Familial aggregation of endometriosis in a large pedigree of rhesus macaques

Krina T.Zondervan1,6, Daniel E.Weeks2, Ricki Colman3, Lon R.Cardon1, Ruth Hadfield4, Joan Schleffler3, Amanda Goudy Trainor3, Christopher L.Coe5, Joseph W.Kemnitz3 and Stephen H.Kennedy4

1Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK, 2Department of Human Genetics and Biostatistics, University of Pittsburgh, Pittsburgh, PA, 3Wisconsin National Primate Research Center and 5Harlow Primate Laboratory, University of Wisconsin-Madison, Madison, WI, USA and 4Nuffield Department of Obstetrics and Gynaecology, University of Oxford, Oxford, UK

6To whom correspondence should be addressed. E-mail: krina.zondervan@well.ox.ac.uk

BACKGROUND: Endometriosis occurs in several non-human primate species that have menstrual cycles. This study investigated the prevalence and familial aggregation of endometriosis in one of those species, the rhesus macaque. METHODS: Between 1978 and 2001, 142 animals with endometriosis were identified from necropsy and surgical records and through the use of magnetic resonance imaging (MRI) at the Wisconsin National Primate Research Center, Madison, USA. All cases were used to build one large multigenerational pedigree and nine nuclear families comprising 1602 females in total. By 2002, the pedigrees contained 124 cases diagnosed at necropsy; 17 at surgery and three at MRI. Female animals that had died aged ≥10 years without endometriosis, had both ovaries until at least 1 year prior to death, and had a full necropsy, were considered unaffected. RESULTS: The prevalence of endometriosis among necropsied animals aged ≥10 years in the colony was 31.4% [95% confidence interval (CI) 26.9–35.9%]; prevalence increased with rising age and calendar age at death. Familial aggregation of endometriosis was strongly suggested by a significantly higher average kinship coefficient among affecteds compared with unaffecteds (P < 0.001) and a higher recurrence risk for full sibs (0.75; 95% CI 0.45–1.0) compared with maternal half sibs (0.26; 95% CI 0.10–0.41) and paternal half sibs (0.18; 95% CI 0.02–0.34). The segregation ratio among affected mothers (44.2%) was not significantly higher compared with unaffected mothers (36.6%). CONCLUSIONS: The results support familial aggregation of endometriosis in the rhesus macaque, and indicate that this is a promising animal model for the investigation of mode of inheritance, the location of potential genetic susceptibility loci and the influence of environmental factors.

Key words: animal model/endometriosis/genetic epidemiology/prevalence/rhesus macaque

Introduction
Endometriosis is a common disease among women (Eskenazi and Warner, 1997), although its exact prevalence in the general population is difficult to estimate because of the need for surgical assessment to establish the diagnosis. While its aetiology is uncertain, there is increasing evidence in humans to suggest that the disease has a genetic basis (Zondervan et al., 2001). The involvement of genetic factors has been implicated by: (i) a large twin study, in which 51% of the variance of susceptibility to endometriosis was attributed to genetic factors (Treloar et al., 1999); (ii) four case–control studies showing that the first-degree relatives of affected women were at three to nine times increased risk for developing the disease compared with first-degree relatives of controls (Simpson et al., 1980; Lamb et al., 1986; Coxhead and Thomas, 1993; Moen and Magnus, 1993); and (iii) a study of 750 women with endometriosis from the entire Icelandic population who were found to be significantly more related to each other than matched control groups (Stefansson et al., 2002). Environmental factors believed to be involved in the aetiology of endometriosis include increased exposure to prolonged and heavy menstruation, and oestrogen (Eskenazi and Warner, 1997), but involvement of other factors remains unclear (Zondervan et al., 2002).

