Genetic burden associated with varying degrees of disease severity in endometriosis

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ABSTRACT: Endometriosis is primarily characterized by the presence of tissue resembling endometrium outside the uterine cavity and is usually diagnosed by laparoscopy. The most commonly used classification of disease, the revised American Fertility Society (rAFS) system to grade endometriosis into different stages based on disease severity (I to IV), has been questioned as it does not correlate well with underlying symptoms, posing issues in diagnosis and choice of treatment. Using two independent European genome-wide association (GWA) datasets and top-level classification of the endometriosis cases based on rAFS [minimal or mild (Stage A) and moderate-to-severe (Stage B) disease], we previously showed that Stage B endometriosis has greater contribution of common genetic variation to its aetiology than Stage A disease. Herein, we extend our previous analysis to four endometriosis stages [minimal (Stage I), mild (Stage II), moderate (Stage III) and severe (Stage IV) disease] based on the rAFS classification system and compared the genetic burden across stages. Our results indicate that genetic burden increases from minimal to severe endometriosis. For the minimal disease, genetic factors may contribute to a lesser extent than other disease categories. Mild and moderate endometriosis appeared genetically similar, making it difficult to tease them apart. Consistent with our previous reports, moderate and severe endometriosis showed greater genetic burden than minimal or mild disease. Overall, our results provide new insights into the genetic architecture of endometriosis and further investigation in larger samples may help to understand better the aetiology of varying degrees of endometriosis, enabling improved diagnostic and treatment modalities.

Key words: endometriosis / rAFS classification system / genetic burden / polygenic prediction / genome-wide association studies

Introduction

Endometriosis is a complex gynaecological disease that affects 6–10% of women during reproductive age (Treloar et al., 1999; Montgomery et al., 2008) and 20–50% of women with infertility (Gao et al., 2006). The disease is primarily characterized by the presence of tissue resembling endometrium outside the uterine cavity, most commonly the pouch of Douglas, ovaries and peritoneum. The most common symptoms include severe pelvic pain, heavy or irregular menstrual bleeding and pain during intercourse and exercise; however, some women remain asymptomatic. Additional symptoms include infertility, lower abdominal and back pain, diarrhoea and/or constipation and chronic fatigue. Even though endometriosis is generally regarded as a benign disease, it can exhibit some characteristics of malignancy in terms of its progression and invasion of surrounding tissue (Giudice et al., 1998; Thomas and Campbell, 2000; Campbell and Thomas, 2001) and in rare cases can undergo malignant transformation (Heaps et al., 1990; Nyiraneza et al., 2010). Risk factors of endometriosis include age, increased exposure to menstruation (shorter cycle length, longer duration of flow and nulliparity) and other factors related to estrogen levels, including...
decreased body mass index and smoking history (Missmer et al., 2004, 2010).

Diagnosis of endometriosis is based on clinical suspicion, pelvic examination, ultrasound and magnetic resonance imaging, but can only be confirmed by laparoscopy, an invasive surgical procedure (Duleba, 1997; Spaczynski and Duleba, 2003). Based on location, diameter and depth of lesions and density of adhesions, there are varying degrees of endometriosis. According to the revised American Fertility Society (rAFS) classification system (American Fertility Society, 1985; American Society for Reproductive Medicine, 1997), the disease is classified into one of four stages (I—minimal, II—mild, III—moderate and IV—severe). Women with minimal or mild endometriosis have superficial implants and very few adhesions whereas moderate or severe endometriosis is generally characterized by chocolate cysts and more severe adhesions. However, despite this rAFS standardization, there is a lack of correlation between clinical symptoms and surgical findings, posing a significant challenge in disease diagnosis and choice of therapeutic modalities (Ripps and Martin, 1992; Kelz and Olive, 1993; Olive and Schwartz, 1993; Stovall et al., 1997). Patients presenting with minimal clinical symptoms may have advanced disease and conversely, women with infertility may have very few endometrial lesions. Consequently, the relevance—and biological basis for—the disease has an estimated total heritability of around 0.51 from twin studies (Treloar et al., 1999) and a common single nucleotide polymorphism (SNP) based heritability of 0.26 (Lee et al., 2013). Several independent genome-wide association (GWA) studies, including two by the International Endogene Consortium (IEC) (Painter et al., 2011; Nyholt et al., 2012), have corroborated the involvement of genetic factors in endometriosis. Earlier, we investigated the genetic burden of combined endometriosis stages [Stage A (rAFS Stage I or II) and Stage B (rAFS Stage III or IV)], using data from our GWA study for endometriosis (Painter et al., 2011). The proportion of variance in case–control status explained by all variants tagged by the common variants assayed in the GWA data (i.e. common SNP-based heritability) was significantly higher for Stage B endometriosis (0.35) than that of Stage A disease (0.15) (Painter et al., 2011). Similar results were observed when this ‘genetic loading’ was assessed by genetic risk scores derived from increasingly large SNP sets ranked on their statistical significance (Painter et al., 2011), indicating substantially greater genetic loading for Stage B disease.

