extended past 1986, when multiple morphologic types first were described and nonpigmented lesions were recognized as being commonly endometriotic (61). Diagnostic practices may not have changed uniformly; hence, some confounding of our diagnostic data is possible. In this study, we did not ask physicians for their staging of endometriosis as defined by the American Society for Reproductive Medicine criteria. This is in place for current work.

Genetic risk may be due to a single major gene or more probably to a number of genes acting multiplicatively on the risk scale. The sibling genetic relative risk of 2.34 and the MZ/DZ ratio of approximately 2 are similar to those of other common diseases, including asthma and breast cancer, both of which have proved amenable to gene searching. Recent

research has suggested a role for several factors, including uterotubal factors that affect retrograde menstruation, myometrial dysfunction, mechanisms underlying cellular invasion, steroids, growth factors, cytokines, enzymes, enzyme inhibitors, and other effector molecules in the pelvic cavity (1), which are under potential genetic influence.

Evidence of genetic influence on endometriosis does not diminish the importance of environmental influences on its onset or pathologic progression—it makes them even more important in terms of prevention. Nevertheless, our findings, together with evidence from other studies, support the hypothesis that genes influence liability to endometriosis, leading us into a current linkage and association study of endometriosis as part of the Australian Cooperative Research Centre for Discovery of Genes for Common Human Diseases.

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