Endometriosis is regarded as a complex trait, in which multiple gene loci conferring susceptibility interact with each other and environmental factors to produce the phenotype. Studying the disease in women is complicated for many reasons. The need for surgical diagnosis means that patient groups are often highly selected relative to the general population in terms of the environmental, and possibly genetic, background. This necessitates careful selection of control
groups so as not to cause spurious environmental and genetic associations, a point that not much attention has been paid to in studies to date (Zondervan et al., 2002). In addition, the influence of environmental exposures is difficult to assess as they should have occurred prior to disease onset, which is likely to have preceded diagnosis by many years (Hadfield et al., 1996). An animal model such as the rhesus monkey (Macaca mulatta) could be a valuable tool to investigate the genetic epidemiology of endometriosis. Colonies of captive rhesus macaques have smaller gene pools and greater genetic homogeneity, and thus may possess unique or high-frequency risk alleles for endometriosis. Moreover, they live in a controlled and monitored environment, which allows a more accurate retrospective analysis of environmental exposures that may have occurred during the animals’ life span than is possible in humans (although, as in humans, the age at onset of endometriosis is unknown in the rhesus macaque).

Endometriosis develops spontaneously in the rhesus macaque and the endometrial implants are morphologically identical to its human counterpart. Clinical manifestations include altered behaviour likely related to discomfort, abdominal distension due to a pelvic mass, and occasionally cachexia because of full thickness bowel involvement (MacKenzie and Casey, 1975). The rhesus macaque shares many aspects of its anatomy and physiology with humans, in particular reproductive physiology. Menarche in the rhesus monkey occurs at ~10 years of age, the length of the menstrual cycle is ~28 days, menstrual bleeding lasts for ~4 days and ovarian function diminishes from the age of ~25 years onwards. The rhesus macaque is a seasonal breeder in nature (Catchpole and van Wagenen, 1975), but this seasonality disappears when housed indoors on a constant light/dark and temperature/humidity regimen. The average life span is 25–29 years but some animals live to 40 years of age (Colman and Kemnitz, 1998).

We previously reported on the high prevalence of spontaneous endometriosis in the rhesus macaque colony of the Wisconsin National Primate Research Center (WNPRC) at the University of Wisconsin-Madison (Hadfield et al., 1997). Briefly, necropsy records from 399 female rhesus monkeys that died ≥4 years of age (the age at which sexual maturity is reached) between 1981 and 1995 were examined for evidence of endometriosis. Eighty-one of the animals (20%) were found to have disease at necropsy. All diseased animals were aged ≥10 years; prevalence among animals that had reached at least the age of 10 years was 29%. Comparison between 62 cases and 62 controls matched on age and year of death showed that cases were more likely to have been exposed to estradiol (E2) and hysterotomy. Moreover, animals in the case group had a higher sum of kinship coefficients (4.375) than did controls (1.6875), suggesting a higher degree of relatedness. In the present paper, we investigate this familial aggregation further, using pedigree information and incorporating colony information updated since 1995.

### Methods

#### Colony description

The WNPRC and the nearby Harlow Primate Laboratory at Madison, WI, USA, have maintained research colonies of rhesus monkeys for over four decades. Most animals in the Center and Laboratory live indoors throughout life in varied housing conditions ranging from simple caging to social groups of 10 or more. Animal room temperature is maintained at ~21°C and 50–60% humidity, with a 12:12 or 16:8 h light/dark schedule. The WNPRC is fully accredited by the American Association for the Accreditation of Laboratory Animal Care.

Until 1978, WNPRC and the Harlow Primate Laboratory used one, paper-based, recording system for the animals. Since then, records have been maintained separately. The Harlow Primate Laboratory has continued to use written records in which information on necropsies, surgical and experimental procedures, and familial relationships of animals living in their colony is recorded. From 1981, WNPRC has held computerized records (back-dated to 1978) of the clinical, experimental and environmental histories of all animals for their time of residence in the colony, as well as their familial relationships. Since animals were often moved between WNPRC and the Harlow Primate Laboratory for breeding purposes, the computerized database contains animals that may have lived at either institute. In the remainder of the paper, we will refer to this group of ≥9000 animals for which data are registered on the database as ‘the colony’. After death, all animals undergo necropsy, the records of which are also held on the computerized database. Parentage is known for ~90% of animals because of arranged mating and post-birth DNA checks. Inbreeding is avoided by checking the kinship between animals prior to every mating.