Here, we extend our previous analysis by examining the genetic loading from common genetic variation for more refined categories of endometriosis stage [rAFS Stage I (minimal), rAFS Stage II (mild), rAFS Stage III (moderate) and rAFS Stage IV (severe) disease] and compare the overlap in genetic burden among varying degrees of endometriosis. Considering the significant contribution of genetic factors to risk of endometriosis, this investigation may improve our current understanding of disease heterogeneity and the underlying genetic architecture of different endometriosis stages.

Materials and Methods

GWA datasets

We used two GWA datasets of European ancestry from our previous multi-ethnic GWA meta-analysis for endometriosis (Nyholt et al., 2012). A detailed description of these datasets is presented elsewhere (Painter et al., 2011; Nyholt et al., 2012). Briefly, endometriosis cases (n = 3181) were recruited by the IEC (Australia (QIMR) = 2262; UK (OX) = 919) and all cases had a surgically confirmed diagnosis of endometriosis based on the medical records at the time of diagnosis. Disease stages were assessed retrospectively from surgical records by two independent gynaecologists with extensive experience in surgically diagnosing the disease (each for Australian and UK cases), following the rAFS classification system (American Fertility Society, 1985; American Society for Reproductive Medicine, 1997). Based on retrospectively assessed surgical records, Australian cases were assigned to different disease stages rASRM Stage I, II, III and IV. Because of uncertainty in ability to distinguish in particular between stages I and II based on retrospective assessment of records, the UK cases were grouped into Stage A (defined as peritoneal implants only) and Stage A+ (defined as some ovarian disease with some adhesions), and Stage B (rAFS III/IV). Both gynaecologists subsequently agreed there had been remarkable consistency in the way they had interpreted the clinical records, and that stage as recorded in the datasets could be combined by using the Stage B (rAFS III/IV) and Stage A (rAFS I/II or some ovarian disease with adhesions) classification. European ancestry-matched population controls (n = 8075) in the GWA data were from an Australian adolescent twin study (Wright and Martin, 2004), the Hunter Community Study (HCS) (McEvoy et al., 2010) and the Wellcome Trust Case–control Consortium 2 (WTCCC2) [combined Australia–HCS (QIMR-HCS) = 2924; UK (OX) = 5151]. The QIMR-HCS and OX samples were genotyped on Illumina 670Quad (cases) and 610Quad (controls) using the services from deCODE genetics. HCS controls were genotyped at the University of Newcastle using Illumina 610Quad Beadchips. The WTCCC2 controls were genotyped at the Welcome Trust Sanger Institute on Illumina HumanHap I M Beadchips.

Standard quality control measures were applied to individual QIMR-HCS and OX GWA datasets, as described earlier (Painter et al., 2011; Nyholt et al., 2012).