#### Identification of animals with endometriosis in the colony (1978 to September 2001)

Unequivocal information on the presence or absence of endometriosis could only be reliably obtained retrospectively through necropsy records. Surgical records were also searched, but rarely contained information about the presence of endometriosis (see Table I), because the pelvis was not routinely inspected for the condition (although it is fair to assume that severe disease, if present, would have been commented on). Most operations were performed for experimental purposes linked to reproductive medicine/fetal projects.

In the 1995 study, only computerized necropsy records of all female rhesus macaques that had died at WNPRC aged 4 years or over (the age at which the animal reaches sexual maturity) between 1981 and 1995 had been audited (Hadfield et al., 1997). For the present study, the search was extended to records of all females in the colony that had died aged 4 years or over, or had undergone pelvic surgery, from 1978 up to September 2001. In addition, a very small number of cases were identified in a sub-study investigating the use of magnetic resonance imaging (MRI) in rhesus macaques that had died aged ≥9 years between 1995 and 1997. Sensitivity analyses showed that all animals with radiographic evidence of pelvic lesions ≥3 cm at MRI were...
subsequently confirmed to have endometriosis at surgery or necropsy (results not shown, but available on request). Thus, females in the colony were classified as endometriosis cases if they had macroscopic evidence of the disease at necropsy and/or surgery \((n = 139)\), or had one or more lesions \(>3\,\text{cm}\) observed at MRI \((n = 3)\). Histological confirmation was obtained in 57% of the cases. Table I shows the breakdown of the 142 cases identified in the whole colony between 1978 and September 2001.

Stage of disease was determined as minimal-mild or moderate-severe according to the rAFS classification system (The American Fertility Society, 1985) using the description contained in the necropsy or surgery records. This staging system has similarly been used in other endometriosis studies of non-human primates (Schenken et al., 1987; D’Hooghe et al., 1992), and its use presented little difficulty because animals tended to have either a few isolated foci or a severely affected pelvis.

**Definition of unaffected animals**

All affected animals identified in the colony were aged 10 years or older at diagnosis or death. Other female animals in the colony database were only considered unaffected if: (i) they had died at WNPCR or the Harlow Primate Laboratory aged 10 years or older; (ii) they had not had a bilateral ovariectomy \(>1\) year prior to death; and (iii) a full necropsy report was available providing evidence of absence of endometriosis. The criterion of having at least one intact ovary was applied because ectopic endometriotic tissue is not viable in ovariectomized monkeys (DiZerega et al., 1980). Note that lack of endometriosis reported at pelvic surgery did not constitute sufficient evidence for a female to be considered unaffected. Thus, 284 females were diagnosed as unaffected at death between 1978 and September 2001.

**Construction of pedigrees (followed up from September 2001 to present)**

To investigate the degree of familial clustering of endometriosis, pedigrees were constructed using all 142 cases identified in the whole colony up to September 2001. All founding ancestors of the cases were identified from the colony database, and all female descendants and any inter-related males were included. Since September 2001, only living females which are part of the pedigrees are being followed up to determine disease status at necropsy or surgery. The last update of disease information in the pedigrees, on which the recurrence risk (RR) analyses in the present paper were based, was in June 2002.

**Statistical analyses**

Differences in age at diagnosis or death between affected and unaffected animals were assessed using a \(t\)-test. Prevalence rates of endometriosis in the colony could only be calculated among females in which presence or absence of endometriosis was unequivocal, i.e., those that had died aged \(\geq 10\) years up to September 2001, had not had a bilateral ovariectomy more than 1 year prior to death and for which a full necropsy report existed \((n = 414)\). Significance of the observed rise in prevalence rates with age (in categories) and with calendar year (in categories) was investigated using a \(\chi^2\)-test for trend. A multivariate logistic regression model was used to assess the effect of calendar year of diagnosis adjusted for age at diagnosis.

Kinship among all 130 affected in the colony diagnosed at necropsy up to September 2001 was compared with that among a random sample of 130 unaffected that were frequency-matched to affecteds on age [categories (years): 10–15 and 16+] and calendar year of death (categories: up to 1980, 1981–1985, 1986–2001). Matching was employed because age and year of death affected both risk of disease and likelihood of having offspring. Using genealogical data from the entire WNPRC colony, the program Kinmean (S.A.Sholl) was used to calculate kinship coefficients for each unique pair within the groups of affected and unaffected animals (Boyce, 1983). For example, a kinship coefficient of 0.25 indicated a full sib or parent–child relationship and 0.125 a half sib or aunt–niece pair. Differences in the distribution of these values between groups of affected and unaffecteds were compared using the non-parametric Mann–Whitney \(U\)-test. Crude segregation ratios (the proportion of affected daughters among all daughters with known disease status) in the colony were compared between affected and unaffected mothers using a \(\chi^2\)-test.

Using pedigree information up to June 2002, RRs (the probability of an affected female’s relative being affected) were calculated for full sibs and half sibs using the method described by Olson and Cordell (2000). This method calculates RR among sibling pairs adjusting for sibship size when proband status is unknown:

\[
RR = \frac{\sum_{s=1}^{\infty} \sum_{a=1}^{\infty} W_s(a-1)n_{(a)}}{\sum_{s=1}^{\infty} \sum_{a=1}^{\infty} W_s(s-1)n_{(a)}}
\]

where \(a = \text{number of affecteds in sibship; } W_s = \text{weight given to the sibship (in our situation of complete ascertainment of cases } W_a = a); s = \text{sibship size; and } n_{(a)} = \text{the number of sibships of size } s \text{ with } a \text{ affecteds. Recurrence risks for mother–daughter and grandmother–granddaughter pairs were calculated as the proportion of affected (grand)daughters among all (grand)daughters of known disease status given that (grand)mothers were affected. Differences between RRs were assessed using Fisher’s exact test.

**Results**

**Prevalence of endometriosis in the colony**

Between 1978 and September 2001, 142 affected and 284 unaffected animals were identified in the whole colony. Of the affecteds, 122 were first diagnosed at necropsy, 17 at surgery (eight of which had diagnosis confirmed at necropsy) and three at MRI (Table I). Disease stage was known for 126 of the 142 affecteds: 101 (80%) had moderate-severe disease and 25 (20%) had minimal-mild disease. Unaffected animals were on average younger at death (17.9 years; SD 6.1) than affected animals were at diagnosis (20.7 years; SD 5.8) \((P < 0.001)\). Among the affecteds, mean age at diagnosis was 18.4 years (SD 4.1) for the 17 animals first diagnosed by surgery, 20.9 years (SD 6.0) for the 107 animals first diagnosed at necropsy and 23.3 years (SD 2.5) for the three animals only diagnosed at MRI \((P = 0.2)\).

The prevalence of endometriosis among all females in the colony that died aged \(\geq 10\) years up to September 2001, without bilateral ovariectomy and with a full necropsy, was 130/414 = 31.4% [95% confidence interval (CI) 26.9–35.9%]. Prevalence of moderate-severe endometriosis only was 98/407 = 24.1% [95% CI 19.9–28.2%]. Prevalence (all stages) increased significantly (Figure 1A) from \(\sim 10\%\) among those that died aged 10–12 years to \(\sim 40\%\) among those aged 15 years or older at death \((\chi^2_{\text{trend}} = 23.0; P < 0.001)\). A similar pattern with age was observed for moderate-severe disease only. Total pedigree prevalence rates also differed significantly with calendar time (Figure 1B) from 7% pre-1981 to 30–40% in 1986–2001 \((\chi^2 = 12.1; P = 0.02)\), again with a similar pattern for moderate-severe disease only. The prevalence among the
females diagnosed since 1995 was 49/135 (36.3%; 95% CI 28.2–44.4%), which was not significantly different ($P = 0.1$) from the prevalence reported previously for the pre-1995 data (Hadfield et al., 1997) (29.0%; 95% CI 23.7–34.4%). Mean age at death was lowest (17.7 years; SD 5.0) in animals that died between 1981 and 1985. The difference in diagnostic rates over the years became non-significant after adjustment for differences in age at death ($P = 0.07$).

**Familial aggregation of endometriosis in the colony**

The distribution of kinship coefficients among all 130 affected females diagnosed at death in the colony was significantly different from that among the randomly selected group of 130 unaffecteds ($Z = -5.7; P < 0.001$), with mean values of $2.28 \times 10^{-3}$ among affecteds and $1.06 \times 10^{-3}$ among unaffecteds. The total sums of kinship coefficients were 18.77 and 8.73, respectively.