Genetic burden analysis

The aim of our genetic burden analysis was to evaluate the aggregate effects of many variants of small effect, using a prediction approach (International Schizophrenia Consortium et al., 2009). We summarized variation across nominally associated loci into quantitative genetic risk scores and related the scores to disease status in independent samples. Although variants of small effect (for example, with genotype relative risk of 1.05) are unlikely to achieve even nominal significance, increasing proportions of true effects will be detected at increasingly liberal P-value thresholds, for example, P < 0.1 (~10% of all SNPs). Using such thresholds, we defined large sets of allele-specific scores in the discovery sample of the QIMR-HCS case–control set, by stratifying the total endometriosis cases (n = 2262) into one of four disease stages [rAFS Stage I (n = 832), rAFS Stage II (n = 491), rAFS Stage III (n = 482) and rAFS Stage IV (n = 267) disease] to generate risk scores for individuals in the target sample of the OX case–control set, by stratifying the total cases (n = 919) into one of three disease stages [Stage A (n = 199), Stage A+ (n = 114) and Stage B (n = 380) disease]. As a result, the QIMR-HCS and OX datasets consisted of four and three subsets, respectively. Endometriosis cases in both the QIMR-HCS and OX case–control sets with unknown disease stages were excluded from the analysis. Furthermore, for each of the subsets created from the QIMR-HCS and OX datasets, we used 2924 and 5151 common controls, respectively.
The term risk score is used instead of risk, as it is impossible to differentiate the minority of true risk alleles from the non-associated variants. In the discovery sample, we selected sets of allele-specific scores for SNPs with the following levels of significance: $P < 0.01, 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9$ and $1.0$. For each individual in the target sample, we calculated the number of scored alleles that they possessed, each weighted by the log odds ratio (OR) from the discovery sample. To assess whether the aggregate scores reflect endometriosis risk, we tested for a higher mean score in cases compared with controls. Logistic regression was used to assess the relationship between target sample disease status and aggregate risk score, without any covariates. Nagelkerke’s pseudo $R^2$ from the logistic regression analysis was used to assess the variance explained. Considering males in our control population, prediction was performed using only 488,833 autosomal SNPs overlapping in the QIMR-HCS and OX GWA datasets to avoid potential bias due to varying number of alleles in X chromosome between males and females. We also performed the predictions in reverse, using risk scores from the OX sample to predict affected status in the QIMR-HCS case–control set. Additional prediction analysis was conducted using potentially independent SNPs ($n \approx 128,000$) obtained from $P$-value based linkage-disequilibrium (LD) clumping in Plink (-clump-p1 1 -clump-p2 1 -clump-r2 0.2 -clump-kb 500) to see if the results are biased by SNPs in high LD.

### Association analysis excluding minimal endometriosis

We further examined if true genetic effects are enriched after excluding endometriosis cases with minimal disease. As an illustrative example, we used the seven previously implicated SNPs in endometriosis (rs7521902, rs13394619, rs4141819, rs7739264, rs12700667, rs1537377 and rs10859871) (Nyholt et al., 2012). We performed allelic association tests for these SNPs in the QIMR-HCS case–control dataset, after excluding endometriosis cases with minimal disease (rAFS Stage I disease). Similar analysis after excluding Stage A disease was also conducted in the OX case–control dataset.

### Results

The genetic risk scores derived from different endometriosis stages in the discovery sample of the QIMR-HCS case–control set significantly predicted case–control status in the corresponding disease stages in the target sample of the OX case–control set and vice-versa (Figs 1 and 2; and Table I). The prediction results using all ($n = 488,833$) and potentially independent SNPs ($n \approx 128,000$) (data not shown) were not fundamentally different and hence results from all SNPs are presented. Stage A

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**Figure 1** Allele-specific score prediction for endometriosis, using Stages A, A+, and B in the OX case–control set as the target population and the QIMR-HCS case–control set as the discovery population. The proportion of variance explained in the target dataset on the basis of allele-specific scores derived from the discovery dataset is represented by Nagelkerke’s pseudo $R^2$, with its statistical significance ($P$). Among the 12 significance thresholds used in each prediction analysis, the most significant result (i.e. best $P$-value) is represented by arrow with corresponding $R^2$ (above) and its $P$-value (below). The colour of the arrows is graded based on the $R^2$ significance—the darker the arrow, the stronger the $R^2$ significance.
endometriosis cases in the OX case–control set were significantly predicted by rAFS Stage I disease in the QIMR-HCS case–control set, with the smallest $P$-value ($P = 3.15 \times 10^{-23}$) obtained for a genetic risk score including $\approx 10\%$ of the SNPs (Fig. 1 and Supplementary data, Fig. S1a). The result was significant, although the proportion of variance explained was small (maximum Nagelkerke’s pseudo $R^2$ of 0.006; 0.60% of the variance). Genetic risk scores generated from other disease stages in the QIMR-HCS case–control set (rAFS Stage II–IV) did not predict the Stage A endometriosis cases in the OX case–control set (Fig. 1 and Supplementary data, Fig. S1b–d).