---

**Figure 1.** Prevalence rates of endometriosis in females that died aged ≥10 years in the colony, by disease stage, (A) age at death and (B) year of death in 5-year bands (errors bars indicate 95% CIs).
Segregation ratios were calculated for all affected and unaffected mothers in the colony up to September 2001 (Table II). The segregation ratio for affected mothers (44.2%) was not significantly higher than for unaffected mothers (36.6%; $P = 0.4$); this translated into an odds ratio (OR) of endometriosis of 1.4 (95% CI 0.6–2.1) for daughters with affected compared with those with unaffected mothers. When the disease definition was limited to moderate-severe only (not shown), the segregation ratios were 38.7 and 29.7%, respectively ($P = 0.3$), corresponding to an OR of 1.5 (95% CI 0.6–3.5). Mothers were more likely than daughters to have died in the years up to 1985 when observed prevalence rates in the colony were reduced. Of the 101 unaffected mothers contributing to the segregation ratios, 30 (29.7%) had died pre-1986; of the 88 unaffected daughters, four (4.5%) had died pre-1986.

### Pedigree analyses

In total, 133 of the 142 animals identified with endometriosis in the whole colony up to September 2001 were part of one large, multigenerational, pedigree consisting of 1831 animals. The remaining nine animals were sporadic cases (not related to any other diseased animal), and were part of very small pedigrees varying in size between one and four animals. The 10 pedigrees together contained 549 founders (both parents unknown), 146 animals with a known mother only, and 1154 with a known mother and father. There were 1602 females in the pedigrees. At the last disease update (June 2002), they comprised 144 affected (two more females had been diagnosed since September 2001) and 235 unaffected. A breakdown of disease status for all females in the pedigrees is given in Table III.

Using the pedigree information, RRs were calculated for female relative pairs with different kinship coefficients (Table IV). There were only three concordant and two discordant affected full sib pairs (kinship coefficient 0.25) in the pedigrees, giving an RR of 0.75 (95% CI 0.45–1.0) for full sibs. The RR for maternal half sib pairs (kinship coefficient 0.125) was significantly lower (0.26; 95% CI 0.10–0.41) than that of full sibs ($P = 0.01$). In contrast, the RR for paternal half sib pairs was not significantly lower than that of full sibs (0.50; 95% CI 0.45–0.56; $P = 0.3$), and was significantly higher than that for maternal half sib pairs ($P = 0.02$). The RRs for mother–daughter (kinship coefficient 0.25) and grandmother–granddaughter (kinship coefficient 0.0625) pairs were 0.43 (95% CI 0.41–0.46) and 0.17 (0.04–0.29), respectively. The prevalence of different disease stages among affecteds constituting all types of relative pairs reflected the general pattern seen among all cases in the colony, with a predominance of moderate-severe disease. Among affecteds constituting same-generation relative pairs, moderate-severe disease was found in 87.5% of full sibs, 86.2% of paternal half sibs and 91.0% of maternal half sibs.

The 51 maternal half sib pairs were derived from 24 maternal half sibships, comprising two or three daughters per mother. The 126 paternal half sibships were derived from only 28 different fathers, with two to 12 daughters of known disease status per father. Paternal halfsib RR was mainly driven by fathers with a high number of offspring. When analyses were stratified between the 14 fathers with two or three daughters and the 14 fathers with four to 12 daughters, the estimated RRs were 0.18 (95% CI 0.02–0.34) and 0.53 (95%: 0.47–0.58), respectively. The RR among half sibs from fathers with two or three daughters was significantly lower than that among full sibs ($P = 0.004$) and no longer significantly different from that among maternal half sibs.

### Discussion

The Oxford Group is continuing to investigate the genetic epidemiology of endometriosis in humans using linkage and association approaches (Kennedy et al., 2001). Significant linkage has been reported in a collaborative genome-wide screen (Treloar and Kennedy, 2002), and fine-mapping studies are currently being pursued. Studies of endometriosis in humans, however, are subject to many methodological difficulties, especially when environmental factors are being considered. Non-human primates can provide animal models that are highly suited to the study of complex diseases seen in humans (VandeBerg and Williams-Blangero, 1997). In par-

---

**Table II.** Comparison of segregation ratios (SR) between affected and unaffected mothers and those of unknown disease status in the colony

<table>
<thead>
<tr>
<th>Disease status daughter(s)</th>
<th>Disease status mother</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unknown</td>
</tr>
<tr>
<td>Affected</td>
<td>196</td>
</tr>
<tr>
<td>Total</td>
<td>282</td>
</tr>
<tr>
<td>SR (%)</td>
<td>30.5</td>
</tr>
</tbody>
</table>

*aSR = proportion affected daughters/(unaffected + affected daughters); comparing SR(affected mothers) with SR(unaffected mothers): $\chi^2 = 0.72, P = 0.4$.

**Table III.** Disease and follow-up status of all 1602 female animals in the pedigree (June 2002)

<table>
<thead>
<tr>
<th>Disease status</th>
<th>Number of animals</th>
<th>Follow-up status</th>
</tr>
</thead>
<tbody>
<tr>
<td>Affected</td>
<td>124</td>
<td>Ended</td>
</tr>
<tr>
<td>Diagnosed at necropsy</td>
<td>17</td>
<td>Ended</td>
</tr>
<tr>
<td>DIagnosed through MRI only (alive)</td>
<td>3</td>
<td>Continued</td>
</tr>
<tr>
<td>Unaffected</td>
<td>235</td>
<td>Ended</td>
</tr>
</tbody>
</table>

*Of the 17 animals first diagnosed by surgery, eight subsequently had a positive necropsy (follow-up ended), two animals left WNPRC (follow-up ended) and seven are still alive (in follow-up).

* died aged <10 years; bilateral ovariectomy >1 year prior

* Died at unknown age

* Died; incomplete necropsy report

* Left WNPRC/Harlow Primate Laboratory

* Records not found

* Alive at WNPRC/Harlow Primate Laboratory

Total: 1602
The widespread and natural occurrence of endometriosis in the rhesus macaque makes it a unique model to study for assessing the genetic epidemiology of the disorder.

Whilst there is considerable evidence that familial clustering of endometriosis occurs in humans (Zondervan et al., 2001), the present study is the first to show detailed evidence of such clustering in the rhesus macaque using pedigree information. First, the average kinship coefficient among the group of affected animals was significantly higher than among a group of randomly selected unaffecteds in the colony. Secondly, the RRs among various relative pairs of different kinship in the pedigrees generally showed a reduced risk among more distantly related animals. Thirdly, the segregation ratio among affected mothers was higher than among unaffected mothers, although this finding was not significant. It should be noted that most (80%) of the endometriosis found in this colony of rhesus macaques constituted moderate or severe disease, and that this phenotype therefore formed the main basis for our results. Separate analysis of familial aggregation limited to animals with moderate-severe disease did not materially alter the results. Unfortunately, the limited number of animals diagnosed with mild disease did not allow a separate analysis of this phenotype.

The interpretation of RR estimates in this study is complicated by potential confounding factors. First, confounding by diagnostic time-period may have occurred for the calculation of RR among relative pairs spanning different generations, such as mother–daughter or grandmother–granddaughter pairs. Analyses showed that (grand)mothers were more likely to have died prior to 1985 than their (grand)daughters, a period in which prevalence of endometriosis was lower mainly because of a lower average age at death (although the possibility of greater misclassification of endometriosis cases as unaffecteds in earlier years cannot be excluded). RRs for mother–daughter pairs (0.44; kinship coefficient 0.25) and grandmother–granddaughter pairs (0.17; kinship coefficient 0.0625) are therefore likely to be underestimates.