QIMR-HCS rAFS Stage I disease also significantly predicted Stage A+ endometriosis cases in the OX case–control set, with the smallest $P$-value ($P = 2.12 \times 10^{-10}$) obtained for a genetic risk score including $\approx 5\%$ of the SNPs (Fig. 1 and Supplementary data, Fig. S2a), which explained 0.95% of the proportion of variance. Similar prediction was also observed for the top $\approx 10\%$ of the SNPs. Stage A+ cases in the OX case–control set were also predicted by the QIMR-HCS rAFS Stage II and III disease (Fig. 1, Supplementary data, Fig. S2b and c); however, the results were weaker ($P = 0.035$ and 0.051, respectively) and the variance explained was smaller than that of rAFS Stage I endometriosis.
SNPs to RefSeq genes if they were positioned within 20 kb from the transcription start and stop sites of a gene. SNPs outside this region were considered to be intergenic. Accordingly, of the 488 833 autosomal SNPs, 295 424 were assigned to genes, 193 409 were intergenic, 4492 were non-synonymous and 23 362 were rare variants. The proportion of variance explained by genic SNPs was slightly higher [QIMR-HCS predicting OX: $R^2 = 0.67\%$; $P = 1.35 \times 10^{-4}$ and OX predicting QIMR-HCS: $R^2 = 1.3\%$; $P = 2.00 \times 10^{-5}$] as compared with intergenic SNPs (QIMR-HCS predicting OX: $R^2 = 0.25\%$; $P = 0.017$ and OX predicting QIMR-HCS: $R^2 = 0.41\%$; $P = 0.016$) (Supplementary data, Figs S7a and S8a and b). Additional analysis restricted to non-synonymous SNPs did not predict either group (QIMR-HCS predicting OX: $R^2 = 0.29\%$; $P = 0.012$ and OX predicting QIMR-HCS: $R^2 = 0.18\%$; $P = 0.012$; Supplementary data, Figs S7c and S8c), with similar results obtained for rare variants (QIMR-HCS predicting OX: $R^2 = 0.10\%$; $P = 0.13$ and OX predicting QIMR-HCS: $R^2 = 0.31\%$; $P = 0.037$; Supplementary data, Figs S7d and S8d). These data indicate that common SNPs within or close to genes, excluding non-synonymous variants, may contribute the bulk of the variance tagged by genome-wide autosomal SNPs; however these findings should be further investigated in larger sample size. Comparable predictions were obtained when we excluded SNPs within the eight robustly implicated endometriosis risk loci (defined by 2500 kb up- and downstream of the sentinel SNPs) that are polymorphic in Europeans (Nyhoff et al., 2012; Sapkota et al., 2015), indicating that our results were not primarily driven by the few most strongly associated regions (Supplementary data, Fig. S9).

When we tested allelic associations of the top seven SNPs previously implicated in endometriosis after excluding QIMR-HCS cases with minimal disease (rAFS Stage I disease), except for rs13394619, we observed a slight increase in effect sizes compared with the effects estimated using all cases (Table II). These results were consistent in the independent OX case–control dataset, as well as in the combined (QIMR-HCS+OX) dataset.

**Discussion**

Endometriosis is an estrogen-dependent disorder affecting relatively younger women. Based on severity of the disease, endometriosis has been grouped into multiple stages, including minimal, mild, moderate, severe and more severe disease (American Fertility Society, 1985; American Society for Reproductive Medicine, 1997). We previously showed that moderate-to-severe (Stage B) endometriosis cases have higher common genetic variant contribution to aetiology compared with minimal or mild (Stage A) endometriosis (Painter et al., 2011). To investigate the relationship between disease stages in more detail, we re-assessed the genetic loading of endometriosis stages, using more refined disease stages of endometriosis cases in two European GWA datasets from the multi-ethnic GWA meta-analysis for endometriosis (Nyhoff et al., 2012). For the purpose of gene discovery, the original top-level classification, including minimal or mild (Stage A) and moderate-to-severe (Stage B) endometriosis remains the most powerful and appropriate, and prediction between the QIMR-HCS and OX datasets for this classification remains significantly strongest. However, our more detailed results extend our earlier observations and show the genetic burden increases progressively for cases diagnosed from less to more severe disease, in agreement
Table II  Effect sizes of the top seven endometriosis loci, with and without including endometriosis cases with minimal disease.

<table>
<thead>
<tr>
<th>Chr</th>
<th>SNP</th>
<th>Position (bp)</th>
<th>RA</th>
<th>OA</th>
<th>QIMR-HCS All cases OR (95% CI)</th>
<th>P</th>
<th>QIMR-HCS Excluding Stage I cases OR (95% CI)</th>
<th>P</th>
<th>OX All cases OR (95% CI)</th>
<th>P</th>
<th>OX Excluding Stage A cases OR (95% CI)</th>
<th>P</th>
<th>QIMR-HCS + OX All cases OR (95% CI)</th>
<th>P</th>
<th>QIMR-HCS + OX Excluding minimal disease OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
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<tr>
<td>1</td>
<td>rs7521902</td>
<td>22490724</td>
<td>A</td>
<td>C</td>
<td>1.16 (1.06–1.27)</td>
<td>8.89E-04</td>
<td>2.06E-04</td>
<td>1.12 (1.00–1.26)</td>
<td>4.97E-02</td>
<td>7.63E-02</td>
<td>1.15 (1.07–1.23)</td>
<td>1.17</td>
<td>6.83E–05</td>
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<tr>
<td>2</td>
<td>rs13394619</td>
<td>11727507</td>
<td>G</td>
<td>A</td>
<td>1.10 (1.02–1.19)</td>
<td>1.73E-02</td>
<td>2.30E-07</td>
<td>1.16 (1.05–1.29)</td>
<td>4.52E-03</td>
<td>8.46E-03</td>
<td>1.17 (1.10–1.25)</td>
<td>1.24</td>
<td>1.29E–08</td>
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<tr>
<td>2</td>
<td>rs4141819</td>
<td>67864675</td>
<td>C</td>
<td>T</td>
<td>1.17 (1.08–1.27)</td>
<td>7.48E-04</td>
<td>3.81E-04</td>
<td>1.18 (1.06–1.30)</td>
<td>1.33E-03</td>
<td>2.74E-03</td>
<td>1.16 (1.09-1.23)</td>
<td>1.18</td>
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<tr>
<td>6</td>
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<td>19785588</td>
<td>T</td>
<td>C</td>
<td>1.15 (1.05–1.23)</td>
<td>5.35E-06</td>
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<td>3.19E-06</td>
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<td>4.76E-03</td>
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Chr, chromosome; position, chromosomal position (bp) based on Human Build 37 (GRCh37/hg19); RA, risk allele; OA, other allele; OR, odds ratio; CI, confidence interval.
with previous results showing stronger evidence for genetic contribution to severe disease. Stronger results were observed from severe disease (rAFS Stage IV) in QIMR-HCS as discovery sample predicting Stage B disease in OX and the reverse. Despite small sample sizes, results from different disease stages from QIMR-HCS as discovery sample provide the best prediction for equivalent stages in the OX sample.

While there is a general trend for stronger genetic contributions to severe disease, the prediction of Stage A and A+ diseases in the OX from rAFS Stage I in QIMR-HCS suggest that weaker genetic effects in the early stage of endometriosis are not just the consequence of difficulties in diagnosis. There may be differences in genetic architecture between minimal and the more severe stages of disease. The observed weaker genetic effects in minimal disease were further supported by a slight enrichment in effect sizes of previously implicated endometriosis loci, after excluding cases with minimal disease (Table 1).

There is genetic overlap between minimal—mild (Stage A) and moderate-to-severe (Stage B) disease. However, in the current study, the early stages of endometriosis exhibit evidence for different genetic risk compared with the other disease stages. Risk scores derived from the early stage of endometriosis could predict themselves only (Fig. 1 and Supplementary data, Figs S1 and S4). Minimal endometriosis in the QIMR-HCS case—control set predicted mild disease in the OX case—control set (Fig. 1 and Supplementary data, Fig. S2a), indicating some shared aetiology between these early stages of endometriosis that may be different from more severe disease stages. When the analysis was reversed, OX minimal disease provided weaker prediction of minimal endometriosis cases in the QIMR-HCS dataset (Fig. 2 and Supplementary data, Fig. S4a) but not other stages of disease. There is reduced statistical power using the OX dataset for prediction because of the number of minimal endometriosis cases in the OX case—control set (n = 199) compared with the QIMR-HCS case—control set (n = 832). Further investigation with a larger sample size is required to confirm these emerging hypotheses.