RRs among siblings are less likely to have been influenced by generational confounding. Moreover, any misclassification of endometriosis cases as unaffecteds within relative pairs of the same generation, if present, would have resulted in a general reduction of absolute RR values, but would not have affected the validity of comparative analyses. Although based on small numbers, we found an RR for full sibs (kinship coefficient 0.25) of 0.75, compared with RRs for paternal and maternal half sibs (kinship coefficient 0.125) of 0.50 and 0.25, respectively. Paternal half sib RR was based on much larger sibship sizes (two to 12 daughters per father) than maternal half sib risk (two to three daughters per mother). Results showed that paternal half sib risk was mainly driven by high RR among daughters from fathers with a great number of offspring. When we limited the analyses to fathers with up to three daughters, the RR decreased to 0.18, a value similar to that found for maternal half sibs. This implied that paternal half sib risk was overestimated because of the higher risk inferred by a few fathers with a large number of daughters. This could be explained by a scenario in which a few of the fathers carried a high-risk susceptibility gene, which, because they happened to produce a large number of offspring, had a large effect on RR. Interestingly, the father inferring the highest RR among paternal half sibs (an RR of 0.83 based on seven daughters, of which six were affected), also fathered one of the concordant full sib pairs. We were unable to check whether any of the fathers inferring a half sib RR >0.5 were related to each other, as they all had unknown parents in our database.

These observations highlight a further issue complicating RR interpretation in this study, namely that all relative pairs and sibships in the analyses were derived from one interconnected pedigree rather than from independent pedigrees. However, this could only have affected RR estimates if there was true genetic transmission of endometriosis in the pedigree. Suppose a susceptibility gene had been transmitted from a single founder animal to certain animals in the pedigree. All relative pairs that descended from the founder would on average display a higher RR than non-descendants. Within one pedigree, the proportion of relative pairs that happened to descend from the high-risk founder may be different for different types of relative pairs, and will influence the RR estimates. It is unlikely, however, that this bias would completely eradicate any correlation between kinship and RR. If there was no genetic transmission, we would expect RRs among the various relative pairs in the pedigree to be uncorrelated with level of kinship, provided kinship was not correlated with exposure to potential environmental risk factors. The latter assumption holds in our data, as animals are housed individually and level of kinship between animals has never been grounds for exposure to certain environmental risk factors. The general trend of higher RRs among more closely related animals in our data thus supports genetic transmission of endometriosis.

The segregation ratio among affected mothers was at 44.2% only modestly higher than that among unaffecteds (36.6%). This could simply be a reflection of the multifactorial origin of endometriosis. In complex traits, the fact that segregation ratios do not differ between affecteds and unaffecteds does not necessarily imply a lack of genetic influence, merely a lack of a major gene effect. However, it is possible that generational confounding mentioned previously caused differential misclassification of affected mothers as unaffected, which would have reduced the difference in segregation ratios.

### Table IV. RRs among various types of female relative pairs within the pedigree

<table>
<thead>
<tr>
<th>Relative pair (kinship coefficient)</th>
<th>Total number of females constituting relative pairs</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Full sibs (0.250)</td>
<td>5</td>
<td>0.75 (0.45–1.0)</td>
</tr>
<tr>
<td>Mother–daughter (0.250)</td>
<td>39 + 46a</td>
<td>0.43 (0.41–0.56)</td>
</tr>
<tr>
<td>Half sibs (0.125)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal</td>
<td>126</td>
<td>0.50 (0.45–0.56)</td>
</tr>
<tr>
<td>Maternal</td>
<td>51</td>
<td>0.26 (0.10–0.41)</td>
</tr>
<tr>
<td>Grandmother–granddaughter (0.0625)</td>
<td>7 + 7b</td>
<td>0.17 (0.04–0.29)</td>
</tr>
</tbody>
</table>

* Thirty-nine affected mothers with a total of 46 daughters.

aSeven affected grandmothers with a total of seven granddaughters.
The prevalence of endometriosis found among all female animals in the colony that died aged ≥10 years was high, at 31.4% (95% CI 26.9–35.9%). Prevalence increased from the age of 10 years to a peak of ~40% in animals aged 15 years or older at death. The analyses of prevalence by calendar year of diagnosis showed a deficit of diagnosed endometriosis cases in the years up to 1985, which was to a limited extent explained by differences in mean age at death for monkeys dying in those years. It is possible that these differences may also partly be accounted for by different frequencies of experimental procedures predisposing to endometriosis, such as hysterotomy and administration of E2. Another possibility, however, is a greater degree of misclassification of endometriosis cases as unaffecteds in earlier years, followed by an increased awareness and improved reporting of the condition in subsequent years. This is supported by an increased frequency of animals diagnosed with minimal-mild disease in later years. Similar patterns of increased prevalence of endometriosis over time (or rather, underestimated prevalence in earlier years) due to diagnostic bias has been observed in humans (Koninckx, 1998).