Higher shared genetic burden was observed between mild and moderate endometriosis cases. Both mild and moderate endometriosis cases in the QIMR-HCS case—control set predicted multiple disease stages, including mild disease in the OX case—control set (Fig. 1 and Supplementary data, Fig. S2b and c) and vice-versa (Fig. 2 and Supplementary data, Fig. S5b and c). Furthermore, the QIMR-HCS mild disease also predicted moderate-to-severe endometriosis cases in the OX case—control set (Fig. 1 and Supplementary data, Fig. S3b) and similar results were obtained from the reverse analysis (Fig. 1 and Supplementary data, Fig. S6b). While moderate-to-severe (Stage B) disease in the OX sample was predicted by risk scores for mild, moderate and severe diseases, shifts in prediction moved from moderate to severe cases. The OX Stage A+ disease was predicted by risk scores for QIMR-HCS mild (rAFS Stage II) and moderate (rAFS Stage III) disease (Fig. 1 and Supplementary data, Fig. S2a—c). Consistent with our previous results (Painter et al., 2011), moderate-to-severe endometriosis exhibited greater genetic burden, for which the proportion of variance explained by genetic risk scores was higher (up to 1.3%) compared with minimal or mild disease. Both moderate and severe endometriosis cases in the QIMR-HCS case—control set predicted moderate-to-severe disease in the OX case—control set (Fig. 1, Supplementary data, Fig. S3c and d) and the reverse analysis corroborated these results (Fig. 2 and Supplementary data, Fig. S6c and S6d).

These results should be interpreted in consideration of several limitations. First, disease stages of endometriosis cases were scored retrospectively, which may be less accurate than a prospective grading system. Second, the rAFS scores used to classify disease stage do not correlate well with all clinical features and that this classification system, even though the most commonly used, has obvious drawbacks and that another classification system often used—peritoneal versus ovarian (and rectovaginal) is confounded with the rAFS system. As suggested by the fact that Stage A+ in OX is more strongly predictive of rAFS Stage III, not Stage II in QIMR-HCS, we may be looking at genetic differences between peritoneal versus ovarian disease. In addition to the traditional rAFS classification system, other grading systems including the ENZIAN classification (Tuttles et al., 2005) and the endometriosis fertility index (Adamson and Pasta, 2010) have been suggested, which may improve endometriosis staging. Genetic marker data can provide insights into similarities and differences in disease architecture, hence, there is a need for systematic studies on large samples with detailed clinical and/or disease presentation including prospective data on rAFS stage. Finally, although current sample sizes lack power to identify enough genetic risk factors to enable accurate prediction, they have good power to identify associations that provide valuable insight into the underlying genetics of complex diseases, including endometriosis (Dudbridge, 2013). The risk prediction will become more feasible as sample sizes for the association analyses grow several orders of magnitude greater than current endometriosis studies.

In conclusion, extending our analysis of genetic risk scores derived from two large European GWA datasets from our previous multi-ethnic GWA meta-analysis of endometriosis, we show genetic factors contribute to minimal disease and may differ from more severe endometriosis. As shown by the higher proportion of variance explained, more severe endometriosis cases exhibited greater genetic loading than minimal or mild disease. The genetic burden generally increased from less severe (minimal) to more severe disease, consistent with disease progression. There was significant prediction for minimal disease between independent datasets, suggesting the minimal disease may be ‘disease’ rather than a physiological phenomenon; however, the genetic contribution to this group was weaker and hence excluding these minimal endometriosis cases may improve association signals in GWA studies for endometriosis. Consequently, well-defined diagnostic and treatment modalities considering both the genetic differences and overlaps among the different stages of endometriosis may be required for a better management of the disease.

**Supplementary data**

Supplementary data are available at http://molehr.oxfordjournals.org/online.

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Authors’ roles

Y.S.: design (QIMR part), analysis and interpretation, drafting manuscript and final approval; J.A.: design (HCS part), revision for critical content and final approval; S.D.G.: design (QIMR part), revision for critical content and final approval; A.K.H.: design (QIMR part), revision for critical content and final approval; E.G.H.: design (HCS part), revision for critical content and final approval; N.R.: design (OX part), revision for critical content and final approval; S.M.: design (QIMR part), revision for critical content and final approval; N.G.M.: design and sample collection (QIMR part), revision for critical content and final approval; M.M.: design (HCS part), revision for critical content and final approval; A.P.M.: design (OX part), revision for critical content and final approval; R.J.S.: design (HCS part), revision for critical content and final approval; K.T.Z.: conception and design (OX part), revision for critical content and final approval; G.W.M.: conception and design (QIMR part), interpretation, revision for critical content and final approval; D.R.N.: conception and design (QIMR part), interpretation, revision for critical content and final approval.

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Conflict of interest

None declared.

References