We were unable to investigate the effect of (peri)menopause on the results, since we had no menstrual data on the animals. If menopausal onset is most likely to occur after the age of 25 years, then a higher probability of misclassification could have been present in the 30 animals (10%) in this age group out of the 284 unaffecteds in our analyses. Considering that such differential misclassification would be much more likely for minimal-mild disease, which has a much lower prevalence, the impact of this potential bias is likely to be small. Moreover, any such bias would have resulted in an underestimation of the prevalence in the colony, and also of the familial aggregation indicators.

We were also unable to assess exposure to environmental factors in the present study, as exposure information between 1995 and 2002 was not yet available. Using data up to 1995, we previously reported an increased risk of endometriosis with increased exposure to hysterotomy and E2; no association was found with parity or number of laparoscopies (Hadfeld et al., 1996) and dioxin (Rier et al., 1993). Radiation-induced endometriosis in Macaca mulatta (Fanton and Golden, 1991) is unlikely to have contributed to disease prevalence in the colony, and also of the familial aggregation indicators.

We were also unable to assess exposure to environmental factors in the present study, as exposure information between 1995 and 2002 was not yet available. Using data up to 1995, we previously reported an increased risk of endometriosis with increased exposure to hysterotomy and E2; no association was found with parity or number of laparoscopies (Hadfeld et al., 1996) and dioxin (Rier et al., 1993). Radiation-induced endometriosis in Macaca mulatta (Fanton and Golden, 1991) is unlikely to have contributed to disease prevalence in the colony, and also of the familial aggregation indicators.

We were unable to investigate the effect of (peri)menopause on the results, since we had no menstrual data on the animals. If menopausal onset is most likely to occur after the age of 25 years, then a higher probability of misclassification could have been present in the 30 animals (10%) in this age group out of the 284 unaffecteds in our analyses. Considering that such differential misclassification would be much more likely for minimal-mild disease, which has a much lower prevalence, the impact of this potential bias is likely to be small. Moreover, any such bias would have resulted in an underestimation of the prevalence in the colony, and also of the familial aggregation indicators.

We were also unable to assess exposure to environmental factors in the present study, as exposure information between 1995 and 2002 was not yet available. Using data up to 1995, we previously reported an increased risk of endometriosis with increased exposure to hysterotomy and E2; no association was found with parity or number of laparoscopies (Hadfeld et al., 1996) and dioxin (Rier et al., 1993) and ionizing radiation (Fanton and Golden, 1991) are unlikely to have contributed to disease prevalence in the colony, and also of the familial aggregation indicators.

We were unable to investigate the effect of (peri)menopause on the results, since we had no menstrual data on the animals. If menopausal onset is most likely to occur after the age of 25 years, then a higher probability of misclassification could have been present in the 30 animals (10%) in this age group out of the 284 unaffecteds in our analyses. Considering that such differential misclassification would be much more likely for minimal-mild disease, which has a much lower prevalence, the impact of this potential bias is likely to be small. Moreover, any such bias would have resulted in an underestimation of the prevalence in the colony, and also of the familial aggregation indicators.

Acknowledgements
We thank Dr Amy Usborne and Mr Les Sander at the Wisconsin Primate Research Center, and Ms Melissa Luck at the Harlow Primate Laboratory for their help in updating pedigree information. We pay tribute to Dr Lodewijk Sandkuyt for his valuable ideas and comments on preliminary drafts of this paper. K.T.Z. is supported by an MRC Special Training Fellowship in Bioinformatics. The MRI studies were supported by Oxagen Ltd, Oxn, UK, with an educational grant. The WNPRC is supported by grant RR00167 from the National Institutes of Health and this is publication number 43-011 of the WNPRC.

References
Hadfield R, Mardon H, Barlow DH and Kennedy SH (1996) Delay in...
diagnosis of endometriosis: a survey of women from the USA and the UK. Hum Reprod 11,878–880.


Submitted on May 27, 2003; accepted on October 2, 2003